

Enhancing the Mechanical Properties of Supramolecular Guanosine Hydrogels with Guanosine-Containing Polymers

*Amanda E. Way,¹ Angie B. Korpusik,^{1,2} Taylor B. Dorsey,² Lauren E. Buerkle,¹ Horst Von
Recum,^{1,2} Stuart J. Rowan^{1,2,*}*

¹ Department of Macromolecular Science and Engineering, Case Western Reserve University,
2100 Adelbert Road, Cleveland, OH 44106, USA

² Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH
44106, USA

Table of Contents:	S1-3
Materials	S4
Instrumentation	S4
VT-NMR	S5
Figure S1 VT-NMR of 1 wt.% co-gel of 8OMeTAcG and 1	S6
Figure S2 VT-NMR of 1 wt.% co-gel of 8OMeTAcG and 2	S7
Figure S3 Stress sweep experiments of 100 mM NaCl gels of 8OMeTAcG:1 and 8OMeTAcG:2	S8
Figure S4 Stress vs. Strain curves of 100 mM NaCl gels of 8OMeTAcG:1 and 8OMeTAcG:2 from stress sweeps	S8
Table S1 Polymerization Data for 3_x and 4₄	S9
Table S2 Polymer co-gel wt.% compositions	S9
Figure S5 VT-NMR of 1 wt.% co-gel of 8OMeTAcG and 3₄	S10
Figure S6 VT-NMR of 1 wt.% co-gel of 8OMeTAcG and 4₄	S11
Figure S7 TEM images of (a) 8OMeTAcG:2 and (b) 8OMeTAcG:4₄ .	S12
Figure S8 AFM images of 8OMeTAcG:1 and (b) 8OMeTAcG:3₄	S13

Figure S9 ^1H NMR (D_2O) 1:0.25 8OMeTAcG:3₄ , 3₇ , 3₁₀ , 1:1 8OMeTAcG:3₀ , 3₇ , 3₁₀ , and 1:1.5 8OMeTAcG:3₄ , 3₇ , 3₁₀	S14
Figure S10 Comparisons of the storage modulus (G') of (a) 1:0.25 8OMeTAcG: 3_x ; (b) 1:0.5 8OMeTAcG: 3_x and (c) 1:1.5 8OMeTAcG:3_x co-gels in 100 mM NaCl.	S15
Figure S11 Stress sweep experiments of 100 mM NaCl gels of 8OMeTAcG:4₄ and b) 8OMeTAcG:3₄ ; (c) 8OMeTAcG:3₇ ; (d) 8OMeTAcG:3₁₀	S16
Figure S12 Stress vs. strain curves of 100 mM NaCl gels of (a) 8OMeTAcG:4₄ ; (b) 8OMeTAcG:3₄ ; (c) 8OMeTAcG:3₇ ; (d) 8OMeTAcG:3₁₀ from stress sweeps	S17
Synthetic procedures	S18
Figure S13 ^1H NMR of 2',3'-O-Isopropylideneguanosine in DMSO.	S19
Figure S14 ^{13}C NMR of 2',3'-O-Isopropylideneguanosine in DMSO.	S20
Figure S15 MALDI-MS spectra of 2',3'-O-Isopropylideneguanosine	S21
Figure S16 ^1H NMR of 2',3'-O-isopropylidene-guanosine-5'-carboxylic acid (5).	S22
Figure S17 ^{13}C NMR of 2',3'-O-isopropylidene-guanosine-5'-carboxylic acid (5)	S23
Figure S18 MALDI-MS of 2',3'-O-isopropylidene-guanosine-5'-carboxylic acid (5)	S24
Figure S19 ^1H NMR of guanosine-5'-carboxylic acid (6)	S25
Figure S20 ^{13}C NMR of guanosine-5'-carboxylic acid (6)	S26
Figure S21 MALDI-MS of guanosine-5'-carboxylic acid (6)	S27
Figure S22 ^1H NMR of 8-bromoguanosine-5'-carboxylic acid (7)	S28
Figure S23 ^{13}C NMR of 8-bromoguanosine-5'-carboxylic acid (7)	S29
Figure S24 MALDI-MS 8-bromoguanosine-5'-carboxylic acid (7)	S30
Figure S25 ^1H NMR of 8-methoxyguanosine-5'-carboxylic acid (8)	S31
Figure S26 ^{13}C NMR of 8-methoxyguanosine-5'-carboxylic acid (8)	S32
Figure S27 MALDI-MS 8-methoxyguanosine-5'-carboxylic acid (8)	S33
Figure S28 ^1H NMR of 8-methoxy-2',3'-diacetylguanosine-5'-carboxylic (9)	S35
Figure S29 ^{13}C NMR of 8-methoxy-2',3'-diacetylguanosine-5'-carboxylic (9)	S35
Figure S30 MALDI-MS 8-methoxy-2',3'-diacetylguanosine-5'-carboxylic (9)	S36
Figure S31 ^1H NMR of 2',3'-diacetylguanosine-5'-carboxylic (10)	S37
Figure S32 ^{13}C NMR of 2',3'-diacetylguanosine-5'-carboxylic (10)	S37
Figure S33 MALDI-MS 2',3'-diacetylguanosine-5'-carboxylic (10)	S38
Figure S34 ^1H NMR of 1	S40

Figure S35 ^{13}C NMR of 1	S40
Figure S36 MALDI-MS 1	S41
Figure S37 ^1H NMR of 2	S42
Figure S38 ^{13}C NMR of 2	S42
Figure S39 MALDI-MS 2	S43
Table S3 Summary of the complex modulus data for the gels	S44
RAFT Polymerization	S45
Gel Formation	S45
References	S45

Materials

70% Perchloric acid, hydrochloric acid, sodium bicarbonate, and triethylamine were purchased from Fisher Scientific. Iodobenzene diacetate and benzyl chloroformate were purchased from TCI America. 4-Dimethylaminopyridine (DMAP) was purchased from Alfa Aesar. All other chemicals were purchased from Sigma-Aldrich and used without further purification. 4-Cyano-4-(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid was synthesized according to literature.¹ 2,2'-(Ethylenedioxy)bis(ethylamine)mono acrylamide was synthesized according to literature.²

Instrumentation

Rheological measurements were taken on a MARS III rheometer with a 20 mm 2° cone-shaped geometry for all experiments. For oscillatory stress sweeps, the samples were subjected to a τ of 0.1 to 1000 Pa at a ω of 6.28 rad/s⁻¹. Frequency sweeps were performed from .01 to 100 rad/s.. NMR spectra were recorded on a Varian Inova 600 MHz NMR spectrometer (¹³C NMR = 150.8 MHz) using deuterated solvents purchased from Norell, Inc. Mass spectra were obtained using a Bruker AUTOFLEX III MALDI TOF/TOF mass spectrometer using α -cyano-4-hydroxycinnamic acid as a matrix and sodium trifluoroacetate. DSC measurements were completed on a TA Instruments Q2000 DSC instrument in hermetic pans at a heating rate of 3 °C/min. 0.5 wt.% gels were drop-cast onto copper coated grids for 5 mins and stained with palladium (1 nm). TEM was performed using a Zeiss LIBRA 200 FE. 0.5 wt.% gels were drop-cast onto mica and washed with water 2x to remove the salt crystals and analyzed under ambient conditions on a Veeco atomic force microscopy (AFM) instrument in contact mode. The tips were silicon with an oscillating frequency of 150 kHz and a spring constant of 5 N/m. The tips

used had a radius of <10 nm. Both height and phase images were collected. Circular dichroism was run on a Jasco J-810 CD spectrometer from 325-500 nm with 5 scans per sample in a 0.1 mm quartz cuvette.

VT-NMR

All experiments were run in D₂O with 5 μ L of dioxane as an internal standard. 1 wt. % samples were run on a 600 MHz NMR, and scanned every 10°C as the sample cooled from 75°C to 25°C.

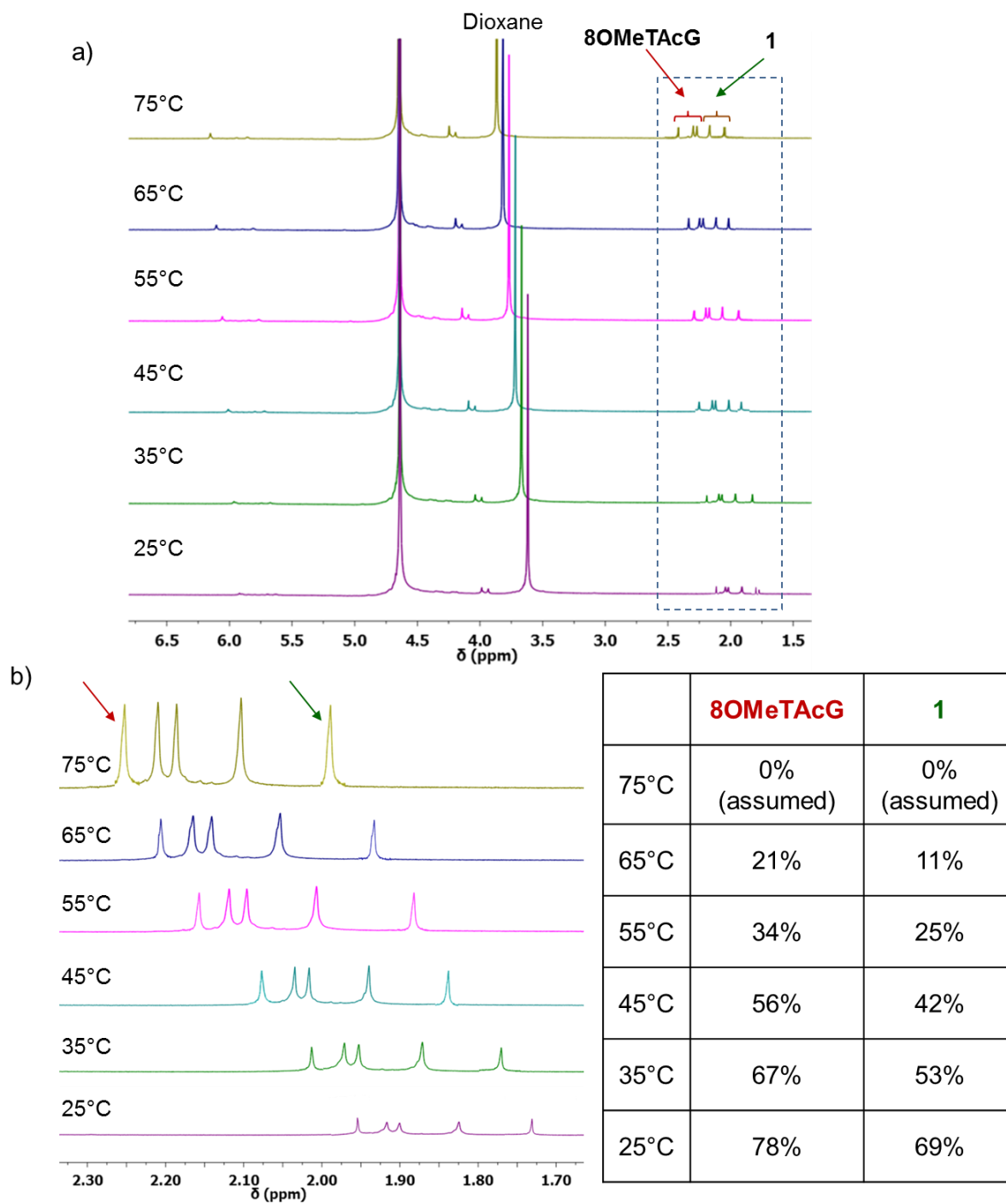


Figure S1. VT-NMR of co-gel of 0.5 wt.% **8OMeTAcG** and **1** (molar ratio 1:1) (a) full view and (b) zoomed in on acetyl groups along with table of % incorporation of guanosine moieties in the gel phase at various temperatures.

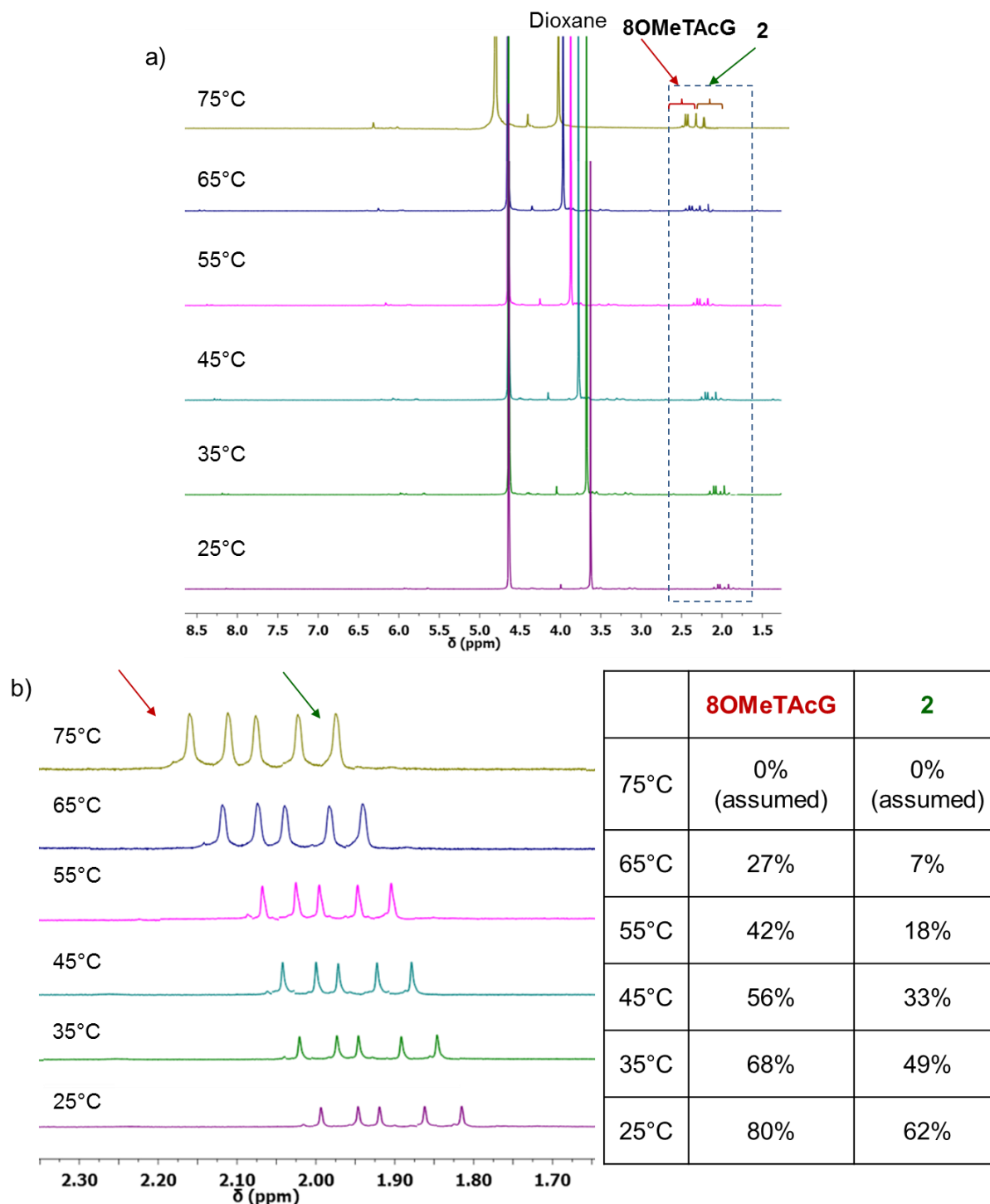


Figure S2. VT-NMR of co-gel of 0.5 wt.% **8OMeTAcG** and **2** (molar ratio 1:1) (a) full view and (b) zoomed in on acetyl groups along with table of % incorporation of the different components in the gel phase at various temperatures.

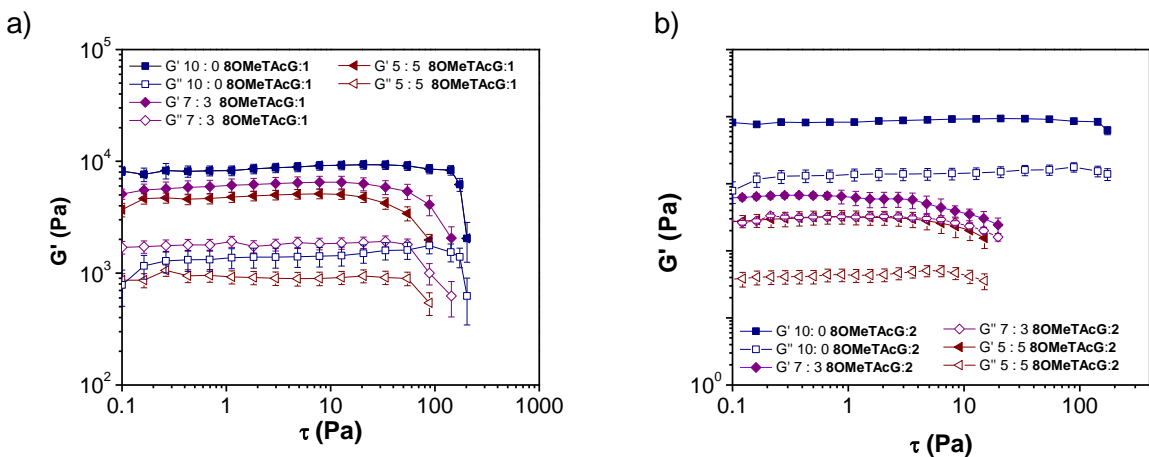


Figure S3. Stress sweep experiments of 100 mM NaCl gels of (a) **8OMeTAcG:1** and (b) **8OMeTAcG:2** showing G' (closed symbols) and G'' (open symbols). $\omega = 6.28 \text{ rad/s}^{-1}$, $T = 25^\circ\text{C}$.

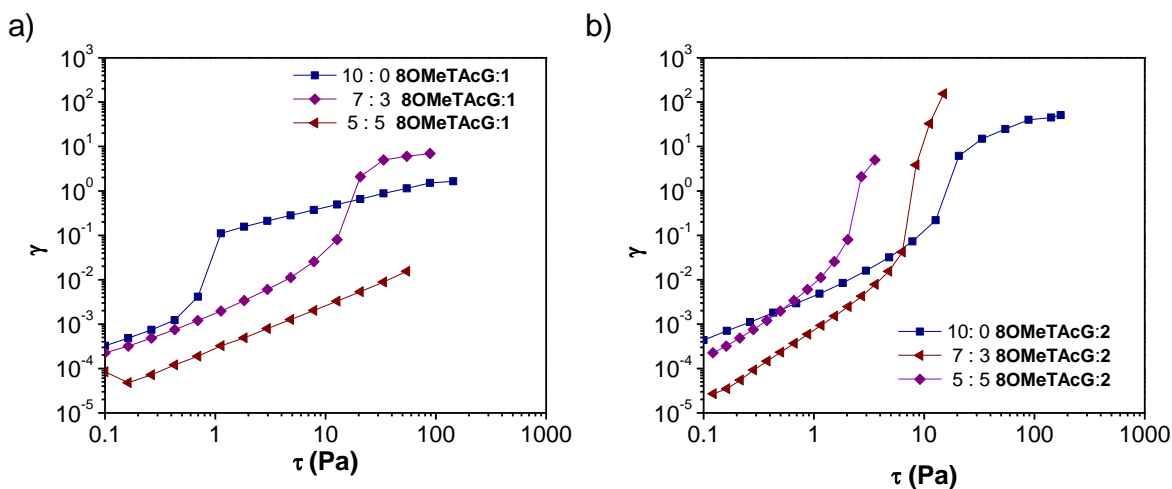


Figure S4. Stress vs. Strain curves of 100 mM NaCl gels of (a) **8OMeTAcG:1** and (b) **8OMeTAcG:2** obtained from stress sweeps showing the yield stress of the materials at maximum strain. $\omega = 6.28 \text{ rad/s}^{-1}$, $T = 25^\circ\text{C}$.

Polymer	M _n , Theory (g/mol)	Feed Ratio G:DMA	M _n , ¹ H NMR (g/mol)	G:DMA (¹ H NMR)
3₀	3,000	-	2,800	0:28
3₄	5,400	5:25	4,500	4:21
3₇	7,900	10:20	6,000	7:18
3₁₀	10,400	15:15	7,400	10:15
4₄	5,300	5:25	4,300	4:21

Table S1. Polymerization Data for **3_x** and **4₄**.

Ratio 8OMeTAcG:3 _x or 4 ₄	Wt. % 8OMeTAcG	Wt. % 3 _x or 4 ₄	Wt. % of G Moieties in Polymer	Molar Equiv. G to 8OMeTAcG
1:1, 3₀	1	2.23	0	0
1:0.25, 3₄	1	0.56	0.36	0.25
1:0.5, 3₄	1	1.12	0.73	0.5
1:1, 3₄	1	2.23	1.45	1
1:1.5, 3₄	1	3.35	2.18	1.5
1:0.25, 3₇	1	0.52	0.36	0.25
1:0.5, 3₇	1	1.04	0.73	0.5
1:1, 3₇	1	2.07	1.45	1
1:1.5, 3₇	1	3.11	2.18	1.5
1:0.25, 3₁₀	1	0.45	0.36	0.25
1:0.5, 3₁₀	1	0.91	0.73	0.5
1:1, 3₁₀	1	1.81	1.45	1
1:1.5, 3₁₀	1	2.72	2.18	1.5
1:0.25, 4₄	1	0.66	0.345	0.25
1:0.5, 4₄	1	1.33	0.69	0.5
1:1, 4₄	1	2.65	1.38	1
1:1.5, 4₄	1	4.00	2.07	1.5

Table S2. Polymer co-gel wt.% compositions.

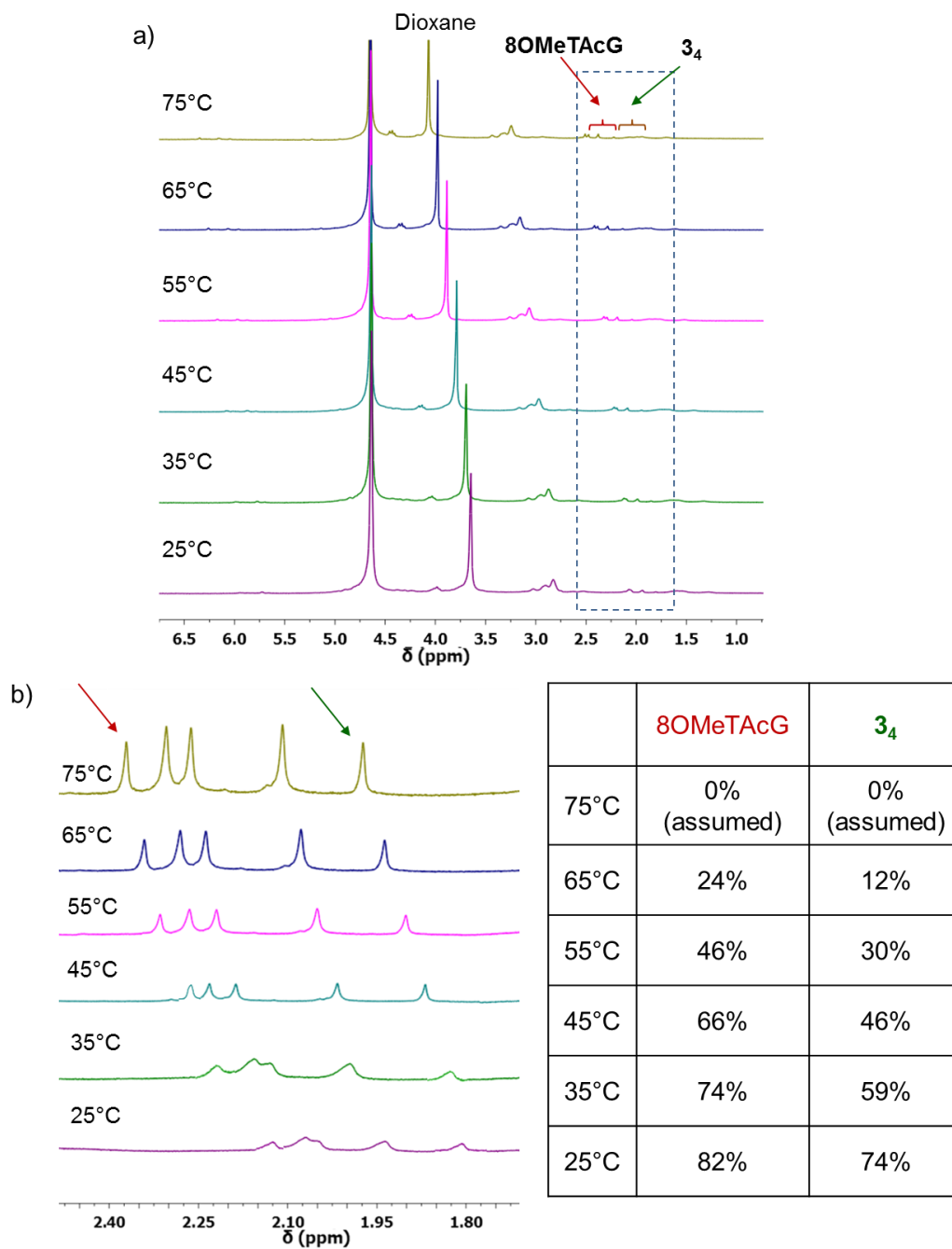


Figure S5. VT-NMR of co-gel of 0.5 wt.% of 8OMeTAcG and **3₄** (ratio 1:1 based on guanosine repeats in **3₄**) (a) full view and (b) zoomed in on acetyl groups along with table of % incorporation in the gel phase at various temperatures.

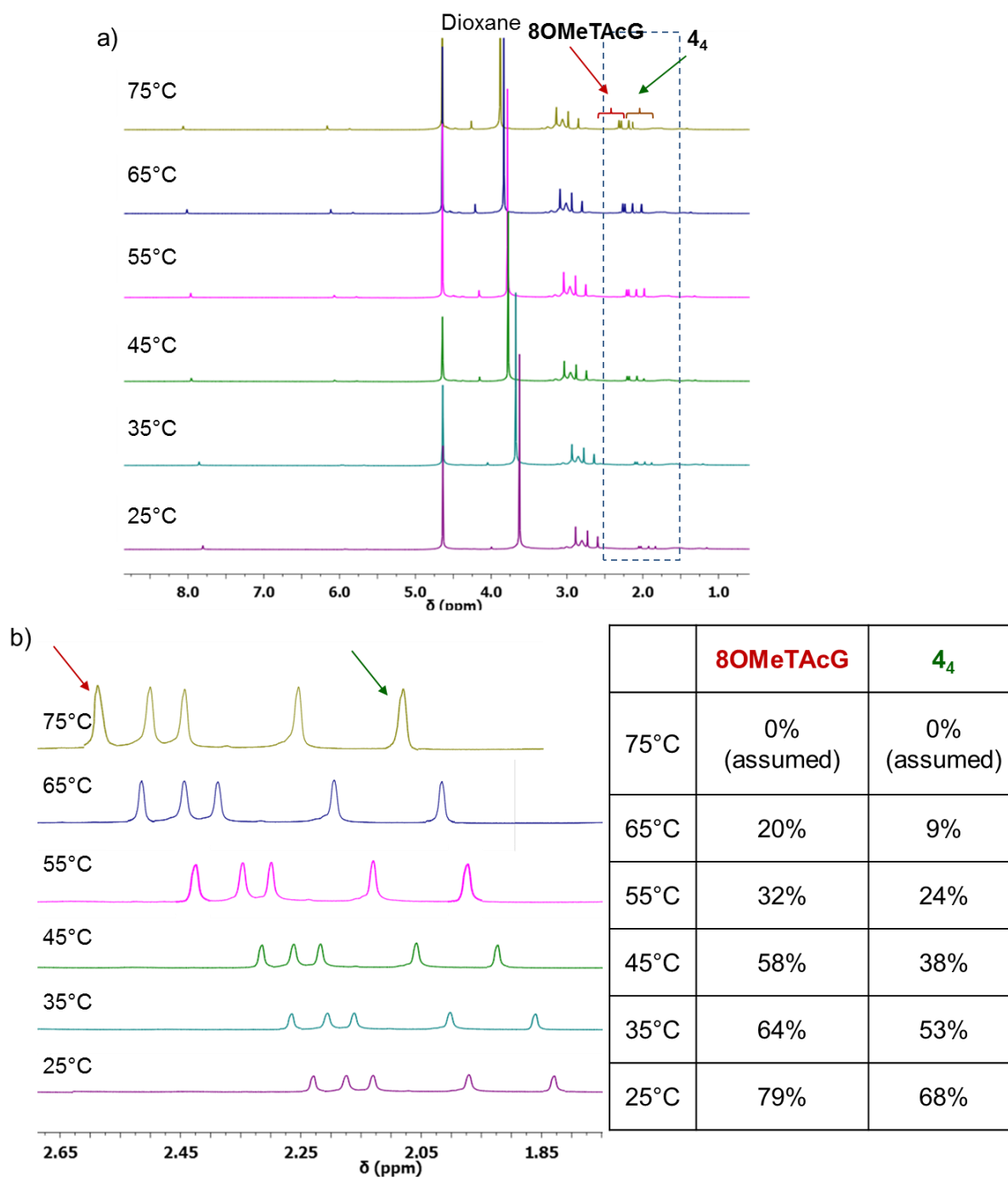


Figure S6. of co-gel of 0.5 wt.% of **8OMeTAcG** and **4₄** (ratio 1:1 based on guanosine repeats in **4₄**) (a) full view and (b) zoomed in on acetyl groups along with table of % incorporation of the components in the gel phase at various temperatures.

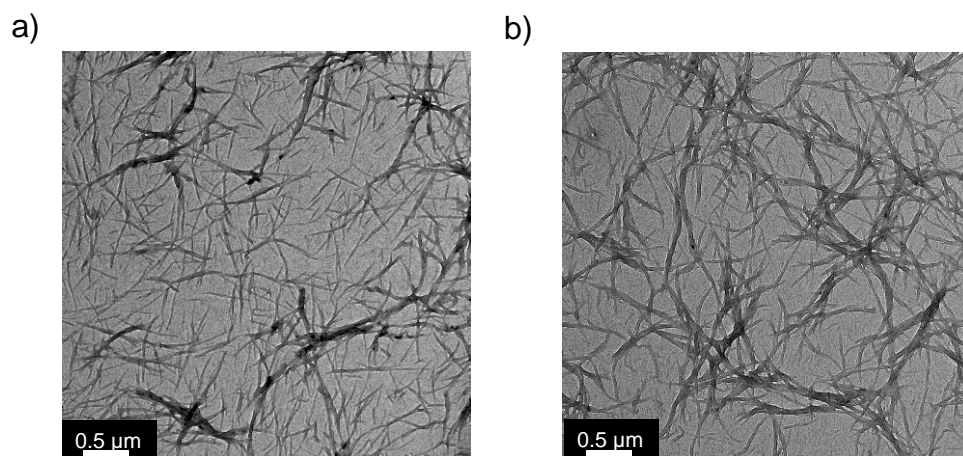


Figure S7. TEM images of (a) **8OMeTAcG:2** and (b) **8OMeTAcG:4₄** in 100 mM NaCl.

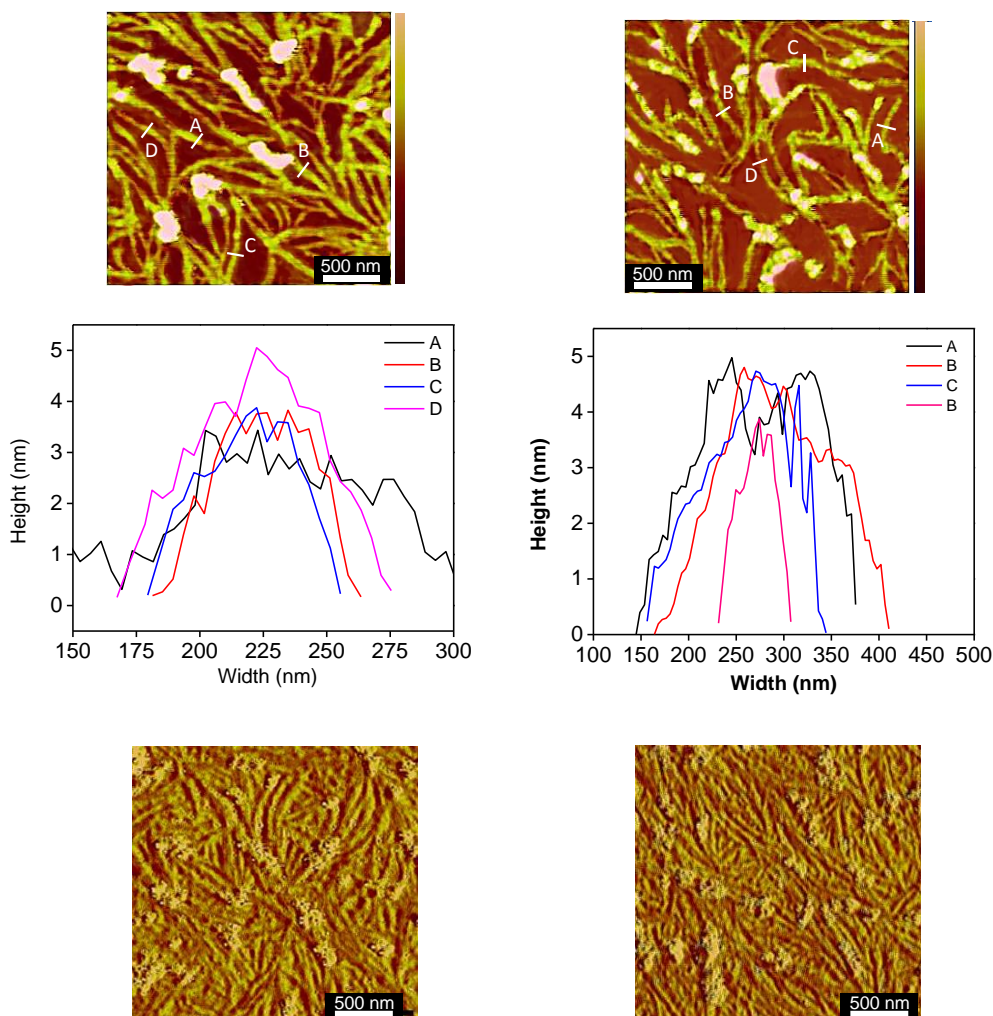
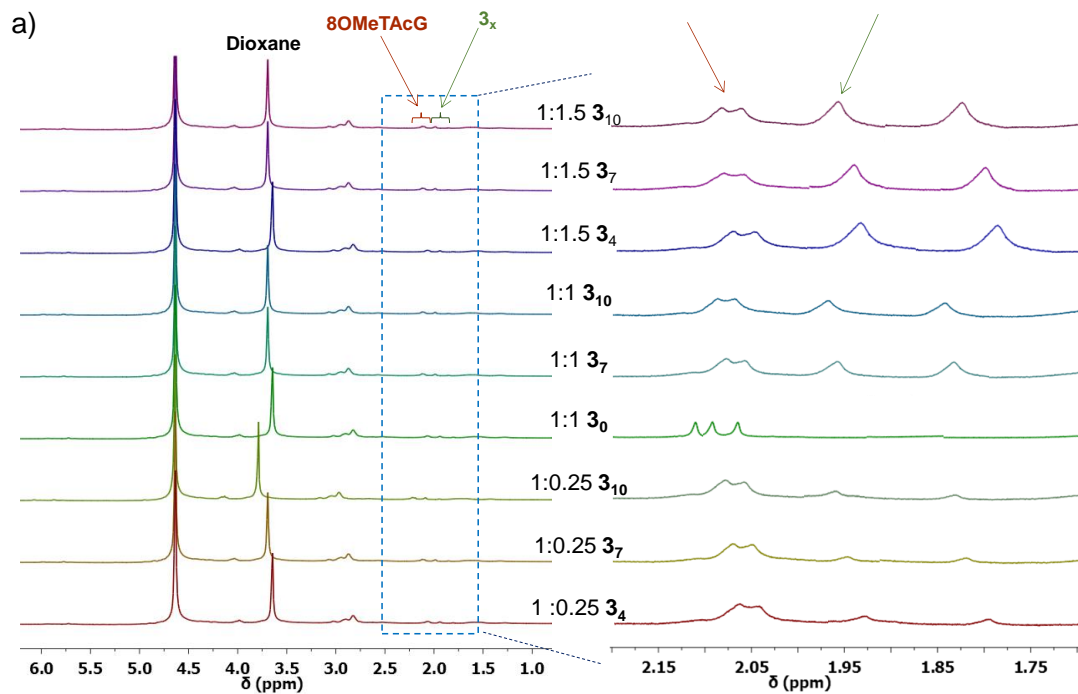


Figure S8. AFM images of (a) 0.5 wt.% **8OMeTAcG:1** (left) (molar ratio 1:1) and 0.5 wt.% **8OMeTAcG:3₄** (molar ratio 1:1) in 100 mM NaCl indicating height averages of 2.97 ± 0.82 nm and 3.14 ± 0.52 nm, respectively; phase images of **8OMeTAcG:1** (left) and 1:1 **8OMeTAcG:3₄** in 100 mM NaCl.



b)

8OMeTAcG :3 _x	8OMeTAcG	3_x
1 0.25 3 ₄	88	72
1:0.25 3 ₇	78	68
1:0.25 3 ₁₀	79	70
1:1 3 ₀	68	0
1:1 3 ₇	85	73
1:1 3 ₁₀	87	75
1:1.5 3 ₄	84	74
1:1.5 3 ₇	87	75
1:1.5 3 ₁₀	89	74

Figure S9. (a) ^1H NMR in D_2O of 1:0.25 **8OMeTAcG**:3₄, 3₇, 3₁₀, 1:1 **8OMeTAcG**:3₀, 3₇, 3₁₀, and 1:1.5 **8OMeTAcG**:3₄, 3₇, 3₁₀ (b) Table of % incorporation of the components in the gel phase at room temperature.

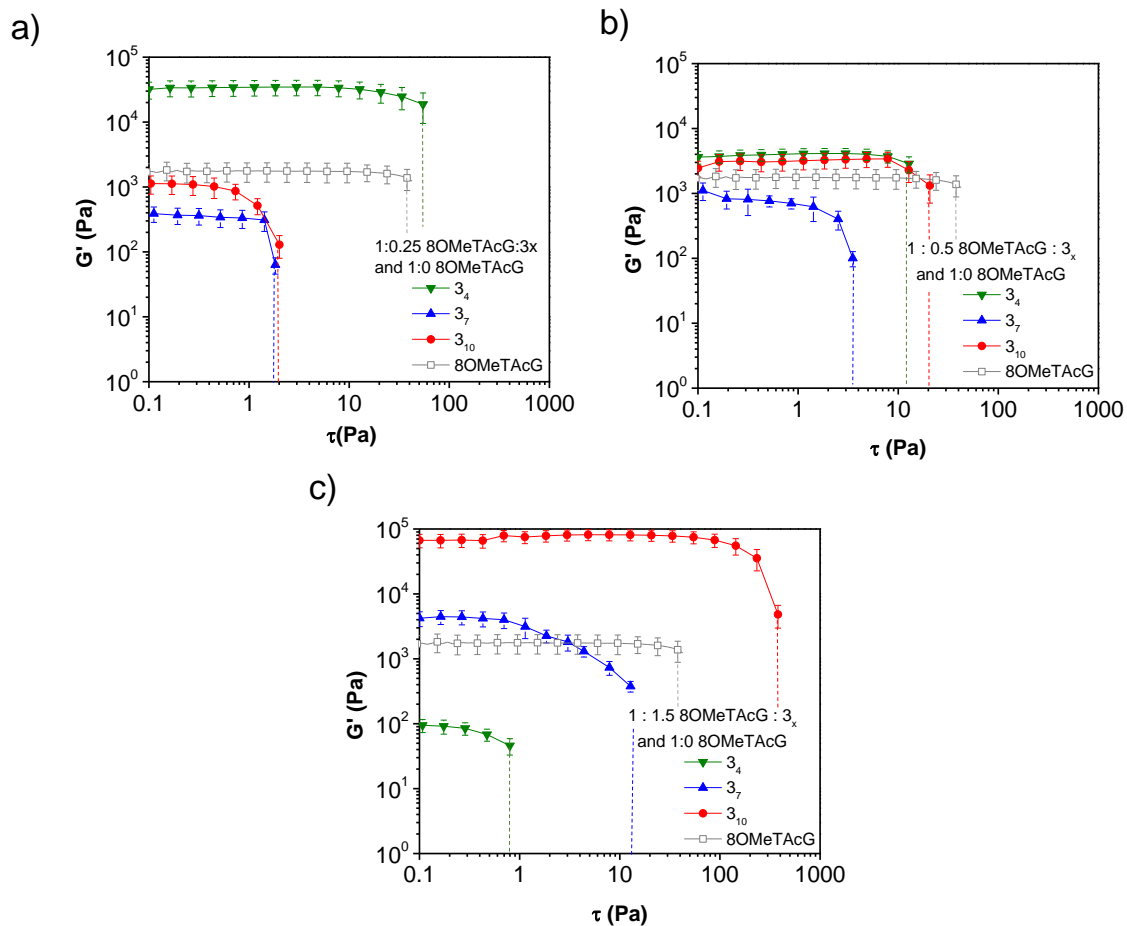


Figure S10. Comparisons of the storage modulus (G') of 1:0 8OMeTAcG and (a) 1:0.25 8OMeTAcG:3_x; (b) 1:0.5 8OMeTAcG:3_x and (c) 1:1.5 8OMeTAcG:3_x co-gels in 100 mM NaCl.

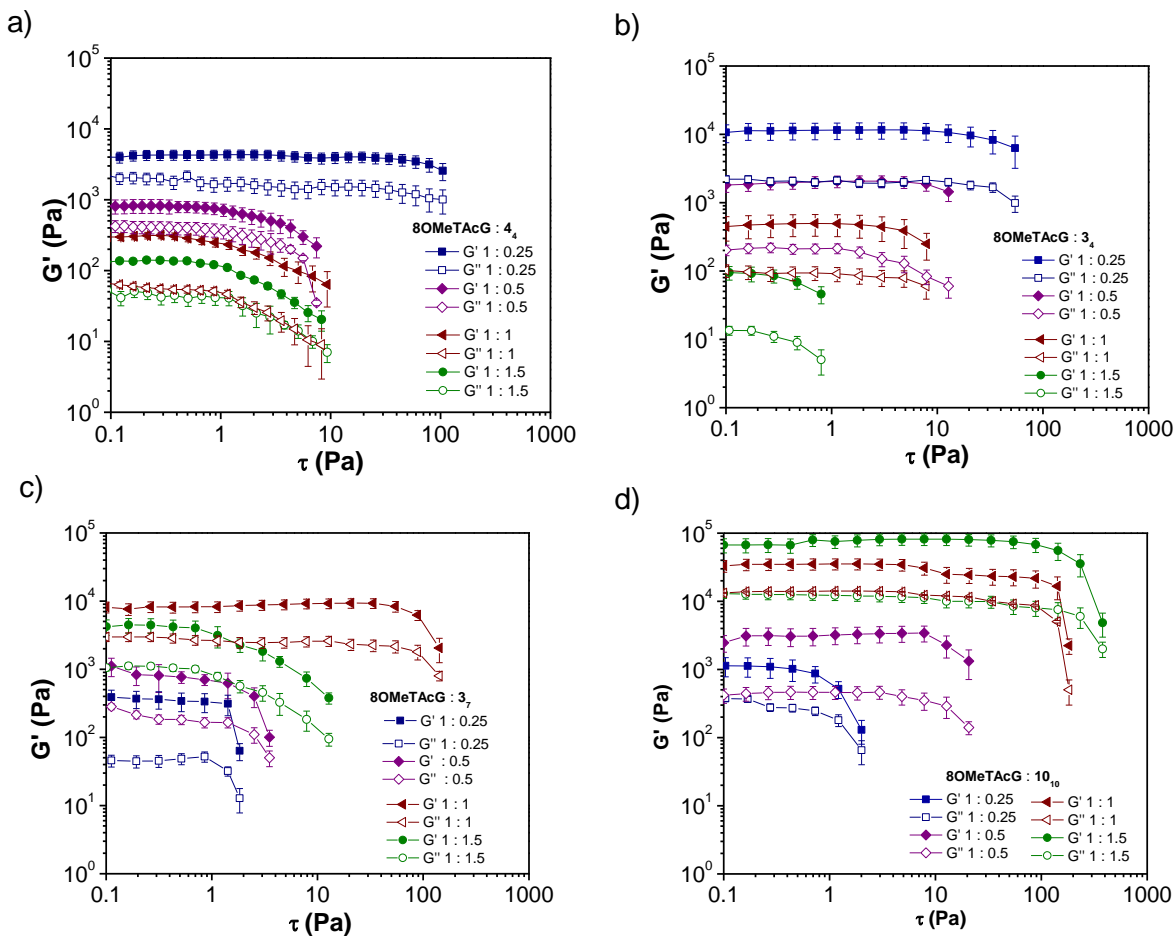


Figure S11. Stress sweep experiments of 100 mM NaCl gels of (a) **8OMeTAcG: 4₄**; (b) **8OMeTAcG: 3₄**; (c) **8OMeTAcG: 3₇**; (d) **8OMeTAcG: 3₁₀** showing G' (closed symbols) and G'' (open symbols). $\omega = 6.28 \text{ rad/s}^{-1}$, $T = 25^\circ\text{C}$.

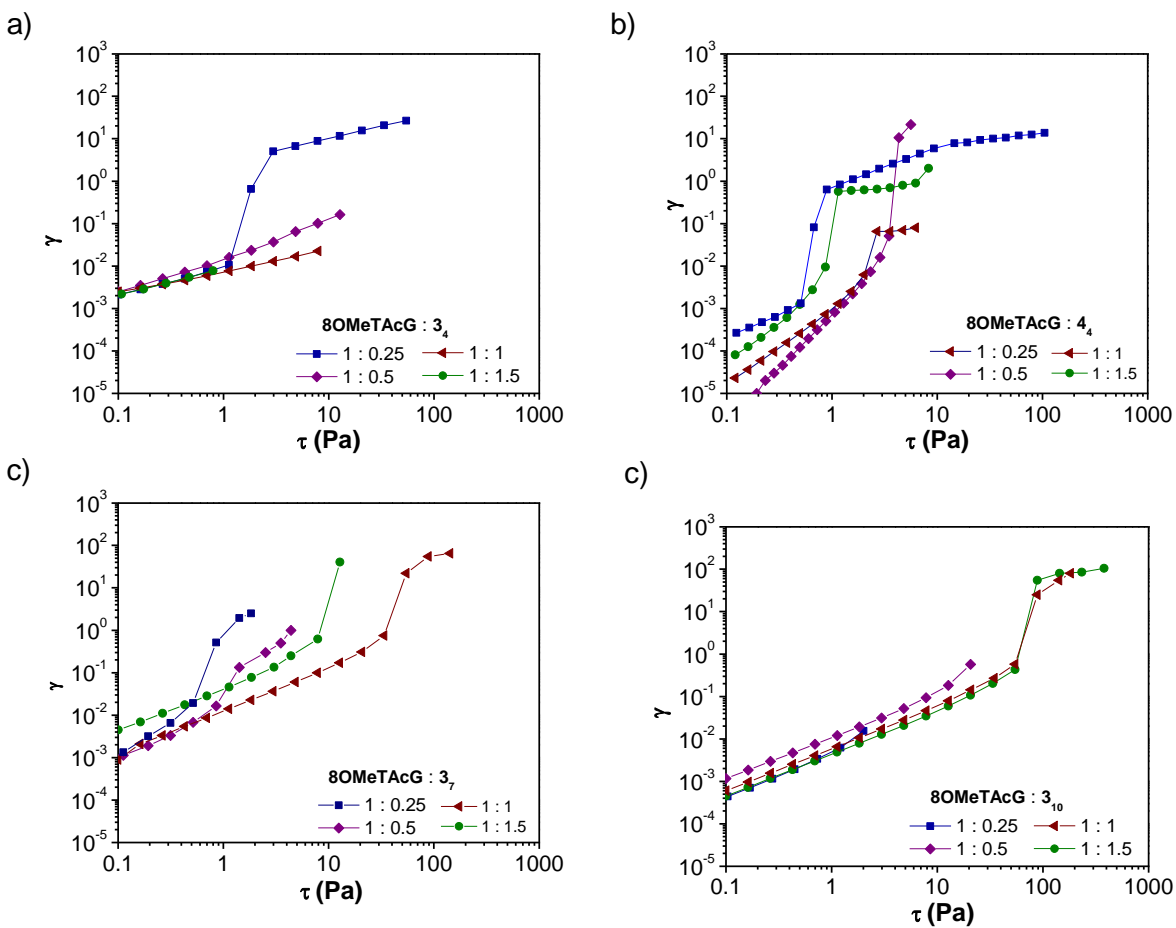


Figure S12. Stress vs. strain curves of 100 mM NaCl gels of (a) **8OMeTAcG:4₄**; (b) **8OMeTAcG:3₄**; (c) **8OMeTAcG:3₇**; (d) **8OMeTAcG:3₁₀** obtained from stress sweeps showing the yield stress of the materials at maximum strain. $\omega = 6.28 \text{ rad/s}^{-1}$.

Synthesis

2',3'-O-Isopropylideneguanosine (**5a**)³

To a suspension of 9.06 g of guanosine (32.0 mmol, 1 eq., 283.24 g/mol) in 375 mL of distilled acetone was added 3.8 mL of 70% perchloric acid (62.9 mmol, 2 eq., 100.46 g/mol, 1.67 g/mL). After 3 hours, 6.4 mL of ammonium hydroxide (163 mmol, 5 eq., 35.05 g/mol, 0.895 g/mL) was added to the reaction mixture and cooled in an ice-water bath. The solid was filtered and then stirred in 100 mL DI H₂O for 3 hours. The reaction mixture was filtered and the white solid was dried over vacuum, 6.0682 g (67%). ¹H NMR (600 MHz, DMSO-d₆): δ 10.57 (s, 1H, NH), 7.86 (s, 1H), 6.42 (s, 2H, NH₂), 5.89 (s, 1H), 5.15 (s, 1H), 4.93 (s, 1H), 4.08 (s, 1H), 3.50 (m, 2H), 1.48 (s, 3H, CH₃), 1.28 (s, 3H, CH₃). ¹³C NMR (600 MHz, DMSO-d₆): δ 157.36 (C=O), 154.34 (C-NH₂), 151.39 (C=C), 136.52 (C=N), 117.37 (C=C), 113.69 (C-CH₃), 89.04 (C-CH₂), 87.28 (C-O), 84.22 (C-O), 81.83 (C-N), 62.25 (CH₂-OH), 27.73 (CH₃-C), 25.89 (CH₃-C). MALDI-MS (α-cyano-4-hydroxycinnamic acid / TFA Na salt): 346.25 [M+Na], 368.45 [M+2Na].

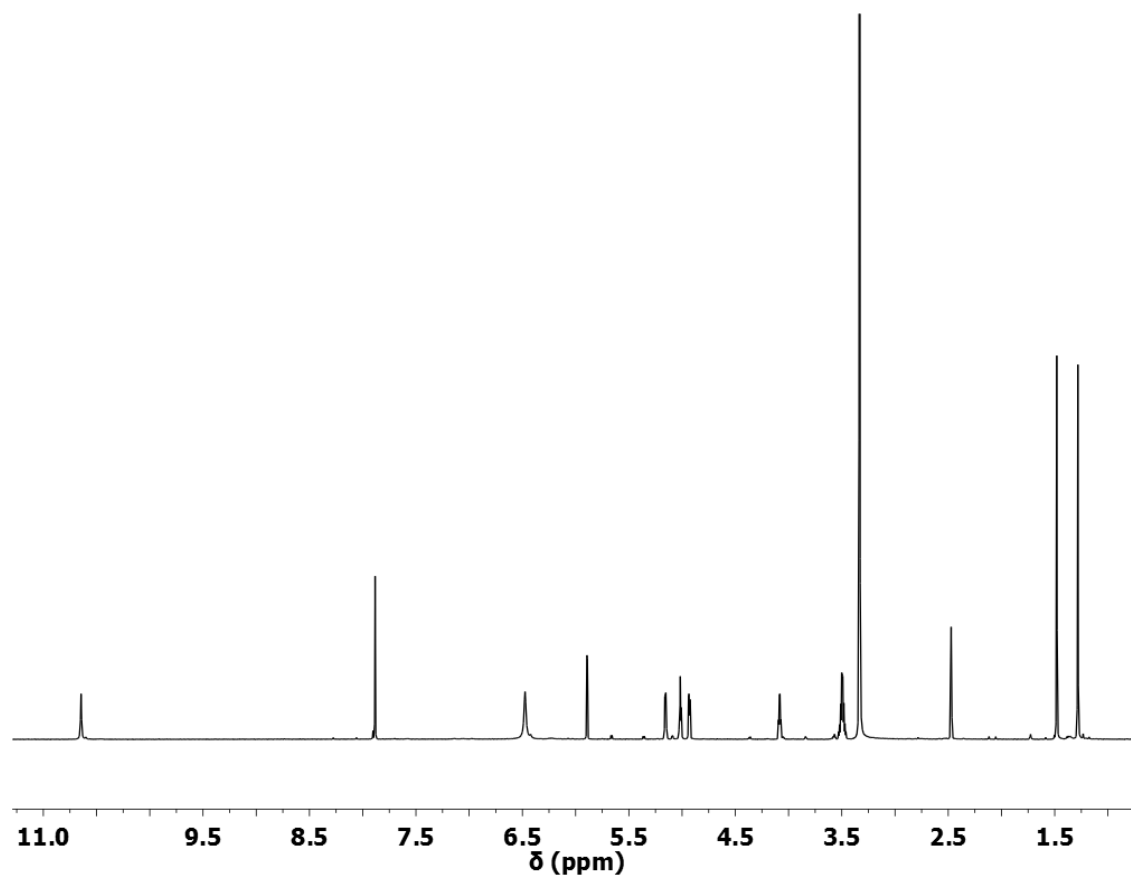


Figure S13. ^1H NMR of 2',3'-O-Isopropylideneguanosine (**5a**) in DMSO.

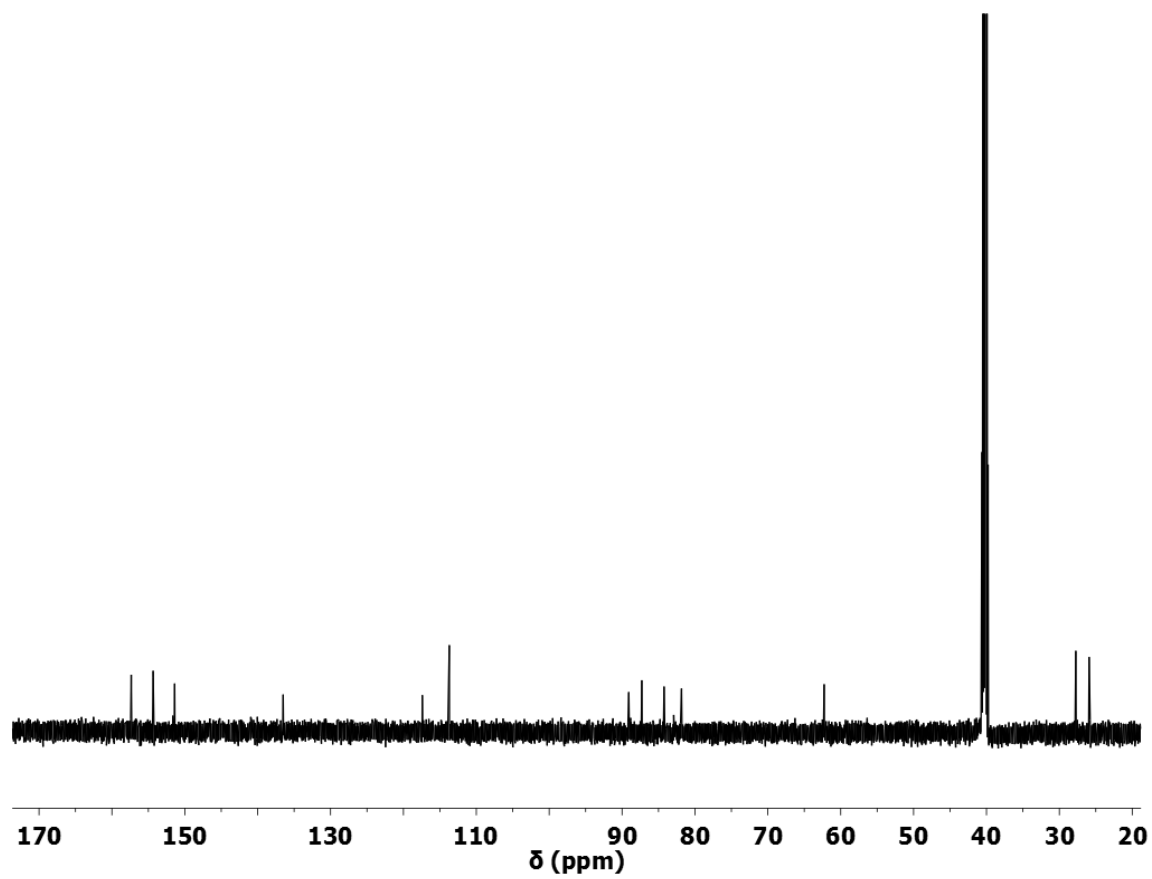


Figure S14. ^{13}C NMR of 2',3'-O-Isopropylideneguanosine (**5a**) in DMSO.

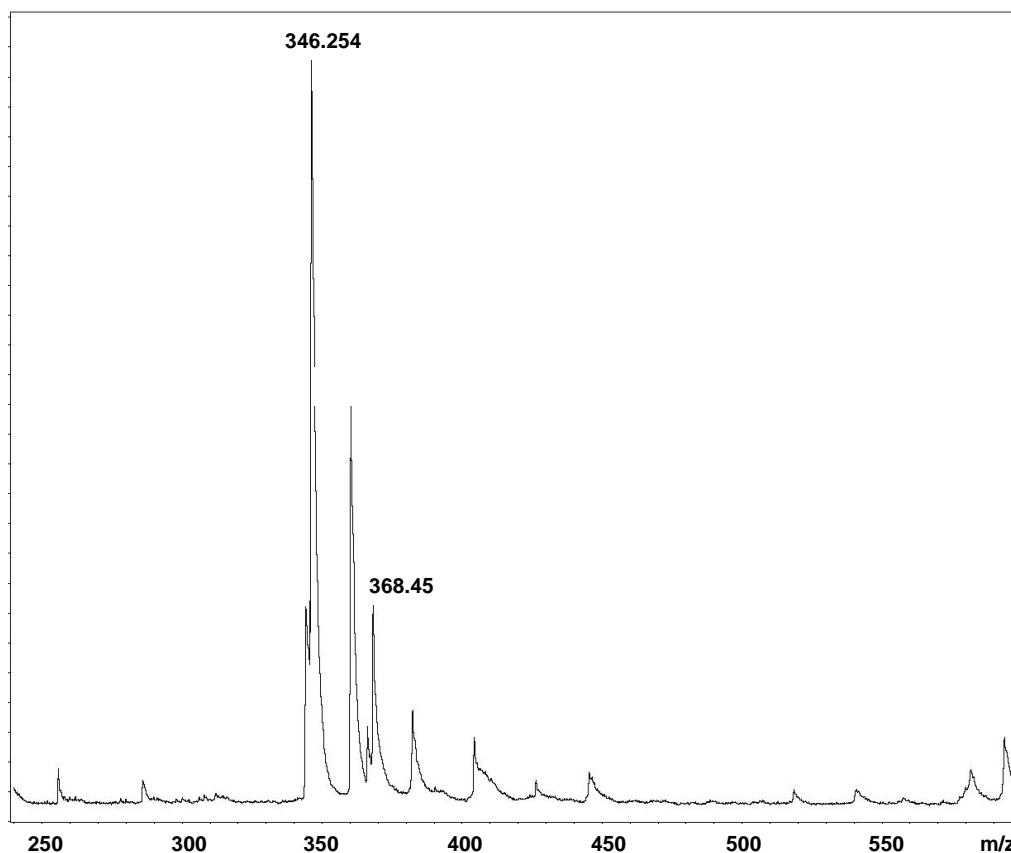


Figure S15. MALDI-MS spectra of 2',3'-O-Isopropylideneguanosine (**5a**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

2',3'-O-isopropylideneguanosine 5'-carboxylic acid, **5⁴**

12.5 g of IPG (323 g/mol, 1 eq. 0.0387 mol), 6.5 g of sodium bicarbonate (84.01 g/mol, 2 eq., 0.0774 mol), 1.21 g of 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) (156.25 g/mol, 0.5 eq., 0.0077 mol), and 27.42 g of iodobenzene diacetate (322.1 g/mol, 2.2 eq., 0.0851 mol) were suspended in 48 mL of acetonitrile and 48 mL of water. The mixture was stirred at room temperature for three hours. The resulting solids were filtered, washed with diethyl ether and acetone, and dried. The solids were then taken up into boiling water, where the product

dissolved in the water and the starting material did not. The solid starting material was filtered off, the water was evaporated, and the resulting product was re-dissolved in 66% acetone and 33% water at 60 °C. The solution was allowed to cool to 4 °C, and the product slowly crystallized out over a period of 72 hours, filtered, dried under vacuum, 7.82 g (60%). ¹H NMR (600 MHz, DMSO-d₆): δ 10.98 (s, 1H, NH), 8.39 (s, 1H), 6.66 (s, 2H, NH₂), 5.89 (s, 1H), 4.97 (m, 1H), 4.88 (m, 1H), 4.24 (s, 1H), 1.46 (s, 3H, CH₃), 1.26 (s, 3H, CH₃). ¹³C NMR (600 MHz, DMSO-d₆): δ 172.11, 157.59, 154.45, 151.49, 137.58, 116.92, 112.82, 90.33, 88.31, 85.67, 84.87, 27.71, 25.77. MALDI-MS (α-cyano-4-hydroxycinnamic acid / TFA Na salt): 360.29 [M+Na].

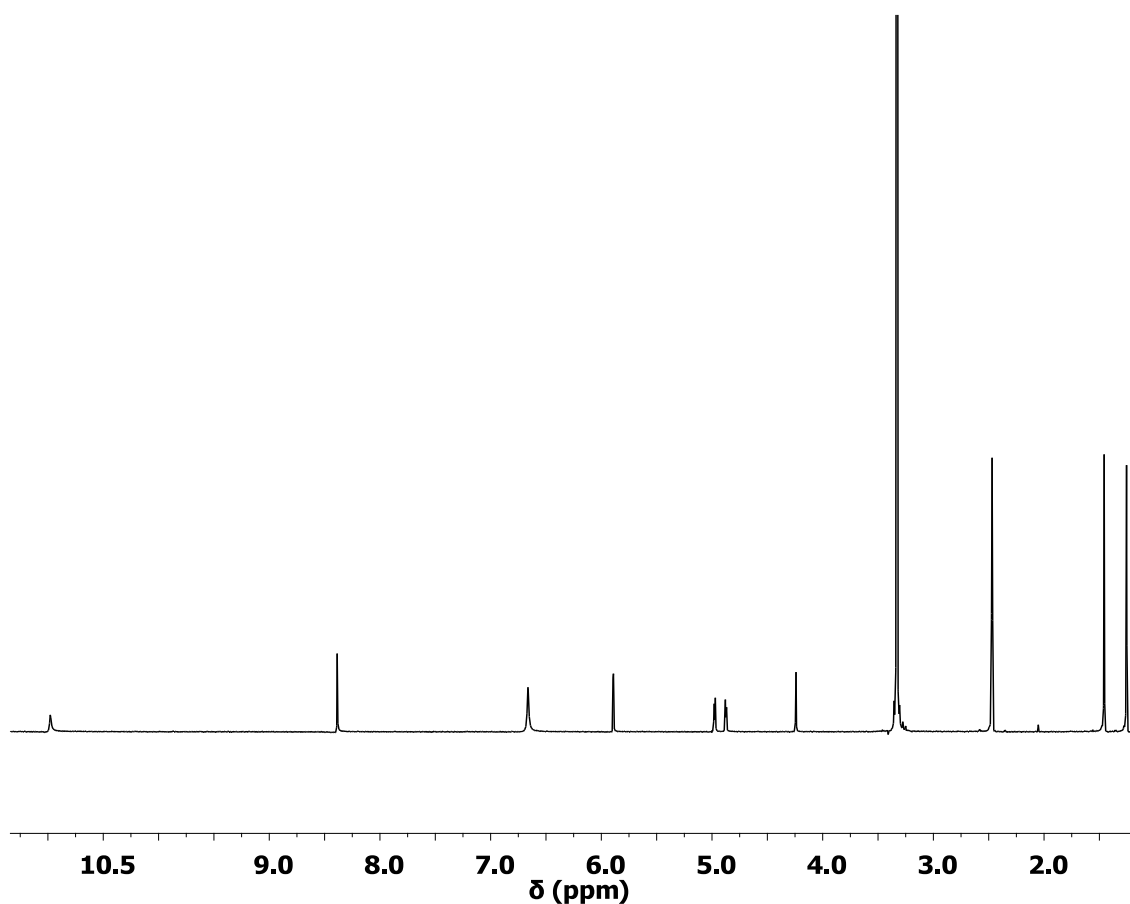


Figure S16. ¹H NMR of 2',3'-O-Isopropylidenguanosine 5'-carboxylic acid (**5**) in DMSO.

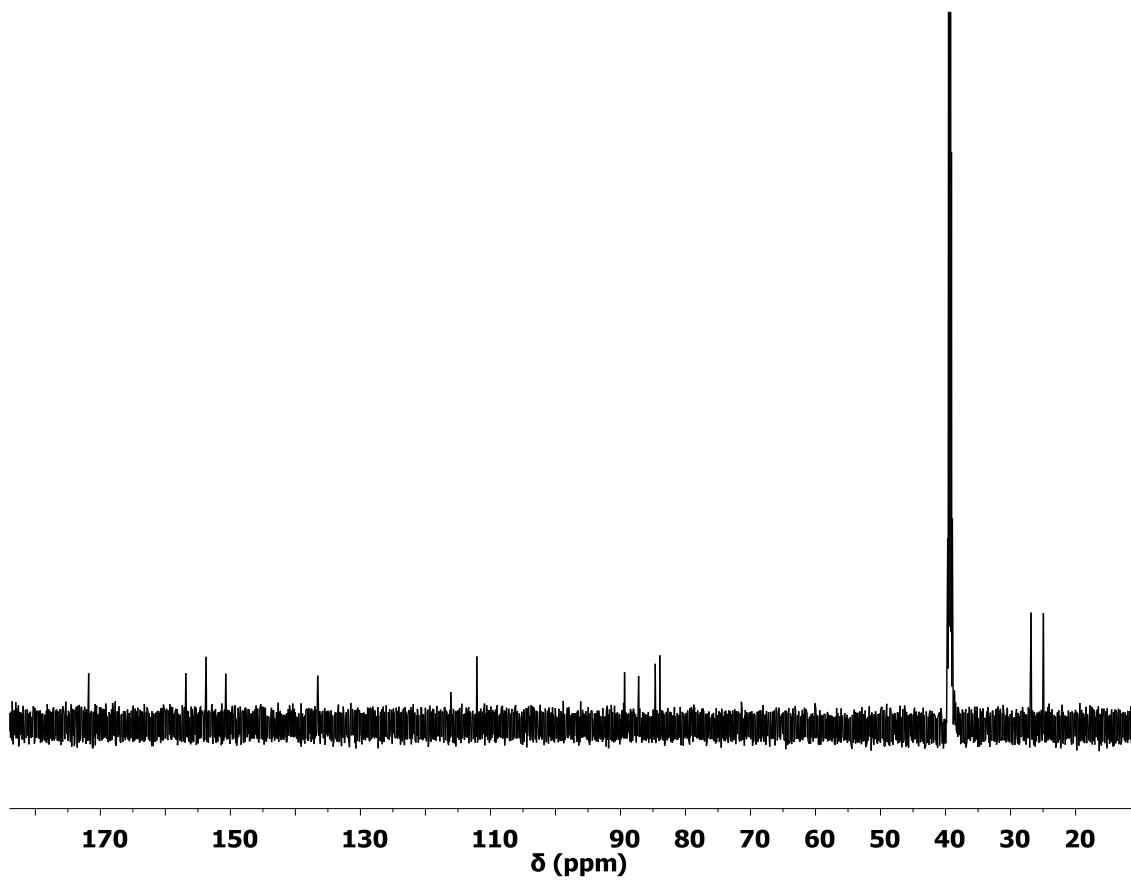


Figure S17. ^{13}C NMR of 2',3'-O-Isopropylideneguanosine 5'-carboxylic acid (**5**) in DMSO.

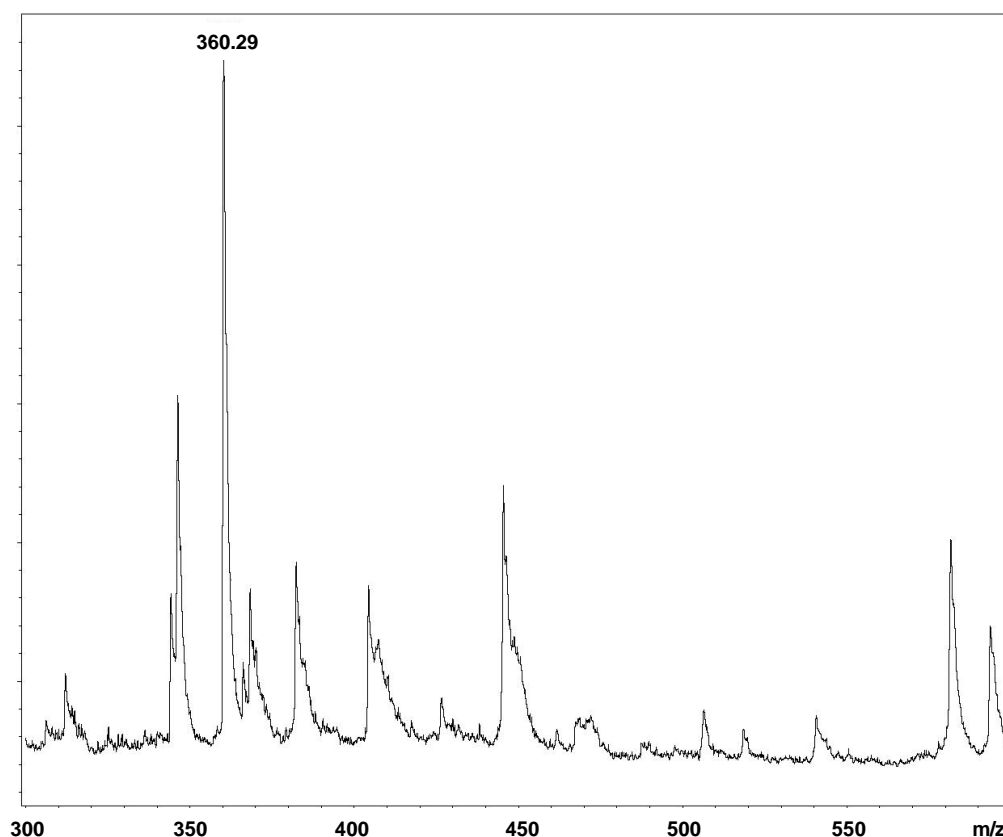


Figure S18. MALDI-MS spectra of 2',3'-O-Isopropylideneguanosine 5'- carboxylic acid (**5**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

Synthesis of Guanosine 5'- carboxylic acid, **6**⁵

150 mL of 1N HCl was added to 7.82 g of IPG acid (337 g/mol, 1 eq., 0.02322 mol) and placed in a 65°C oil bath under N₂. After the solution dissolved, it was left stirring for 1 hr. The solution was cooled and then dripped into acetone to precipitate. The product was filtered and dried over vacuum, 6.5 g (98 %). ¹H NMR (600 MHz, DMSO-d₆): δ 11.21 (s, 1H, COOH), 10.69 (s, 1H, NH), 8.01 (s, 1H, CH), 6.53 (s, 2H, NH₂), 5.83 (d, 1H, CH), 4.34 (m, 2H), 4.22 (s, 1H, CH), 3.36 (s, 2H, OH). ¹³C NMR (600 MHz, DMSO-d₆): δ 172.24, 156.98, 154.10, 151.92,

135.21, 116.46, 86.25, 82.65, 74.22, 73.66. MALDI-MS (α -cyano-4-hydroxycinnamic acid / TFA Na salt): 299.18 [M+], 319.91 [M+Na], 343.38 [M+2Na], 378.44 [M+2K].

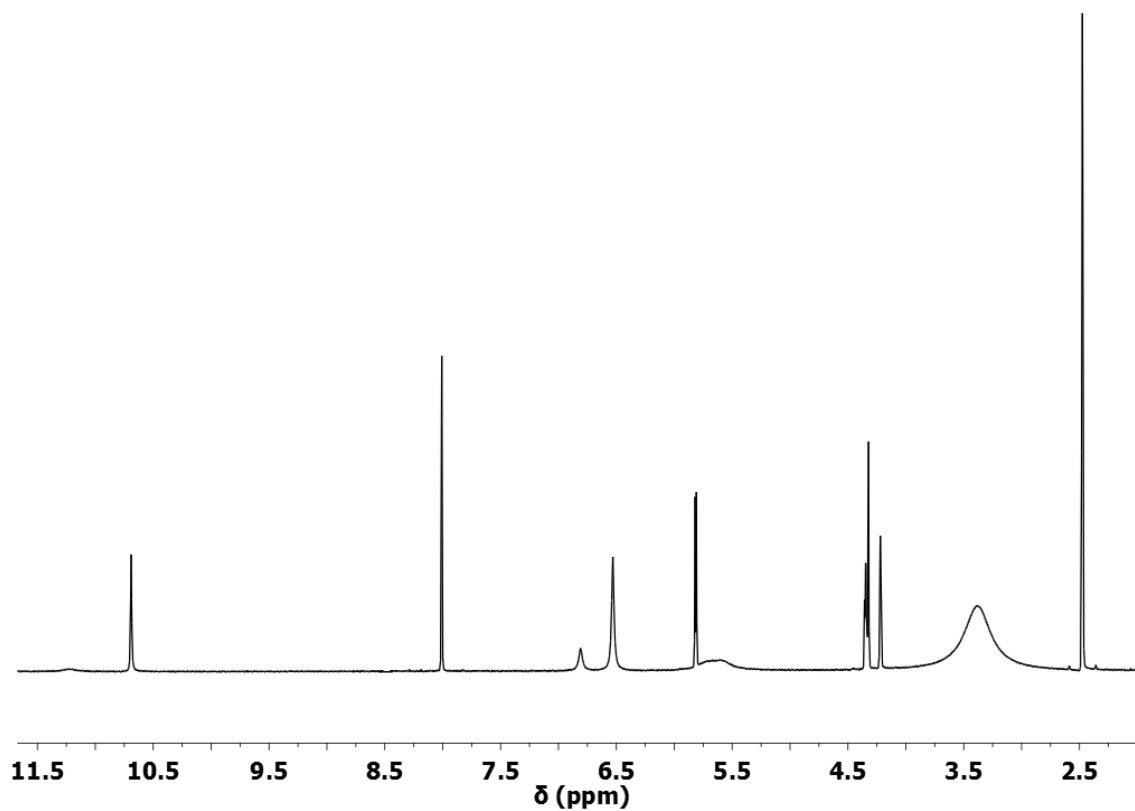


Figure S19. ^1H NMR of Guanosine 5'-carboxylic acid (**6**) in DMSO.

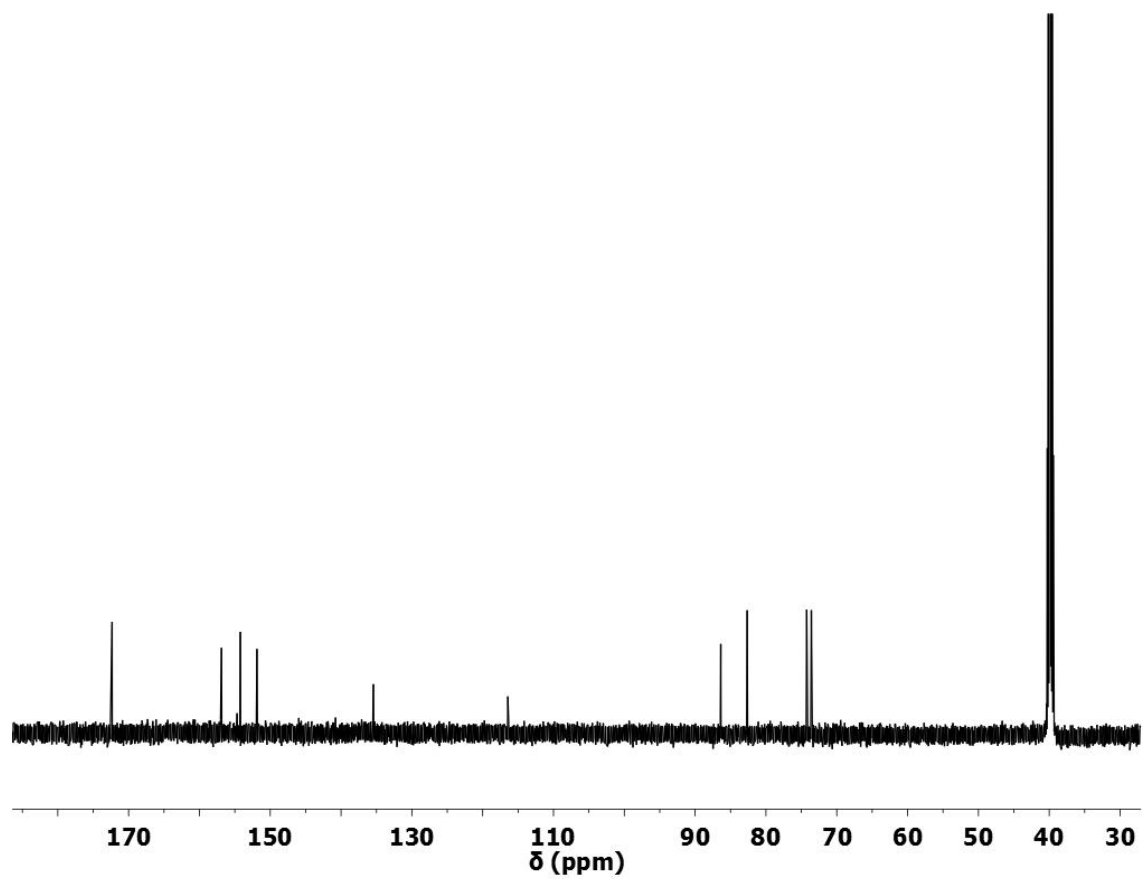


Figure S20. ^{13}C NMR of Guanosine 5'- carboxylic acid (**6**) in DMSO.

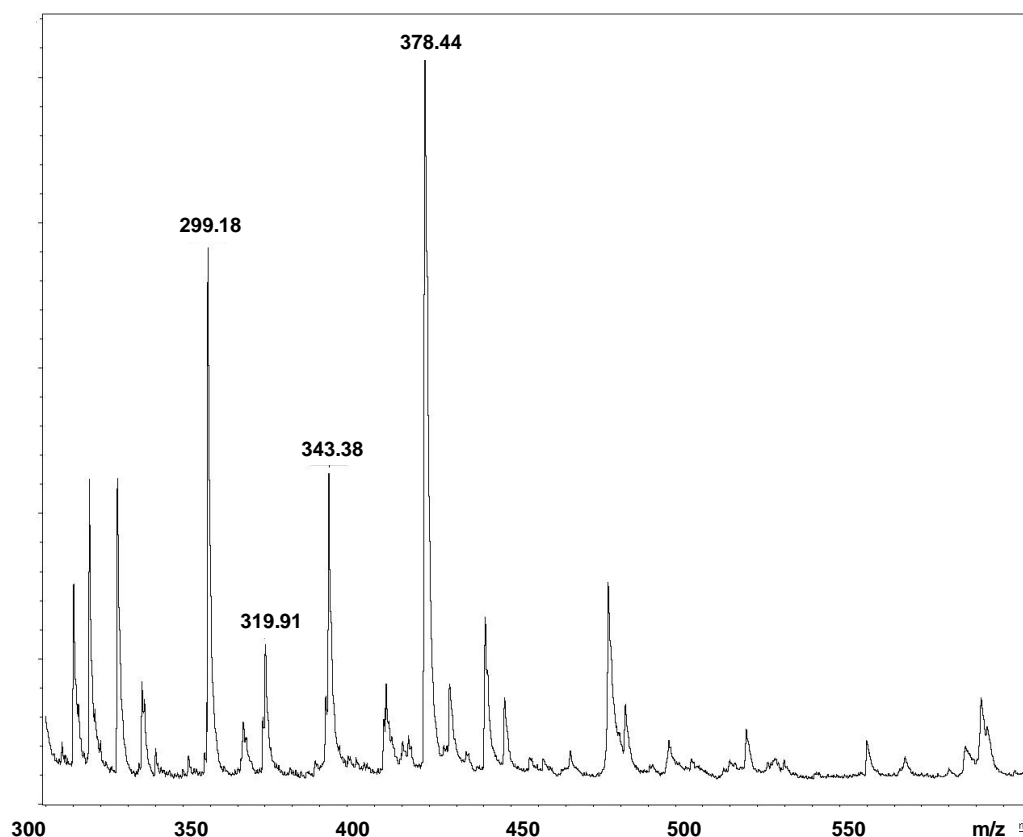


Figure S21. MALDI-MS spectra of guanosine 5'- carboxylic acid (**6**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

Synthesis of 8-bromoguanosine 5'- carboxylic acid, **7⁶**

6 g of guanosine 5'-acid (297 g/mol, 1 eq., 0.0202 mol) was dissolved in 70 mL of DI water. 27 mL of bromine (79.904 g/mol, 3.1028 g/cm⁻³, 5 eq., 0.101 mol) was dissolved in 600 mL of DI water. 10 mL of the bromine water solution was added to the guanosine 5'-acid water solution and the solution was stirred until the yellow color disappeared. 10 mL aliquots were added to the solution until the yellow color remained. The solution was left to stir at room temperature for an additional 1 hr. The reaction was filtered, recrystallized from water, filtered, and dried over

vacuum, 6.07 g (80 %). ^1H NMR (600 MHz, DMSO- d_6): δ 10.86 (s, 1H, NH), 6.68 (s, 2H, NH₂), 6.49 (s, 2H, OH), 5.72, (d, 1H, CH), 5.01 (t, 1H, CH), 4.49 (t, 1H, CH), 4.26 (d, 1H, CH). ^{13}C NMR (600 MHz, DMSO- d_6): δ 170.60, 155.28, 153.41, 151.86, 120.83, 117.22, 89.73, 82.32, 72.20, 70.06. MALDI-MS (α -cyano-4-hydroxycinnamic acid / TFA Na salt): 378.31 [M⁺], 400.39 [M+Na].

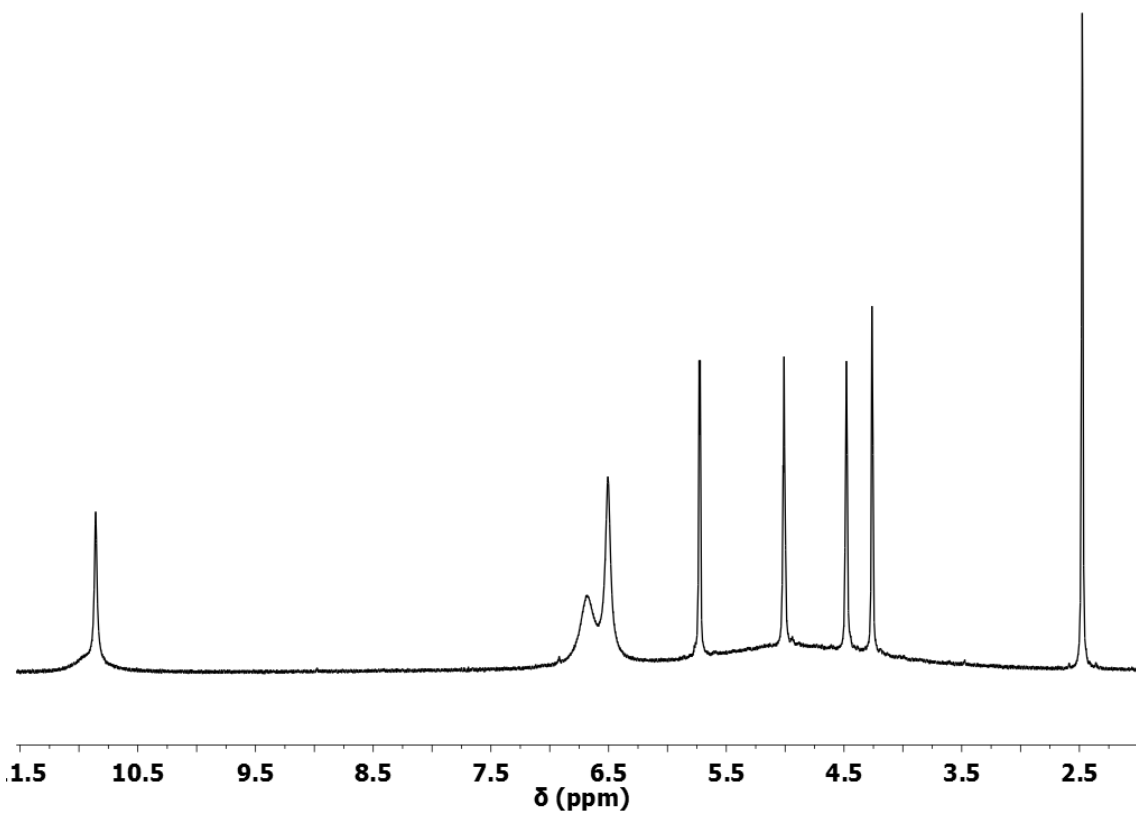


Figure S22. ^1H NMR of 8-bromoguanosine 5'-carboxylic acid (**7**) in DMSO.

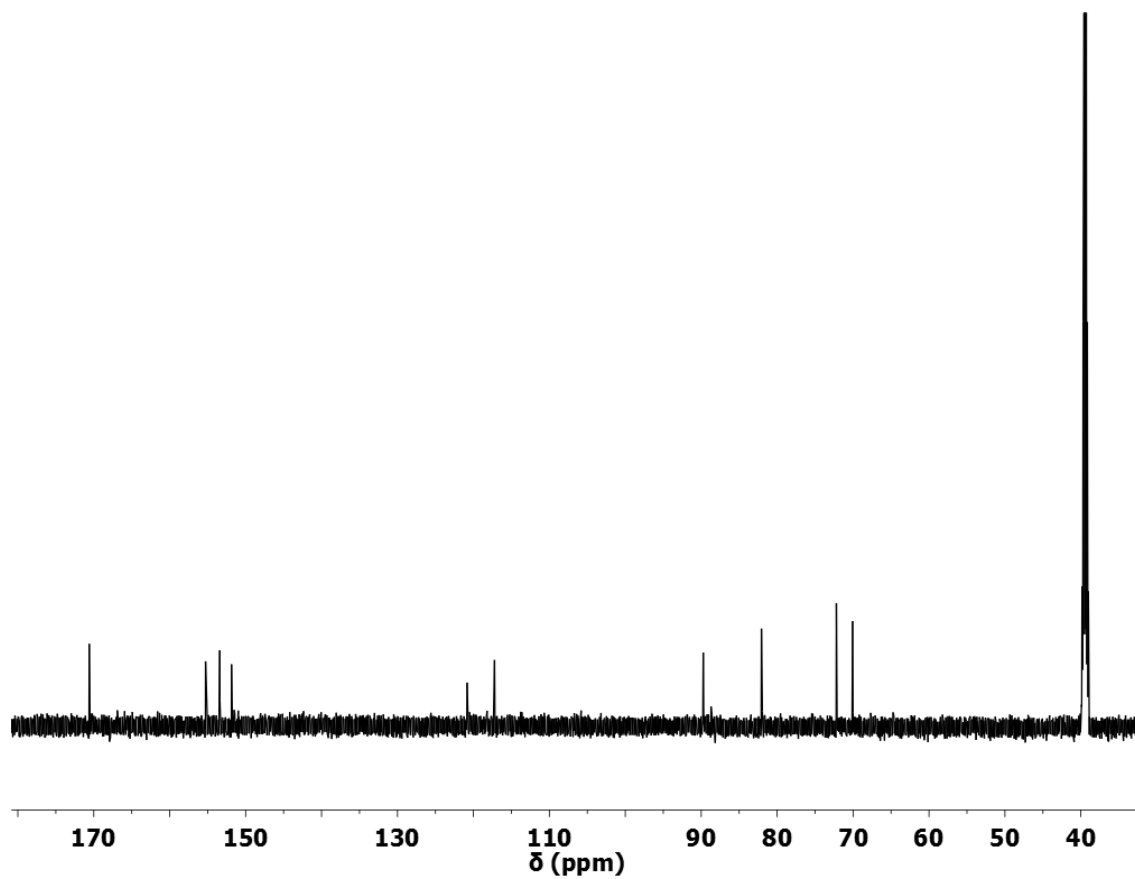


Figure S23. ^{13}C NMR of 8-bromoguanosine 5'- carboxylic acid (**7**) in DMSO.

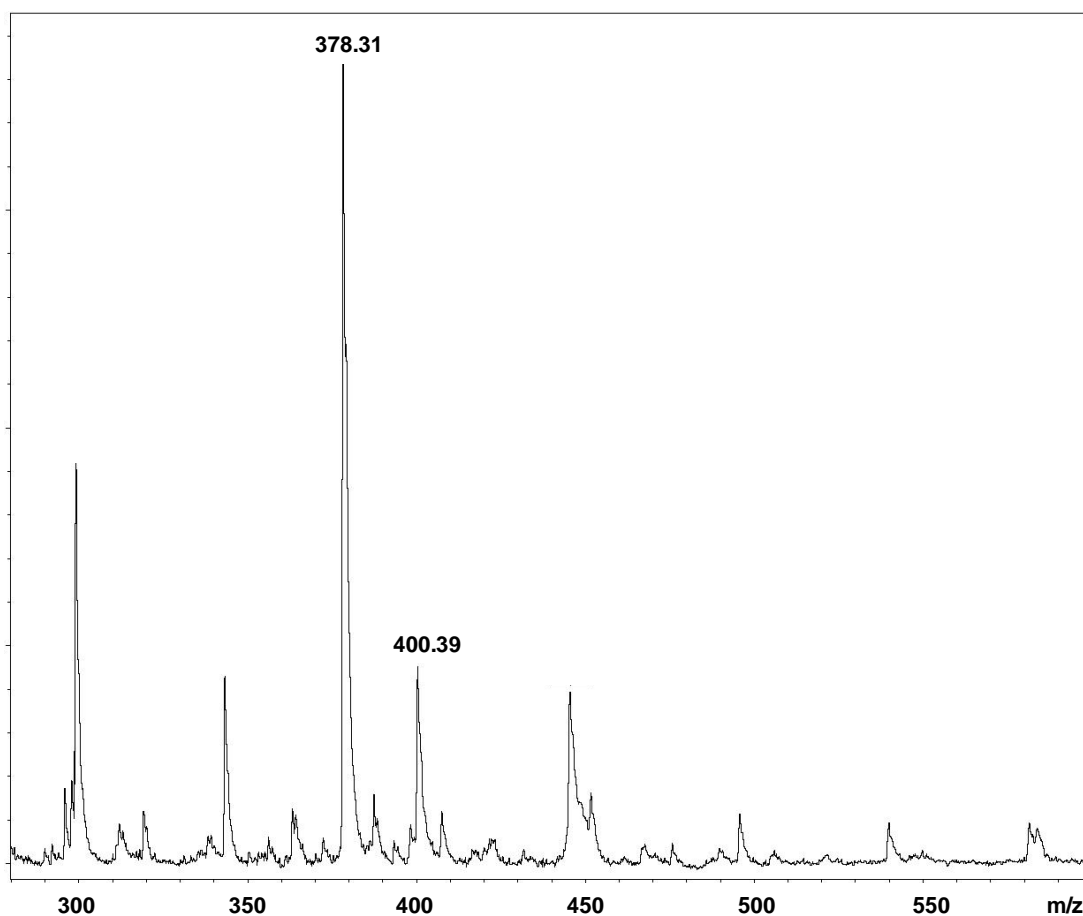


Figure S24. MALDI-MS spectra of 8-bromoguanosine 5'- carboxylic acid (**7**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

Synthesis of 8-methoxyguanosine 5'- carboxylic acid, **8**

6g of 8-bromoguanosine 5'-acid (376.12 g/mol, 1 eq., 0.016 mol) was placed in a flask and purged with argon for 15 minutes. 121 mL of DMSO was added and the mixture was stirred until the reactant was dissolved. 7.4g of sodium methoxide (54.02 g/mol, 8.5eq., 0.137 mol) was dissolved in 65.4 mL of methanol. The sodium methoxide/methanol solution was added to the DMSO solution and the reaction was stirred at 65°C for 15 hours. The reaction was cooled to

room temperature and neutralized with approximately 12 mL of acetic acid. The neutralized solution was added dropwise to diethyl ether, and the resulting precipitate was collected by filtration and washed with cold acetone. The product was recrystallized from distilled water and dried under vacuum, 3.4 g (65%). ^1H NMR (600 MHz, DMSO- d_6): δ 10.69 (s, 1H, NH), 8.48 (s, 2H, NH₂), 6.53 (s, 2H, OH), 5.82 (d, 1H, CH), 4.35 (t, 1H, CH), 4.33 (t, 1H, CH), 4.22 (d, 1H, CH), 3.75 (s, 3H, O-CH₃). ^{13}C NMR (600 MHz, DMSO- d_6): δ 172.03, 156.56, 153.31, 151.33, 135.07, 116.04, 88.55, 85.80, 82.32, 73.79, 47.21. MALDI-MS (α -cyano-4-hydroxycinnamic acid / TFA Na salt): 350.22 [M+Na], 373.38 [M+2Na].

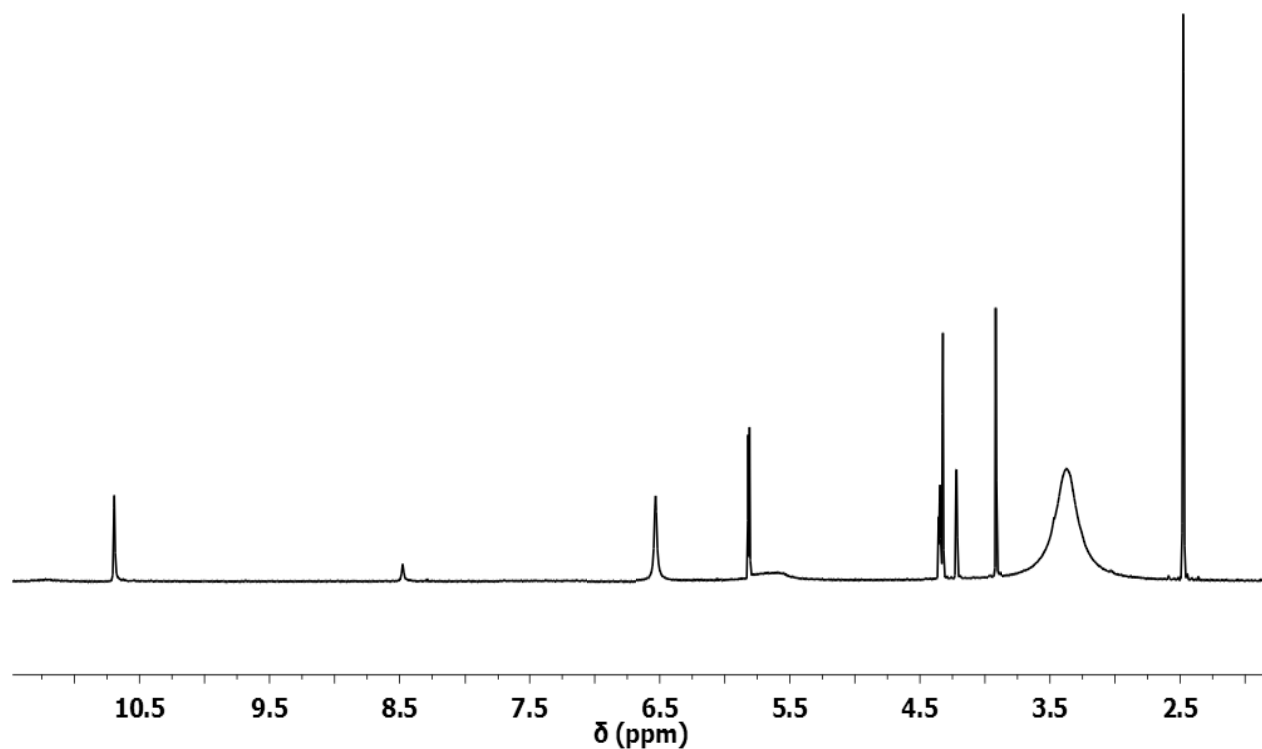


Figure S25. ^1H NMR of 8-methoxyguanosine 5'-carboxylic acid (**8**) in DMSO.

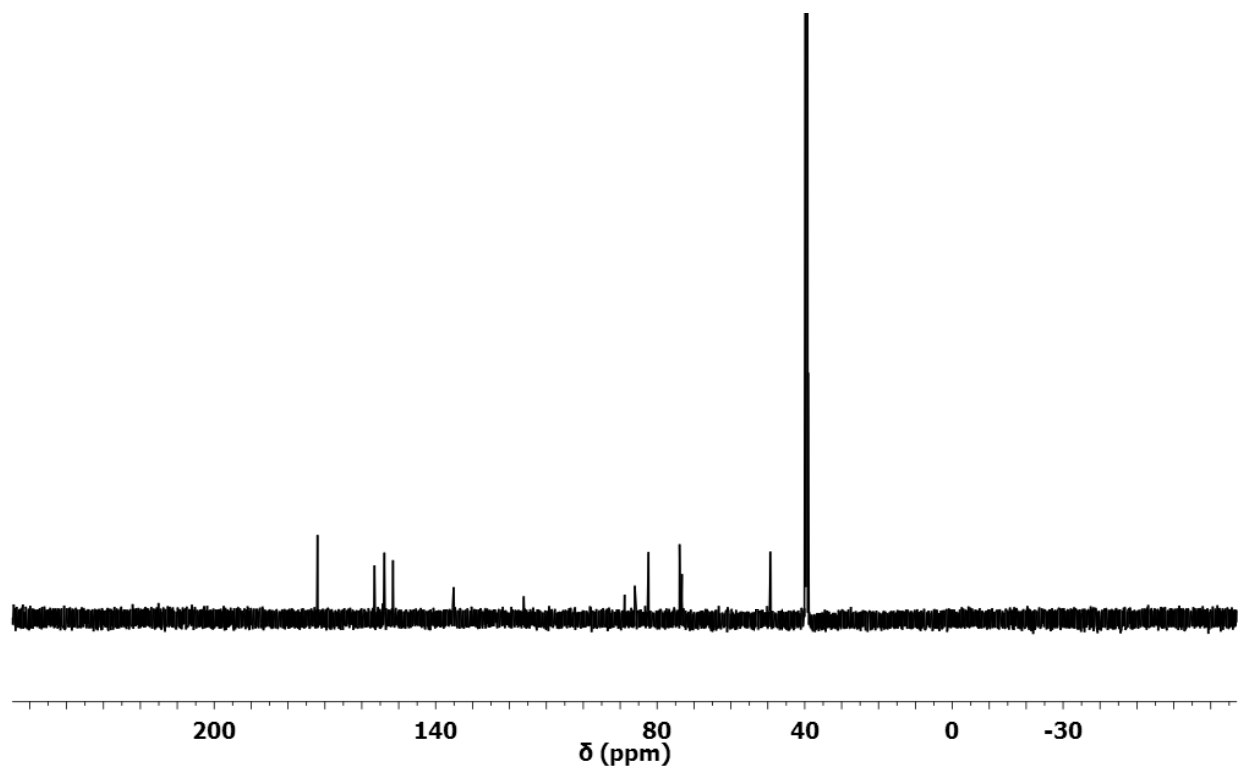


Figure S26. ^{13}C NMR of 8-methoxyguanosine 5'- carboxylic acid (**8**) in DMSO.

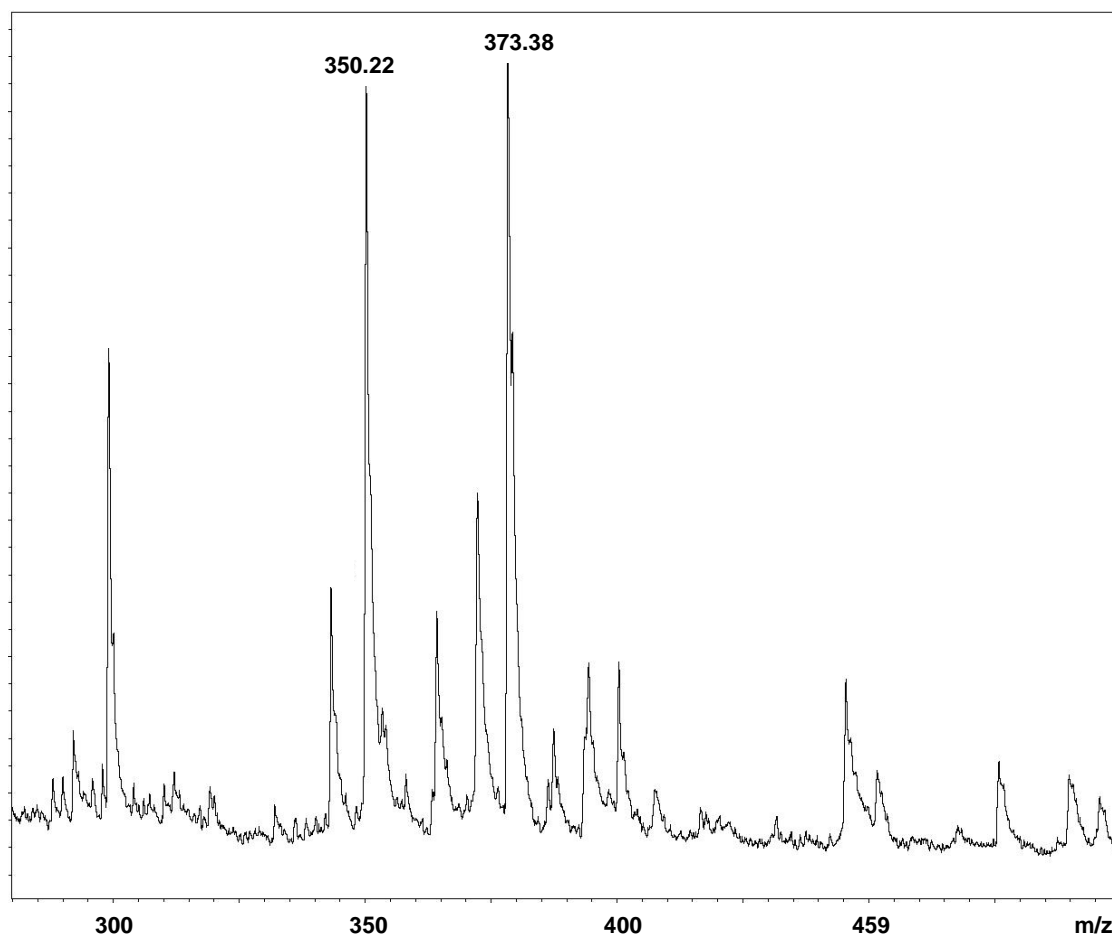


Figure S27. MALDI-MS spectra of 8-methoxyguanosine 5'- carboxylic acid (**8**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

Synthesis of 8-methoxy-2',3'-diacetylguanosine 5'- carboxylic acid, **9** / 2',3'-diacetylguanosine 5'- carboxylic acid, **10**

0.5g 8-methoxyguanosine 5'-acid (327.25 g/mol, 1 eq., 0.0015 mol) and 0.02g DMAP were placed in a flask and purged with argon for 15 minutes. 20 mL of acetonitrile was added, followed by 1.33 mL of triethylamine (101.19 g/mol, 0.7255 g/mL, 6 eq., 0.0095 mol). The mixture was cooled in an ice-water bath and 0.47 mL of acetic anhydride (102.09 g/mol, 1.082

g/mL, 3 eq., 0.005 mol) was added. The reaction was left to stir for 1 hr at 0°C and then warmed to RT and stirred for an additional 3 hr, followed by quenching with 1.2 mL of methanol and evaporation of half the volume of solvent. The rest of the reaction was added dropwise to ether, filtered, recrystallized from 2-propanol, and dried under vacuum, 0.275g (45%).): δ 8.47 (s, 1H, NH), 6.55 (s, 2H, NH₂), 5.79 (d, 1H, CH), 5.43 (t, 1H, CH), 4.16 (s, 1H, CH), 4.13 (s, 1H, CH), 4.07 (d, 1H, CH), 3.57 (s, 3H, OCH₃), 2.04 (s, 3H, CH₃), 1.94 (s, 3H, CH₃). ¹³C NMR (600 MHz, DMSO-d₆): δ 172.13, 169.23, 153.41, 151.61, 150.78, 116.24, 88.66, 85.82, 82.41, 74.24, 74.21, 52.21, 21.04. MALDI-MS (α -cyano-4-hydroxycinnamic acid / TFA Na salt): 434.24 [M+Na], 457.28 [M+2Na].

10: ¹H NMR (600 MHz, DMSO-d₆): δ 8.6 (s, 1H, NH), 8.38 (s, 1H, CH), 6.63 (s, 2H, NH₂), 5.78 (d, 1H, CH), 4.31 (t, 1H, CH), 4.26 (s, 1H, CH), 4.13 (s, 1H, CH), 4.10 (d, 1H, CH), 2.11 (s, 3H, CH₃), 1.89 (s, 3H, CH₃). ¹³C NMR (600 MHz, DMSO-d₆): δ 172.33, 169.45, 153.67, 151.41, 151.13, 135.53, 116.54, 86.45, 83.62, 82.41, 73.53, 73.41, 21.14.

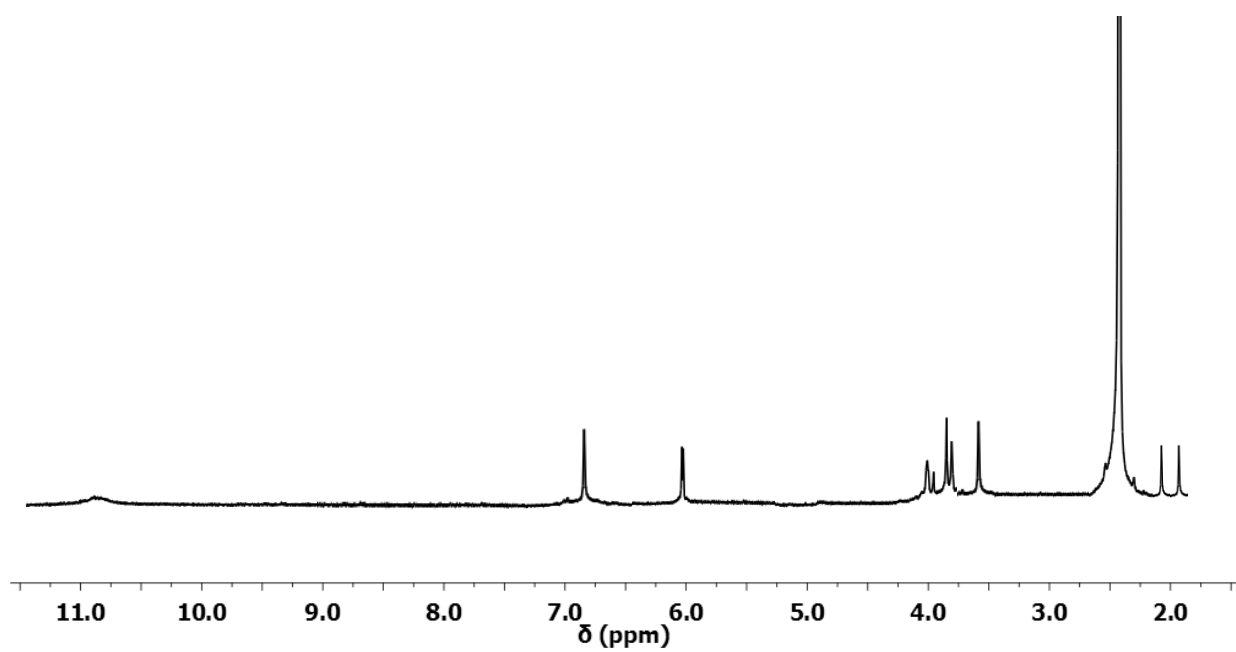


Figure S28. ^1H NMR of 8-methoxy-2',3'-diacetylguanosine 5'-carboxylic acid (**9**) in DMSO.

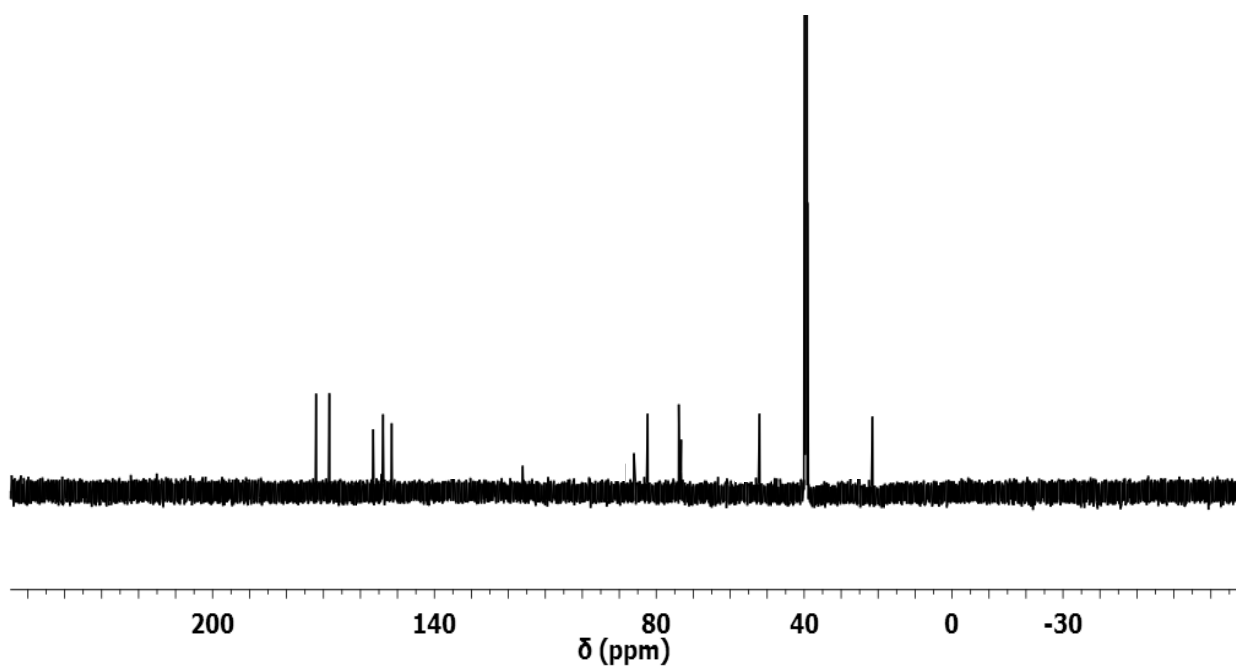


Figure S29. ^{13}C NMR of 8-methoxy-2',3'-diacetylguanosine 5'-carboxylic acid (**9**) in DMSO.

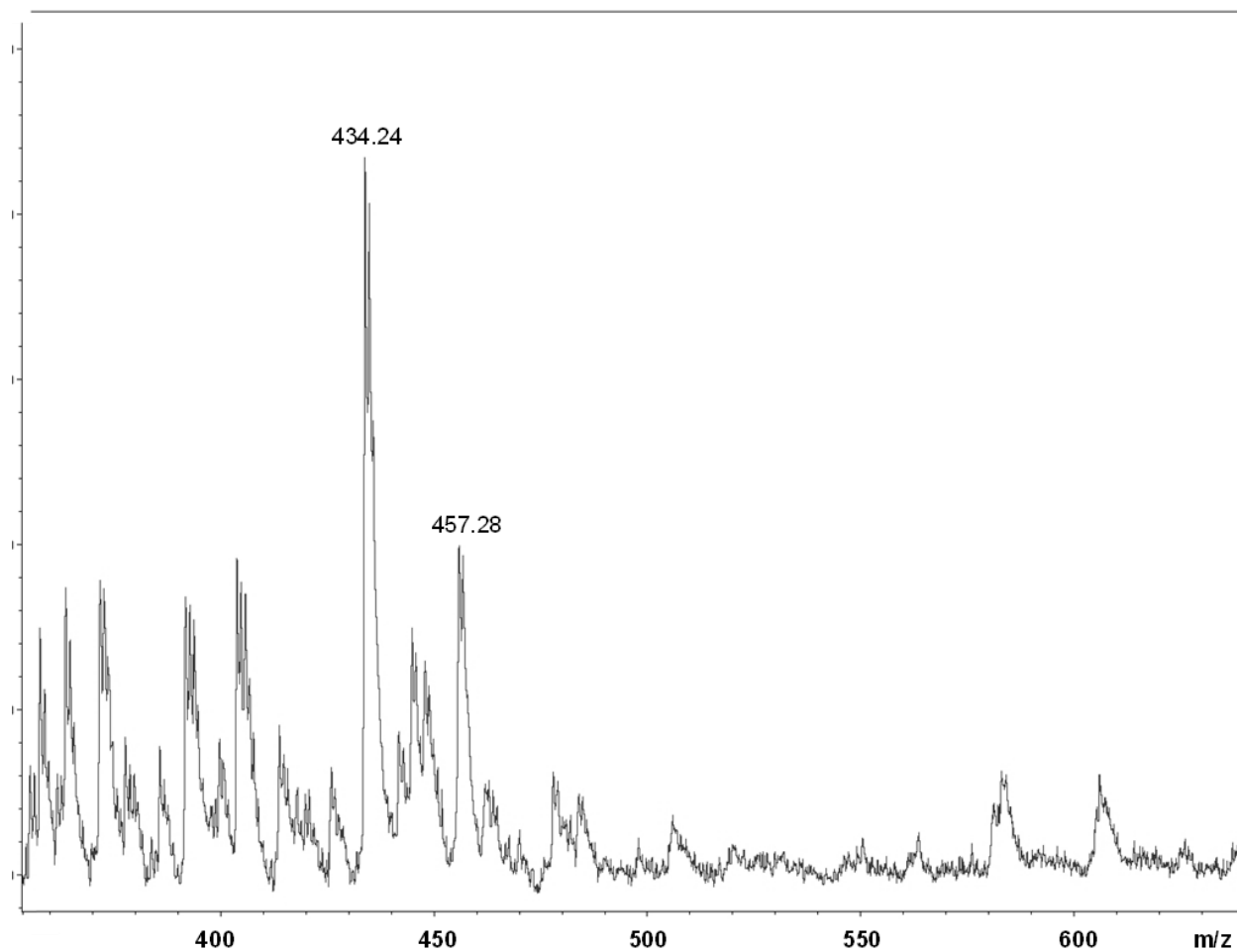


Figure S30. MALDI-MS spectra of 8methoxy-2',3'-diacetylguanosine 5'- carboxylic acid (**9**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

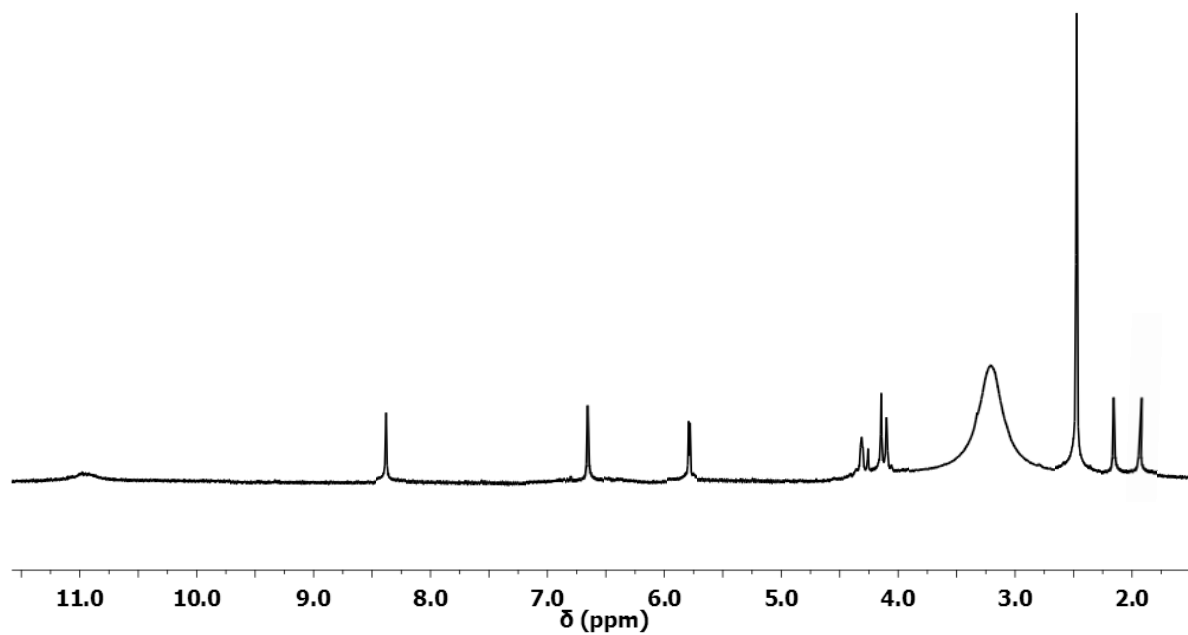


Figure S31. ^1H NMR of 2',3'-diacetylguanosine 5'-carboxylic acid (**10**) in DMSO.

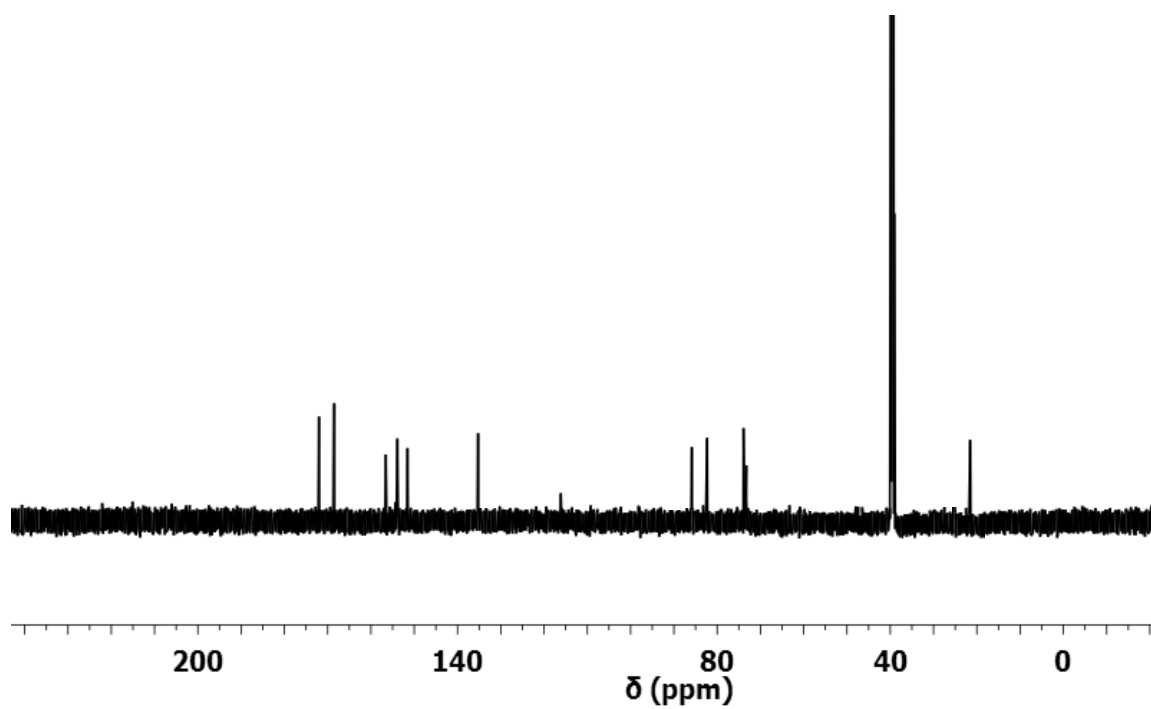


Figure S32. ^{13}C NMR of 2',3'-diacetylguanosine 5'-carboxylic acid (**10**) in DMSO.

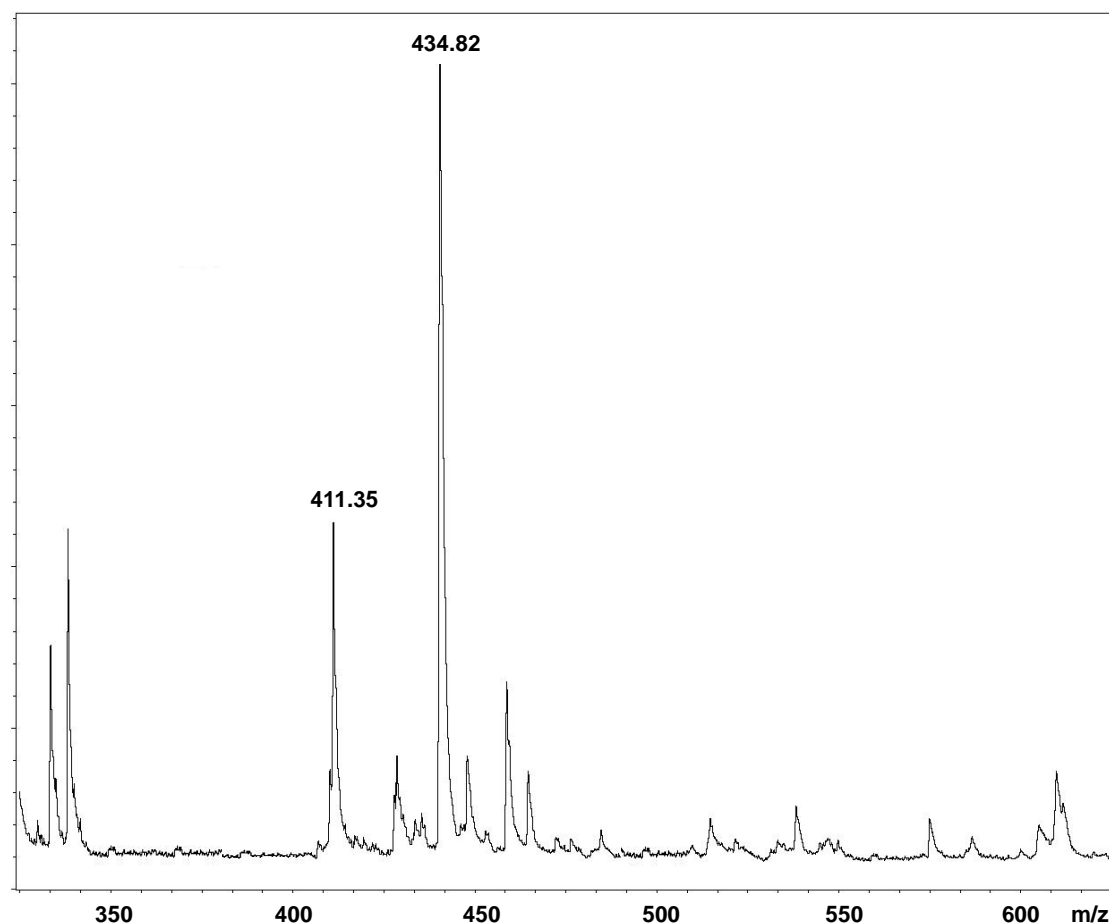


Figure S33. MALDI-MS spectra of 2',3'-diacetylguanosine 5'- carboxylic acid (**10**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

Synthesis of 8-methoxy-2',3'-diacetylguanosine 5'-acrylamide, **1** / 2',3'-diacetylguanosine 5'-acrylamide, **2**

0.275 g 8-methoxy-2',3'-diacetylguanosine 5'-acid (411.33 g/mol, 1 eq., 0.0007 mol), 0.417g of 1H-Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (520.39 g/mol, 1.2eq, 0.0008 mol), and 0.173g N,N-diisopropylethylamine (129.38 g/mol, 2 eq., 0.0013 mol) were dissolved in 10 mL of DMF and stirred for 20 min until dissolved. 0.203g of PEG-

acrylamide (202.25 g/mol, 1.5 eq., 0.001 mol) was then added and the reaction was stirred for 24 hr. The reaction mixture was centrifuged and the remaining solid was washed 3x with methanol and vacuum dried, 0.318g (80%). ^1H NMR (600 MHz, DMSO- d_6): δ 10.72 (s, 1H, NH), 6.65 (m, 2H, NH_2), 6.46 (m, 1H, CH), 6.15 (m, 1H, CH), 5.5 (m, 1H, NH), 5.26 (m, 1H, CH), 4.69 (m, 2H, CH_2), 3-3.8 (m, 6H, CH_2), 2.18 (s, 3H, CH_3), 1.93 (s, 3H, CH_3). ^{13}C NMR (600 MHz, DMSO- d_6): δ 172.54, 170.58, 168.46, 149.35, 148.52, 147.53, 132.53, 129.15, 104.73, 83.42, 79.98, 87.68, 68.24, 77.13, 64.68, 60.04, 52.63, 50.07, 32.24. MALDI-MS (α -cyano-4-hydroxycinnamic acid / TFA Na salt): 657.52 [M+Na+K].

2: ^1H NMR (600 MHz, DMSO- d_6): δ 10.71 (s, 1H, NH), 7.92 (s, 2H, CH, CH), 6.63 (m, 2H, NH_2), 6.46 (m, 1H, CH), 6.1 (m, 1H, CH), 5.24 (m, 1H, NH), 5.19 (m, 1H, CH), 4.67 (m, 2H, CH_2), 3-3.8 (m, 6H, CH_2), 2.2 (s, 3H, CH_3), 1.9 (s, 3H, CH_3). ^{13}C NMR (600 MHz, DMSO- d_6): 171.43, 170.04, 168.14, 149.49, 147.32, 142.13, 134.26, 131.48, 103.84, 84.78, 79.97, 78.14, 68.26, 67.48, 64.32, 61.27, 53.45, 32.28. MALDI-MS (α -cyano-4-hydroxycinnamic acid / TFA Na salt): 565.2 [M+], 588.13 [M+Na].

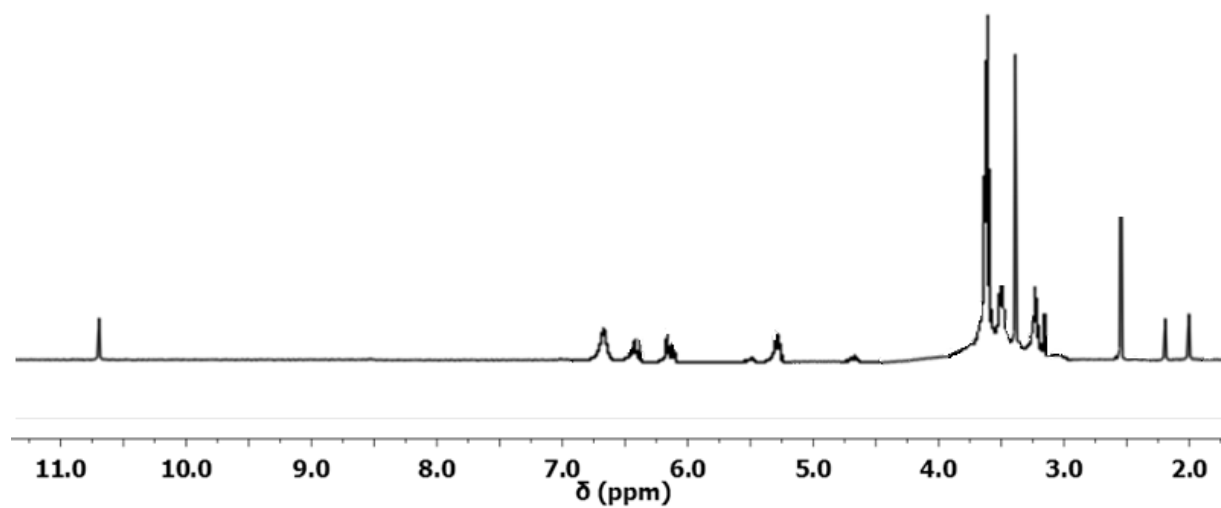


Figure S34. ^1H NMR of 8-methoxy-2',3'-diacetylguanosine 5'-acrylamide (**1**) in DMSO.

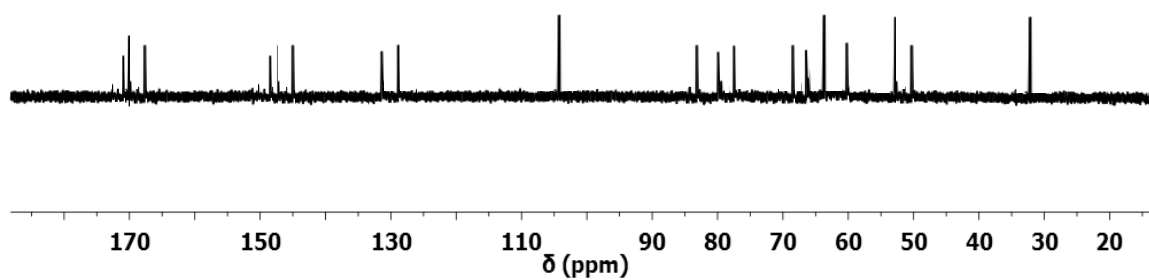


Figure S35. ^{13}C NMR of 8-methoxy-2',3'-diacetylguanosine 5'-acrylamide (**1**) in D_2O .

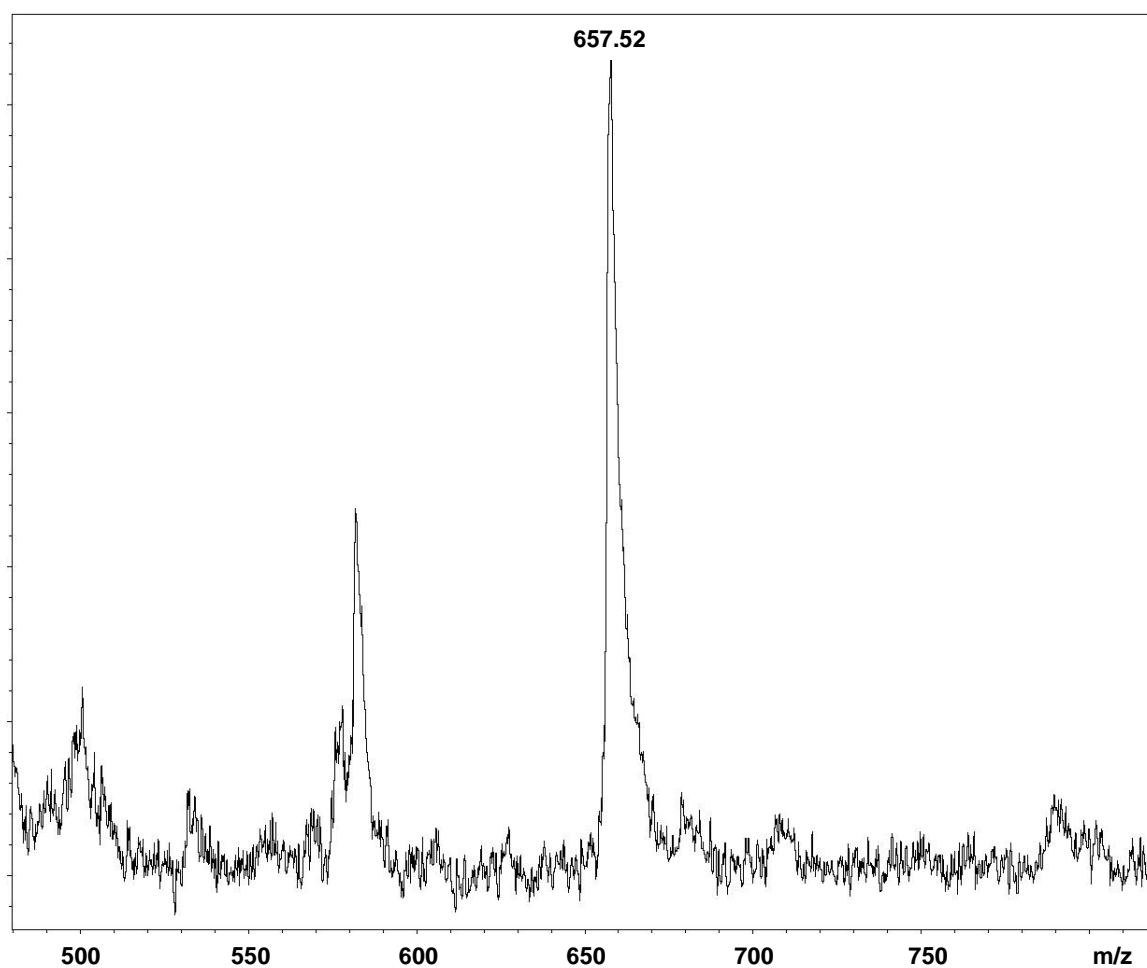


Figure S36. MALDI-MS spectra of 8methoxy-2',3'-diacetylguanosine 5'-acrylamide (**1**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

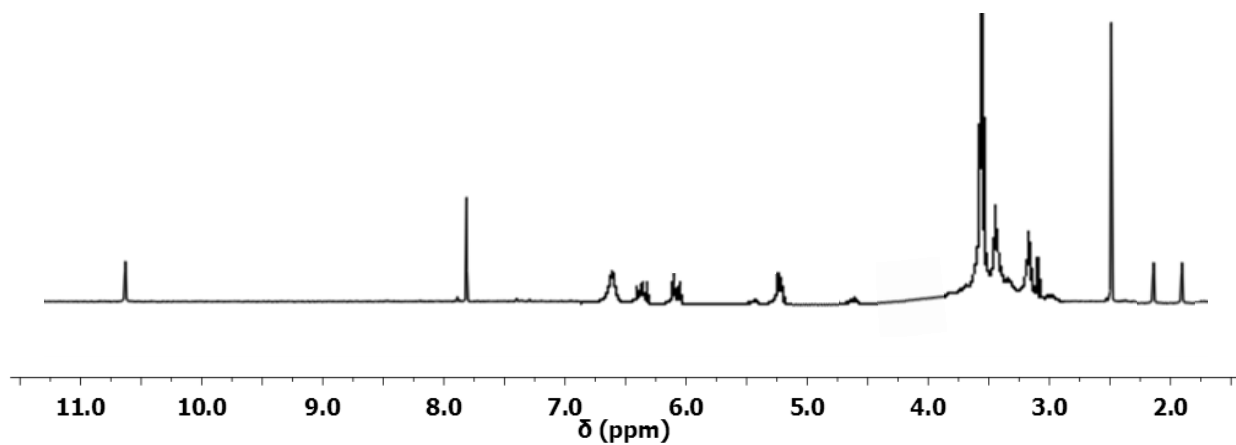


Figure S37. ^1H NMR of 2',3'-diacetylguanosine 5'-acrylamide (**2**) in DMSO.

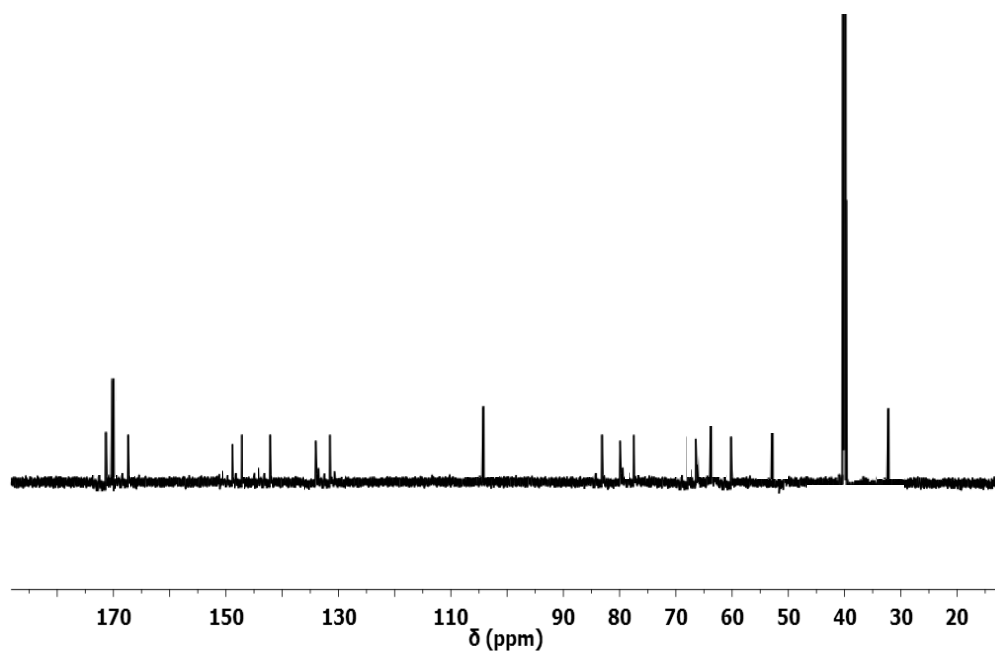


Figure S38. ^{13}C NMR of 8-methoxy-2',3'-diacetylguanosine 5'-acrylamide (**2**) in DMSO.

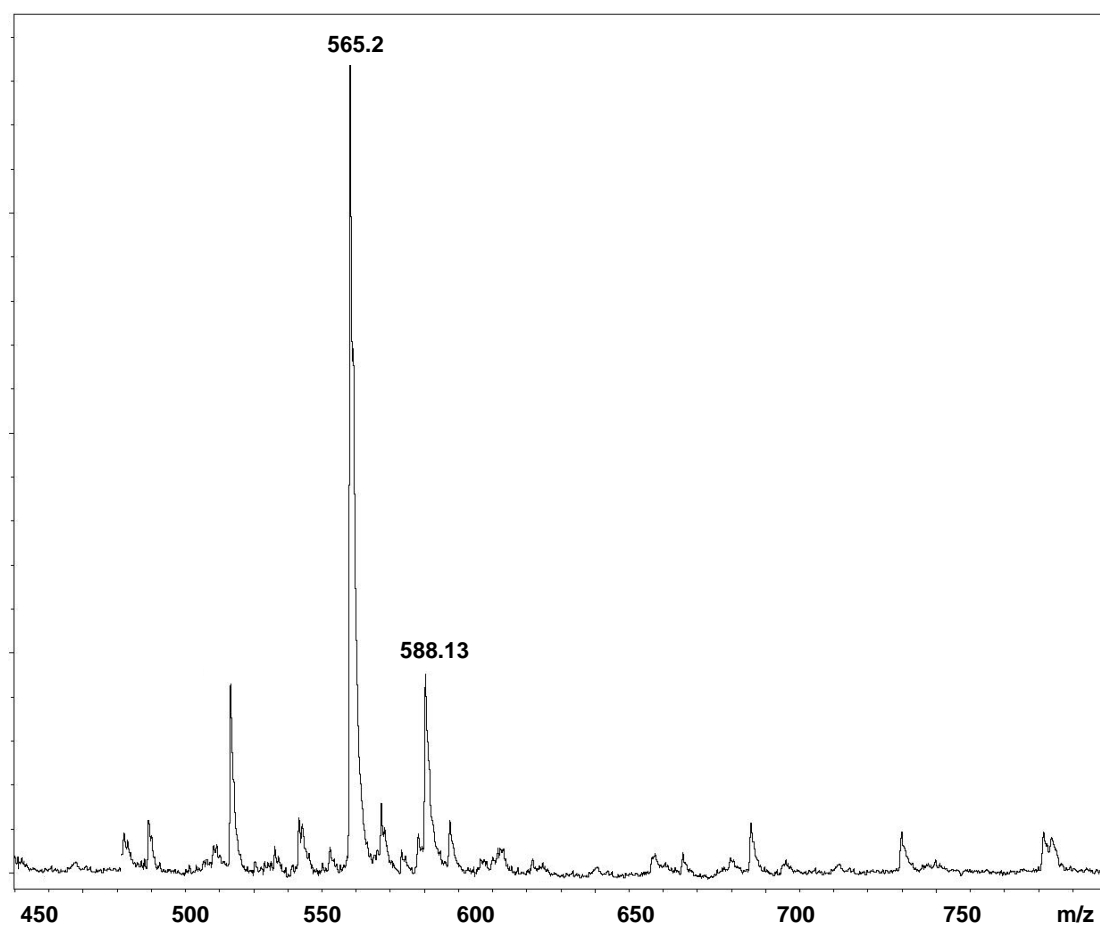


Figure S39. MALDI-MS spectra of 2',3'-diacetylguanosine 5'-acrylamide (**2**) with an α -cyano-4-hydroxycinnamic acid / TFA Na salt matrix.

	G* (Pa) $([G'^2 + G''^2]^{1/2})$
10:0 8OMeTAcG:1	8337
7:3 8OMeTAcG:1	6365
5:5 8OMeTAcG:1	4744
7:3 8OMeTAcG:2	730
5:5 8OMeTAcG:2	318
1:0.25 8OMeTAcG:4₄	4597
1:0.5 8OMeTAcG:4₄	883
1:1 8OMeTAcG:4₄	291
1:1.5 8OMeTAcG:4₄	142
1:0.25 8OMeTAcG:3₄	11587
1:0.5 8OMeTAcG:3₄	2016
1:1 8OMeTAcG:3₄	504
1:1.5 8OMeTAcG:3₄	92
1:0.25 8OMeTAcG:3₇	371
1:0.5 8OMeTAcG:3₇	855
1:1 8OMeTAcG:3₇	8769
1:1.5 8OMeTAcG:3₇	4551
1:0.25 8OMeTAcG:3₁₀	1127
1:0.5 8OMeTAcG:3₁₀	3172
1:1 8OMeTAcG:3₁₀	37535
1:1.5 8OMeTAcG:3₁₀	67433

Table 3. Summary of the complex modulus (G*) for the gels studied

RAFT Polymerization of **1** (**3_x**) or **2** (**4_x**)

For example, **3₄** was synthesized by: Dimethylacrylamide (0.5g, 99.13 g/mol, 5 mmol), **1** (0.5g, 595 g/mol, 0.84 mmol), CTA (0.074g, 406 g/mol, 0.18 mmol), and 5 mL of DMF were purged with argon for 20 minutes. AIBN (3mg, 164 g/mol, 0.018 mmol) was added and the reaction was placed at 75°C for 20 hours. The polymer was precipitated in ether (3x) and dried under vacuum. All other polymerizations were performed in similar a similar manner.

Gel Formation

The appropriate amount of guanosine derivative(s) were added to 1 mL of distilled water in a vial. The suspension was heated until all components dissolved. The sample was then allowed to cool to room temperature upon which gelation occurred within minutes or hours. Gelation was confirmed by turning the vial upside down to see if the gel stayed at the top of the vial or fell to the bottom like a liquid.

REFERENCES

-
- (1) Moad, G.; Chong, Y. K.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polymer* **2005**, 46, 8458–8468.
 - (2) Zhang, R.; Liberski, A.; Khan, F.; Diaz-Mochon, J. J.; Bradley, M. *Chem. Commun.* **2008**, 11, 1317-1319.
 - (3) Zhang, B.; Cui, Z.; Sun, L. *Org. Lett.* **2001**, 3, 275-278.
 - (4) Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, 64, 293-295.
 - (5) Hutchinson, A. J.; Williams, M.; de Jesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.; Webb, R. L.; Zoganas, H. C.; Stone, G. A.; Jarvis, M. F. *J. Med. Chem.* **1990**, 33, 1919-1924.
 - (6) Lappchen, T.; Pinas, V. A.; Hartog, A. F.; Koomen, G-J.; Scaffner-Barbero, C.; Andreu, J. M.; Trambaiolo, D.; Lowe, J.; Juhem, A.; Popov, A. V.; den Blaauwen, T. *Chem. & Biol.* **2008**, 15, 189-199.