Electronic Supplementary Material (ESI) for Chemical Communications.

Sequence-Defined Vinyl Sulfonamide Click Nucleic Acids (VS-CNAs) and Their Assembly into Dynamically Responsive Materials

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1. Materials and Instrumentation

All reagents unless otherwise stated were purchased from Millipore Sigma. Thymine, adenine, acetyl cytosine, 2-amino-6-chloropurine, cysteamine hydrochloride salt, and trityl chloride were all purchased from Chemimpex. 2-chlorosulfonyl chloride was purchased from TCI America. Concentrated HCl and sodium hydroxide pellets was purchase from Fischer Scientific. Thiol functional PEG polymers were purchased from JenKem. A CombiFlash Rf column robot with RediSep Rf Gold columns (Teledyne Isco) was used for small molecule purification. An AV400 and AV600 MHz NMR (Bruker) was used for characterizing small molecule and polymers. Polymers were characterized using a MicroFlex MALDI-TOF (Bruker) in linear mode. Hydrogels were characterized on an AR-G2 rheometer with a Peltier plate and an 8 mm flat plate geometry (TA instruments).

2. Statistical Analysis

Table S1. Statistical analysis was performed using a one-way ANOVA with post-hoc Tukey's analysis for each time point during the degradation study using linear VS-CNAs. Error bars represent standard error and all data points are represented by the mean (n = 3). For each comparison point, p-values were determined where i) $p < 0.05$; ii) $p < 0.01$, iii) $p < 0.001$, n.s. $p > 0.05$

Time point	Comparison			Significance	p-value
2 h	AAA0mMTGT	vs.	AAA1mMTGT	n.s.	p = 0.5
	ACA1mMTGT	vs.	AAA1mMTGT	n.s.	p = 0.2
	ACA1mMTGT	vs.	AAA0mMTGT	i	p = 0.03
	ACA0mMTGT	vs.	AAA1mMTGT	n.s.	p = 0.6
	ACA0mMTGT	vs.	AAA0mMTGT	n.s.	p = 1
	ACA0mMTGT	vs.	ACA1mMTGT	i	p = 0.03
4 h	AAA0mMTGT	vs.	AAA1mMTGT	i	p = 0.01
	ACA1mMTGT	vs.	AAA1mMTGT	n.s.	p = 0.1
	ACA1mMTGT	vs.	AAA0mMTGT	iii	p < 0.001
	ACA0mMTGT	vs.	AAA1mMTGT	ii	p = 0.004
	ACA0mMTGT	vs.	AAA0mMTGT	n.s.	p = 0.8
	ACA0mMTGT	vs.	ACA1mMTGT	iii	p < 0.001
8 h	AAA0mMTGT	vs.	AAA1mMTGT	iii	p < 0.001
	ACA1mMTGT	vs.	AAA1mMTGT	i	p = 0.04
	ACA1mMTGT	vs.	AAA0mMTGT	iii	p < 0.001
	ACA0mMTGT	vs.	AAA1mMTGT	iii	p < 0.001
	ACA0mMTGT	vs.	AAA0mMTGT	n.s.	p = 0.5
	ACA0mMTGT	vs.	ACA1mMTGT	iii	p < 0.001
12 h	AAA0mMTGT	vs.	AAA1mMTGT	iii	p < 0.001
	ACA1mMTGT	vs.	AAA1mMTGT	ii	p = 0.002
	ACA1mMTGT	vs.	AAA0mMTGT	iii	p < 0.001
	ACA0mMTGT	vs.	AAA1mMTGT	iii	p < 0.001
	ACA0mMTGT	vs.	AAA0mMTGT	n.s.	p = 0.09
	ACA0mMTGT	vs.	ACA1mMTGT	iii	p < 0.001

3. Alkylation of Pyrimidines vs. Purines

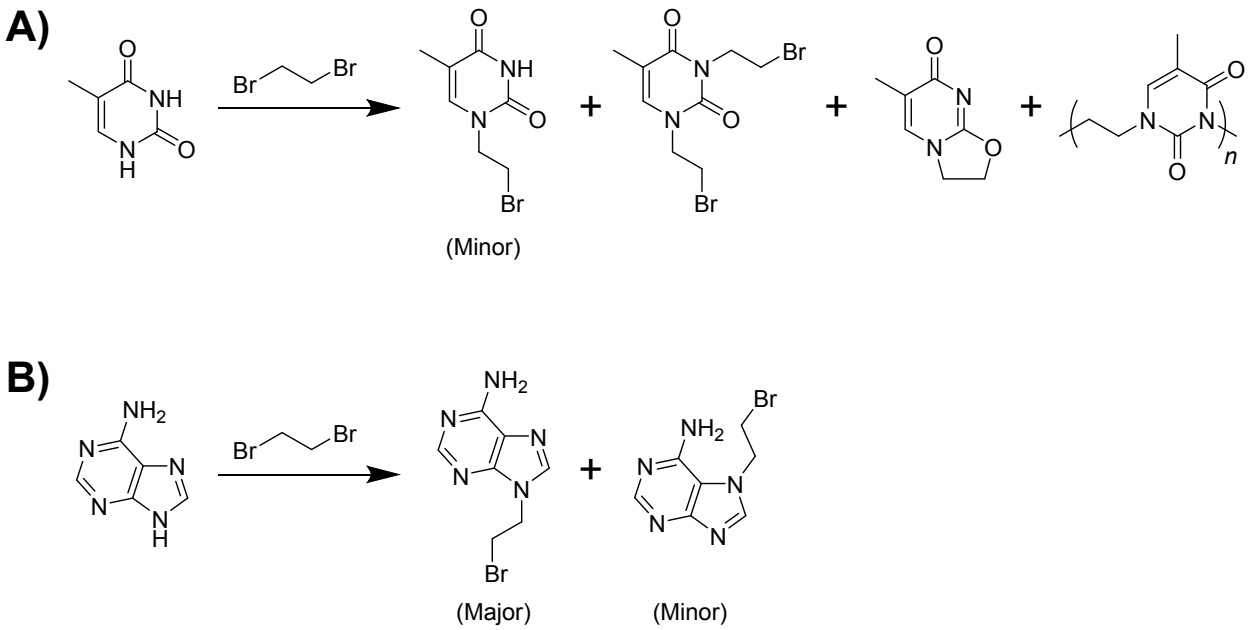
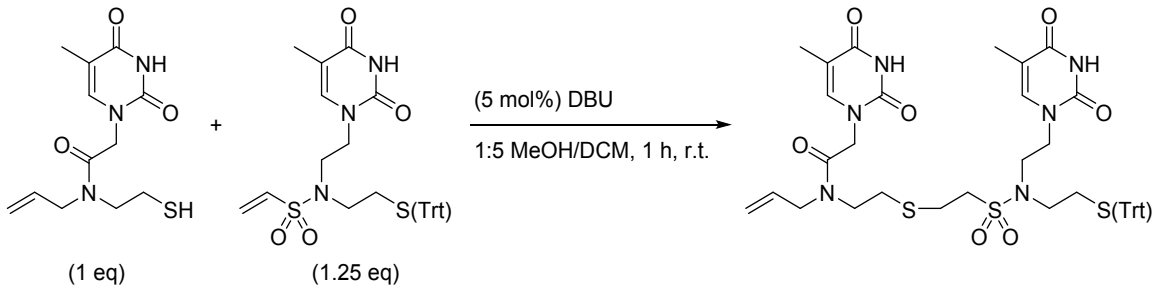


Figure S1. The products obtained from alkylating A) thymine and B) adenine with dibromoethane. Attempts at functionalizing Thymine resulted in minor formation of the desired product along with difunctional, cyclic, and oligomeric species. Functionalizing adenine provided the desired product in high yields with a small amount of a minor impurity that could be removed via column chromatography.

4. Model CNA Sequence Building



To a 50 mL round bottom flask was added **TE-T** (1 eq, 3.6 mmol, 1 g), **TM-T** (1.25 eq, 4.5 eq, 2.52 g), and 25 mL of 5:1 DCM/MeOH. To the stirring solution was added 27 µL of DBU (0.05 eq) and the reaction was left to stir for an hour. The solution was then concentrated to an oil and column purified using a gradient of 0% to 5% MeOH in DCM. The fractions of both the product and the excess TM-T were collected separately to give the dimer product **Allyl-TT-S(Trt)** (2.86 g, 94%) and the excess **TM-T** (0.42 g, 0.21 eq of the initial 1.25 amount) as off-white foams.

^{1}H NMR (600 MHz, DMSO) δ 11.28 (d, *J* = 10.4 Hz, 1H), 11.22 (d, *J* = 4.9 Hz, 1H), 7.48 – 7.01 (m, 17H), 5.93 – 5.83 (m, 1H), 5.71 (dt, *J* = 10.6, 5.4 Hz, 0H), 5.29 – 5.21 (m, 1H), 5.21 – 5.11 (m, 1H), 4.64 (s, 1H), 4.55 (s, 1H), 4.03 (d, *J* = 5.3 Hz, 1H), 3.94 (d, *J* = 5.6 Hz, 1H), 3.58 (q, *J* = 6.0 Hz, 2H), 3.44 (d, *J* = 7.6 Hz, 1H), 3.38 (d, *J* = 7.3 Hz, 1H), 3.23 – 3.13 (m, 4H), 3.04 (dt, *J* = 14.8, 7.5 Hz, 2H), 2.85 – 2.73 (m, 2H), 2.72 – 2.62 (m, 2H), 2.43 (t, *J* = 7.6 Hz, 2H), 1.75 (d, *J* = 4.3 Hz, 3H), 1.71 (s, 3H).

^{13}C NMR (151 MHz, DMSO) δ 167.37, 166.86, 164.89, 164.71, 151.55, 151.50, 151.34, 144.76, 142.76, 141.92, 141.88, 133.83, 133.76, 129.59, 128.58, 127.32, 117.67, 117.58, 108.79, 108.75, 108.45, 67.02, 55.38, 51.32, 49.62, 48.55, 48.07, 47.38, 46.54, 46.28, 46.11, 46.06, 30.58, 30.51, 29.47, 28.93, 24.73, 24.60, 12.39, 12.36.

Mass: $C_{42}H_{48}N_6O_7S_3$ $[M+H]^+$ Calculated: 846.06, Found: 846.34

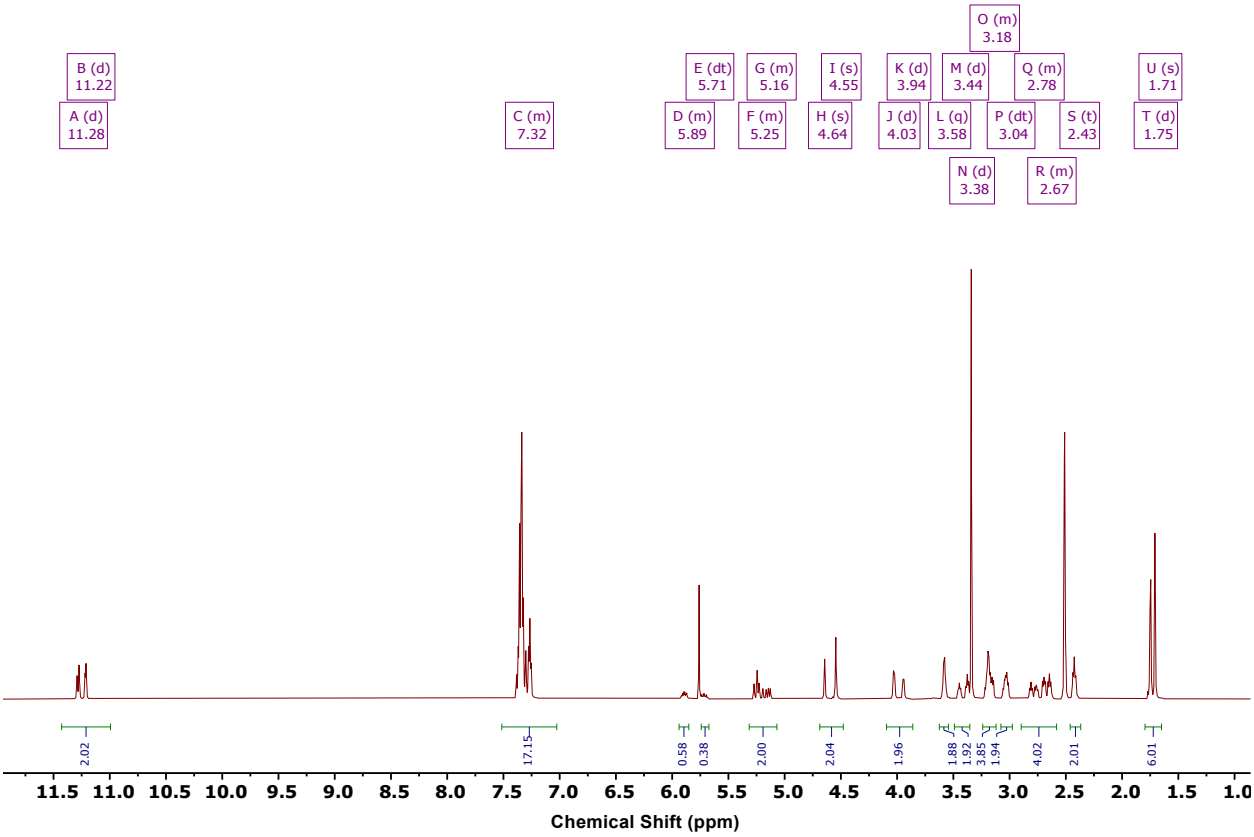


Figure S2. ^{1}H NMR (600 MHz, DMSO) of the dimer product Allyl-TT-S(Trt).

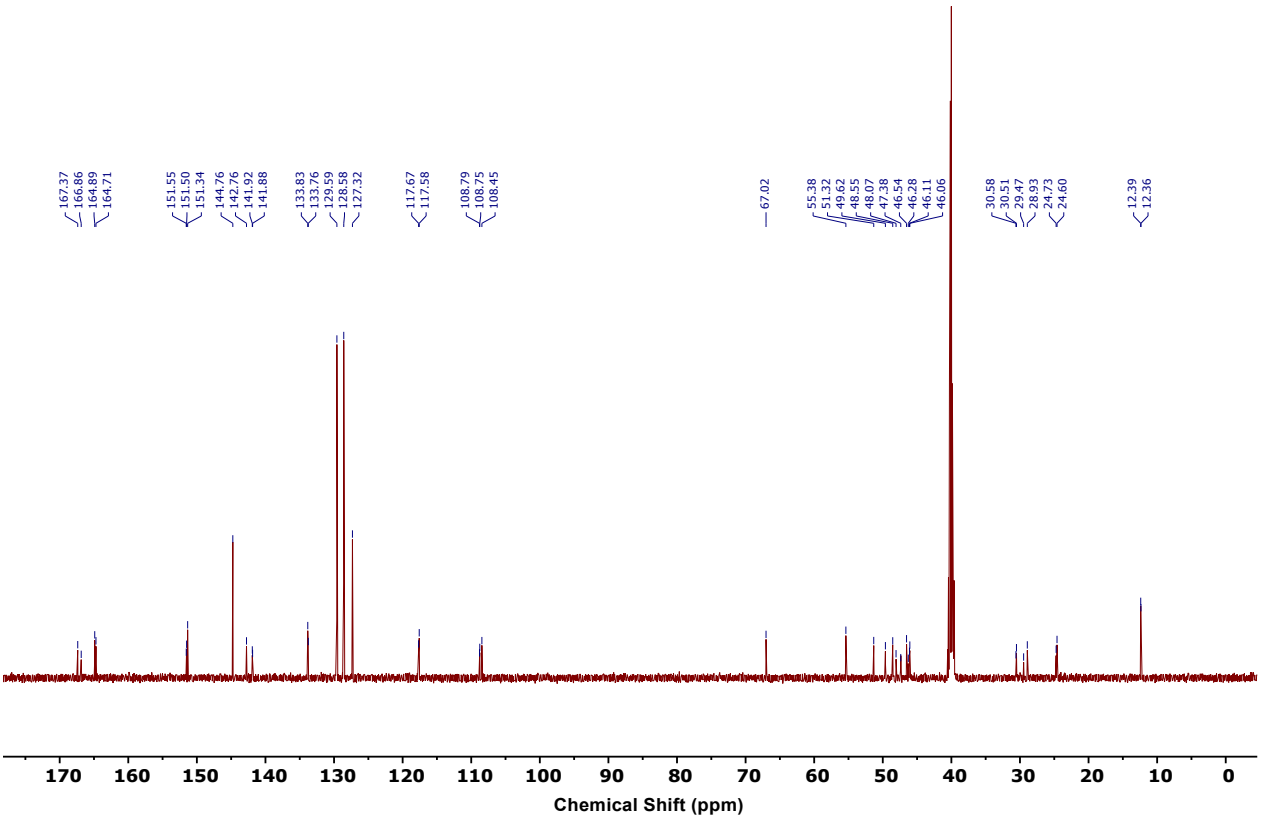


Figure S3. ^{13}C NMR (151 MHz, DMSO) of the dimer product **Allyl-TT-S(Trt)**.

5. General procedures

General procedure for Thiol-Michael coupling to thiol functional linear and multiarm PEG: To a 20 mL vial was added a stir bar and thiol functional linear PEG(5k) or thiol functional 4-arm PEG(20k) (1 g, 0.2 mmol relative to thiol functionality, 1 eq) followed by DMF (10 mL). The polymer was mixed with gentle heating until completely dissolved. Next, the thiol-Michael thymine CNA monomer (TM-T) was added (561 mg, 1 mmol, 5 eq relative to thiol functionality) and the solution was mixed with gentle heating until homogenous. The reaction was initiated with the addition of the base catalyst 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (36 µL, 0.24 mmol, 1.2 eq relative to thiol functionality). The solution was mixed for 1 h before being precipitated into 40 mL of cold diethyl ether. The precipitated polymer was centrifuged at 4000 rpm at room temperature for 5 min and the diethyl ether decanted. The solid polymer was washed with 40 mL of diethyl ether an additional two times. Next, the precipitate was dissolved in 40 mL of hot ethanol and subsequently cooled in an ice bath for 15 min to precipitate the PEG-CNA. The polymer was then centrifuged at 4000 rpm at 4°C for 5 min followed by decanting of the ethanol. The solid polymer was subjected to hot ethanol washes followed by precipitation for a total of five times or until NMR showed no remaining monomer present. ***NOTE:*** *When coupling the thymine monomer to the PEG support, additional washes beyond 5 may be necessary to completely remove the excess monomer. When coupling the guanine monomer to the PEG support, upon dissolving the PEG in hot ethanol, a slight cloudiness sometimes occurred associated with insoluble guanine monomer. To facilitate purification, an initial filtration of the hot ethanol-PEG solution through a fine or medium fret filter removed these impurities. The general purification process can then be subsequently followed to purify the PEG product.* Finally, the solid polymer was washed with 40 mL of diethyl ether once and dried via high vac to produce trityl protected PEG(5k)-Thymine (PEG-T-S(Trt)) (1.064 g, 96%) or trityl protected 4-arm PEG(20k)-Thymine (4PEG-T-S(Trt)) (1.053 g, 95%). This procedure is analogous for all four nucleobase monomers.

General procedure for Trityl (Trt) deprotection of PEG-CNA: To a 20 mL vial was added a stir bar and PEG-T-S(Trt) (1.064 g, 0.191 mmol, 1 eq) or 4PEG-T-S(Trt) (1.050 g, 0.189 mmol relative to thiol functionality, 1 eq). The polymer was dissolved in DCM (5.5 mL) followed by the addition of triethylsilane (TES, 1.1 mL). Then trifluoroacetic acid (TFA, 3.4 mL) was added and the reaction proceeded for 15 min. Upon completion, the solution was precipitated into 40 mL of cold diethyl ether. The precipitated polymer was centrifuged down and the diethyl ether decanted. The solid polymer was washed with 40 mL of diethyl ether an additional two times. Next, the precipitate was dissolved in 40 mL of hot ethanol and subsequently cooled in an ice bath for 15 min to precipitate the PEG-CNA. The polymer was then centrifuged, and the ethanol decanted. The solid polymer was subjected to the hot ethanol wash followed by precipitation one additional time. Finally, the solid polymer was washed with 40 mL of diethyl ether once and dried via high vac to produce thiol functional PEG_{5k}-Thymine (PEG-T-SH) or (973 mg, 96%) or 4-arm PEG_{20k}-Thymine (4PEG-T-SH) (939 mg, 93%). This procedure is analogous for all four nucleobase monomers.

General procedure for CNA-based hydrogel formation: Multiarm PEG-CNAs were dissolved in DMSO at 20 wt%. Equal volumes of 20 wt% tPEG-TTT and 20 wt% tPEG-AAA or tPEG-ACA were combined in a cylindrical mold followed by the addition of water to yield a bulk gel (total volume of 30 µL) with a final polymer concentration of 13.3 wt% (13.3 mM final TTT concentration and 13.3 mM final AAA or ACA concentration). The mold was covered with parafilm and left at 40 °C to gel for 20 h. The polymerized gels were transferred into a 48-well plate and allowed to swell in 1x PBS overnight at room temperature followed by an additional buffer exchange prior to further use in experiments. Hydrogels made with tPEG-

AAA or tPEG-ACA systems swelled to a volume of 46.4 +/- 3.5 mm3 and 52.8 +/- 2.0 mm3, respectively. Error is reported as standard error (n = 6) and the volumes were found to be statistically the same using a two-sided Student's t-test with p = 0.05.

Mechanical testing of CNA-based hydrogels: The mechanical properties of equilibrium swollen PEG-CNA hydrogels were tested using rheology. Rheological testing was conducted on a parallel plate rheometer (AR-G2, TA Instruments, New Castle, DE) equipped with a Peltier plate and an 8 mm flat plate geometry. The storage and loss moduli were recorded with an applied strain of 1% and a frequency of 6 rad s^{-1} unless otherwise noted. Prior to being placed on the bottom plate, the swollen hydrogel diameter was determined using the average of three measurements gathered with Vernier calipers. The height of the hydrogels was determined by lowering the top plate onto the hydrogel until an applied normal force of 0.1 N was achieved. Both the diameter and height were recorded in the rheometer software (TRIOS, TA Instruments) before starting any measurements. Thermal cycling was conducted using dynamic time sweep measurements between 25 ºC and 75 ºC. The initial storage and loss moduli of the hydrogel were determined at 25 ºC for 5 min. Subsequently, the temperature was ramped from 25 ºC to 75 ºC (ramp rate of 5ºC per minute) followed by a hold at 75 ºC for 5 min. The temperature was returned to 25 ºC and held for 60 min to allow for the hydrogel to recover after being subjected to high temperature. This process was repeated for a total of three cycles. Strain cycling measurements were done using dynamic time sweep measurements to monitor the storage and loss moduli of the hydrogel under different strain conditions. In between increased strain application, the hydrogels were monitored at 1% strain for 5 min to assess any recovery. A total of three strain cycles were conducted. To ensure the swollen hydrogel did not slip during the experiment, sandpaper was placed on the bottom plate of the rheometer.

General procedure for CNA-based hydrogel degradation via strand-displacement: Bulk hydrogels were formed as discussed above. After the hydrogels were equilibrium swollen, they were transferred to either a 1 mM linear PEG-TGT solution or fresh PBS. At predefined time points, the hydrogel diameter was determined using three measurements taken with Vernier calipers and the height was measured using the gap height on the rheometer when a 0.1 N force was applied to the hydrogel surface. From these data, the hydrogel volume was calculated at each time point assuming ideal cylindrical geometry. All data was normalized to the average initial volume at time t = 0 for that hydrogel composition. A total of three hydrogels were monitored for each condition over a 12 h period.

6. CNA Polymer and Hydrogel Characterization

Table S2. MALDI-TOF peaks for the evolution of PEG-GATTACA-S(Trt) were evaluated using peak fitting in Origin to determine M_n. The MALDI results were in good agreement between the calculated M_n and the theoretical M_n, further demonstrating the fidelity of the sequence-control process. Peak fitting was performed to calculate the Mn using Origin.

PEG-CNA	Theor. M_n (g/mol)	Calc. M_n (g/mol)	Δ	% Δ
PEG-SH	5000	5005	5	< 0.1%
PEG-G-S(Trt)	5586	5591	5	< 0.1%
PEG-GA-S(Trt)	5914	5895	-19	0.3%
PEG-GAT-S(Trt)	6234	6200	-34	0.5%
PEG-GATT-S(Trt)	6553	6585	32	0.5%
PEG-GATTA-S(Trt)	6881	6843	-38	0.5%
PEG-GATTAC-S(Trt)	7186	7096	-90	1.3%
PEG-GATTACA-S(Trt)	7514	7425	-89	1.2%

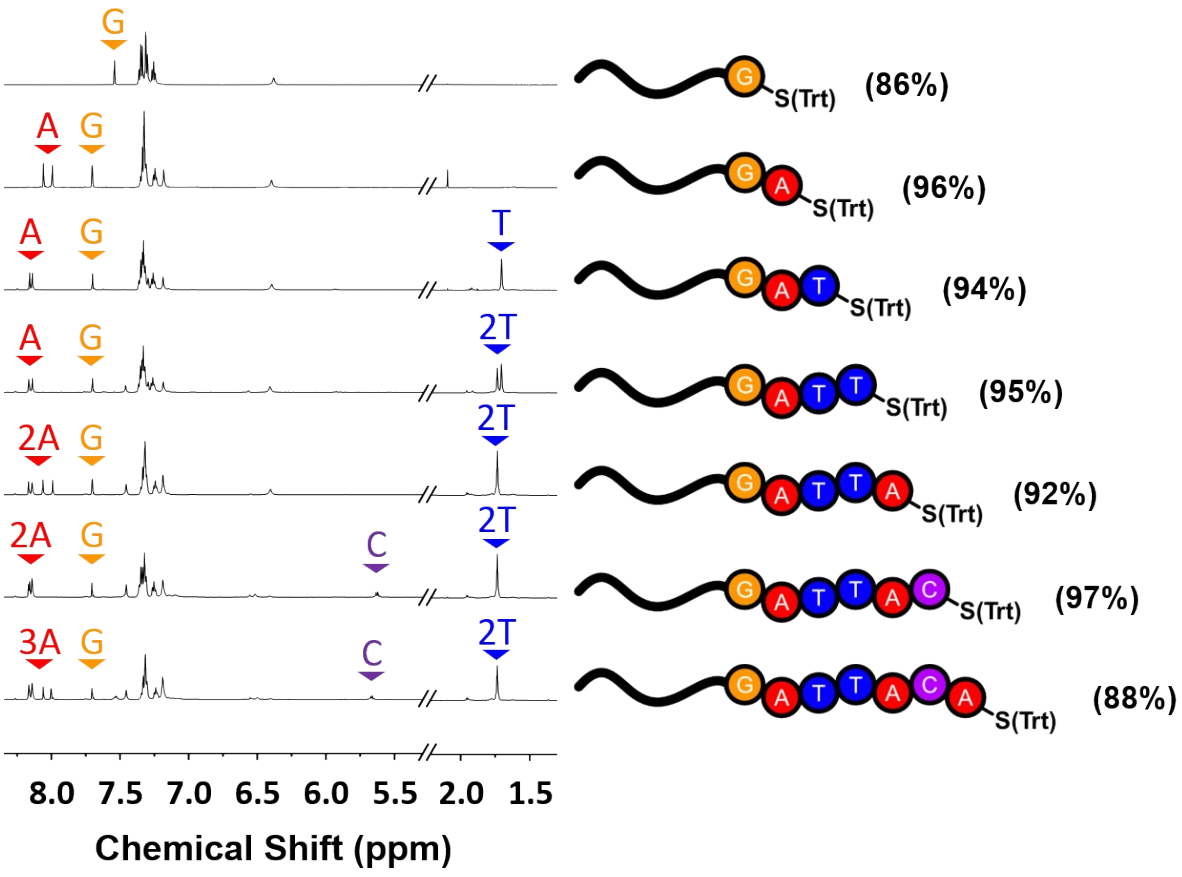


Figure S4. ^{1}H NMR shows the appearance of the characteristic aromatic protons of the nucleobases post conjugation confirming the iterative addition of monomers. The resulting yields of each conjugation and polymer purification are shown to the right of the sequences.

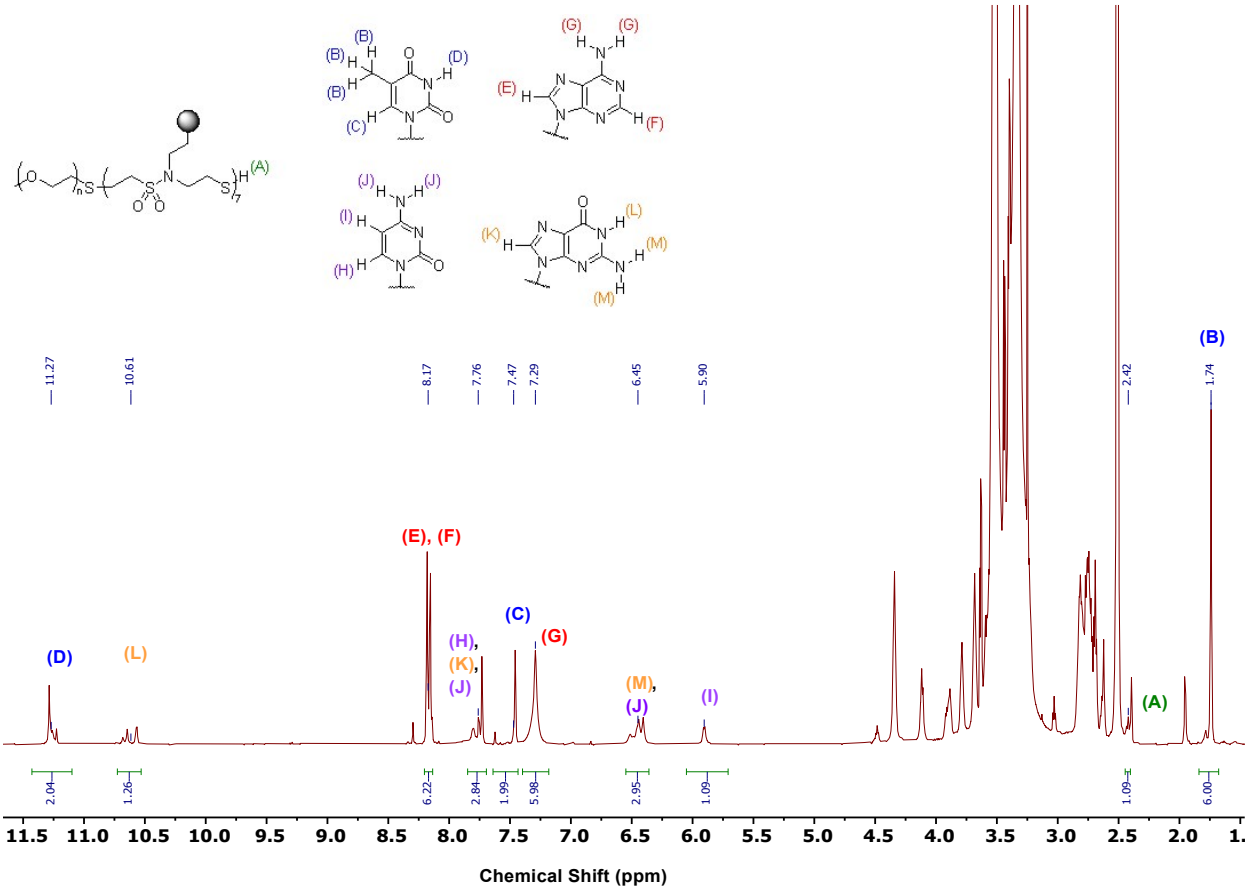


Figure S5. ^{1}H NMR (600 MHz, DMSO) of PEG_{5k}-GATTACA-SH.

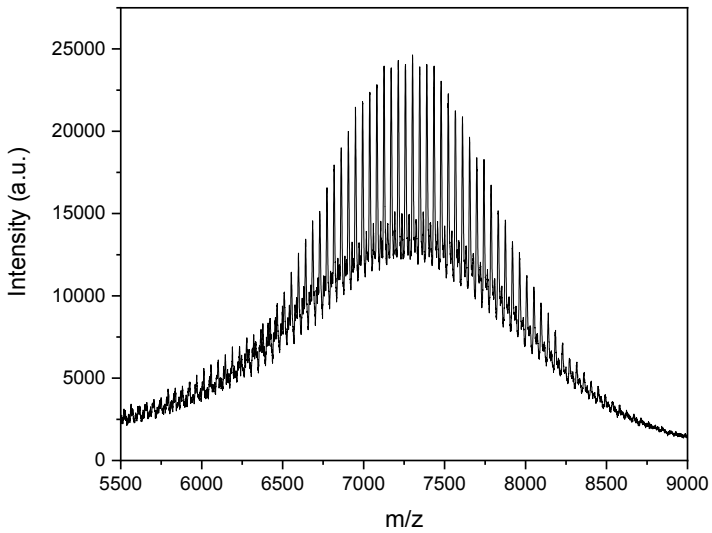


Figure S6. MALDI of PEG_{5k}-GATTACA-SH (Expected M_n = 7263 g/mol, Calculated M_n = 7290 g/mol).

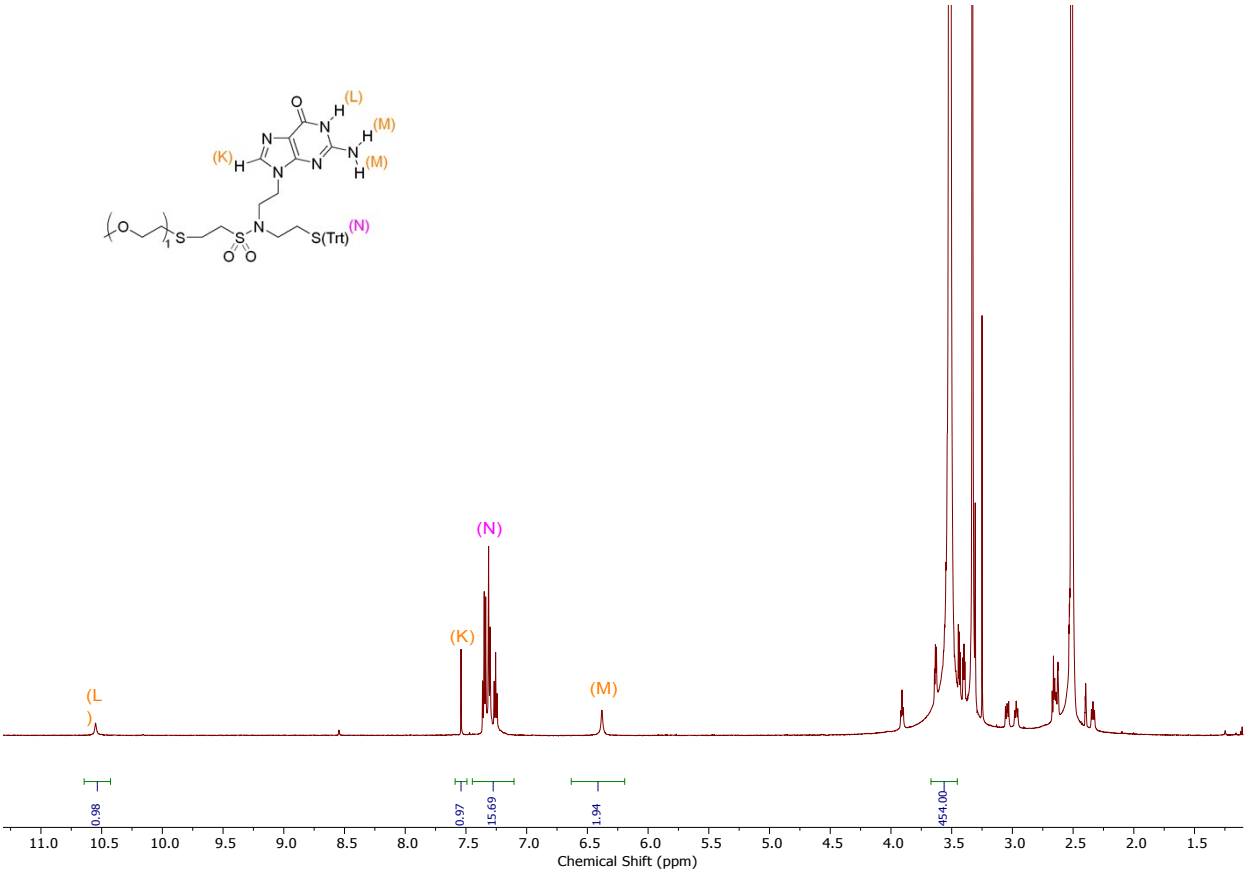


Figure S7. Conversions for the synthesis of PEG(5k)-G-STrt via the conjugation of PEG(5k)-SH with a guanine monomer was determined quantitatively via ^{1}H NMR (600 MHz, DMSO). Using PEG as an internal standard with an average of 454 protons, the guanine peak (K) post conjugation suggests quantitative coupling efficiency (> 97% conversion).

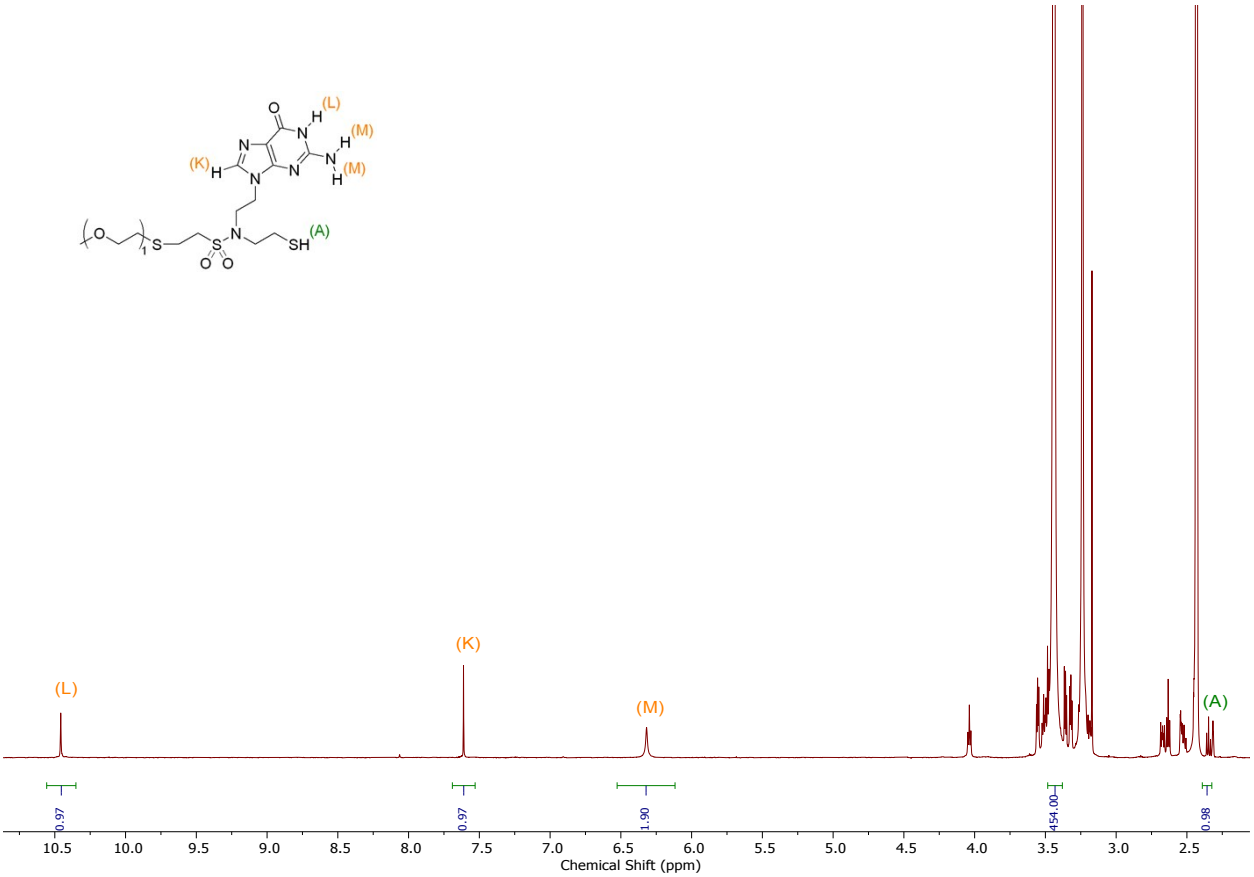


Figure S8. Conversions for the synthesis of PEG(5k)-G-SH via deprotection of the trityl group to regenerate the thiol functional handle determined quantitatively via ^{1}H NMR (600 MHz, DMSO). Using PEG as an internal standard with an average of 454 protons, the thiol peak (A) post deprotection suggests quantitative deprotection efficiency (> 99% conversion).

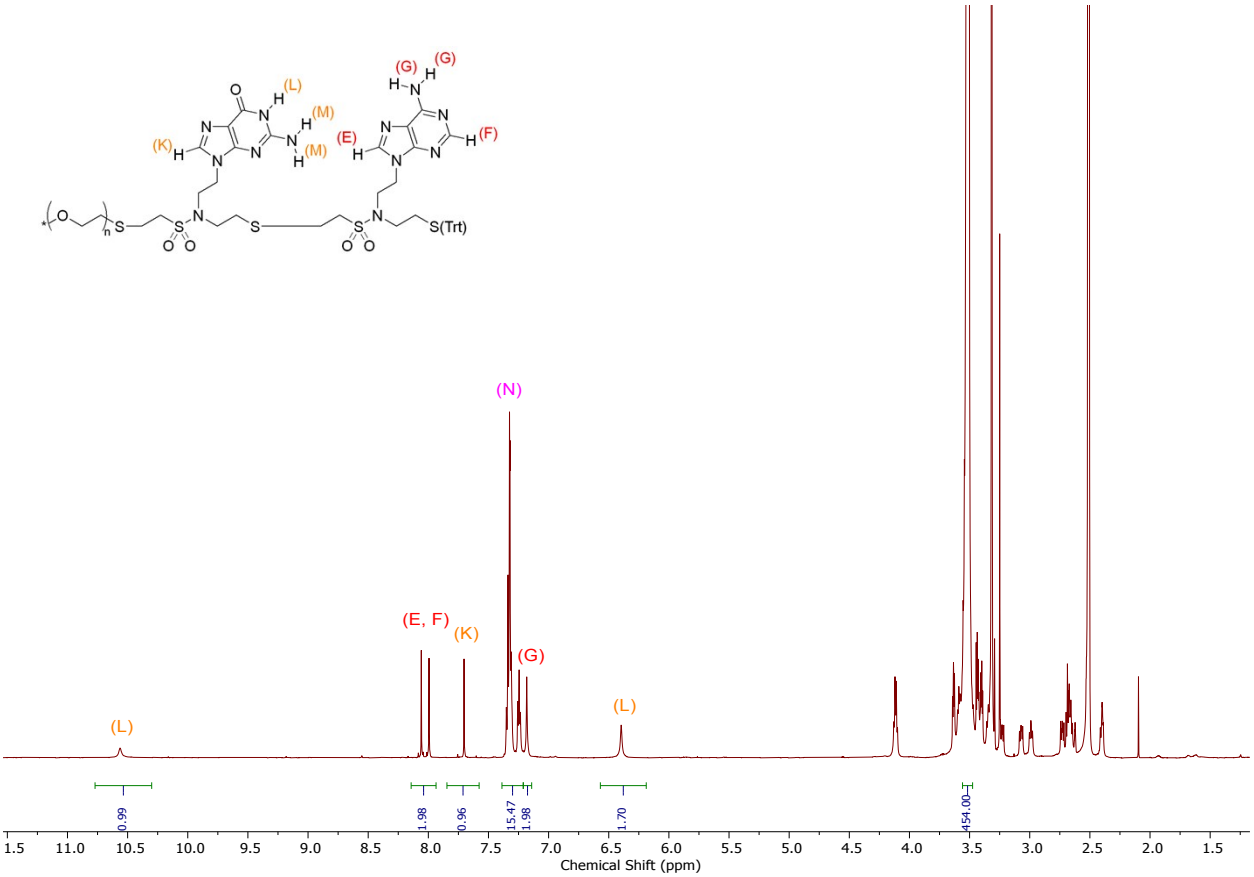


Figure S9. Conversions for the synthesis of PEG(5k)-GA-S(Trt) via the conjugation of PEG(5k)-G-SH with an adenine monomer was determined quantitatively via ^{1}H NMR (600 MHz, DMSO). Using PEG as an internal standard with an average of 454 protons, the average of the two adenine peaks (E and F) post conjugation suggests quantitative coupling efficiency (> 99% conversion).

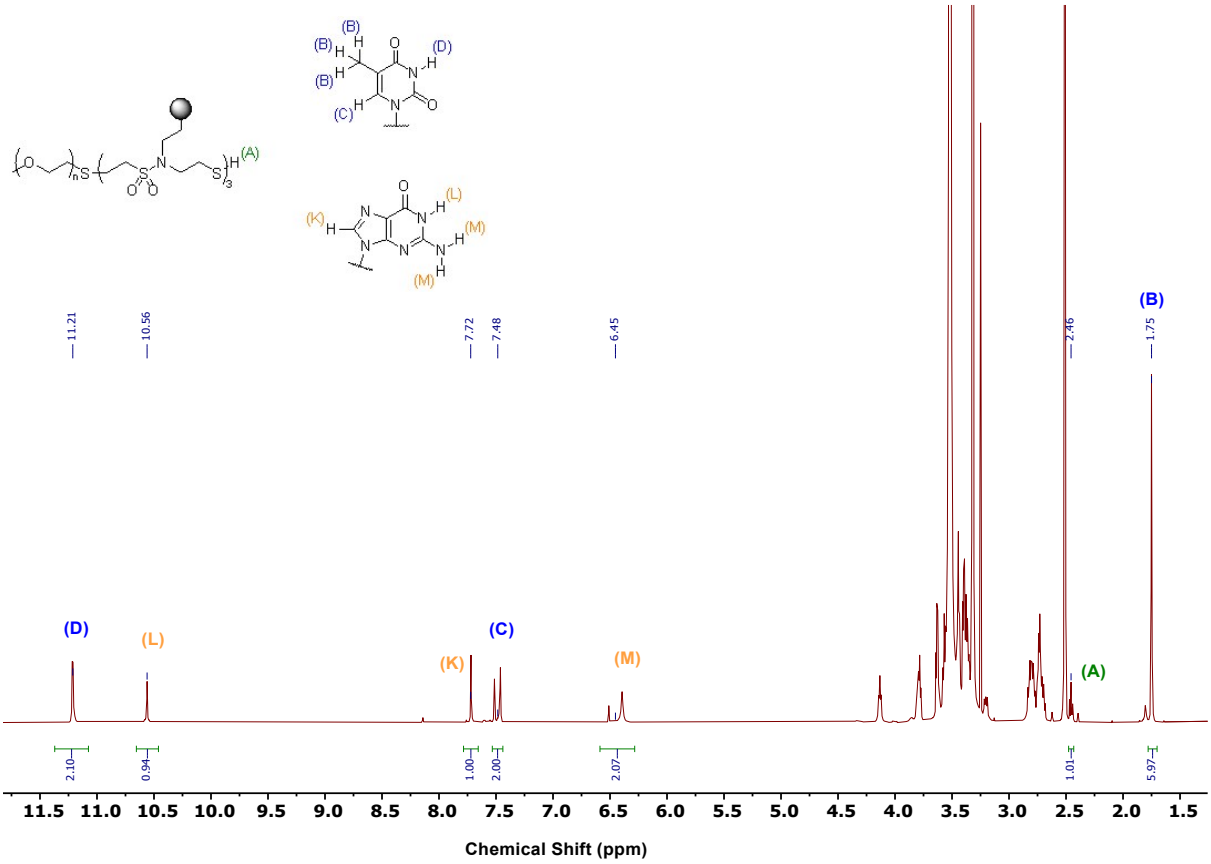


Figure S10. ^{1}H NMR (600 MHz, DMSO) of PEG_{5k}-TGT-SH.

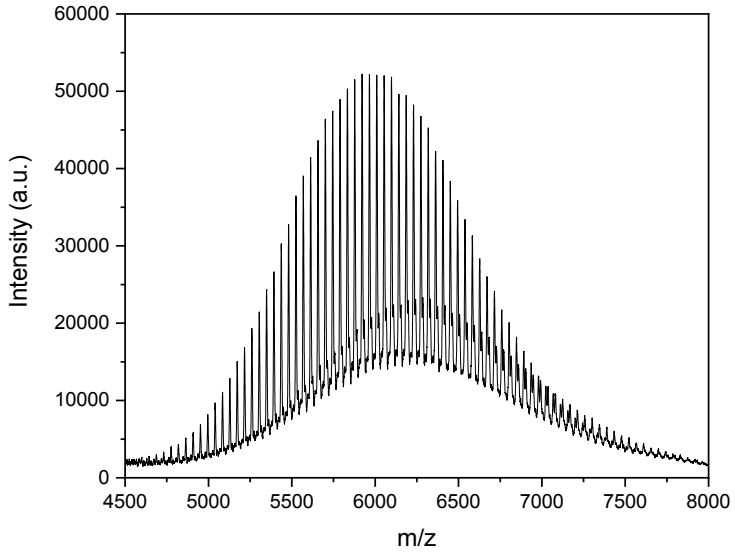


Figure S11. MALDI of PEG_{5k}-TGT-SH (Expected M_n = 5979 g/mol, Calculated M_n = 6023 g/mol).

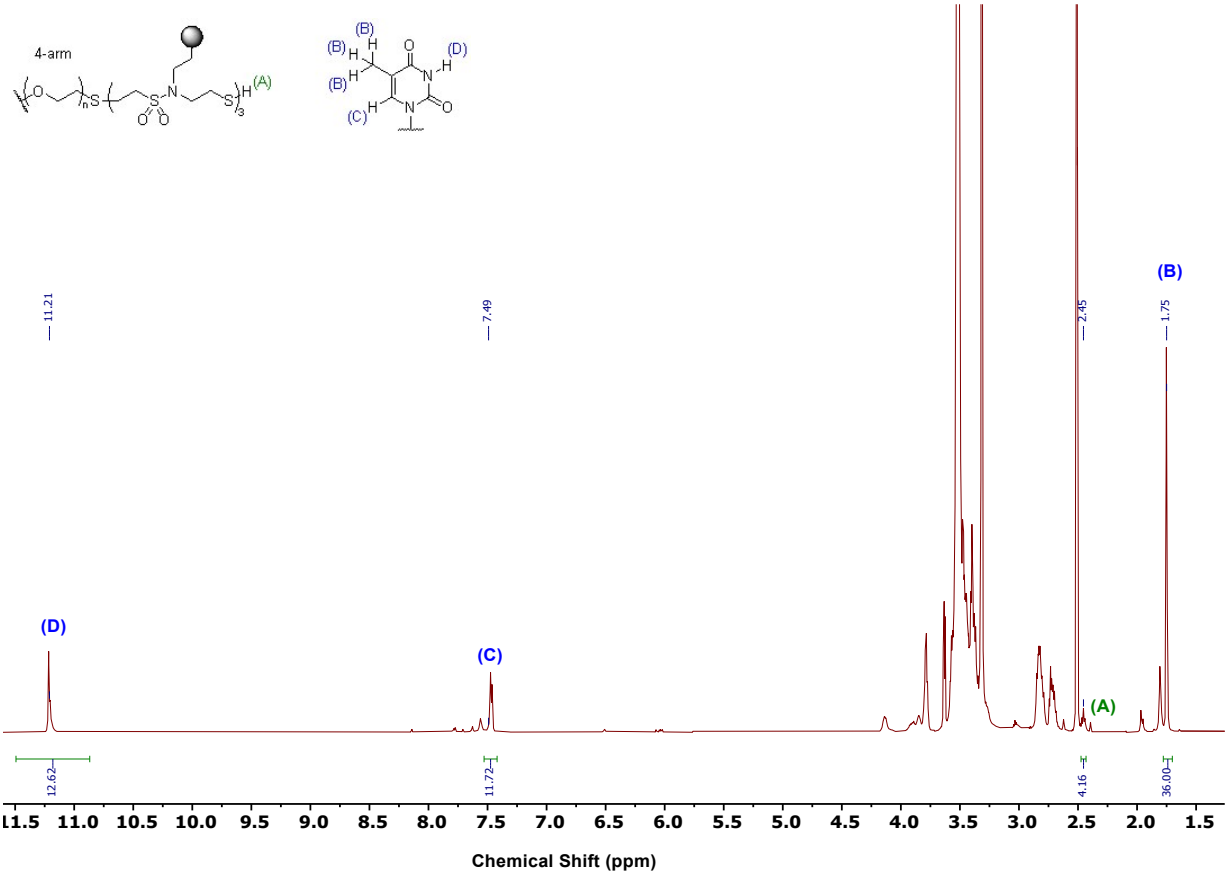


Figure S12. ^{1}H NMR (600 MHz, DMSO) of 4-arm PEG_{20k}-TTT-SH.

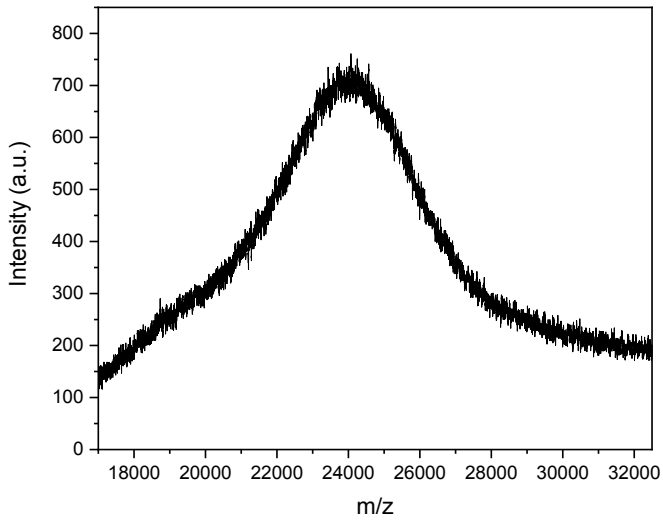


Figure S13. MALDI of 4-arm PEG_{20k}-TTT-SH (Expected M_n = 23,816 g/mol, Calculated M_n = 24174 g/mol).

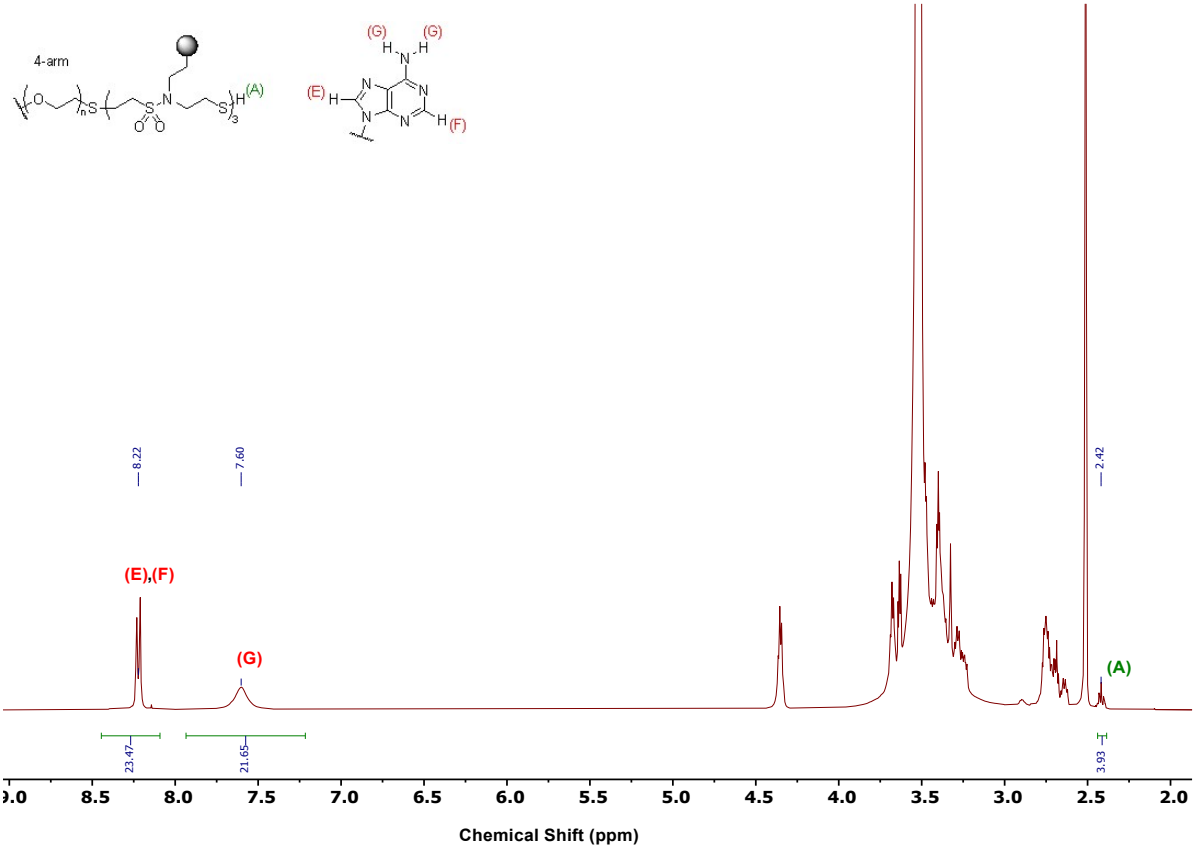


Figure S14. ^{1}H NMR (600 MHz, DMSO) of 4-arm PEG_{20k}-AAA-SH.

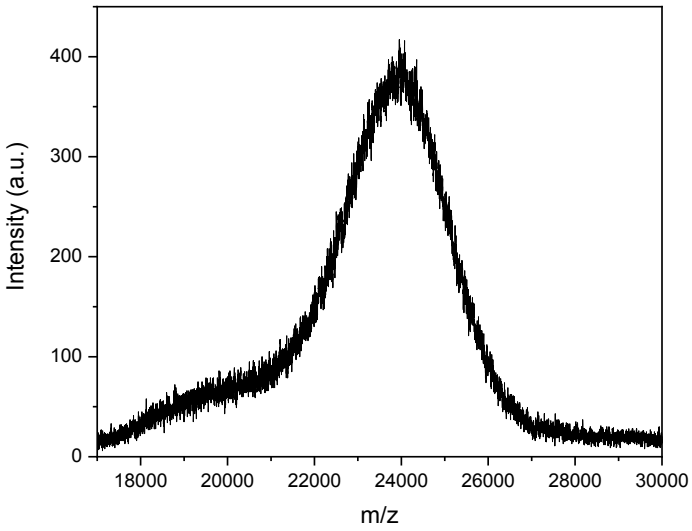


Figure S15. MALDI of 4-arm PEG_{20k}-AAA-SH (Expected M_n = 23,924 g/mol, Calculated M_n = 23,887 g/mol).

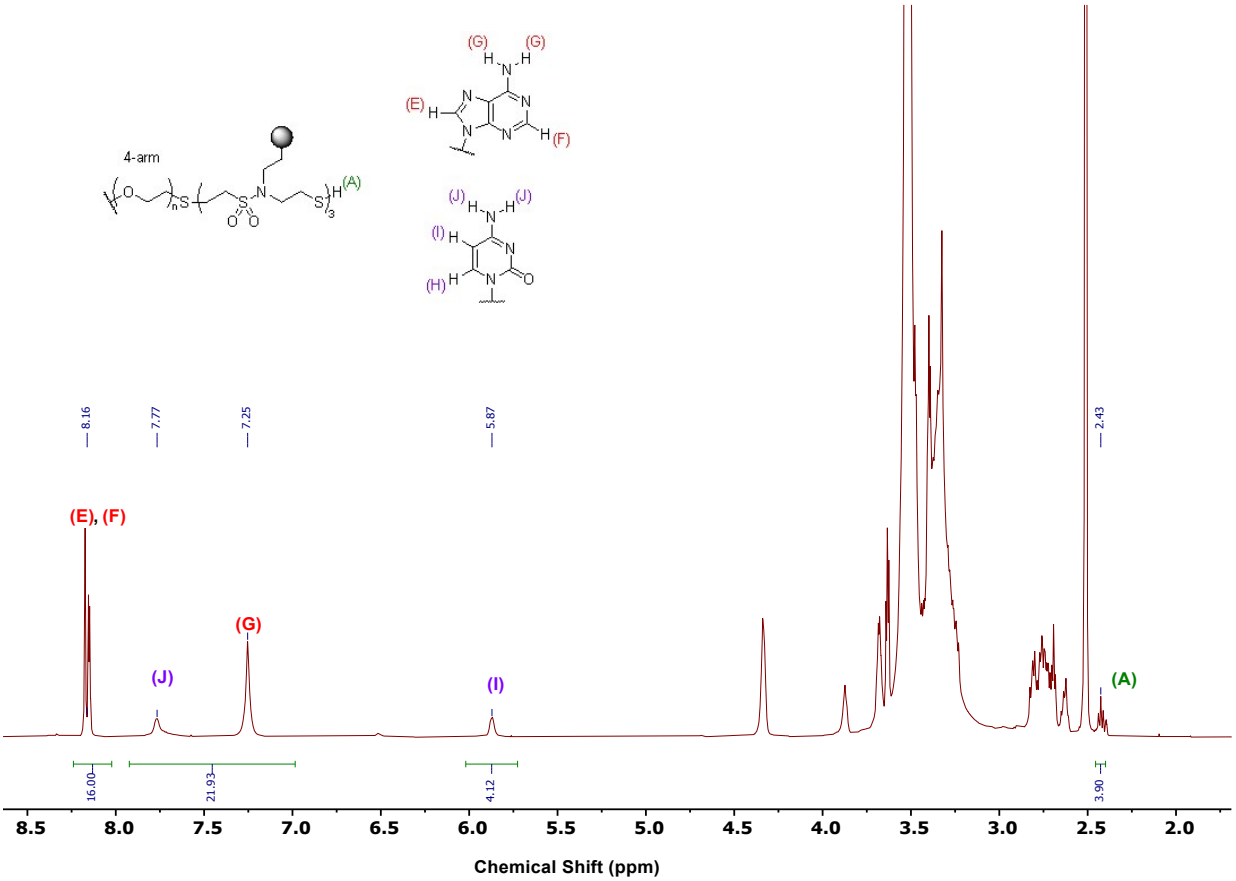


Figure S16. ^{1}H NMR (600 MHz, DMSO) of 4-arm PEG_{20k}-ACA-SH.

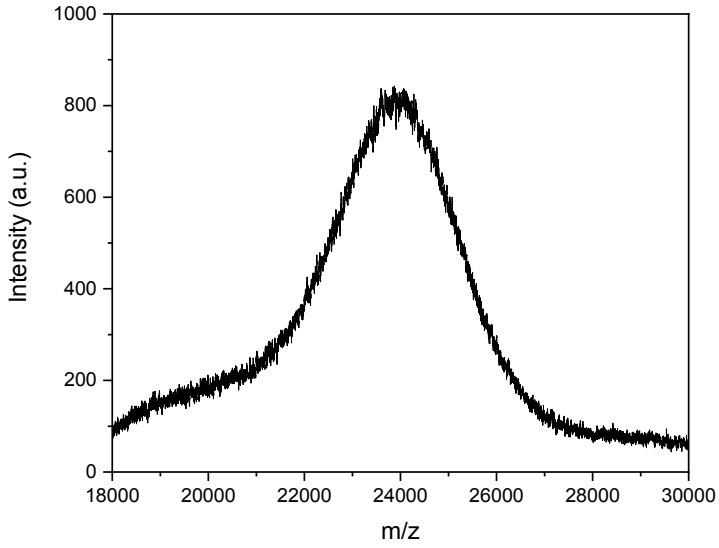
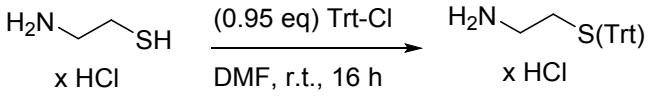


Figure S17. MALDI of 4-arm PEG_{20k}-ACA-SH (Expected M_n = 23,816 g/mol, Calculated M_n = 23,739 g/mol).

7. Synthesis of Backbones for Thiol-Michael and Thiol-ene



1

Cysteamine hydrochloride (87 mmol, 10 g, 1 eq) was added to an oven dried and nitrogen purged 500 mL round-bottom flask followed by 75 mL of anhydrous DMF. Trityl chloride (82.7 mmol, 23 g, 0.95 eq) was subsequently added and the mixture was stirred overnight. Once the reaction was finished, ethyl acetate (100 mL) was added to the round bottom flask. The solution was then poured into 700 mL of deionized water while stirring. A white solid precipitated and was allowed to stir for 15 min. The white colloid was then filtered and the cake was washed with water (100 mL) and diethyl ether (100 mL) to afford compound **1** as a white solid. The material was further dried in a desiccator overnight to remove residual water (30.9 g, 99%).

^{1}H NMR (400 MHz, DMSO) δ 7.98 (s, 3H), 7.48 – 7.14 (m, 16H), 2.47 (s, 4H).

^{13}C NMR (101 MHz, DMSO) δ 144.45, 129.49, 128.70, 127.42, 66.87, 38.10, 28.89.

Mass: $C_{21}H_{21}NS$ $[M+H]^+$ Calculated: 320.46, Found: 320.37.

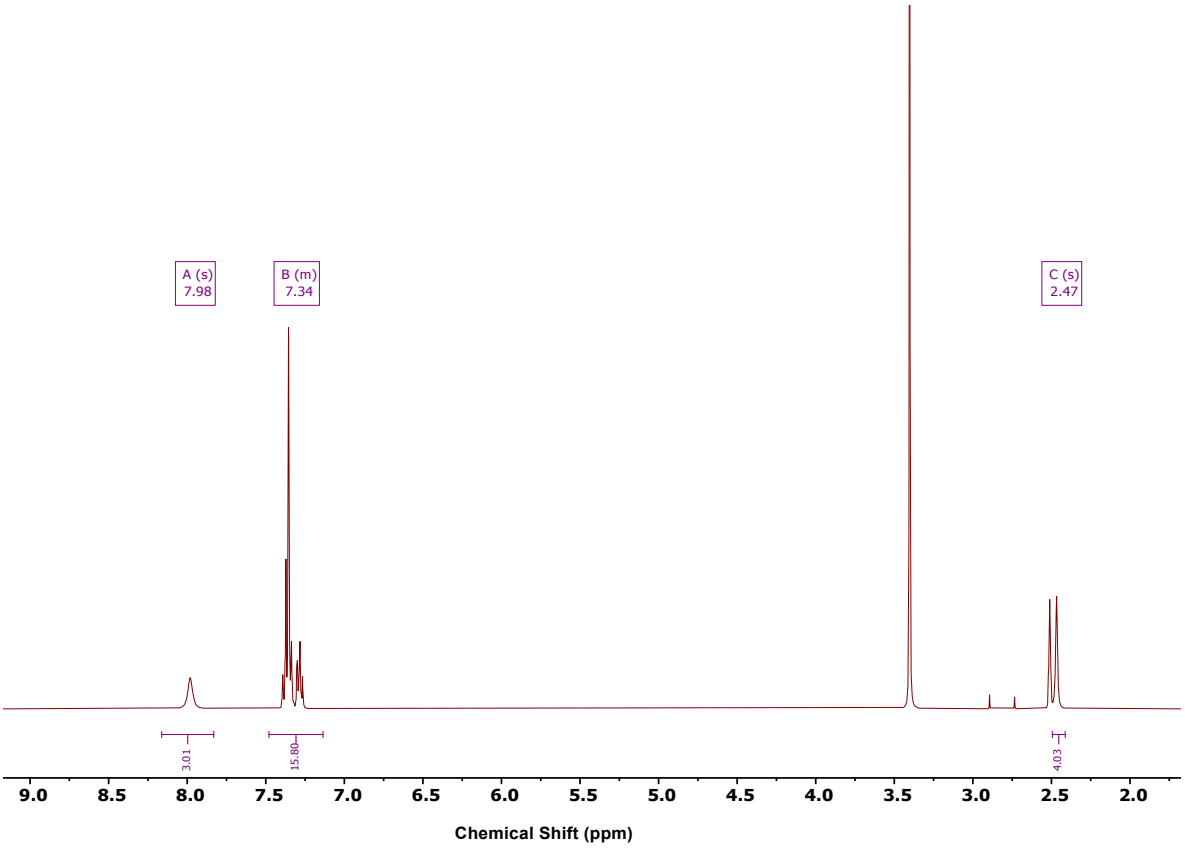


Figure S18. ^{1}H NMR (400 MHz, DMSO) of trityl protected cysteamine (1).

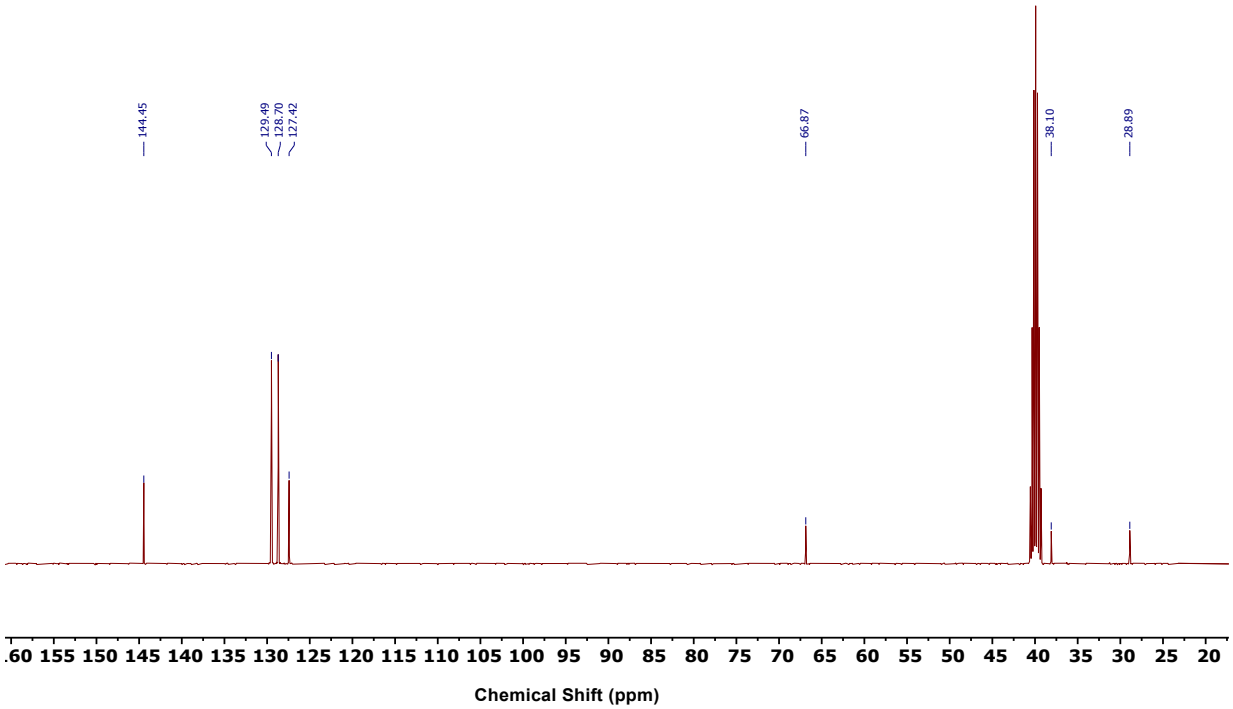
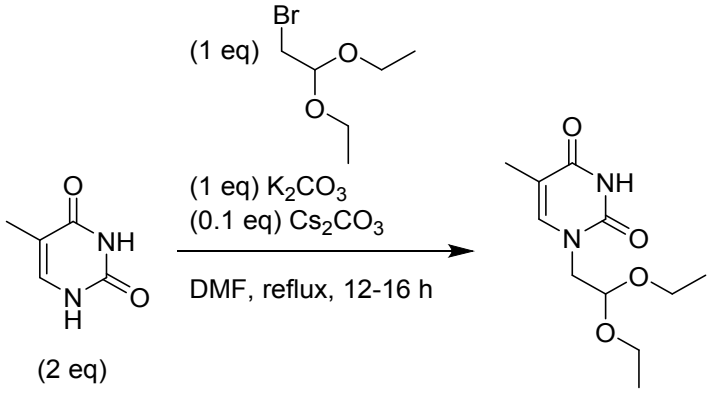


Figure S19. ^{13}C NMR (101 MHz, DMSO) of trityl protected cysteamine (1).

8. Thiol-Michael Thymine Monomer (TM-T) Synthesis



2

To an oven dried N_2 purged round bottom flask was added thymine (159 mmol, 20 g, 2 eq), potassium carbonate (79.4 mmol, 10.96 g, 1 eq), and cesium carbonate (7.94 mmol, 2.58 g, 0.1 eq). Anhydrous DMF (150 mL) was added and allowed to mix for 10 min. Next, bromoacetaldehyde diethyl acetal (79.4 mmol, 11.94 mL, 1 eq) was added in one portion. The flask was then refluxed overnight at 130 ℃. The following day, the solution was filtered while hot to remove unreacted thymine and salts. The cake was washed with minimal DMF before being concentrated via rotary evaporation. Once most of the DMF was removed, the residual oil was dissolved in ethyl acetate (300 mL) and extracted with water (5x 150 mL) to remove residual DMF. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated to dryness to produce a viscous oil that crystallized upon standing in a fridge. After 8 h, the solid was triturated with 2:1 petroleum ether to ether and filtered. The cake was washed with minimal 2:1 petroleum ether to ether, collected, and dried under high vac to produce compound **2** (9.02 g, 47%).

^{1}H NMR (600 MHz, DMSO) δ 11.26 (s, 1H), 7.42 (d, *J* = 1.3 Hz, 1H), 4.64 (t, *J* = 5.5 Hz, 1H), 3.68 (d, *J* = 5.4 Hz, 2H), 3.67 – 3.61 (m, 2H), 3.46 (dq, *J* = 9.8, 7.0 Hz, 2H), 1.75 (d, *J* = 1.2 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (151 MHz, DMSO) δ 164.69, 151.48, 142.80, 108.37, 99.91, 63.04, 50.02, 15.69, 12.29.

Mass: $C_{11}H_{18}N_2O_4$ $[M+H]^+$ Calculated: 243.23, Found: 243.17.

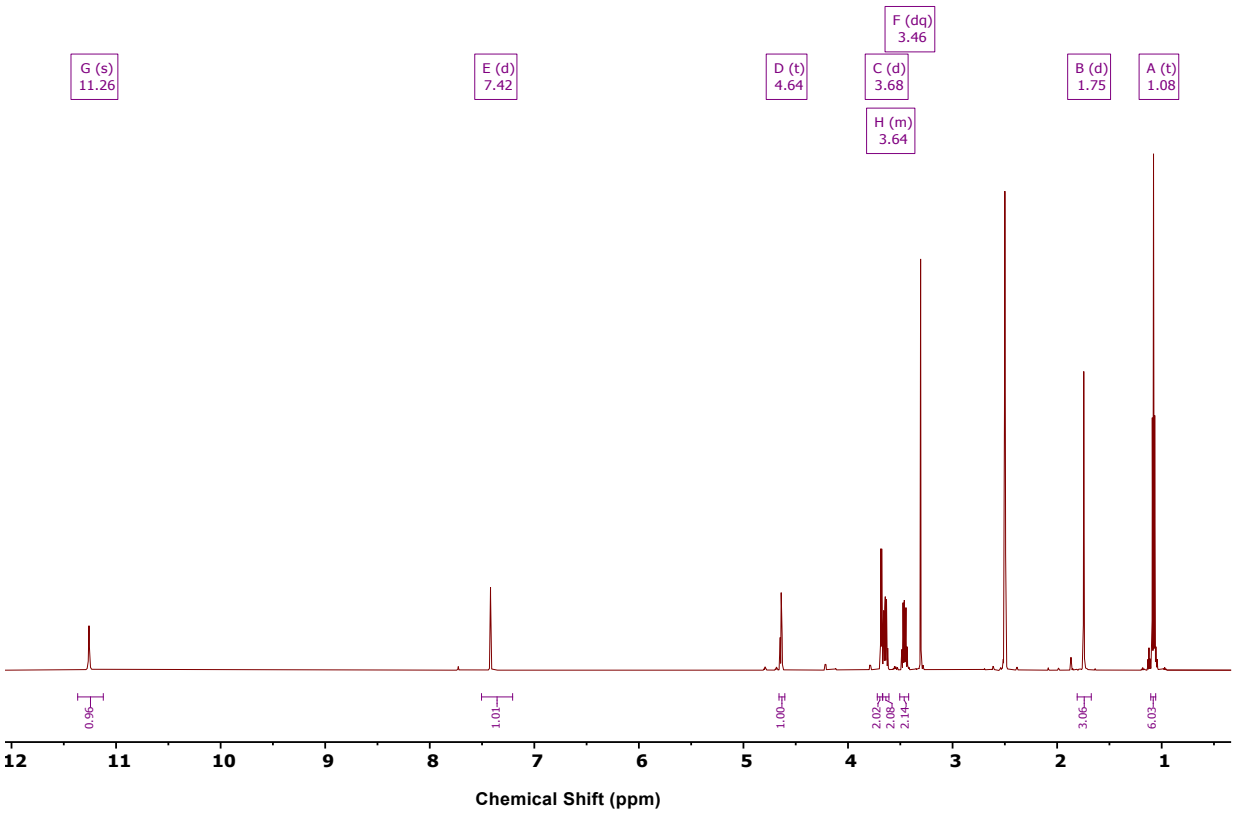


Figure S20. ^{1}H NMR (600 MHz, DMSO) of diethyl acetal functional thymine (2).

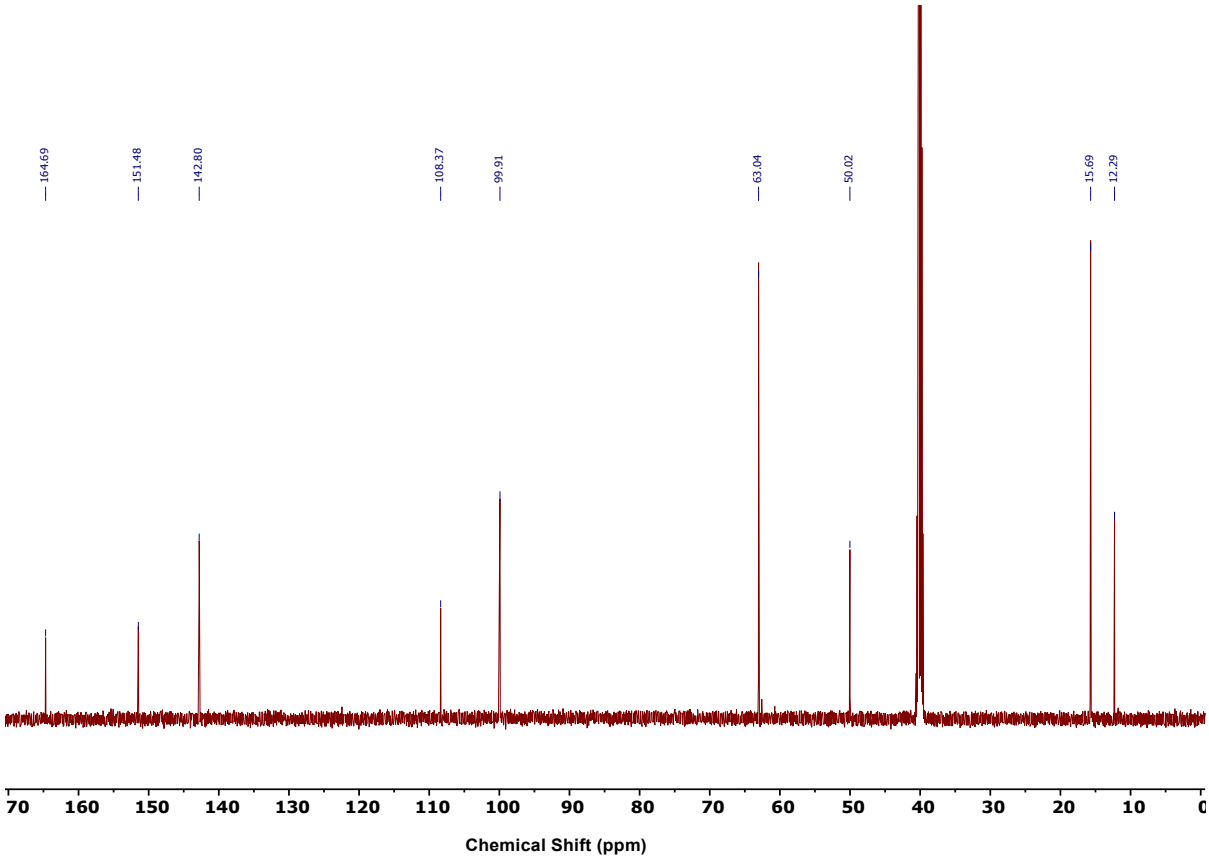
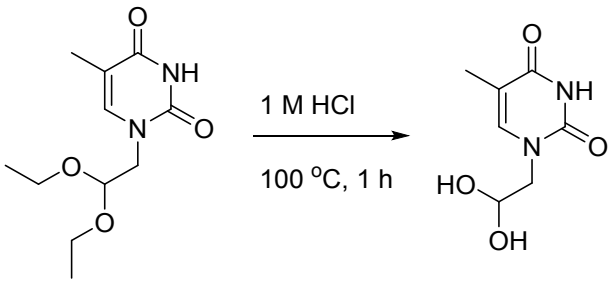


Figure S21. ^{13}C NMR (151 MHz, DMSO) of diethyl acetal functional thymine (2).



2 **3**

Compound **2** (10 g, 41.3, 1 eq) was added to a 500 mL round bottom flask followed by 1 M HCl (100 mL). The solution was then refluxed for 1 h. Following the completion of the reaction, the solution was evaporated to dryness via rotary evaporation producing a white solid. ***NOTE****: Only after the complete removal of water did a white solid form and ending drying prematurely can result in the product redissolving in residual water left in the round bottom flask*. To the white solid was added THF (50 mL) and cooled in an ice bath for 1 h before being filtered and subsequently washed with diethyl ether before being vacuum dried producing 7.6 g (quantitative yield) of the hydrated aldehyde **3** which was used in the next step without further purification.

^{1}H NMR (400 MHz, D_2O) δ 7.32 (d, *J* = 1.3 Hz, 1H), 5.11 (t, *J* = 5.3 Hz, 1H), 3.68 (d, *J* = 5.3 Hz, 2H), 1.73 (d, *J* = 1.1 Hz, 3H).

^{13}C NMR (101 MHz, D_2O) δ 166.95, 152.36, 143.72, 110.32, 87.18, 53.00, 11.17.

Mass: $C_7H_{10}N_2O_4$ $[M+H]^+$ Calculated: 169.15, Found: 169.09, $[M+H_2O]^+$ Calculated: 183.82, Found: 183.15.

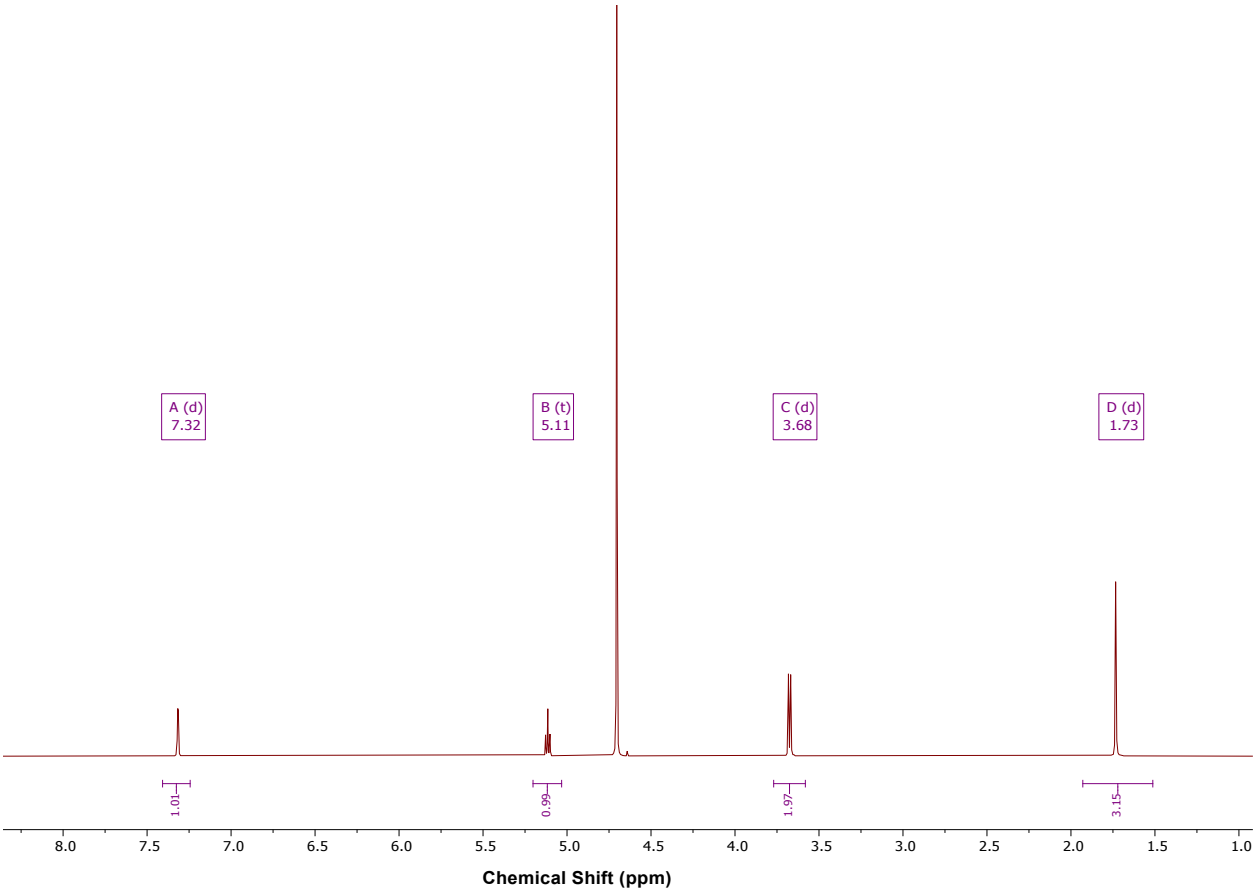


Figure S22. ^{1}H NMR (400 MHz, D_2O) of hydrated aldehyde functional thymine (3).

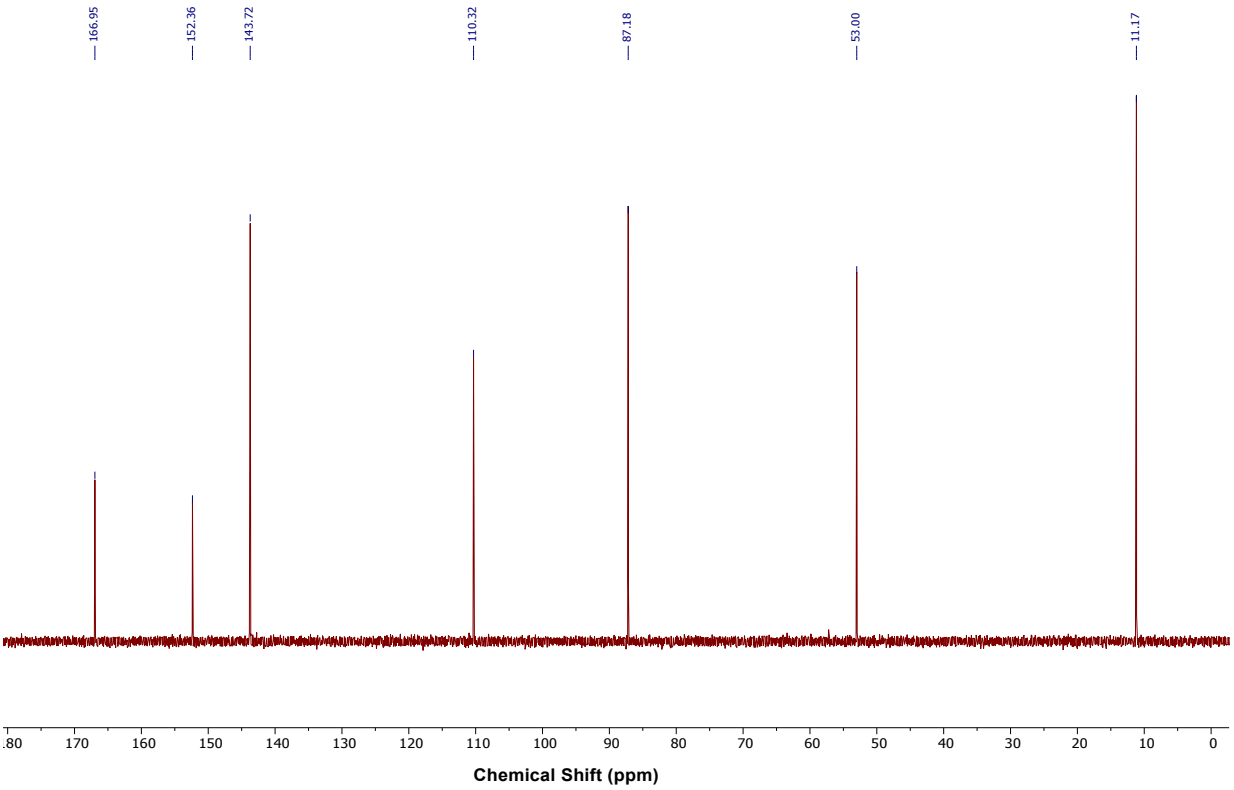
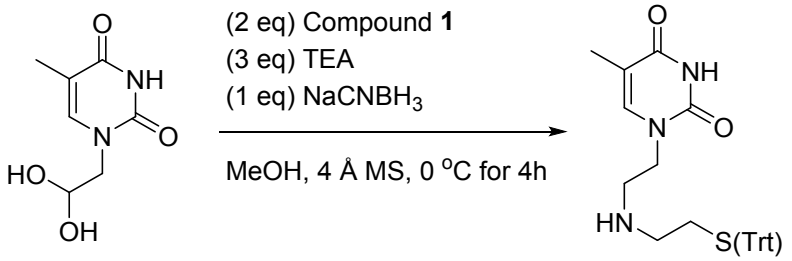


Figure S23. ^{13}C NMR (101 MHz, D_2O) of hydrated aldehyde functional thymine (3).



3 **4**

To an oven dried nitrogen purged 1000 mL round bottom flask was added 4 Å molecular sieves. Compound **1** (11.4 g, 32.2 mmol, 2 eq) was added to the round bottom flask followed by 500 mL of methanol. Anhydrous triethylamine (6.74 mL, 48.4 mmol, 3 eq) and compound **3** (3 g, 16.1 mmol, 1 eq) were added sequentially. The reaction was cooled in an ice bath before sodium cyanoborohydride (1 g, 16.1 mmol, 1 eq) was added to the solution. The reaction was monitored by TLC for 4 h followed by filtration. Once filtered, the cake was washed with 50 mL of methanol and 100 mL of DCM. The solvent was concentrated via rotovap to roughly 200 mL after which the solution was transferred to a separatory funnel. An additional 300 mL of DCM and 300 mL of water were added to separatory funnel, mixed, and the organic layer was collected. The aqueous layer was washed with DCM (2x, 100 mL) and the organic layers were combined, dried over magnesium sulfate, and filtered. The solution was then concentrated and purified by flash chromatography with a gradient of 0 to 5% MeOH in DCM to afford the compound **4** as a white solid (4.62 g, 61%).

^{1}H NMR (600 MHz, DMSO) δ 7.42 (d, *J* = 1.3 Hz, 1H), 7.36 – 7.22 (m, 16H), 3.58 (t, *J* = 6.1 Hz, 2H), 2.55 (t, *J* = 6.1 Hz, 2H), 2.44 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 6.9 Hz, 2H), 1.70 (d, *J* = 1.2 Hz, 3H).

^{13}C NMR (151 MHz, DMSO) δ 164.80, 151.37, 145.09, 142.67, 129.57, 128.44, 127.11, 108.01, 66.34, 47.87, 47.51, 47.47, 32.36, 12.39.

Mass: $C_{28}H_{29}N_3O_2S$ $[M+H]^+$ Calculated: 472.62, Found: 472.31.

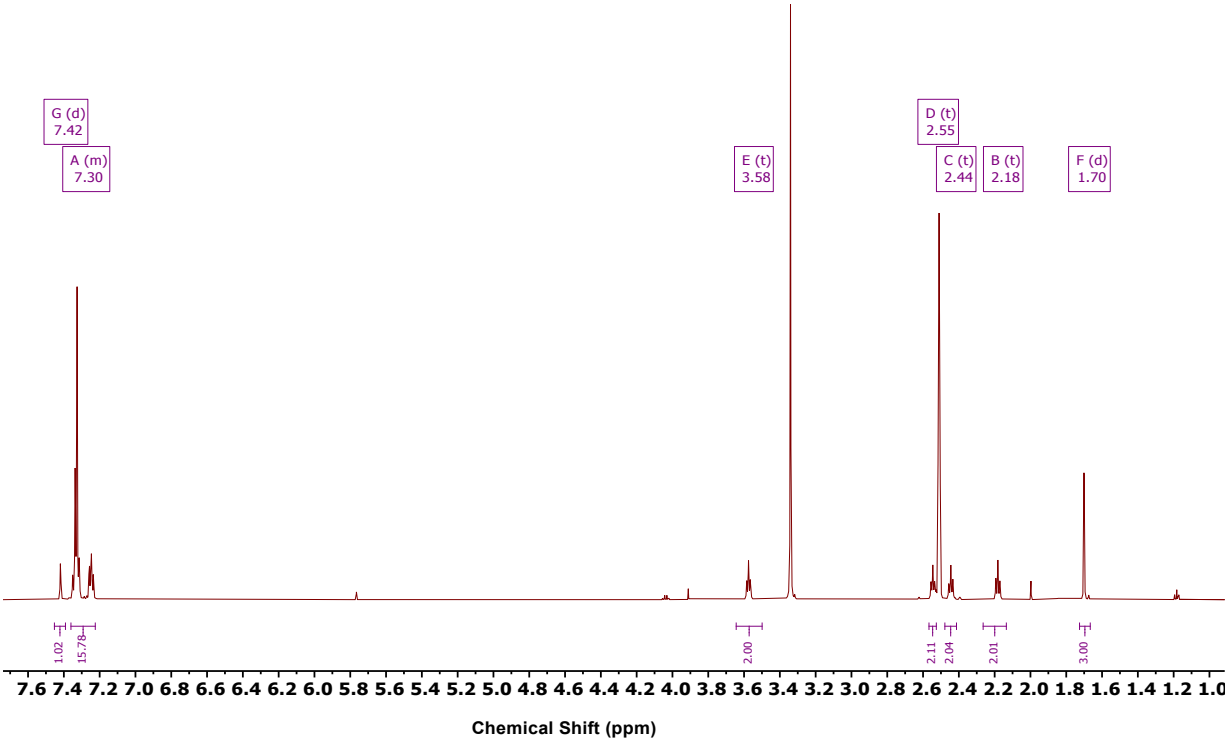


Figure S24. ^{1}H NMR (600 MHz, DMSO) of trityl protected cysteamine functional thymine (4).

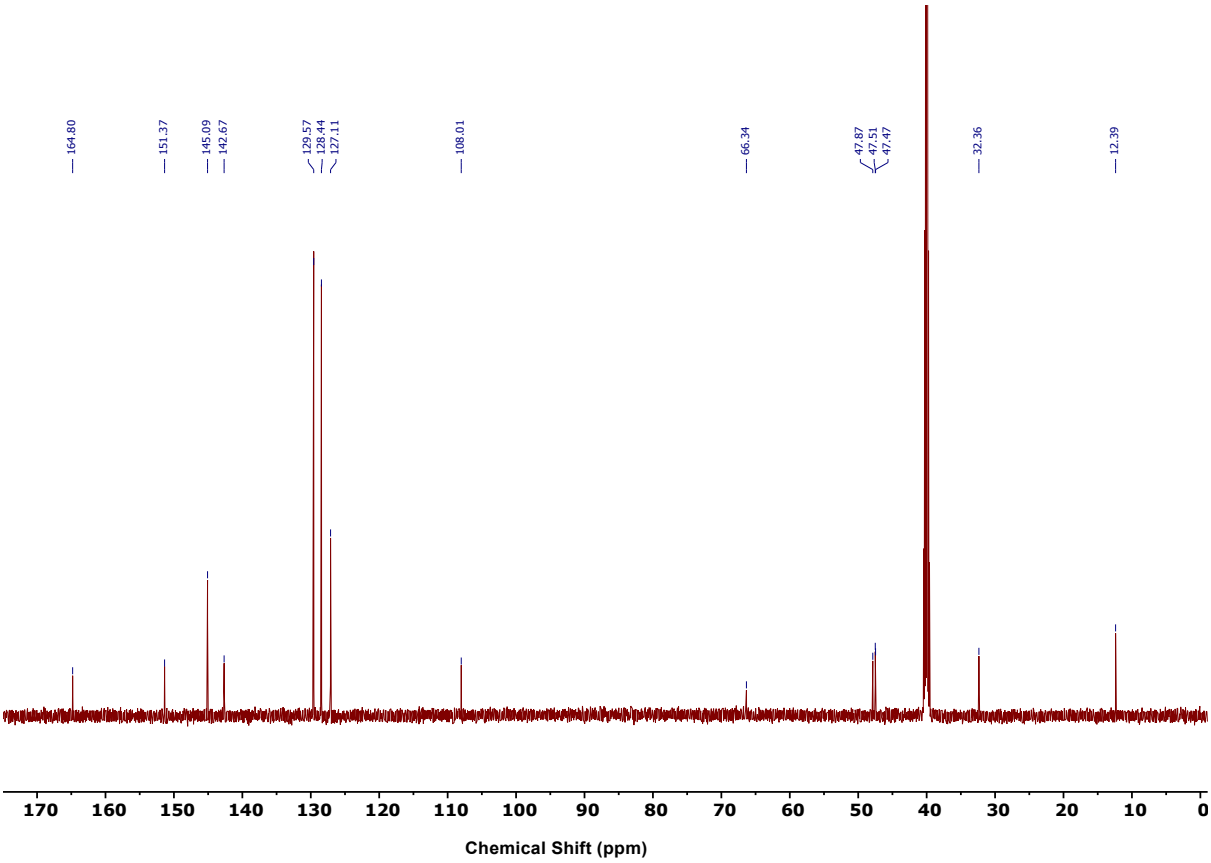
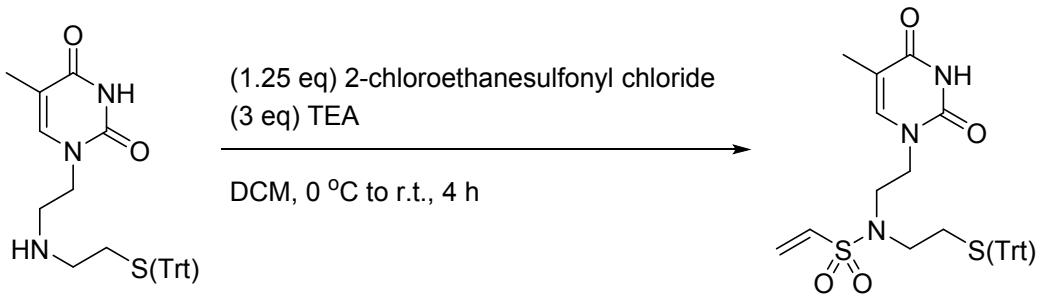


Figure S25. ^{13}C NMR (151 MHz, DMSO) of trityl protected cysteamine functional thymine (4).



4 **5**

To an oven dried N_2 purged round bottom flask was added compound **4** (6.5 g, 13.8 mmol, 1 eq) followed by 300 mL of DCM. Once compound **4** dissolved, the round bottom flask was placed in an ice bath and allowed to cool for 15 min. Next, anhydrous triethylamine (5.76 mL, 41.4 mmol, 3 eq) was added followed by dropwise addition of 2-chloroethylsulfonyl chloride (1.802 mL, 17.25 mmol. 1.25 eq) over the course an hour. Once the addition was finished, the ice bath was removed and the reaction was allowed to stir at room temperature for 4 h. Once finished, 100 mL of water was added to quench the reaction and was transferred to an extraction vessel. An additional 200 mL of DCM was added, and the organic layer was washed with water (2x 100 mL) followed by drying over Na_2SO_4. The Na_2SO_4 was filtered and the organic solution concentrated before being purified by flash chromatography with a gradient of 0% to 5% methanol in DCM to afford product **5** (5.19 g, 67%) as a tan solid.

^{1}H NMR (600 MHz, DMSO) δ 11.22 (s, 1H), 7.42 – 7.20 (m, 18H), 6.60 (dd, *J* = 16.4, 9.9 Hz, 1H), 6.01 – 5.88 (m, 2H), 3.60 (t, *J* = 6.0 Hz, 2H), 3.11 (t, *J* = 6.0 Hz, 2H), 2.94 (t, *J* = 7.7 Hz, 2H), 2.45 – 2.35 (m, 2H), 1.71 (d, *J* = 1.2 Hz, 3H).

^{13}C NMR (151 MHz, DMSO) δ 164.72, 151.34, 144.77, 141.95, 134.91, 129.58, 128.57, 127.74, 127.31, 108.77, 67.03, 55.36, 47.73, 46.73, 46.33, 30.44, 12.37.

Mass: $C_{30}H_{31}N_3O_4S_2$ $[M+H]^+$ Calculated: 562.72, Found: 562.19.

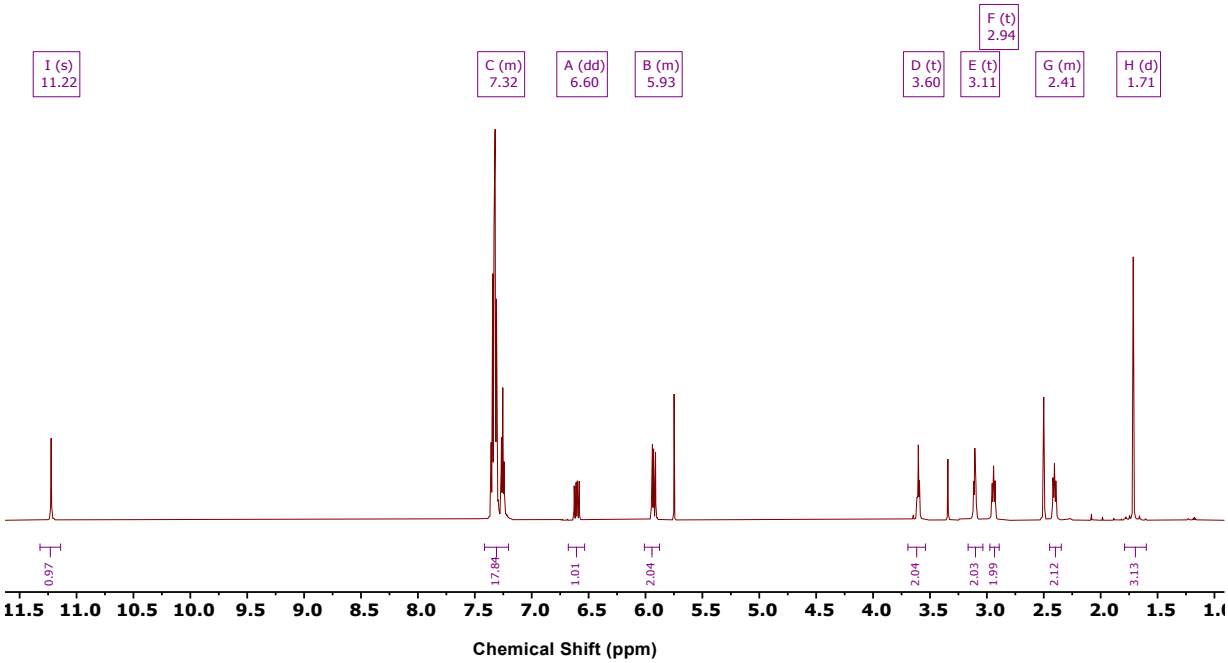


Figure S26. ^{1}H NMR (600 MHz, DMSO) of the thiol-Michael thymine (TM-T) monomer (5).

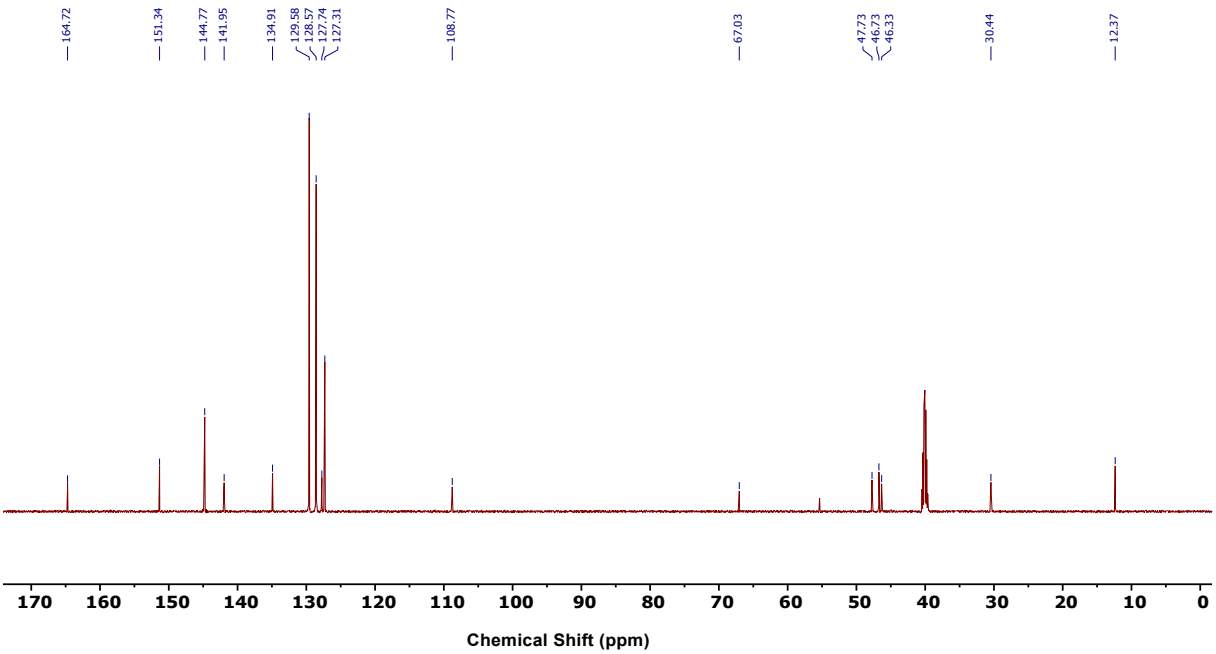
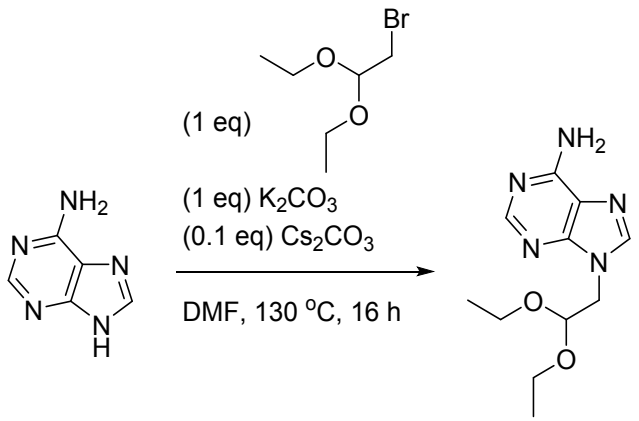


Figure S27. ^{13}C NMR (151 MHz, DMSO) of the thiol-Michael thymine (TM-T) monomer (5).

9. Thiol-Michael Adenine Monomer (TM-A) Synthesis



6

Adenine (74.1 mmol, 10g, 1 eq) was added to an oven-dried round bottom flask that had been purged with nitrogen gas. Anhydrous potassium carbonate (74.1 mmol, 10.2 g, 1 eq) and cesium carbonate (7.41 mmol, 2.41 g, 0.1 eq) were added followed by 150 mL anhydrous DMF. After stirring for 15 minutes, bromoacetaldehyde diethyl acetal (74.1 mmol, 11.14 mL, 1 eq) was added in one portion. The reaction was then refluxed at 130 ℃ for 16 h. Upon completion of the reaction, the flask contents were filtered while hot. The cake was washed with an additional 50 mL of DMF and the solvent was subsequently evaporated using a rotary evaporator. Once dry, 200 mL of ethanol was added to the flask. The flask was stirred at 85 ℃ until all product was dissolved, leaving only insoluble salts as the precipitate. The solution was then filtered while hot over a medium fret and the eluent was refrigerated overnight to precipitate the product. The solid was filtered once more and the cake was washed with 100 mL of ethanol and 250 mL of diethyl ether. The product was collected and dried in vacuo to produce compound **6** as a white solid (9.9 g, 53%).

^{1}H NMR (400 MHz, DMSO) δ 8.11 (d, *J* = 35.3 Hz, 2H), 7.25 (s, 2H), 4.85 (t, *J* = 5.4 Hz, 1H), 4.23 (d, *J* = 5.4 Hz, 2H), 3.81 – 3.39 (m, 4H), 1.03 (t, *J* = 7.0 Hz, 7H).

^{13}C NMR (101 MHz, DMSO) δ 156.38, 152.94, 150.11, 141.73, 118.81, 99.88, 62.68, 45.68, 15.59.

Mass: $C_{11}H_{17}N_5O_2$ $[M+H]^+$ Calculated: 252.30, Found: 252.49.

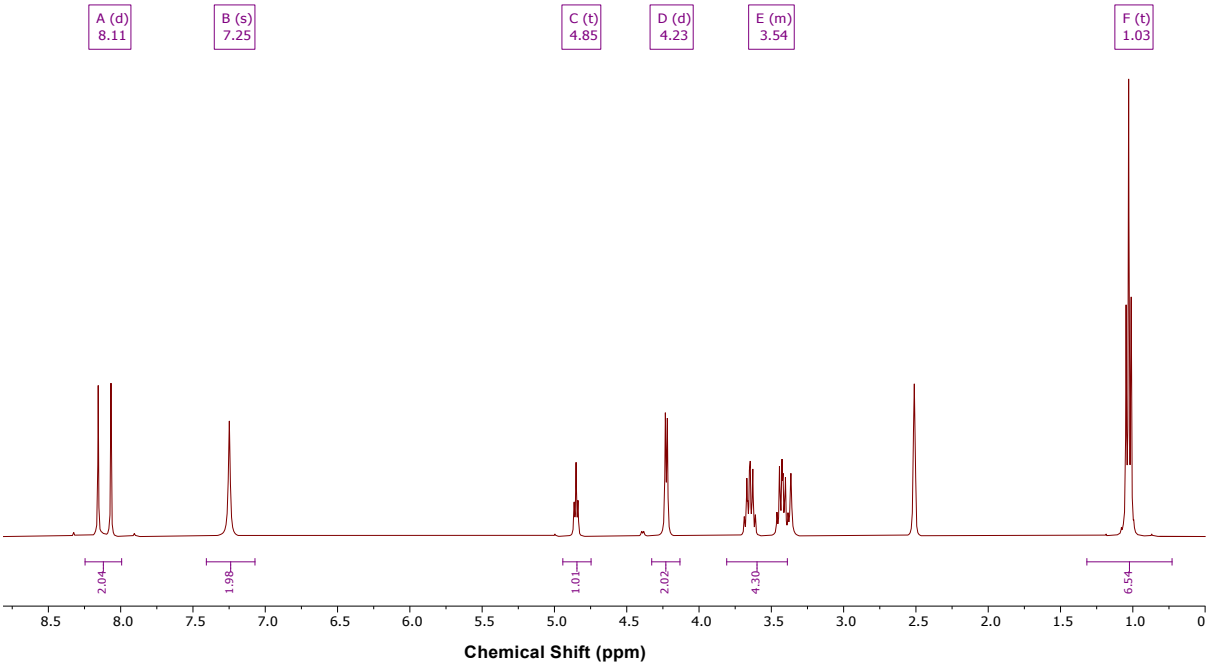


Figure S28. ^{1}H NMR (400 MHz, DMSO) of diethyl acetal functional adenine (6).

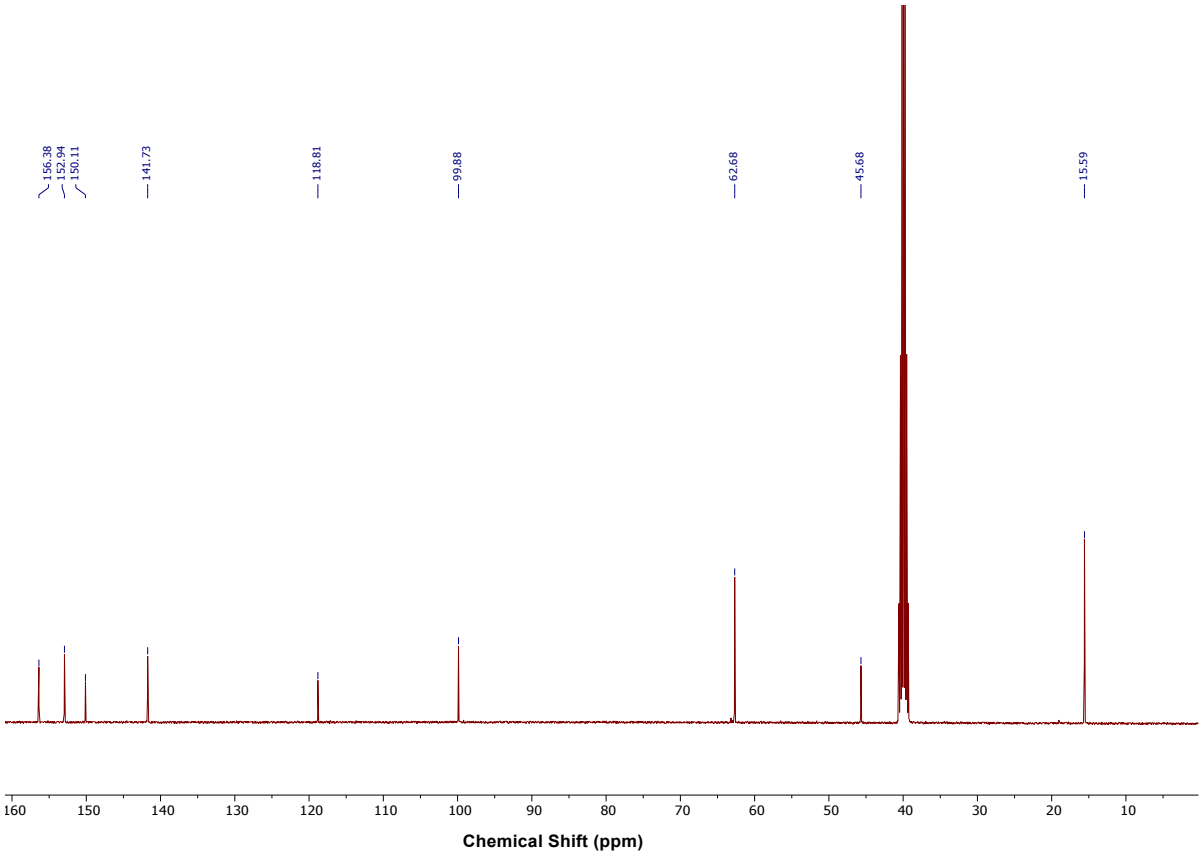
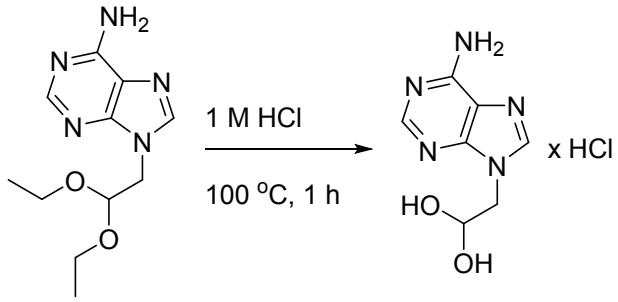


Figure S29. ^{13}C NMR (101 MHz, DMSO) of diethyl acetal functional adenine (6).



6 **7**

Compound **6** (8 g, 31.8 mmol, 1 eq) was added to a round bottom flask that had been purged N_2. 100 mL of 1 M HCl was then added and the round bottom flask was refluxed for 1 h at 100 °C in the dark. While still in the dark, the water was completely removed via rotary evaporation. ***NOTE****: Only after the complete removal of water did a white foam form and ending drying prematurely can result in the product redissolving in residual water left in the round bottom flask*. Upon drying, an off-white solid formed. To the solid was added 125 mL of tetrahydrofuran and the mixture was cooled in an ice bath for 1 h. The solid was filtered and washed with diethyl ether. The solid was dried under high vac in the dark to afford compound **7** as an off-white solid (6.97 g, 94%). The product was found to be isolated as the hydrate hydrochloride salt and was used without any further purification.

^{1}H NMR (400 MHz, D_2O) δ 8.28 (s, 1H), 8.21 (s, 1H), 5.29 (t, *J* = 4.7 Hz, 1H), 4.26 (d, *J* = 4.7 Hz, 2H).

^{13}C NMR (101 MHz, D_2O) δ 149.85, 148.78, 145.24, 144.41, 117.75, 87.25, 49.09.

Mass: $C_7H_9N_5O_2$ $[M+H]^+$ Calculated: 178.17, Found: 178.11, $[M+H_2O]^+$ Calculated:196.18, Found: 196.21.

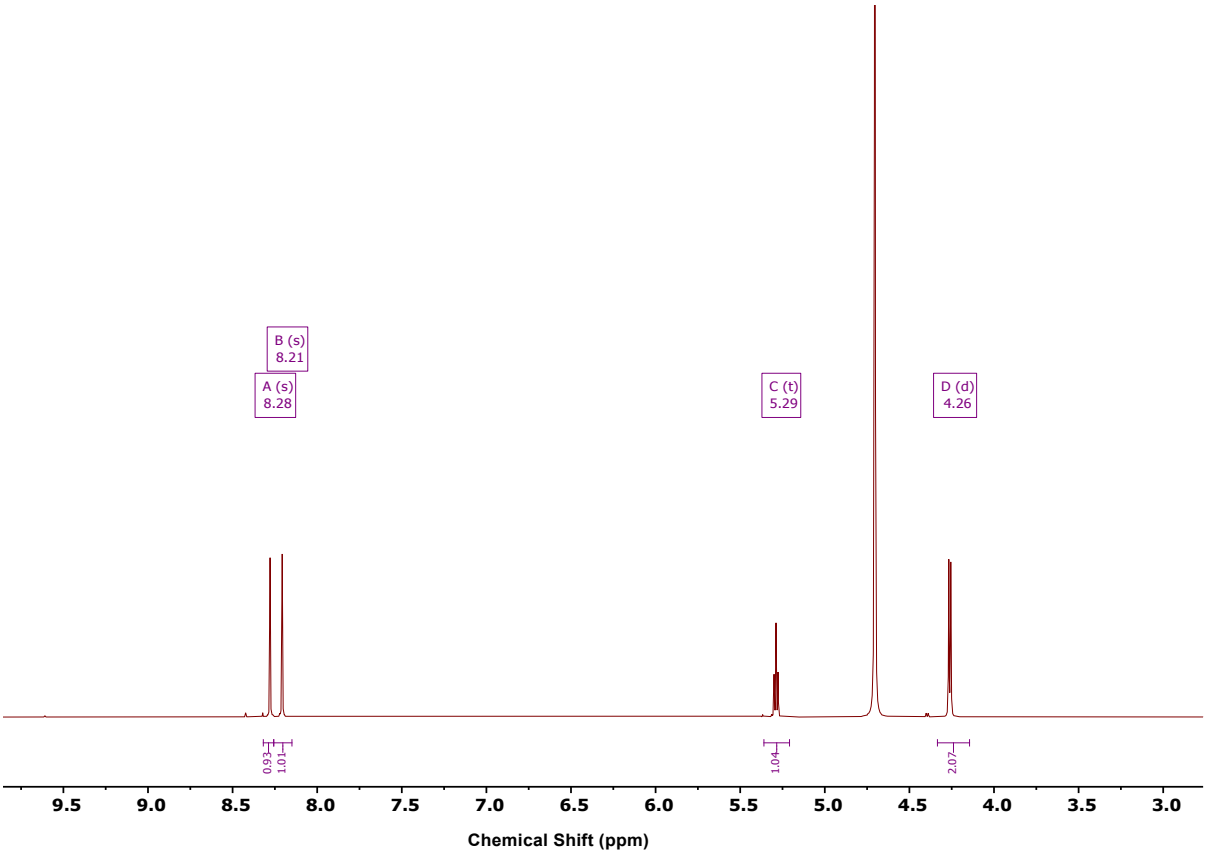


Figure S30. ^{1}H NMR (400 MHz, D_2O) of hydrated aldehyde functional adenine (7).

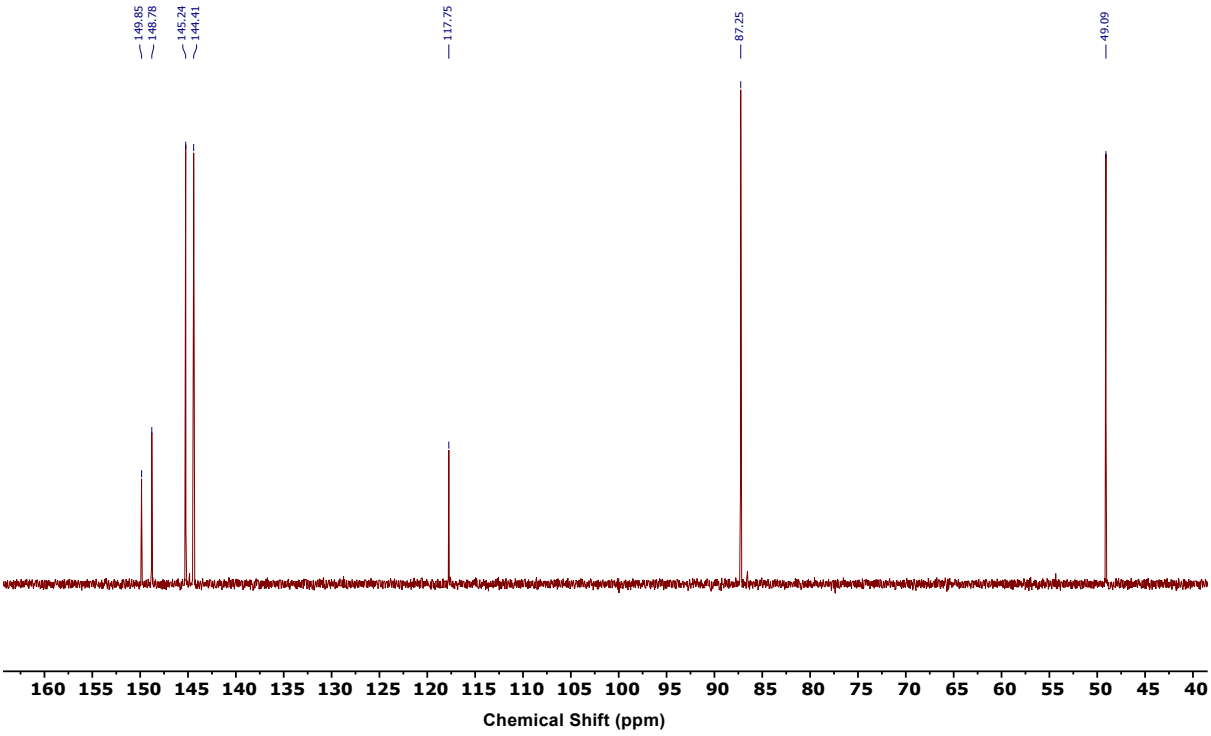
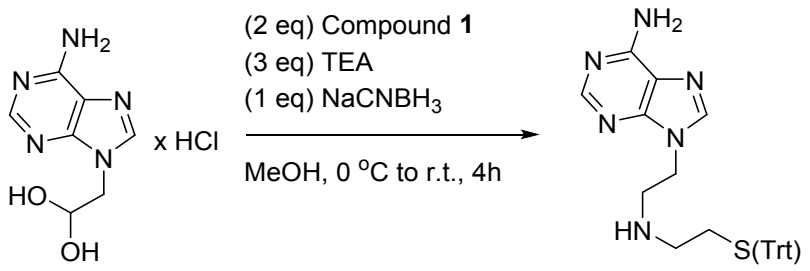


Figure S31. ^{1}H NMR (101 MHz, D_2O) of hydrated aldehyde functional adenine (7).



7 **8**

To an oven dried nitrogen purged 1000 mL round bottom flask was added 4 Å molecular sieves. Compound **1** (9.23 g, 26 mmol, 2 eq**)** was added to the round bottom flask followed by 500 mL of methanol. Anhydrous triethylamine (5.43 mL, 39 mmol, 3 eq) and compound **7** (3 g, 13 mmol, 1 eq) were added sequentially. The reaction was cooled in an ice bath before sodium cyanoborohydride (0.82 g, 13 mmol, 1 eq) was added to the solution. The reaction proceeded over ice for 1 h followed by room temperature for 3 h. Filtration of the reaction was performed once the reaction was completed, which was determined by TLC. Once filtered, the cake was washed with 50 mL of methanol and 100 mL of DCM. The solvent was concentrated via rotovap to roughly 200 mL after which the solution was transferred to a separatory funnel. An additional 300 mL of DCM and 300 mL of water were added to separatory funnel and the organic layer was collected. The aqueous layer was washed with DCM (2x, 100 mL) and the organic layers were combined, dried over magnesium sulfate, and filtered. The solution was then concentrated and purified by flash chromatography with a gradient of 0 to 10% MeOH in DCM to afford the compound **8** as a white solid (3.1 g, 50%).

^{1}H NMR (400 MHz, DMSO) δ 8.08 (d, *J* = 21.1 Hz, 2H), 7.64 – 6.93 (m, 18H), 4.08 (t, *J* = 6.1 Hz, 2H), 2.73 (t, *J* = 6.1 Hz, 2H), 2.43 (t, *J* = 6.9 Hz, 2H), 2.16 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 156.34, 152.68, 149.95, 145.03, 141.62, 129.55, 128.45, 127.10, 119.07, 66.32, 48.27, 47.74, 43.31, 32.25.

Mass: $C_{28}H_{28}N_6S$ $[M+H]^+$ Calculated: 481.63, Found: 481.22.

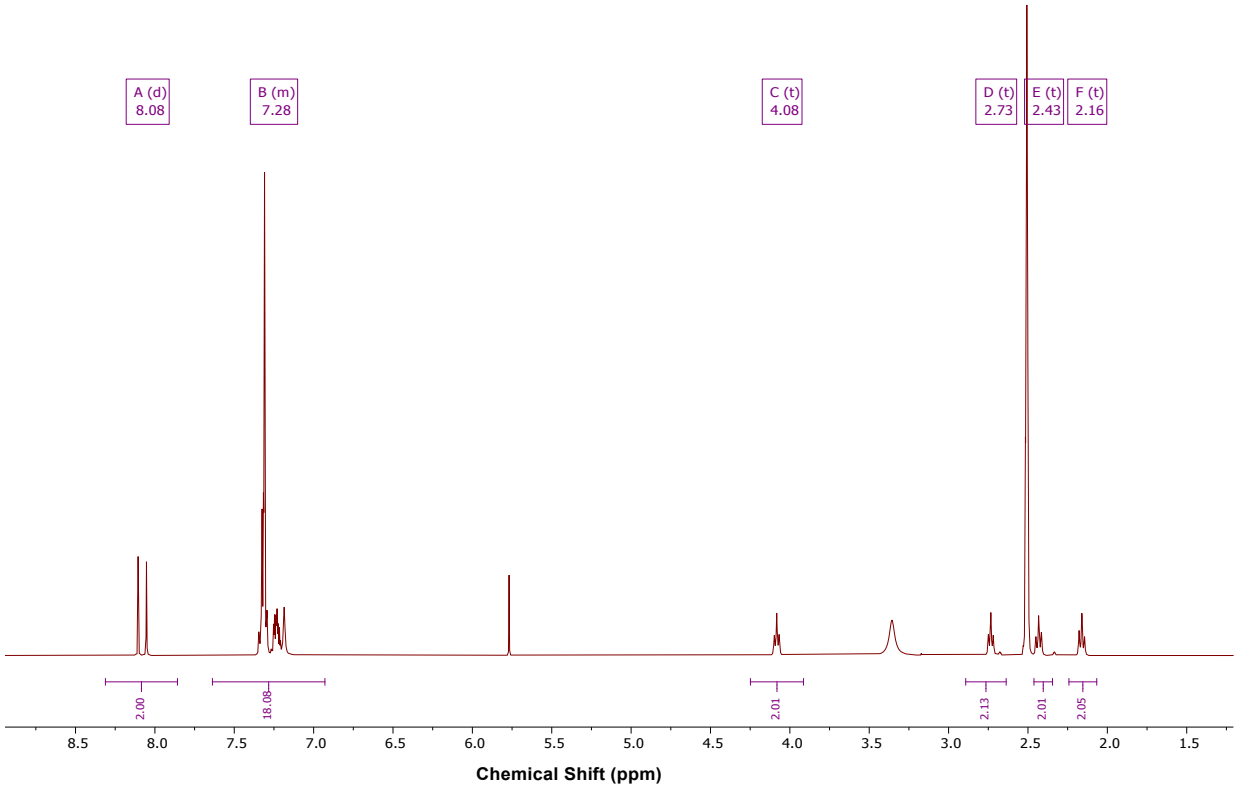


Figure S32. ^{1}H NMR (400 MHz, DMSO) of trityl protected cysteamine functional adenine (8).

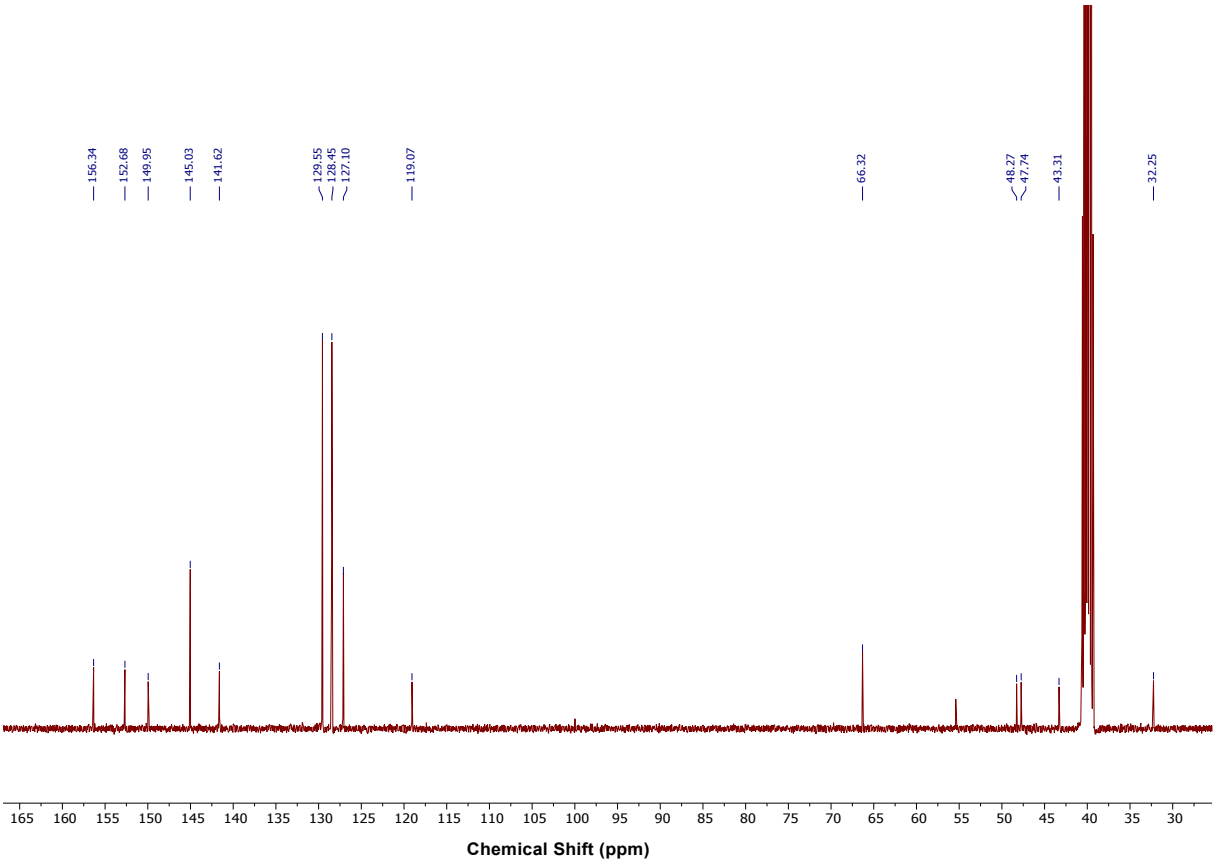
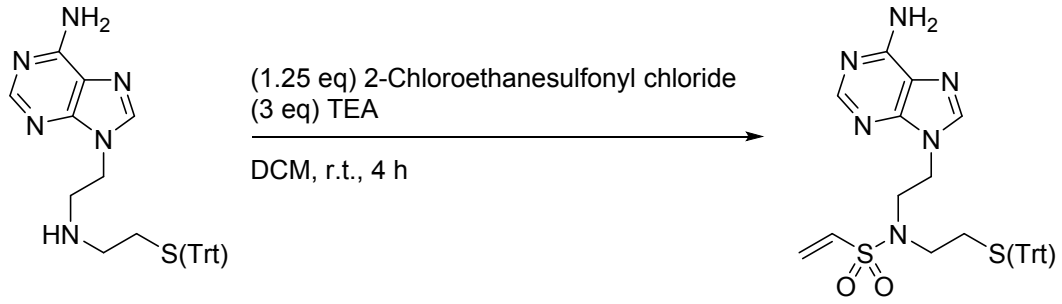


Figure S33. ^{13}C NMR (101 MHz, DMSO) of trityl protected cysteamine functional thymine (8).



8 **9**

To an oven dried N_2 purged round bottom flask was added compound **8** (3 g, 6.25 mmol, 1 eq) followed by 300 mL of DCM. Once compound **8** dissolved, the round bottom flask was placed in an ice bath and allowed to cool for 15 min. Next, anhydrous triethylamine (2.61 mL, 18.75 mmol, 3 eq) was added followed by dropwise addition of 2-chloroethylsulfonyl chloride (0.81 mL, 7.81 mmol. 1.25 eq) over the course an hour. Once the addition was finished, the ice bath was removed and the reaction was allowed to stir at room temperature for 4 h. Once finished, 100 mL of water was added to quench the reaction and was transferred to an extraction vessel. An additional 200 mL of DCM was added and the organic layer was washed with water (2x 100 mL) followed by drying over Na_2SO_4. The Na_2SO_4 was filtered and the organic solution concentrated before being purified by flash chromatography with a gradient of 0% to 5% methanol in DCM to afford product **9** (1.8 g, 50.5%) as a slightly yellow solid.

^{1}H NMR (400 MHz, DMSO) δ 8.03 (d, *J* = 25.1 Hz, 2H), 7.45 – 7.09 (m, 18H), 6.49 (dd, *J* = 16.4, 9.9 Hz, 1H), 5.97 – 5.81 (m, 2H), 4.13 (t, *J* = 6.2 Hz, 2H), 3.33 (s, 2H), 2.86 (t, *J* = 7.8 Hz, 2H), 2.35 (dd, *J* = 8.8, 6.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 156.38, 152.85, 149.99, 144.73, 141.15, 134.47, 129.56, 128.56, 127.86, 127.28, 119.07, 66.92, 47.66, 47.32, 42.26, 30.42.

Mass: $C_{30}H_{30}N_6O_2S_2$ $[M+H]^+$ Calculated: 571.73, Found: 571.97.

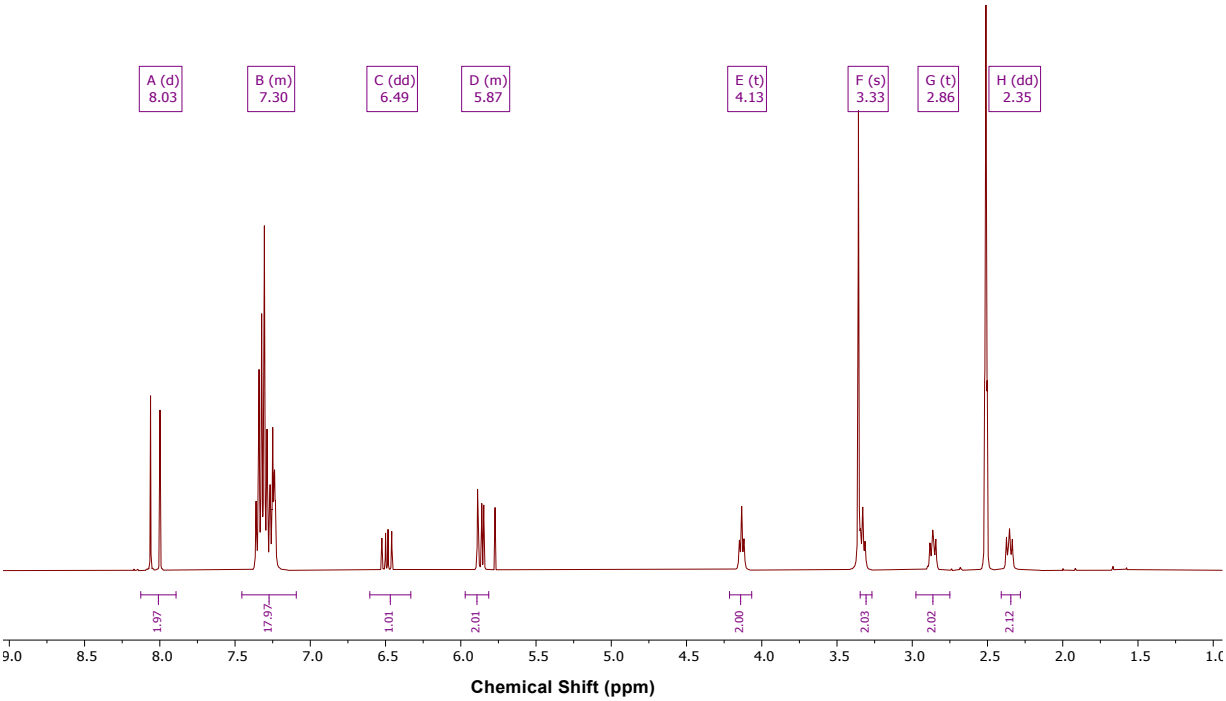


Figure S34. ^{1}H NMR (400 MHz, DMSO) of the thiol-Michael adenine (TM-A) monomer (9).

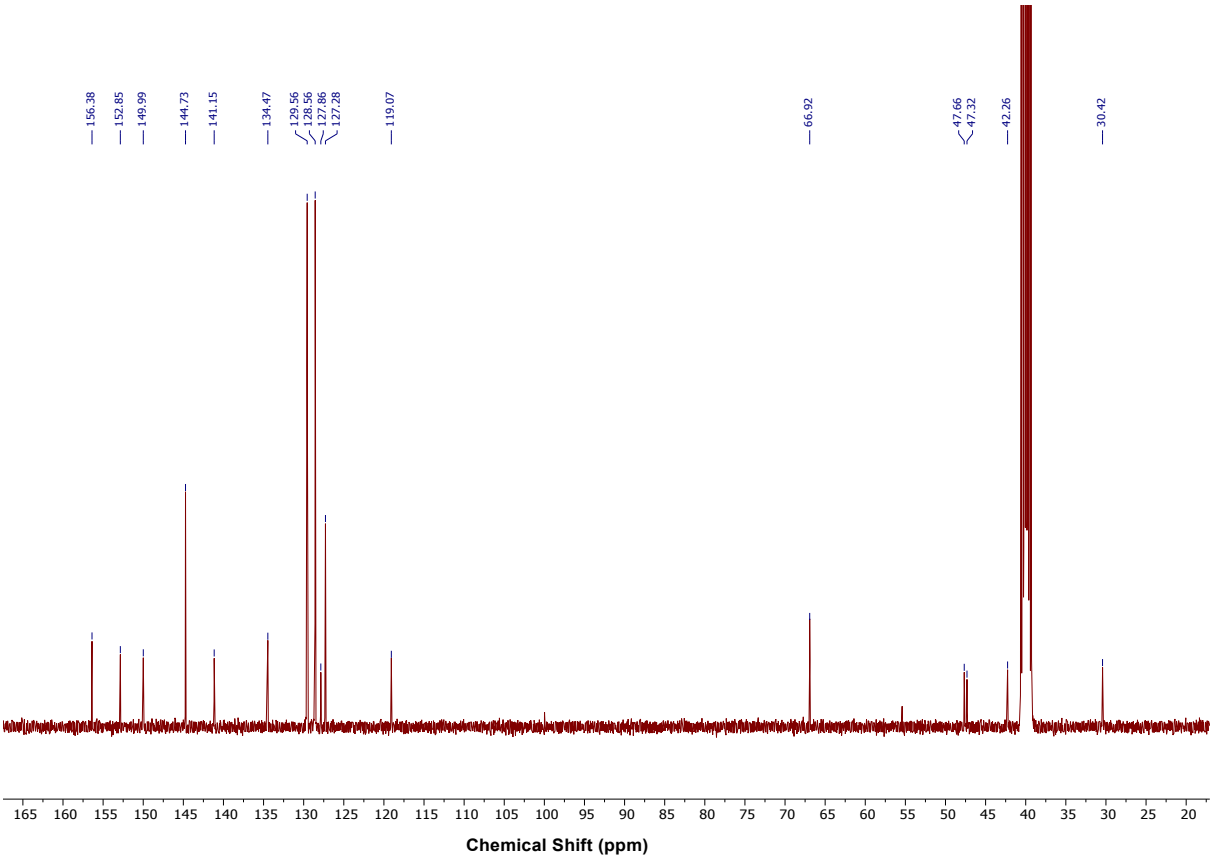
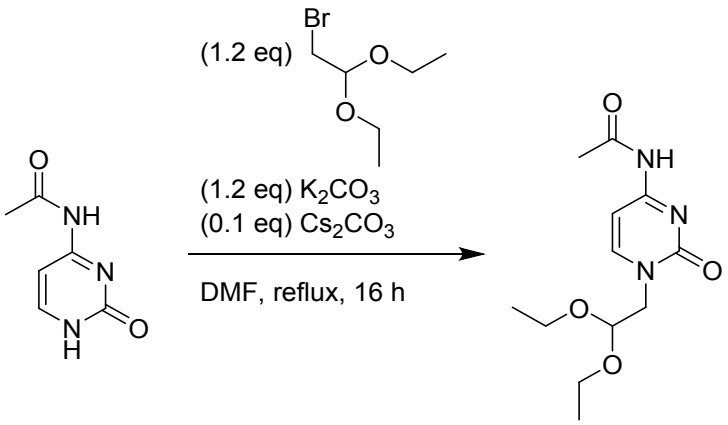


Figure S35. ^{1}H NMR (101 MHz, DMSO) of the thiol-Michael adenine (TM-A) monomer (9).

10. Thiol-Michael Cytosine Monomer (TM-C) Synthesis



10

N4-acetylcytosine (65 mmol, 10 g, 1 eq) was added to an oven-dried N_2 purged round bottom flask. Anhydrous potassium carbonate (78 mmol, 10.8 g, 1.2 eq) and cesium carbonate (6.5 mmol, 2.11 g, 0.1 eq) were then added. Anhydrous DMF (150 mL) was then added followed by one portion of bromoacetaldehyde diethyl acetal (78 mmol, 11.73 mL, 1.2 eq). The flask was then refluxed at 130 ℃ for 16 h. Once the reaction was finished, the flask was filtered while hot. The filtered cake was washed with minimal DMF before the eluent was concentrated via rotary evaporation resulting in a brown solid. The crude product was then dissolved in chloroform (300 mL) and was extract with water (3x, 200 mL). The organic layer was then collected and dried over anhydrous sodium sulfate. The chloroform was then filtered and evaporated completely under reduced pressure resulting in a brown solid. Once completely dry the flask was charged with 60 mL of diethyl ether. The mixture was cooled in a fridge over night before being filtered. The cake was then washed with 100 mL diethyl ether and was collected and dried under high vac to afford compound **10** as a white solid (8.1 g, 46%).

^{1}H NMR (400 MHz, DMSO) δ 10.86 (s, 1H), 7.97 (d, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 7.3 Hz, 1H), 4.72 (t, *J* = 5.4 Hz, 1H), 3.85 (d, *J* = 5.4 Hz, 2H), 3.55 (ddq, *J* = 83.6, 9.7, 7.0 Hz, 4H), 2.09 (s, 3H), 1.06 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, DMSO) δ 171.40, 162.99, 155.82, 151.80, 99.43, 95.12, 63.20, 52.50, 24.79, 15.68.

Mass: $C_{12}H_{19}N_3O_4$ $[M+H]^+$ Calculated: 270.30, Found: 270.09

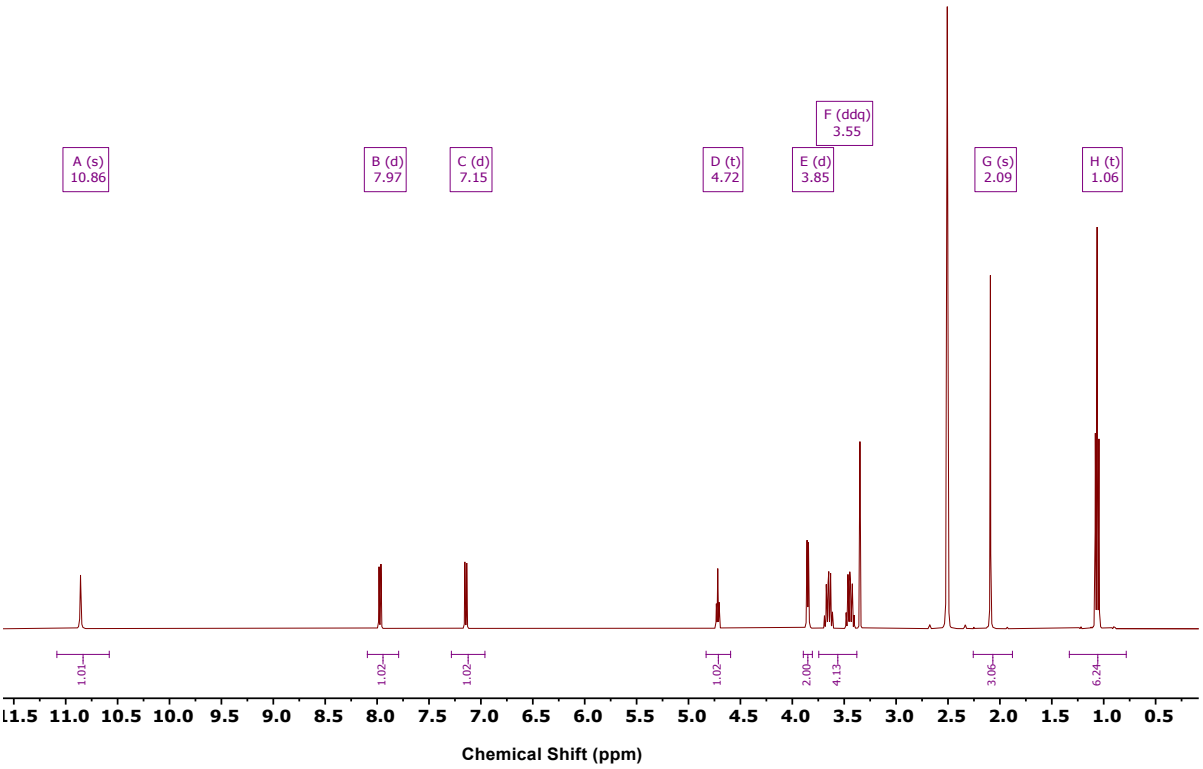


Figure S36. ^{1}H NMR (400 MHz, DMSO) of diethyl acetal functional (Ac)cytosine (10).

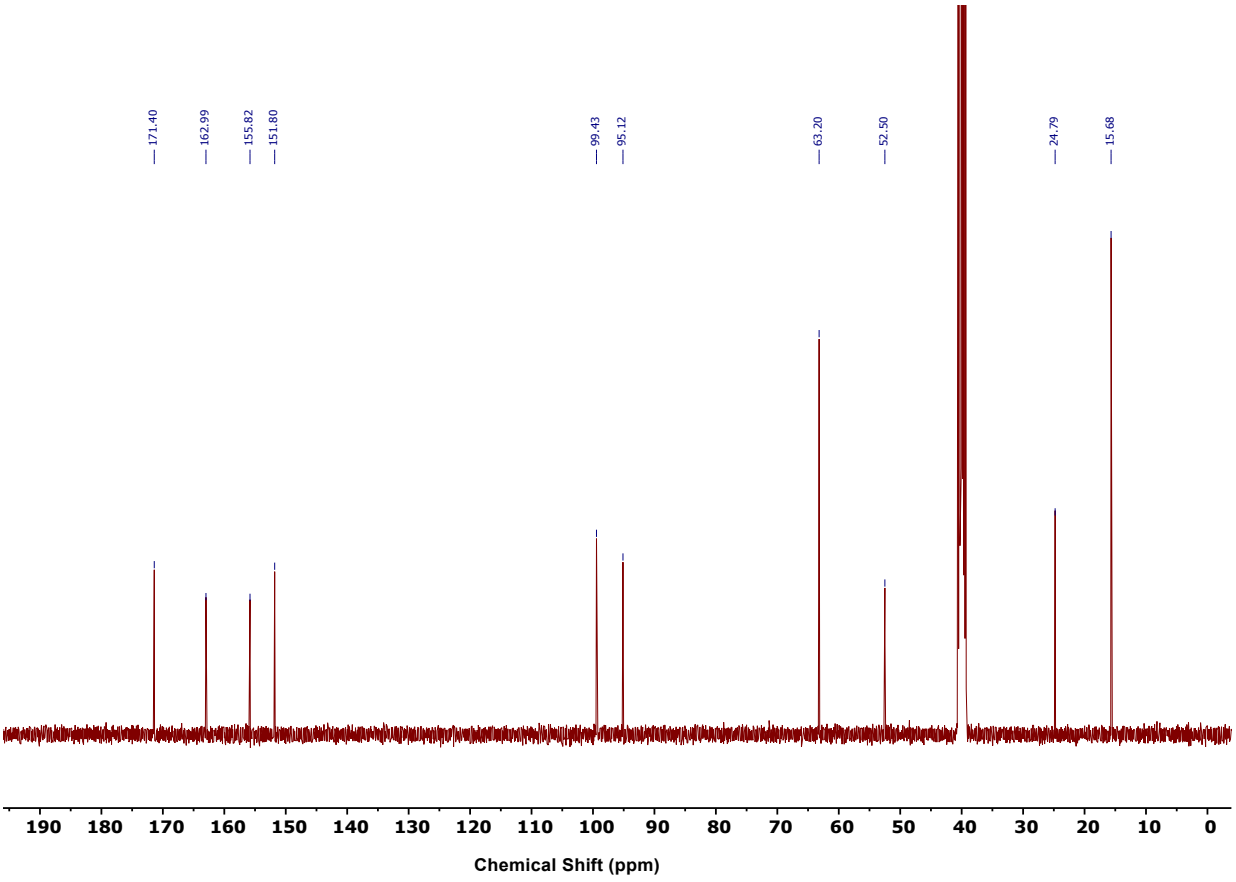
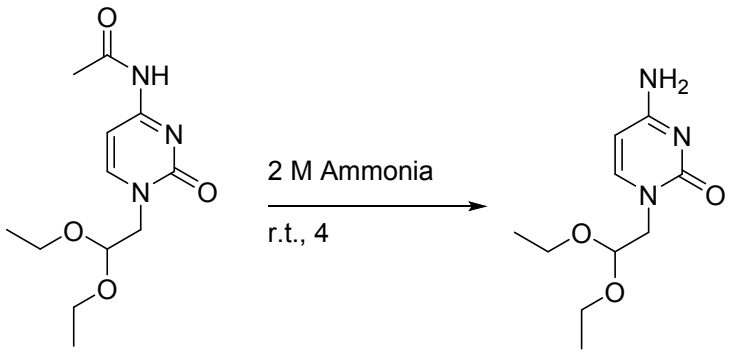


Figure S37. ^{13}C NMR (101 MHz, DMSO) of diethyl acetal functional (Ac)cytosine (10).



10 **11**

Compound **10** (8.2 g, 30 mmol, 1 eq) was added to a 500 mL round bottom flask that had been oven dried and N_2 purged. A solution of 2 M ammonia in methanol (160 mL) was added and the reaction stirred for 4 h at room temperature. The flask was then evaporated at a reduced pressure to dryness and diethyl ether (100 mL) was added. The contents were then filtered and the cake was washed with 50 mL diethyl ether. The solid was collected and dried under high vac to produce compound **11** as a white solid (6.8 g, 98%).

^{1}H NMR (400 MHz, DMSO) δ 7.45 (d, *J* = 7.2 Hz, 1H), 7.06 (d, *J* = 25.1 Hz, 2H), 5.62 (d, *J* = 7.2 Hz, 1H), 4.63 (t, *J* = 5.4 Hz, 1H), 3.76 – 3.37 (m, 6H), 1.07 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, DMSO) δ 166.54, 156.37, 147.51, 100.09, 93.30, 63.07, 51.96, 15.72.

Mass: $C_{10}H_{17}N_3O_3$ $[M+H]^+$ Calculated: 228.26, Found: 228.83

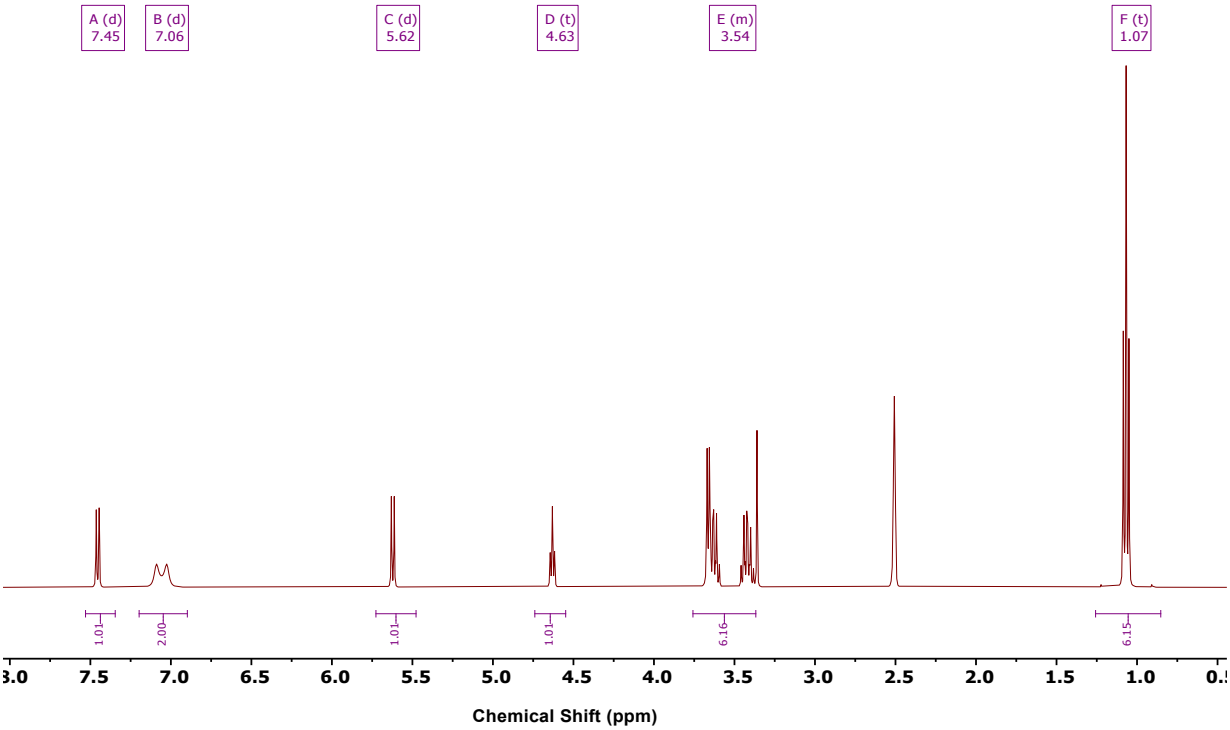


Figure S38. ^{1}H NMR (400 MHz, DMSO) of diethyl acetal functional cytosine (11).

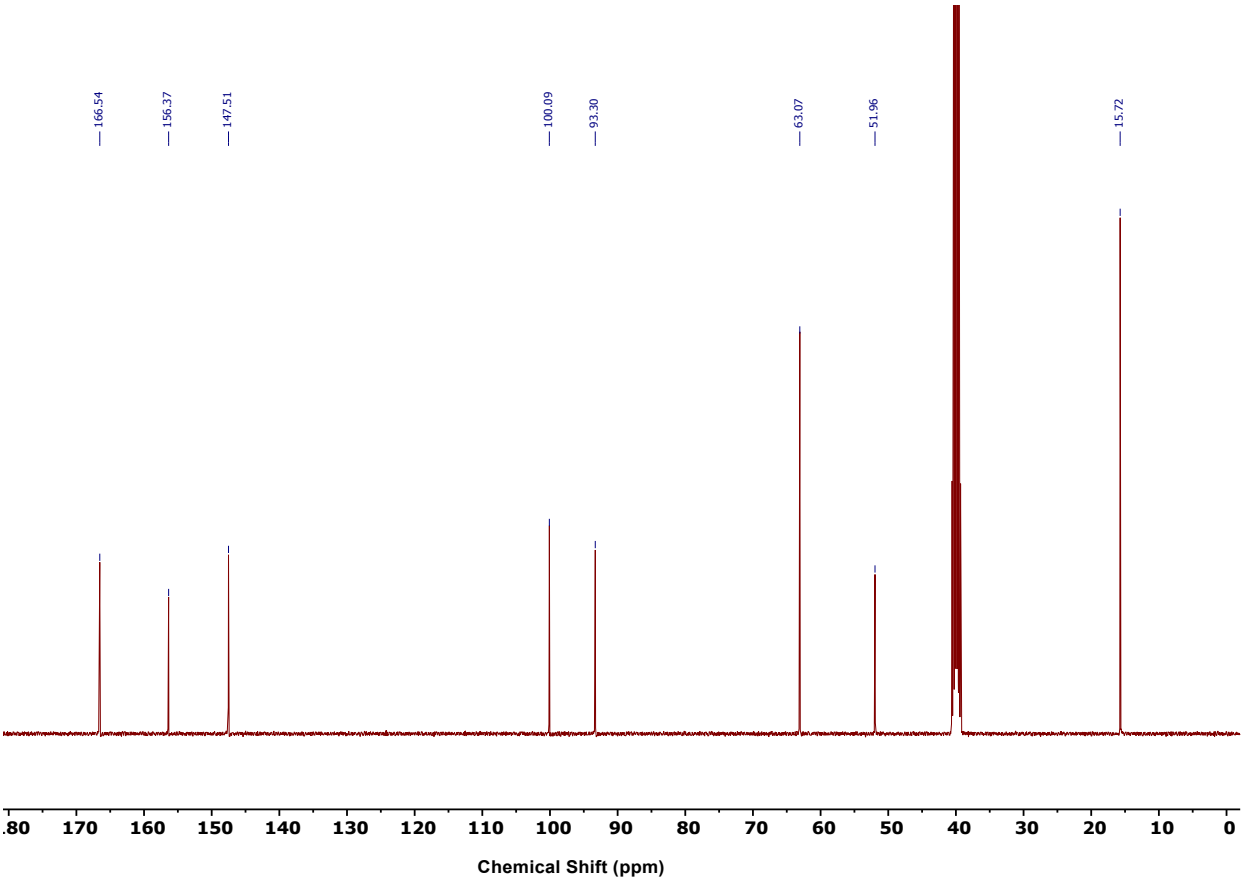
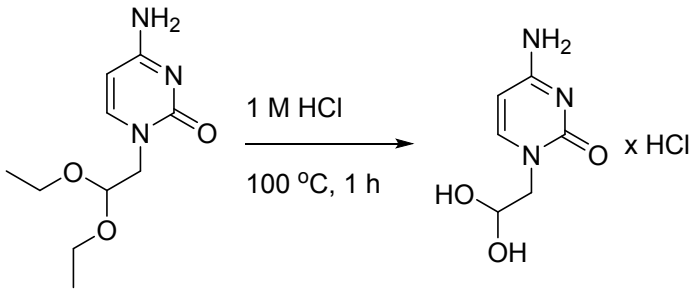


Figure S39. ^{1}H NMR (101 MHz, DMSO) of diethyl acetal functional cytosine (11).



11 **12**

Compound **11** (6.6 g, 29.1 mmol, 1 eq) was added to a round bottom flask that had been purged with nitrogen gas. 100 mL of 1 M HCl was then added and the round bottom flask was refluxed for 1 h at 100 °C. Upon completion of the reaction, the water was completely removed via rotary evaporation producing a white foam. ***NOTE****: Only after the complete removal of water did a white foam form and ending drying prematurely can result in the product redissolving in residual water left in the round bottom flask*. The solid was added 100 mL of tetrahydrofuran and the mixture was cooled in an ice bath for 1 h. The solid was filtered and washed with diethyl ether. The solid was dried under high vac to afford compound **12** as a white solid (4.87 g, 98%). The product was found to be isolated as the hydrate hydrochloride salt and was used without any further purification.

^{1}H NMR (400 MHz, D_2O) δ 7.67 (dd, *J* = 7.8, 3.9 Hz, 1H), 6.01 (dd, *J* = 7.7, 1.9 Hz, 1H), 5.26 – 4.96 (m, 1H), 4.00 – 3.63 (m, 2H).

^{13}C NMR (101 MHz, D_2O) δ 159.40, 150.35, 150.25, 149.13, 149.03, 94.07, 94.01, 89.39, 86.75, 53.72, 52.69.

Mass: $C_6H_9N_3O_3$ $[M+H]^+$ Calculated: 154.14, Found: 154.69, $[M+H_2O]^+$ Calculated: 172.15, Found: 172.38.

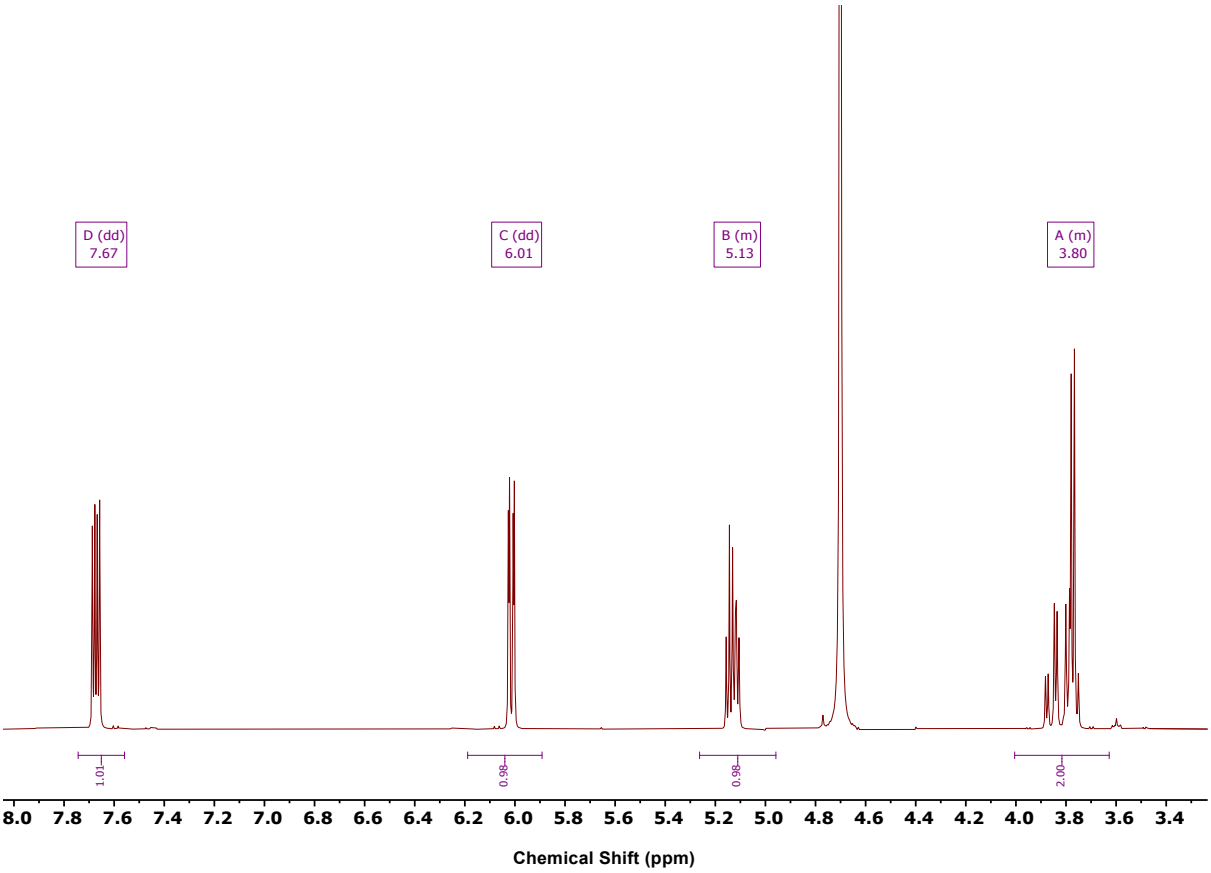


Figure S40. ^{1}H NMR (400 MHz, D_2O) of hydrated aldehyde functional cytosine (12).

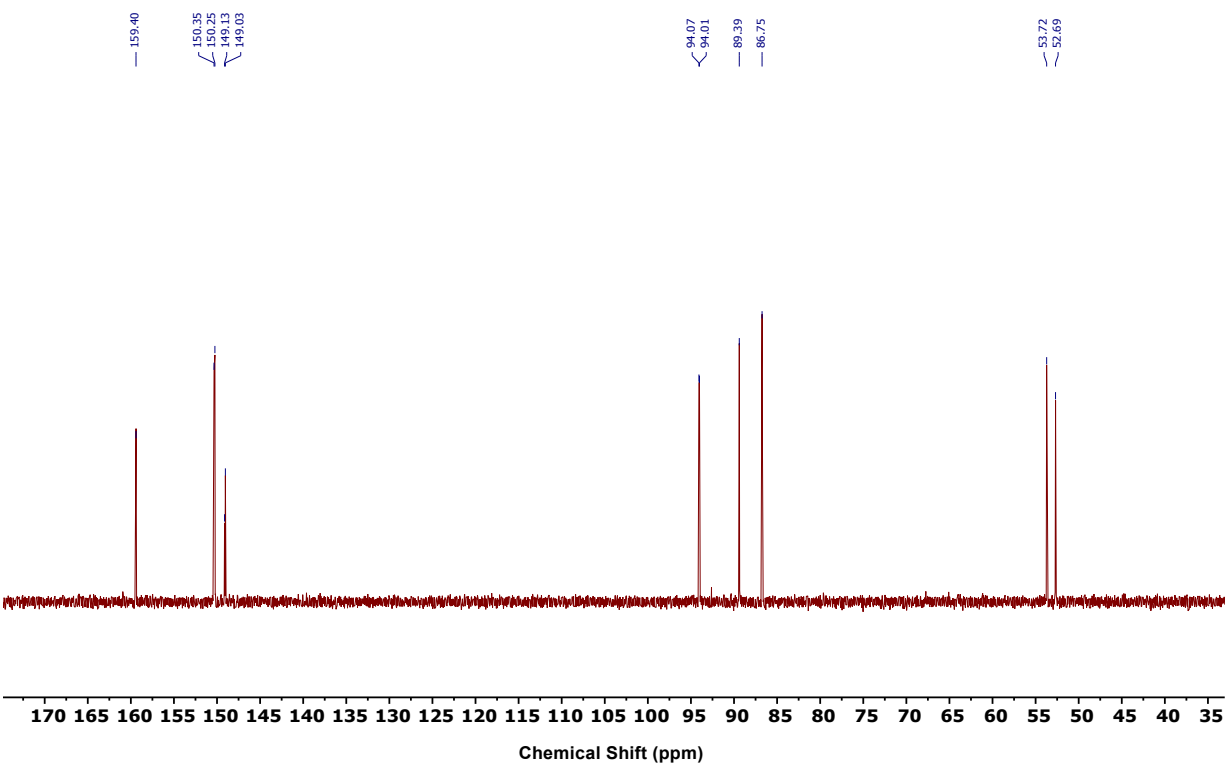
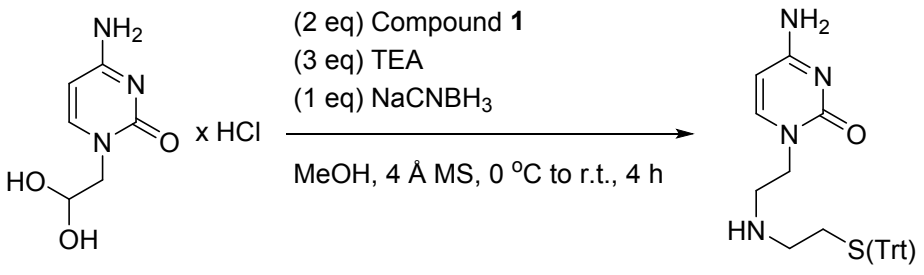


Figure S41. ^{13}C NMR (101 MHz, D_2O) of hydrated aldehyde functional cytosine (12).



12 **13**

To an oven dried N_2 purged 1000 mL round bottom flask was added 4 Å molecular sieves. Compound **1** (10.2 g, 28.9 mmol, 2 eq**)** was added to the round bottom flask followed by 500 mL of methanol. Anhydrous triethylamine (6.1 mL, 43.5 mmol, 3 eq), and compound **12** (3 g, 14.5 mmol, 1 eq) were added sequentially. The flask was then submerged in an ice bath followed by the addition of sodium cyanoborohydride (0.91 g, 14.5 mmol, 1 eq) was then added in one portion to the flask. The reaction was reacted on ice for one hour and then at room temperature 3 h. After 4 h, the reaction was filtered. Once filtered, the cake was washed with 50 mL of methanol and 100 mL of DCM. The solvent was concentrated via rotovap to roughly 200 mL after which the solution was transferred to a separatory funnel. An additional 300 mL of DCM and 300 mL of water were added to separatory funnel and the organic layer was collected. The aqueous layer was washed with DCM (2x, 100 mL) and the organic layers were combined, dried over magnesium sulfate, and filtered. The solution was then concentrated and purified by flash chromatography with a gradient of 0 to 12.5% MeOH in DCM to afford compound **13** as a white solid (3.4 g, 53%).

^{1}H NMR (400 MHz, DMSO) δ 7.45 (d, *J* = 7.1 Hz, 1H), 7.40 – 7.19 (m, 15H), 6.95 (d, *J* = 34.6 Hz, 2H), 5.57 (d, *J* = 7.1 Hz, 1H), 3.58 (t, *J* = 6.1 Hz, 2H), 2.55 (d, *J* = 6.0 Hz, 2H), 2.43 (t, *J* = 6.9 Hz, 2H), 2.18 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 166.38, 156.29, 147.06, 145.02, 129.56, 128.48, 127.13, 99.99, 93.04, 66.35, 48.89, 47.90, 47.75, 32.09.

Mass: $C_{27}H_{28}N_4OS$ $[M+H]^+$ Calculated: 457.61, Found: 457.30.

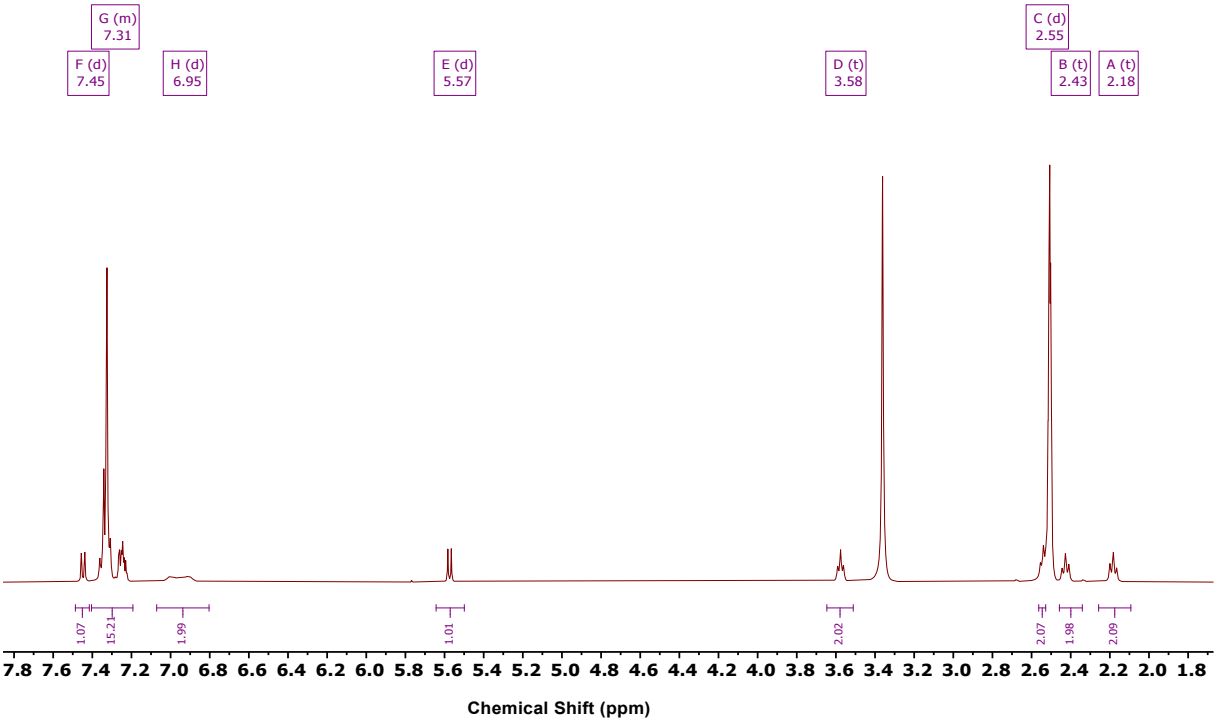


Figure S42. ^{1}H NMR (400 MHz, DMSO) of trityl protected cysteamine functional cytosine (13).

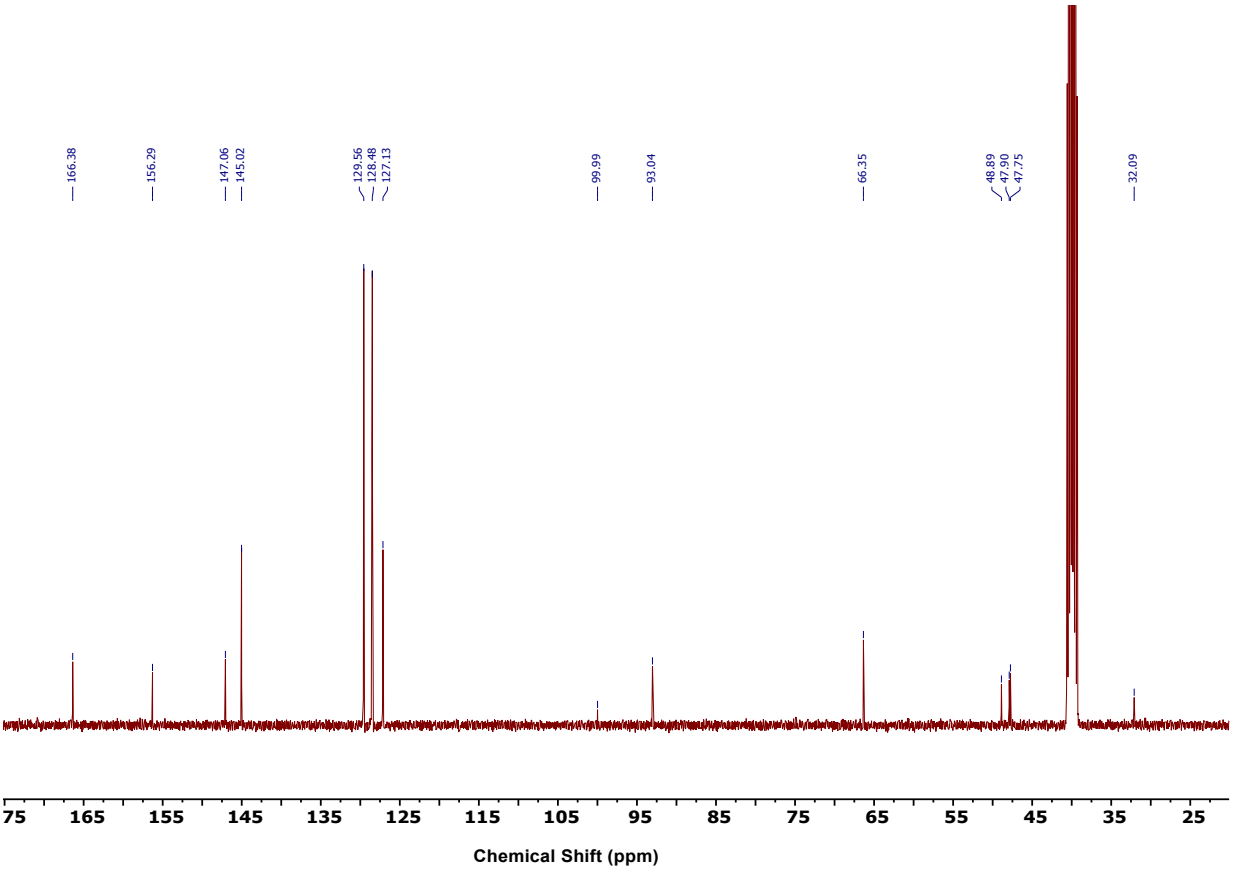
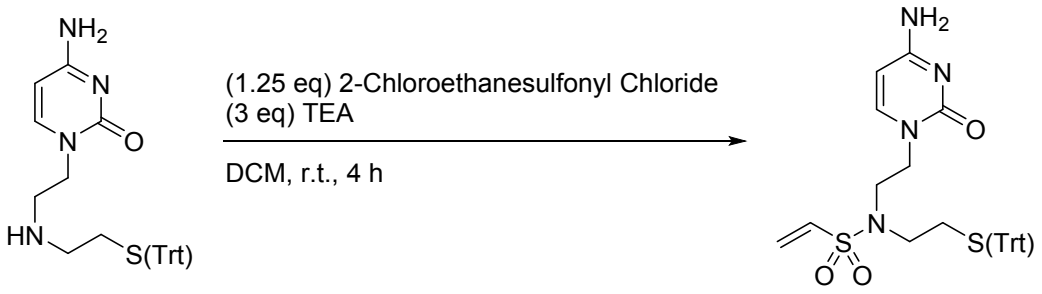


Figure S43. ^{13}C NMR (101 MHz, DMSO) of trityl protected cysteamine functional cytosine (13).



13 **14**

To an oven dried N_2 purged round bottom flask was added compound **13** (4.93 g, 10.8 mmol, 1 eq) followed by 100 mL of DCM. Next, anhydrous triethylamine (4.51 mL, 32.4 mmol, 3 eq) was added followed by dropwise addition of 2-chloroethylsulfonyl chloride (1.36 mL, 13 mmol. 1.2 eq) over the course an hour. Once the addition was finished, the reaction was stirred at room temperature for 4 h. Once finished, 100 mL of water was added to quench the reaction and was transferred to an extraction vessel. An additional 200 mL of DCM was added and the organic layer was washed with water (2x 100 mL) followed by drying over Na_2SO_4. The Na_2SO_4 was filtered and the organic solution concentrated before being purified by flash chromatography with a gradient of 0% to 10% methanol in DCM to afford product **14** (3.1 g, 52%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ 7.48 – 7.18 (m, 18H), 7.03 (d, *J* = 35.2 Hz, 2H), 6.60 (dd, *J* = 16.4, 9.9 Hz, 1H), 6.01 – 5.86 (m, 2H), 5.60 (d, *J* = 7.2 Hz, 1H), 3.60 (t, *J* = 6.0 Hz, 2H), 3.10 (t, *J* = 6.1 Hz, 2H), 2.88 (dd, *J* = 8.7, 6.8 Hz, 2H), 2.37 (dd, *J* = 8.8, 6.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 166.47, 156.11, 146.56, 144.73, 134.73, 129.56, 128.59, 127.82, 127.29, 93.71, 66.94, 48.36, 47.82, 46.61, 30.43.

Mass: $C_{29}H_{30}N_4O_3S_2$ $[M+H]^+$ Calculated: 547.70, Found: 547.34

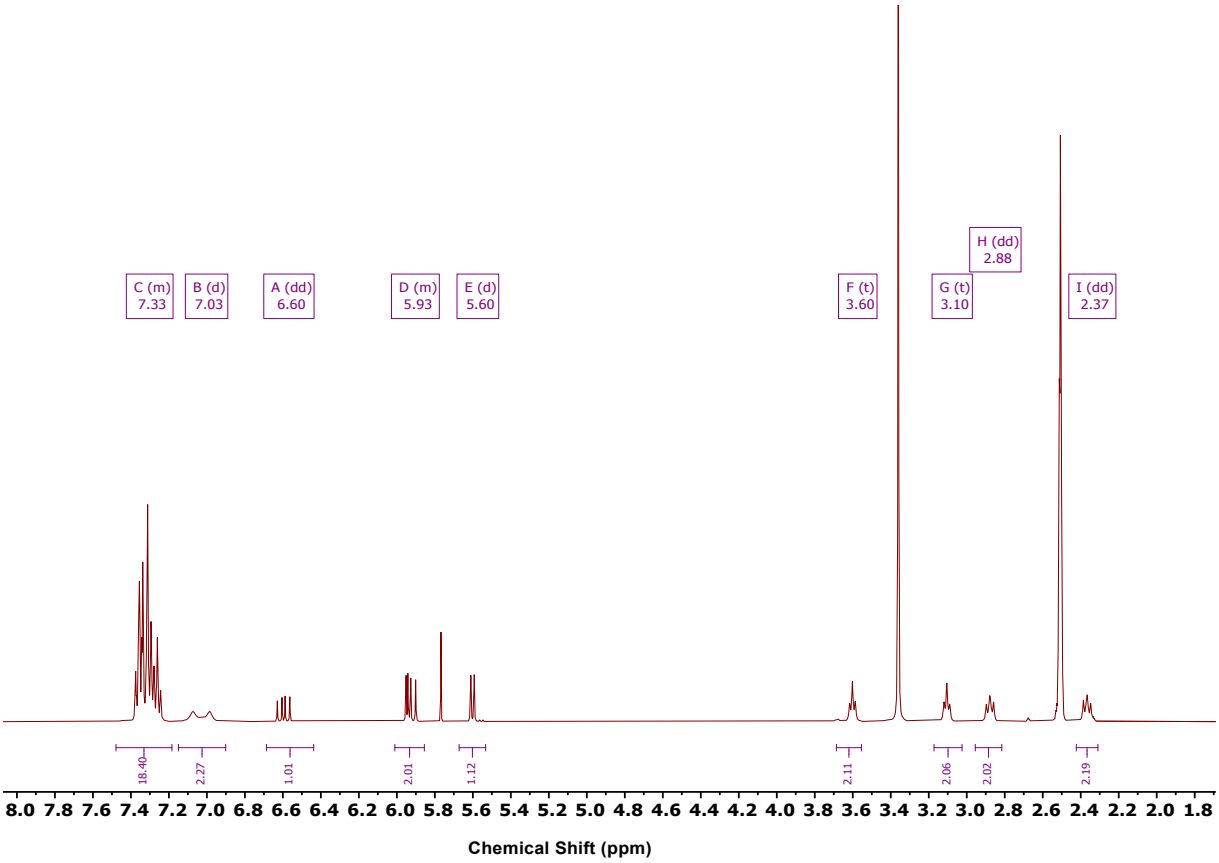


Figure S44. ^{1}H NMR (400 MHz, DMSO) of the thiol-Michael cytosine (TM-C) monomer (14).

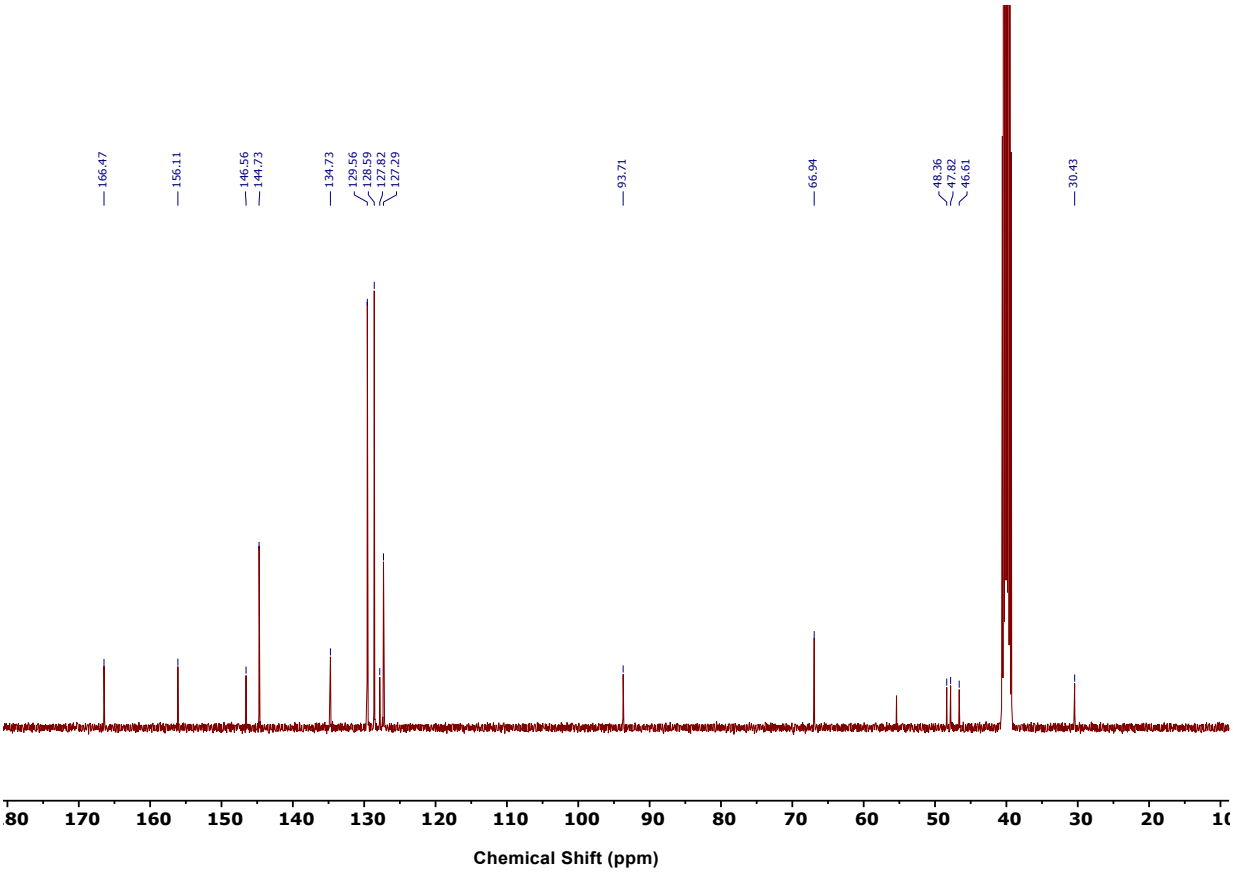
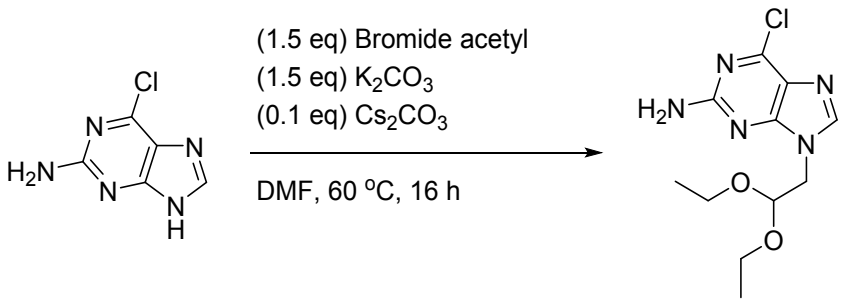


Figure S45. ^{13}C NMR (101 MHz, DMSO) of the thiol-Michael cytosine (TM-C) monomer (14).

11. Thiol-Michael Guanine Monomer (TM-G) Synthesis



15

2-amino-6-chloropurine (59 mmol, 10 g, 1 eq) was added to an oven-dried round bottom flask that has been purged with nitrogen gas. Anhydrous potassium carbonate (88.5 mmol, 12.2 g, 1.5 eq) and cesium carbonate (5.9 mmol, 1.9 g, 0.1 eq) were added to the flask. Anhydrous DMF (150 mL) was added followed by bromoacetaldehyde diethyl acetal (88.5 mmol, 13.3 mL, 1.2 eq) in one portion. The flask was then heated at 60 ℃ overnight. Upon completion of the reaction, the flask was filtered while hot. The cake was washed with minimal DMF and the eluent was evaporated under reduced pressure to produce a reddish oil. The resulting oil was dissolved in chloroform (300 mL) and washed with water (5x, 200 mL). The organic layer was collected and dried over anhydrous magnesium sulfate. After filtering the magnesium sulfate the chloroform was evaporated resulting in an orange oil. The resulting oil was then dry loaded onto silica. The dry loaded silica was put over a silica plug and was washed with 1 L of 75% EtOAc in hexanes. Additional 1 L washes were performed until the product stopped appearing in the eluent, as determined by TLC. The organics were combined, concentrated, and dried under high vac producing compound **15** (8.8 g, 52%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ 8.05 (s, 1H), 6.97 (s, 2H), 4.81 (t, *J* = 5.3 Hz, 1H), 4.14 (d, *J* = 5.4 Hz, 2H), 3.65 (dq, *J* = 9.6, 7.0 Hz, 2H), 3.43 (dq, *J* = 9.6, 7.0 Hz, 2H), 1.04 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 162.75, 160.30, 154.75, 149.76, 144.03, 123.47, 99.69, 62.80, 45.77, 36.24, 31.25, and 15.60 ppm

Mass: $C_{11}H_{16}N_5O_2Cl$ $[M+H]^+$ Calculated: 286.73, Found: 286.09

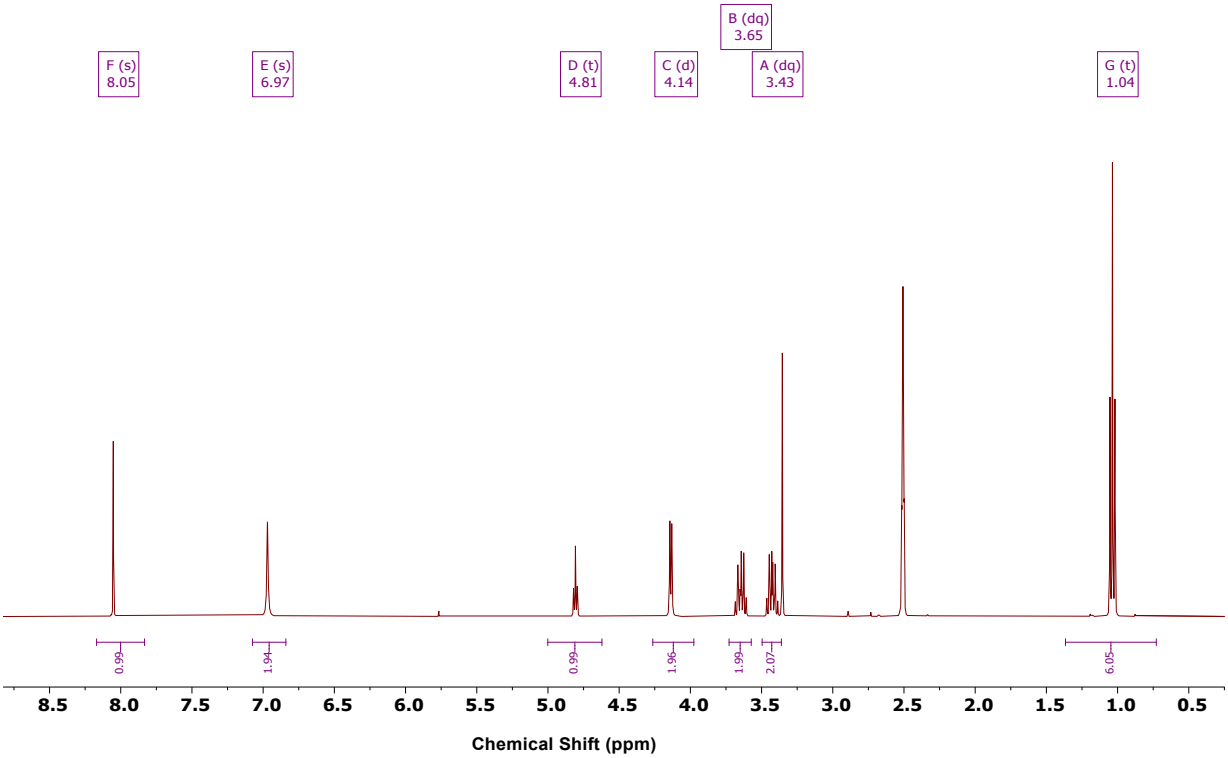


Figure S46. ^{1}H NMR (400 MHz, DMSO) of diethyl acetal functional chloroguanine (15).

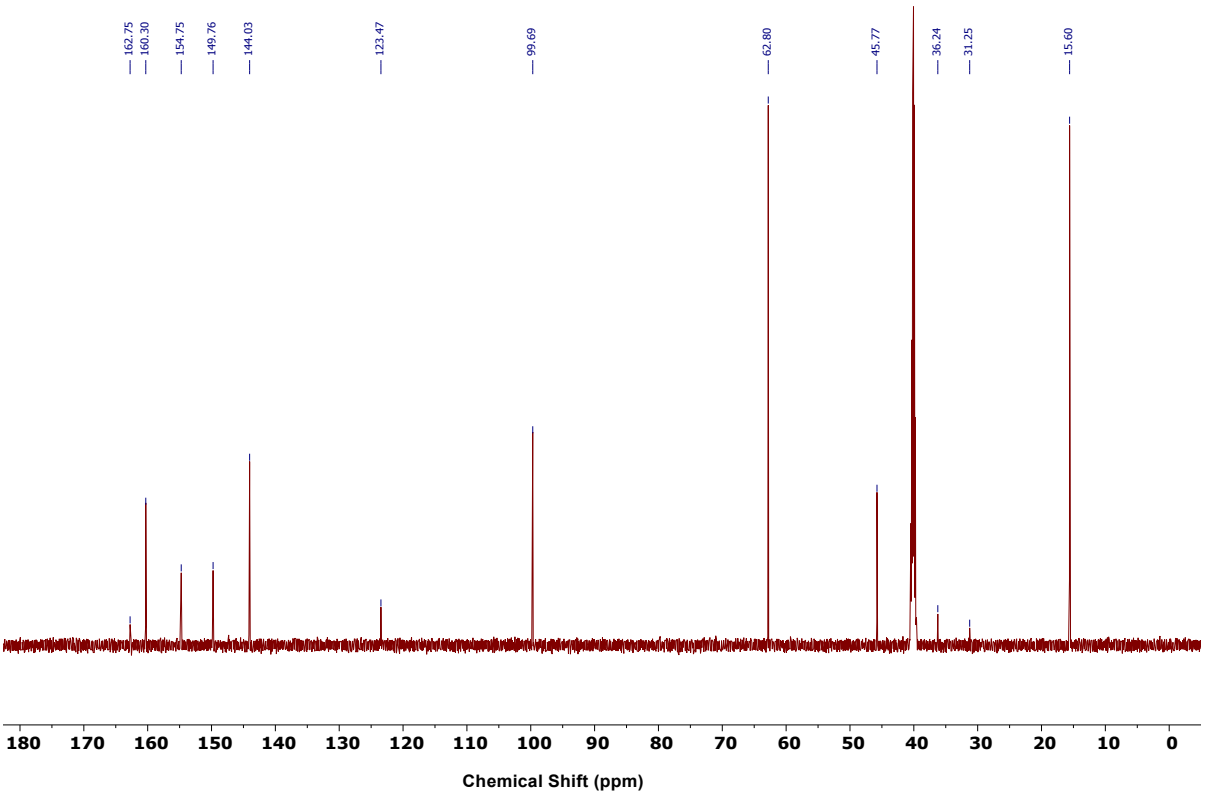
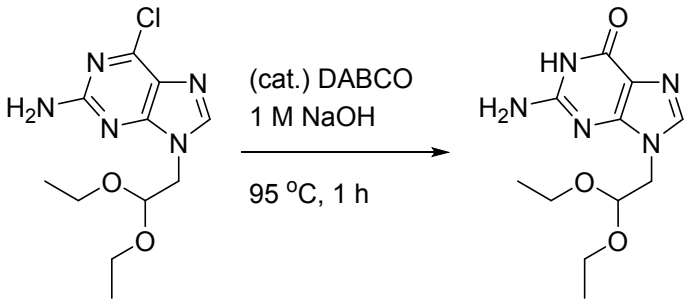


Figure S47. ^{13}C NMR (151 MHz, DMSO) of diethyl acetal functional chloroguanine (15).



15 **16**

Compound **15** (15.6 mmol, 4.45 g, 1 eq) was added to an oven-dried and N_2 purged round bottom flask followed by 1,4-diazabicyclo[2.2.2]octane (0.156 mmol, 17 mg, 0.01 eq) and 50 mL of 1M NaOH. The flask was then heated to 95 °C for one hour. Upon completion of the reaction, the flask was cooled in an ice bath for 1 h. The pH was then adjusted to seven while stirring by dropwise addition of concentrated HCl. Once pH 7 was reached, a white precipitate was formed. The solution was then filtered and the cake was washed with a minimal amount of water and 50 mL diethyl ether. The solid product was then dried via high vac to produce compound **16** as a white solid (3.8 g, 92%).

^{1}H NMR (400 MHz, DMSO) δ 10.59 (s, 1H), 7.61 (s, 1H), 6.48 (s, 2H), 4.74 (t, *J* = 5.4 Hz, 1H), 4.02 (d, *J* = 5.4 Hz, 2H), 3.64 (dq, *J* = 9.6, 7.1 Hz, 2H), 3.45 – 3.37 (m, 2H), 1.04 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, DMSO) δ 157.26, 154.03, 151.79, 138.36, 116.59, 100.04, 62.69, 45.59, 15.62.

Mass: $C_{11}H_{17}N_5O_3$ $[M+H]^+$ Calculated: 268.29, Found: 268.04

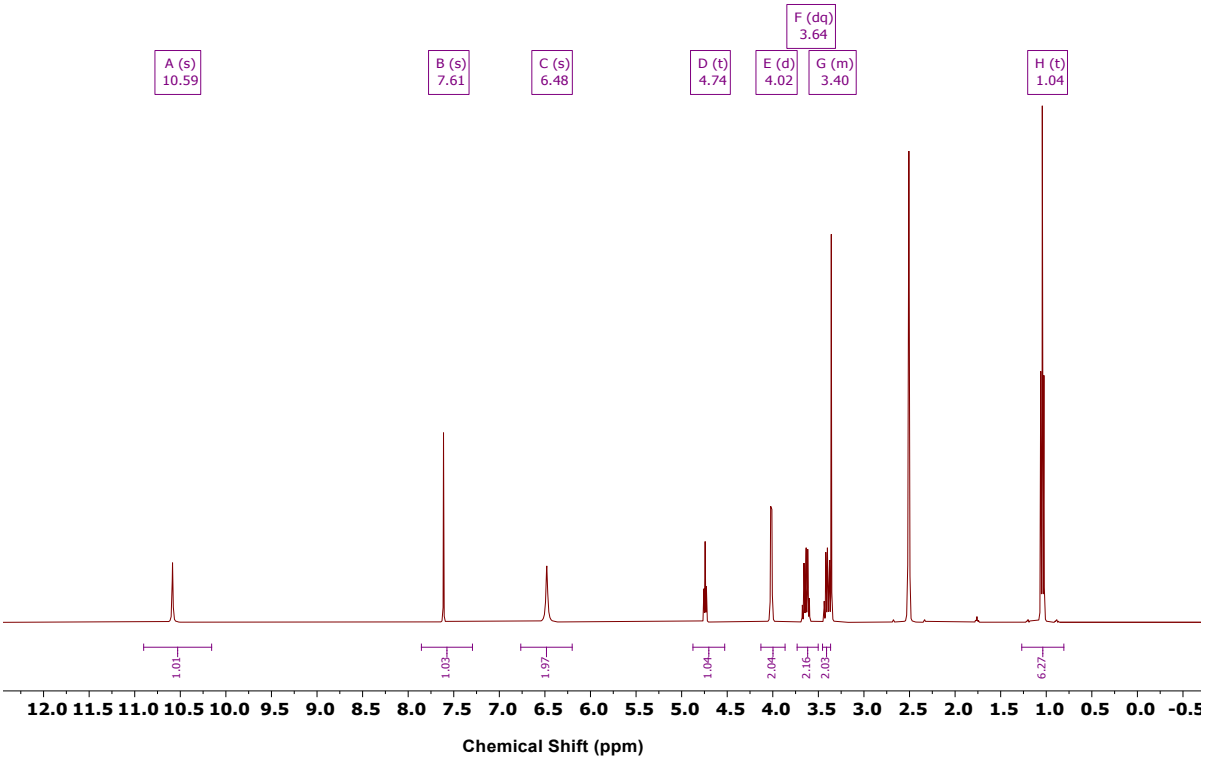


Figure S48. ^{1}H NMR (400 MHz, DMSO) of diethyl acetal functional guanine (16).

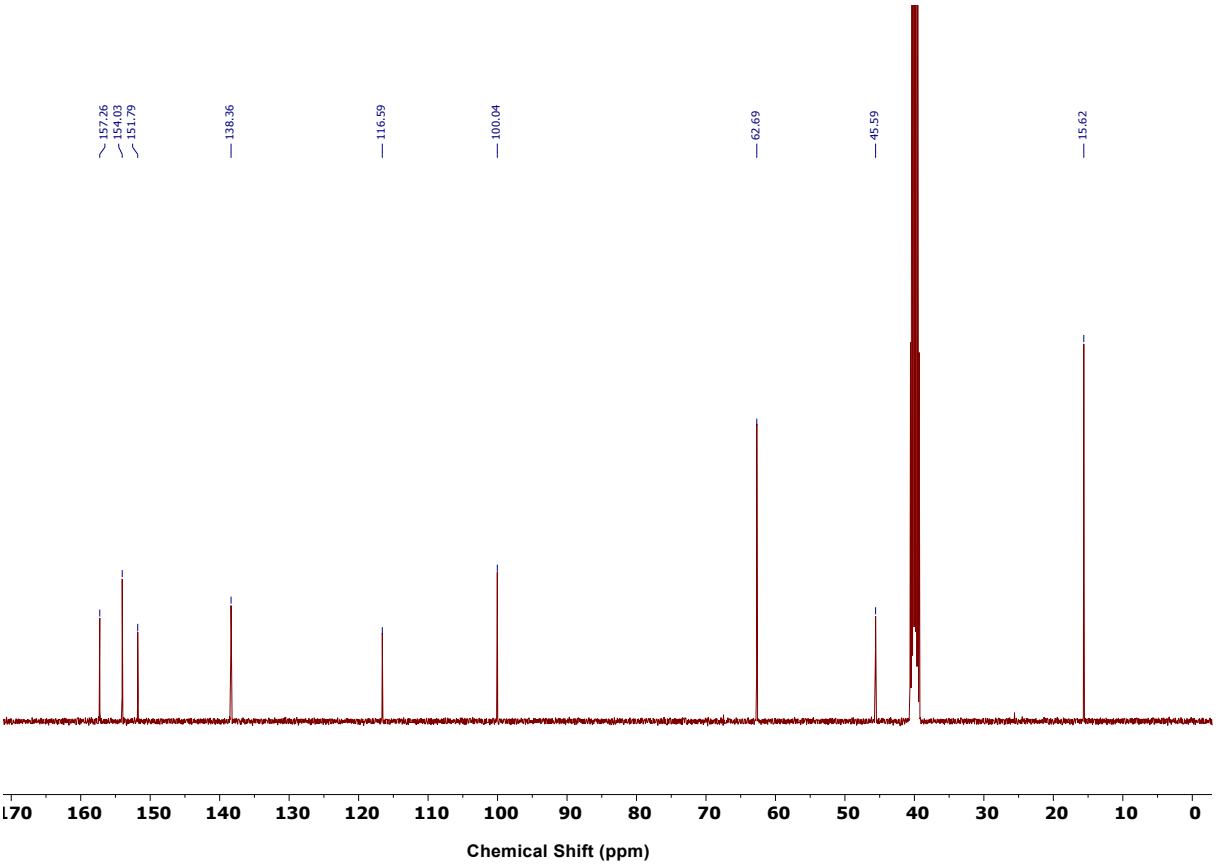
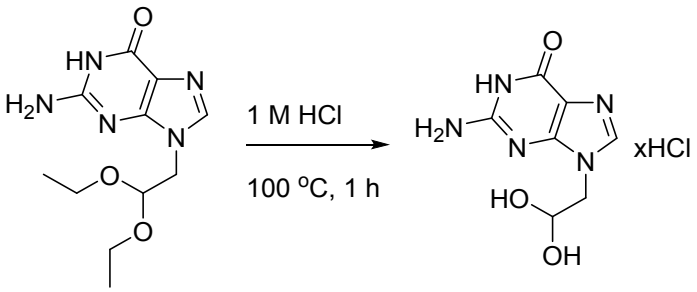


Figure S49. ^{13}C NMR (101 MHz, DMSO) of diethyl acetal functional guanine (16).



16 **17**

Compound **16** (51.5 mmol, 13.8 g) was added to a 250 mL oven-dried round bottom flask that had been purged with nitrogen gas followed by 125 mL of 1 M HCl. The reaction was refluxed at 100 ℃ in the dark for one hour. Once the reaction was complete, the water was then evaporated completely via rotary evaporation in the dark producing an off-white foam. ***NOTE****: Only after the complete removal of water did a white foam form and ending drying prematurely can result in the product redissolving in residual water left in the round bottom flask*. Subsequently, 50 mL of tetrahydrofuran was added to the round bottom flask and the mixture was cooled in an ice bath for one hour. The solid was then filtered and the cake was washed with diethyl ether before being dried under high vac to afford compound **17** as an off-white/yellow foam (12.6 g, 99%). The product was isolated as the hydrate hydrochloride salt and was used directly without further purification.

^{1}H NMR (400 MHz, D_2O) δ 8.77 (s, 1H), 5.29 (t, *J* = 4.5 Hz, 1H), 4.18 (d, *J* = 4.7 Hz, 2H).

^{13}C NMR (151 MHz, D_2O) δ 155.35, 150.32, 138.28, 107.55, 86.78, 73.22, 49.32.

Mass: $C_7H_9N_5O_3$ $[M+H]^+$ Calculated: 194.17, Found: 194.12, $[M+H_2O]^+$ Calculated: 212.18, Found: 212.02

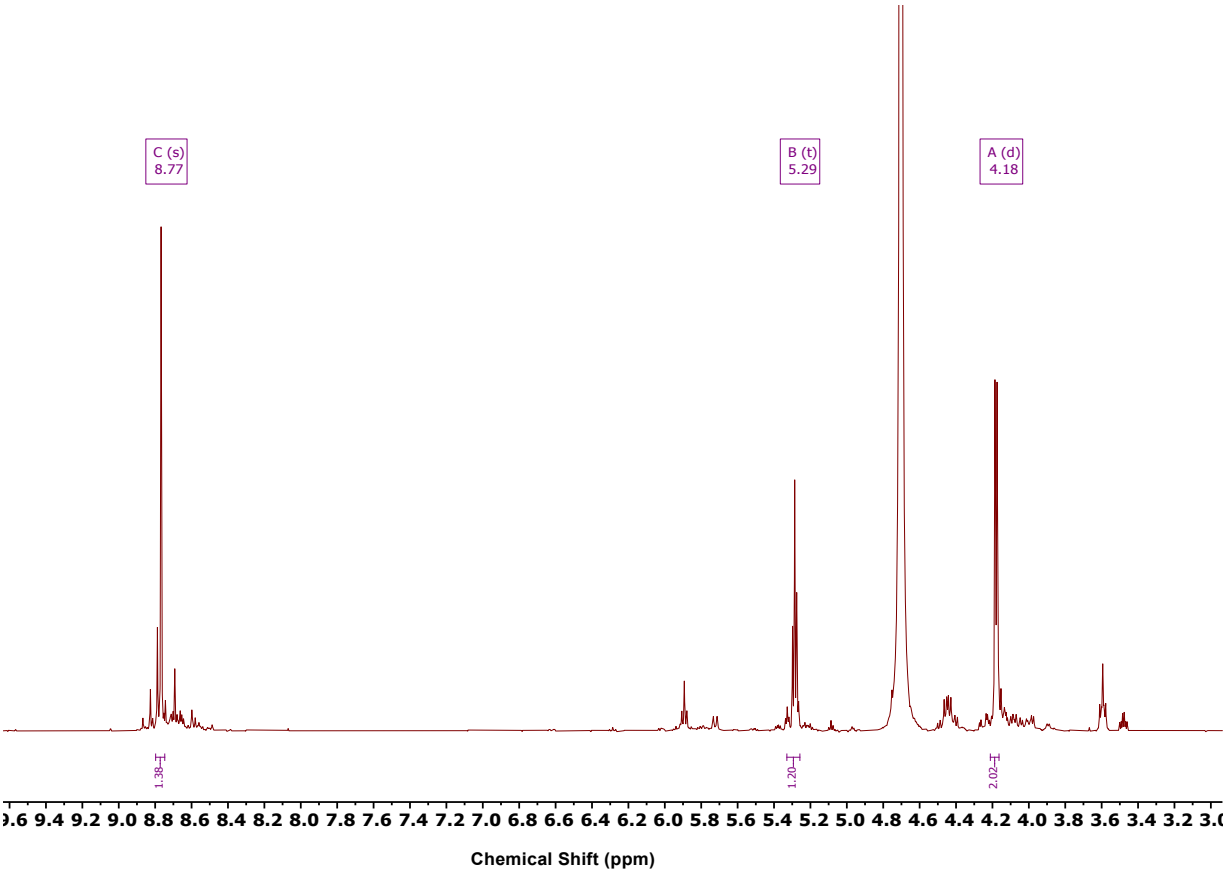


Figure S50. ^{1}H NMR (400 MHz, D_2O) of the hydrochloride salt hydrated aldehyde functional guanine (17).

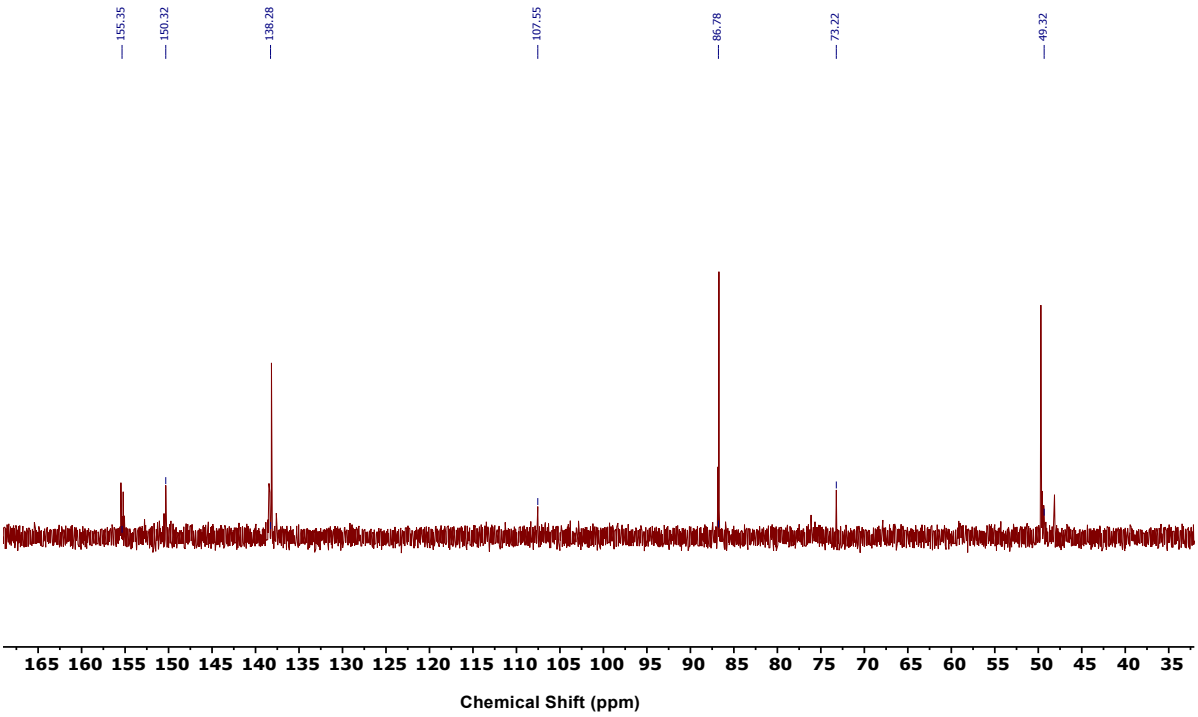
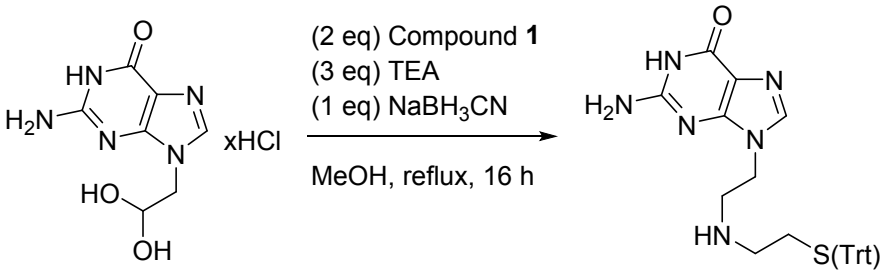


Figure S51. ^{13}C NMR (151 MHz, D_2O) of the hydrochloride salt hydrate aldehyde functional guanine (17).



17 **18**

To a 500 mL round bottom flask that had been oven dried and N_2 purged was added molecular sieves. Compound **1** (8.6 g, 24.3 mmol, 2 eq), 500 mL of methanol, and anhydrous triethylamine (5.1 mL, 36.4 mmol, 3 eq) were subsequently added followed by mixing for 15 min. Compound **17** (3 g, 12.1 mmol, 1 eq) was then added followed by sodium cyanoborohydride (0.76 g, 12.1 mmol, 1 eq). After the addition, the round bottom flask was heated to reflux at 75 °C overnight. Upon completion of the reaction, the mixture was filtered while hot and the cake was washed with 100 mL DCM. The eluent was collected and concentrated to roughly 200 mL and transferred to an extraction vessel in which an additional 300 mL of DCM was added. The organic solution was extracted with water (400 mL). The water layer was then washed twice with 200 mL DCM. The DCM layers were combined and were subsequently dried over sodium sulfate and filtered. The solution was then concentrated via rotary evaporation. Flash chromatography was used to purify the product using a gradient from 0% MeOH in DCM to 15% MeOH in DCM. The solvent was collected and evaporated to produce compound **18** as a white solid (1.86 g, 30%).

^{1}H NMR (400 MHz, DMSO) δ 10.56 (s, 1H), 7.61 (s, 1H), 7.45 – 7.10 (m, 16H), 6.45 (s, 2H), 3.89 (t, *J* = 6.2 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 2H), 2.44 (t, *J* = 6.9 Hz, 2H), 2.18 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO) δ 157.27, 153.88, 151.55, 145.00, 138.28, 129.55, 128.47, 127.12, 116.88, 66.36, 48.29, 47.78, 43.03, 32.07.

Mass: $C_{28}H_{28}N_6O_5S$ $[M+H]^+$ Calculated: 497.63, Found: 497.68

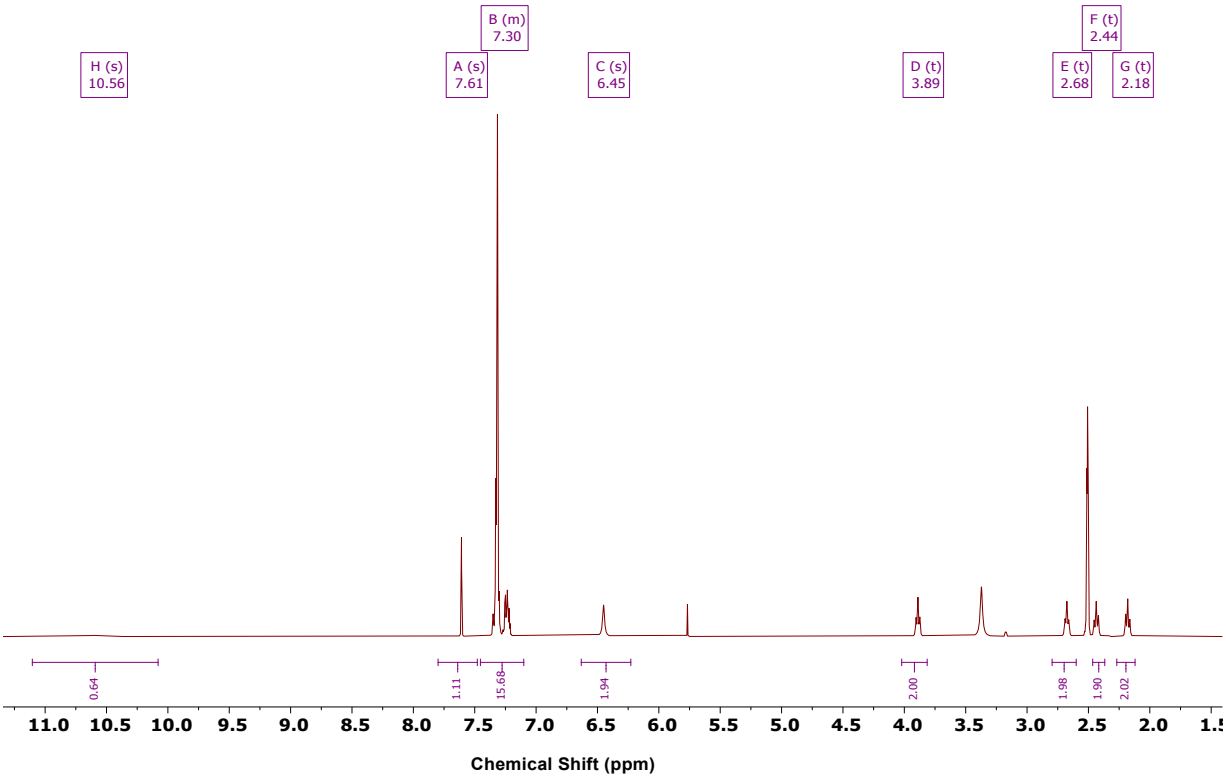


Figure S52. ^{1}H NMR (400 MHz, DMSO) of trityl protected cysteamine functional guanine (18).

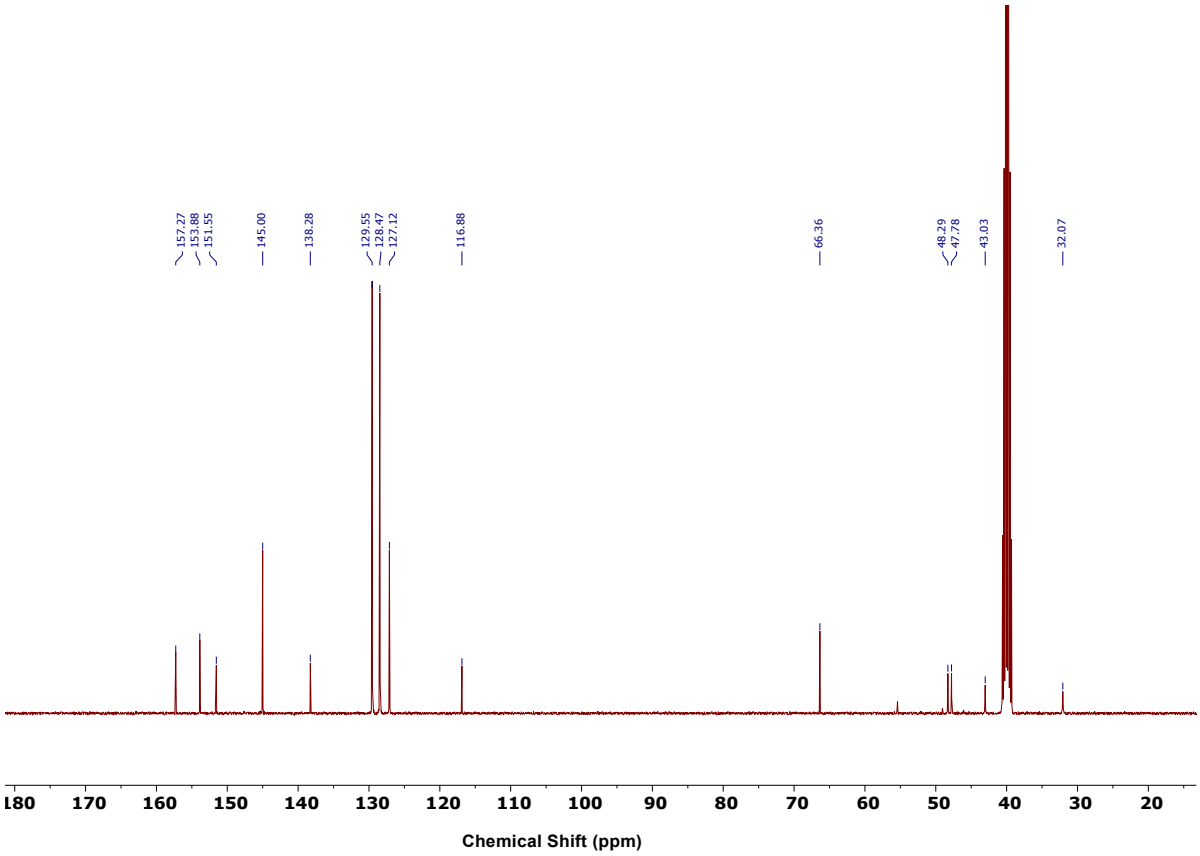
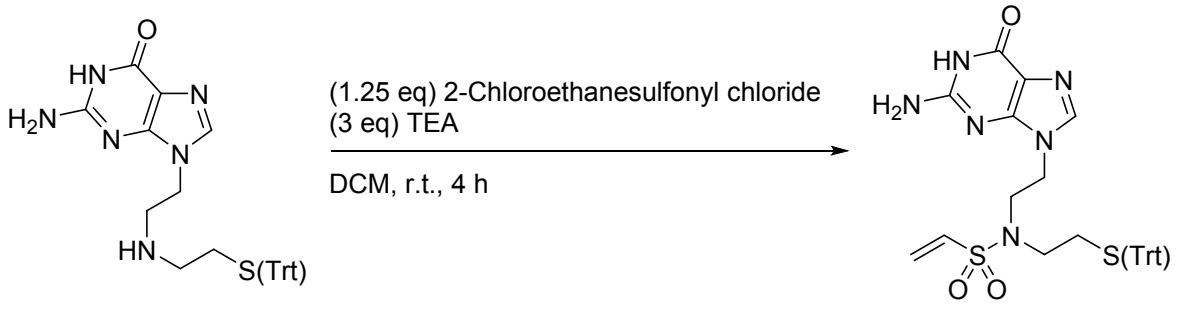


Figure S53. ^{13}C NMR (101 MHz, DMSO) of trityl protected cysteamine functional guanine (18).



18 **19**

Compound **18** (3 g, 6.05 mmol, 1 eq) was added to a 500 mL round bottom flask that had been oven dried and N_2 purged. Anhydrous DCM (300 mL) and anhydrous triethylamine (2.53 mL, 18.15 mmol, 3 eq) were added. After mixing for 15 min, 2-Chloroethanesulfonyl chloride (790 µL, 7.56 mmol, 1.25 eq) was added dropwise over the course of one hour. Once the addition was finished, the mixture was reacted for 4 h before water (100 mL) was added to quench the reaction. The organic phase was extracted, dried over Na_2SO_4, filtered, and concentrated via rotary evaporation. The resulting oil was column purified with a gradient of 0% to 12.5% methanol in DCM to produce compound **19** as a white solid (1.83 g, 52%).

^{1}H NMR (400 MHz, DMSO) δ 10.56 (s, 1H), 7.53 (s, 1H), 7.41 – 7.14 (m, 17H), 6.63 – 6.29 (m, 3H), 5.90 (dd, *J* = 13.1, 3.4 Hz, 2H), 3.92 (t, *J* = 6.0 Hz, 2H), 3.25 (t, *J* = 6.0 Hz, 2H), 2.90 – 2.79 (m, 2H), 2.36 – 2.21 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 157.22, 153.92, 151.60, 144.69, 137.78, 134.53, 129.54, 128.57, 127.93, 127.29, 116.98, 66.93, 47.52, 47.20, 42.09, 30.35.

Mass: $C_{30}H_{30}N6O_3S_2$ $[M+H]^+$ Calculated: 587.73, Found: 587.50

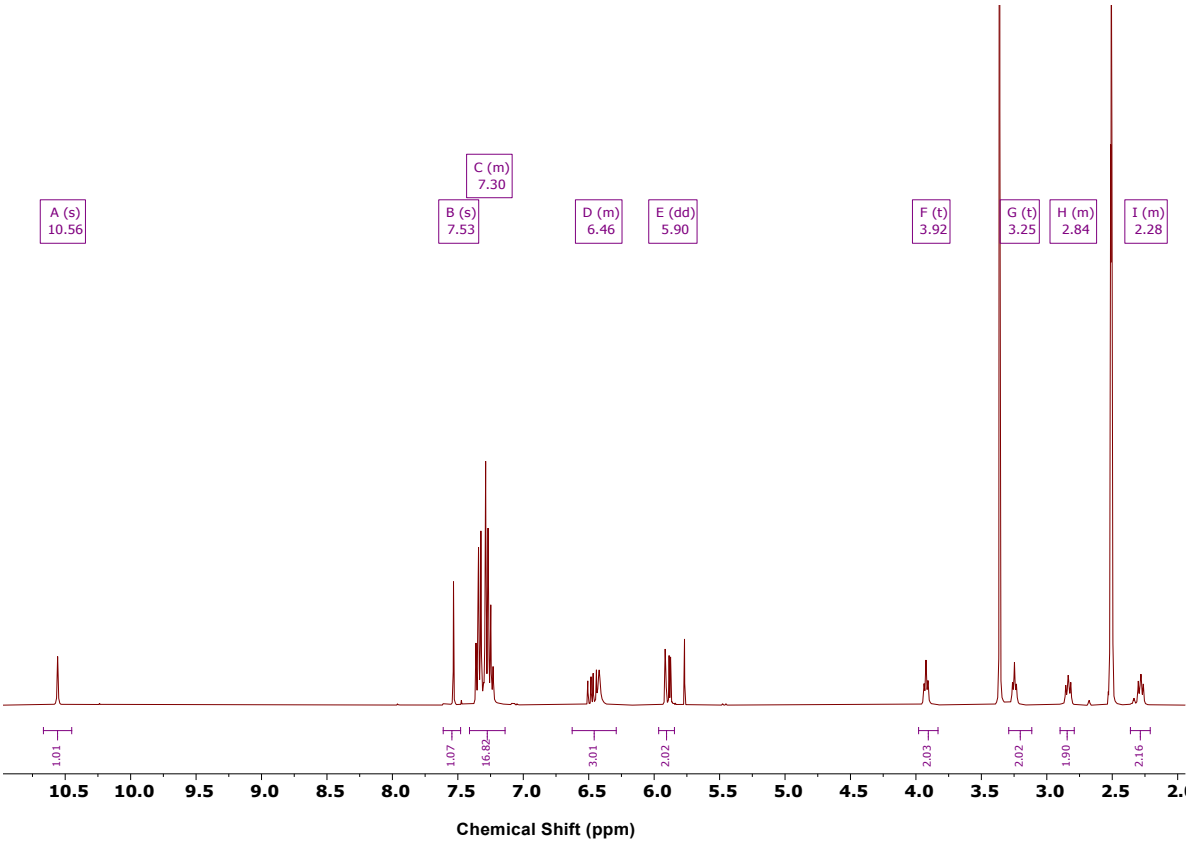


Figure S54. ^{1}H NMR (400 MHz, DMSO) of the thiol-Michael guanine (TM-G) monomer (19).

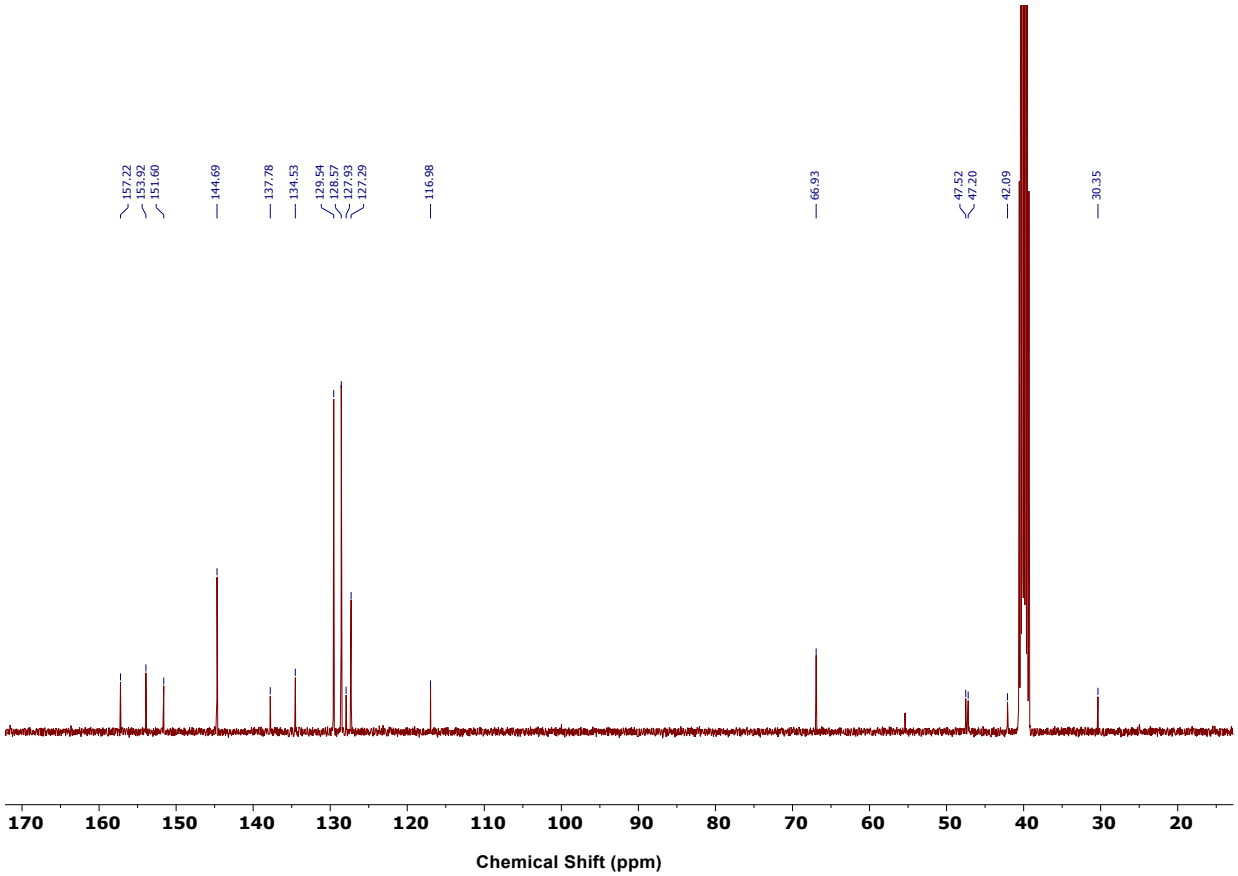
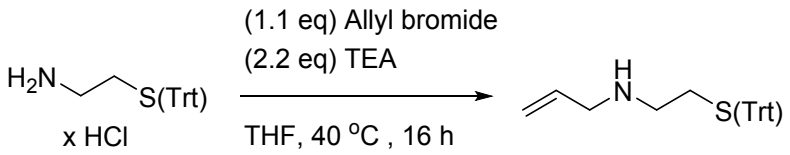


Figure S55. ^{13}C NMR (101 MHz, DMSO) of the thiol-Michael guanine (TM-G) monomer (19).

12. Thiol-ene Thymine Monomer (TE-T) Synthesis



1 **20**

To an oven dried and N_2 purged 500 mL RBF was added compound **1** (30 g, 84.5 mmol, 1 eq) and anhydrous THF (375 mL). To the mixture was added triethylamine (26 mL, 187.8 mmol, 2.2 eq) followed by dropwise addition of allyl bromide (8 mL, 93.9, 1.1 eq) over the course of 30 min. The reaction was heated to 40 °C overnight. The reaction was then filtered to remove the triethylamine salts and concentrated via rotary evaporation. Flash chromatography was used to purify the resulting oil using a gradient of 5% to 30% ethyl acetate in hexanes. Fractions containing the product were collected and evaporated to produce compound **20** as a yellow oil that solidified upon standing (19.9 g, 65%).

^{1}H NMR (400 MHz, DMSO) δ 7.51 – 7.04 (m, 15H), 5.77 – 5.64 (m, 1H), 5.16 – 4.92 (m, 2H), 2.97 (d, *J* = 5.8 Hz, 2H), 2.42 (dd, *J* = 7.8, 6.3 Hz, 2H), 2.21 (dd, *J* = 7.7, 6.3 Hz, 2H), 1.77 (s, 1H).

^{13}C NMR (101 MHz, DMSO) δ 145.08, 137.92, 129.57, 128.43, 127.09, 115.55, 66.32, 51.59, 47.63, 32.26.

Mass: $C_{24}H_{25}NS$ $[M+H]^+$ Calculated: 360.53, Found: 360.09

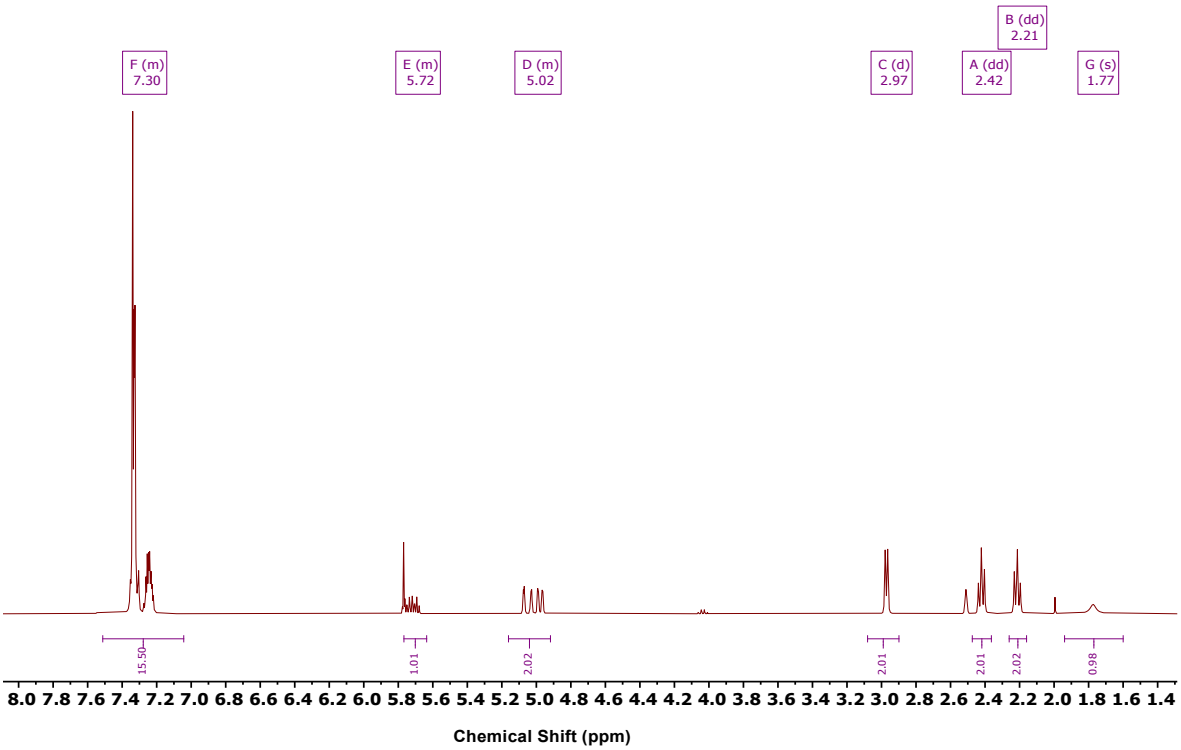


Figure S56. ^{1}H NMR (400 MHz, DMSO) of allyl functional trityl protected cysteamine (20).

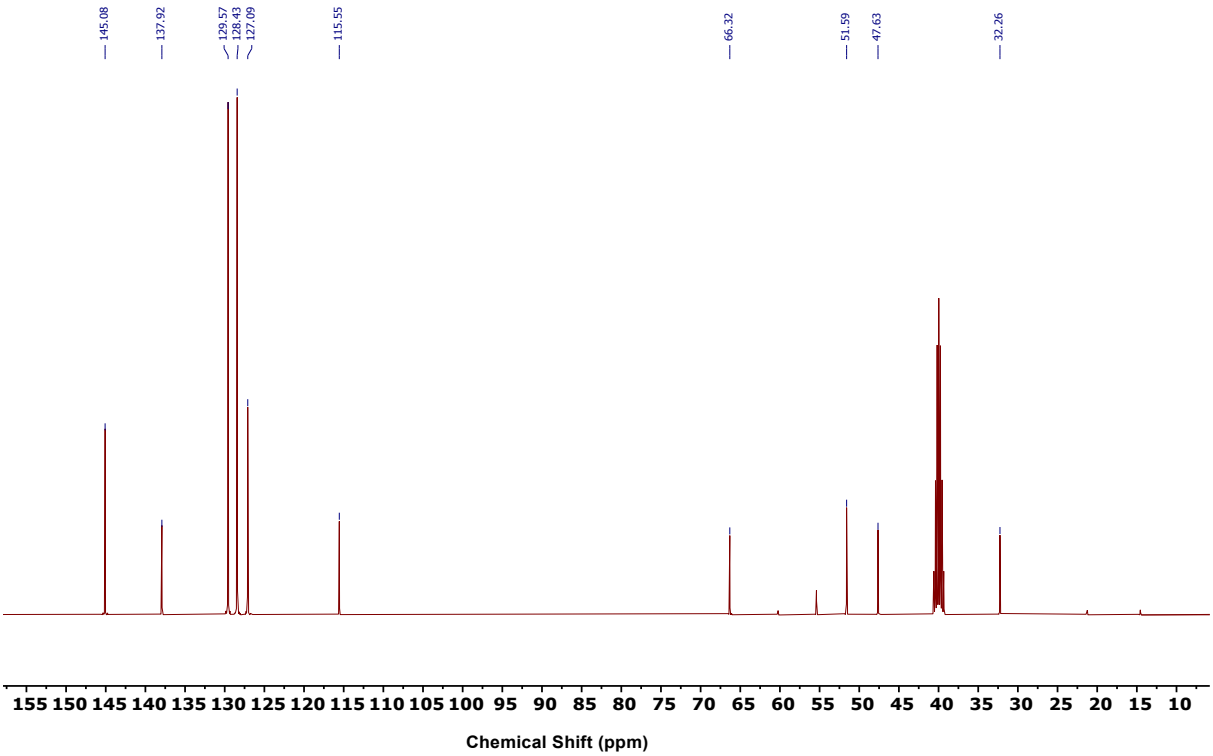
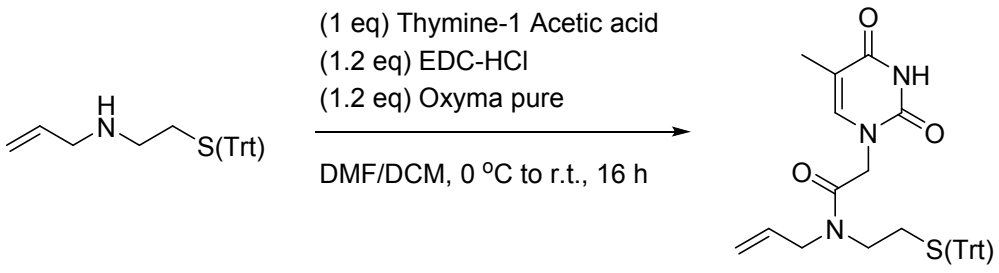


Figure S57. ^{13}C NMR (101 MHz, DMSO) of allyl functional trityl protected cysteamine (20).



20 **21**

To an oven-dried 100 mL round bottom flask was added (2.15 g, 11.7 mmol, 1 eq) of thymine-1-acetic acid, which was dissolved in 25 mL of DCM and 30 mL of anhydrous DMF. The solution was cooled in an ice bath. To the solution was then added Oxyma pure (2 g, 14 mmol, 1.2 eq) and EDC-HCl (2.69, 14 mmol, 1.2 eq). Following 15 min of stirring at 0 °C, compound **20** (4.2 g, 11.7 mmol, 1 eq) was added dropwise over 15 min. After finishing the addition, the reaction was allowed to warm to room temperature and was left to react for 24 h. Upon completion of the reaction, the solution was concentrated, and the residue was dissolved in ethyl acetate (300 mL). The solution was extracted with water (4x 200 mL) and saturated sodium bicarbonate (2x 100 mL). The organic layer was collected, dried over sodium sulfate, and filtered. The eluent was concentrated via rotary evaporation resulting in an oil that was purified using flash chromatography with a gradient of 20% to 100% ethyl acetate in hexanes. Fractions containing the product were evaporated to produce compound **21** as an off-white solid (5.6 g, 92%).

^{1}H NMR (400 MHz, DMSO) δ 11.28 (d, *J* = 5.0 Hz, 1H), 7.54 – 7.09 (m, 17H), 5.83 – 5.41 (m, 1H), 5.17 – 4.89 (m, 2H), 4.34 (d, *J* = 78.5 Hz, 2H), 3.65 (dd, *J* = 40.8, 5.3 Hz, 2H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.55 (d, *J* = 8.6 Hz, 1H), 2.32 (dd, *J* = 8.8, 6.3 Hz, 1H), 1.74 (d, *J* = 1.2 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 167.15, 166.59, 164.89, 151.44, 151.36, 144.76, 142.75, 133.45, 129.63, 128.58, 127.32, 117.22, 108.36, 99.98, 67.01, 49.61, 48.28, 45.94, 31.23, 12.39.

Mass: $C_{31}H_{31}N_3O_3S$ $[M+H]^+$ Calculated: 526.67, Found: 526.29

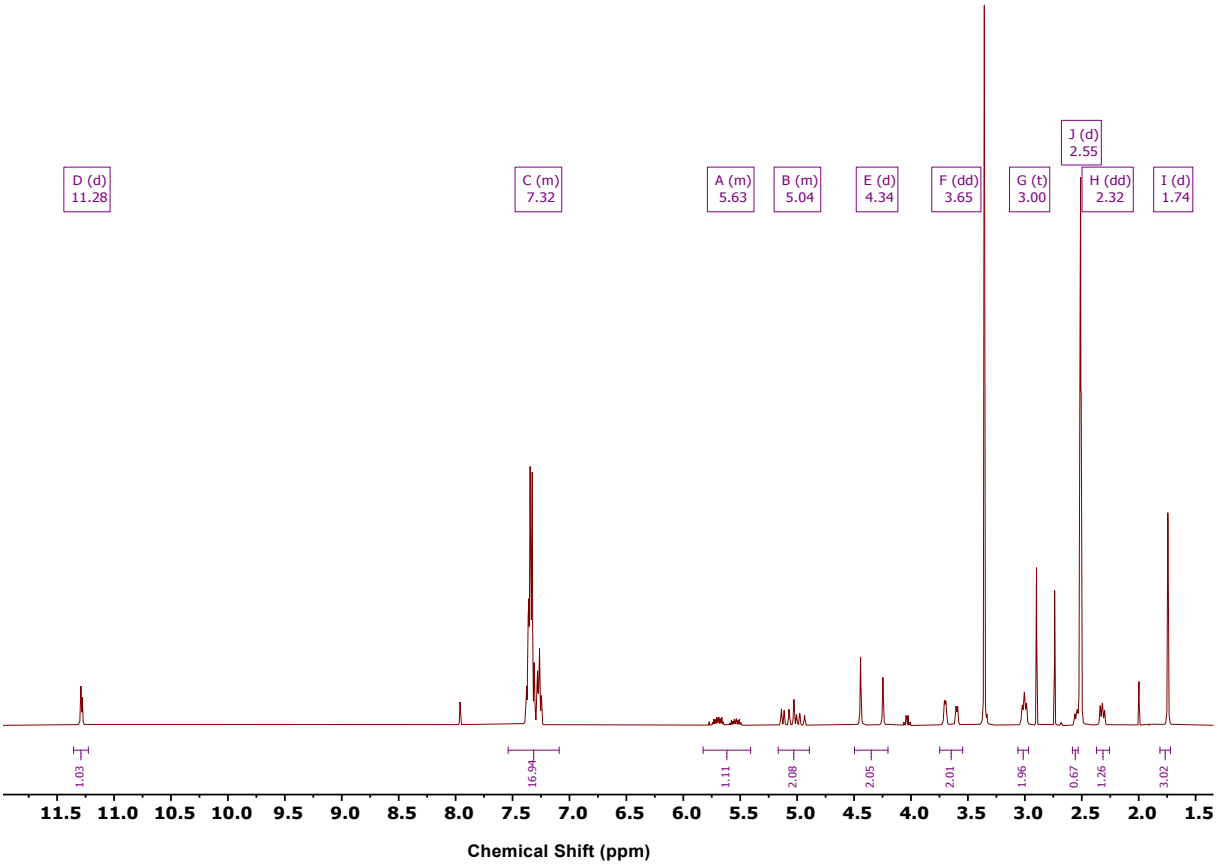


Figure S58. ^{1}H NMR (400 MHz, DMSO) of the trityl protected Thiol-ene thymine monomer (21).

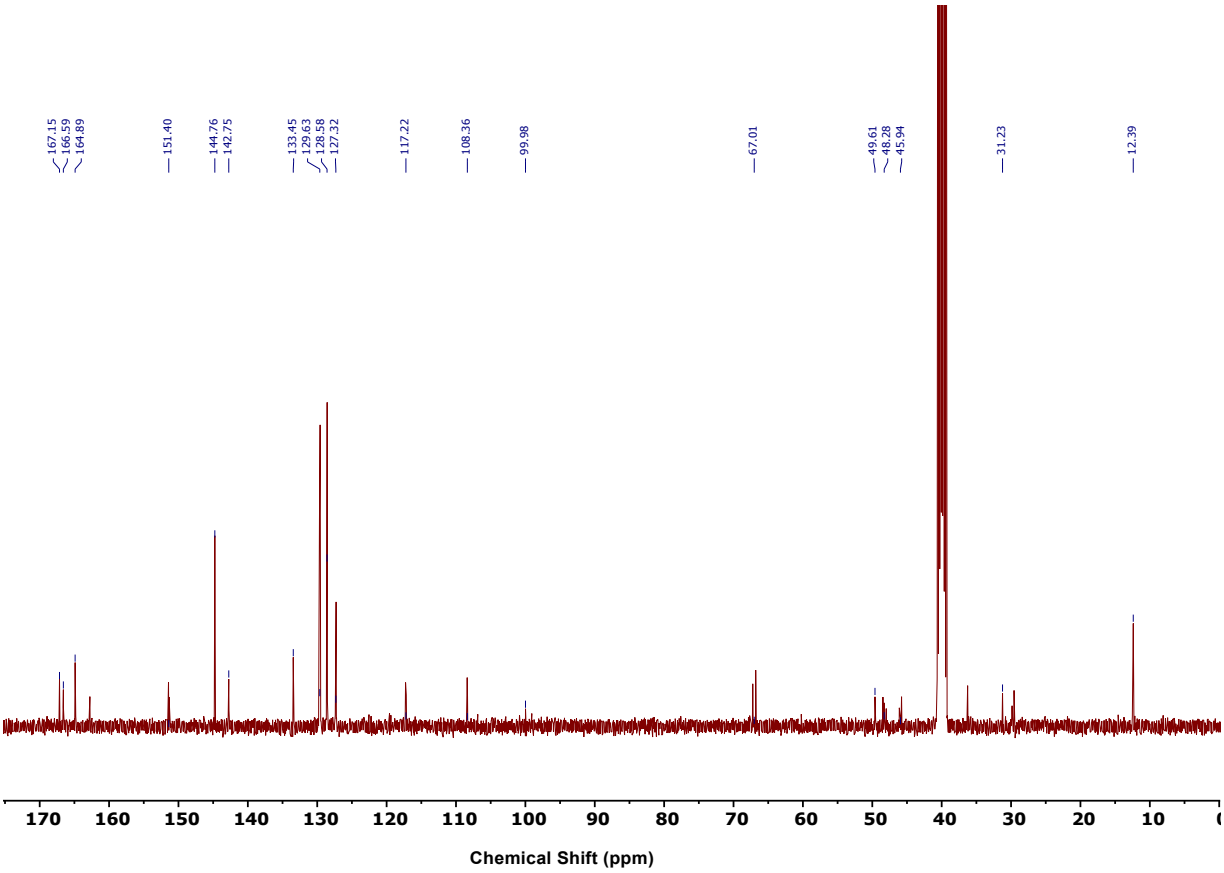
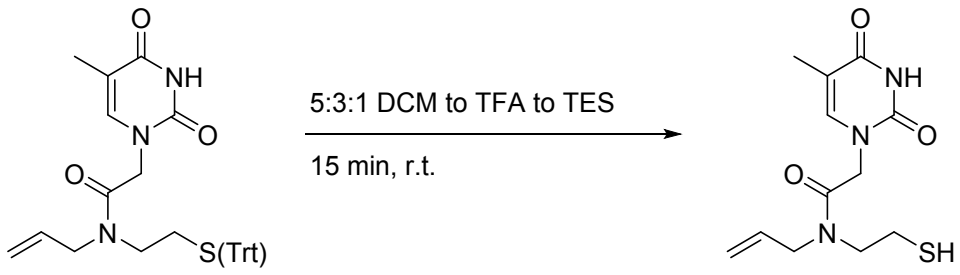


Figure S59. ^{13}C NMR (101 MHz, DMSO) of the trityl protected Thiol-ene thymine monomer (21).



21 **22**

To an oven-dried 100 mL round bottom flask was added **21** (8.56 g, 16.3 mmol, 1 eq) followed by DCM (90 mL). Once the solid had dissolved, TES (18 mL) and TFA (54 mL) was added to the round bottom flask, and the reaction was allowed to proceed for 15 min. Once complete, the solution was concentrated via rotary evaporation and diluted with 300 mL of DCM. The solution was neutralized with sat. $NaHCO_3$ (200 mL) and the organic phase was extracted. The aqueous phase was washed with DCM (2x, 100 mL) before combining the organic phases and drying them over Na_2SO_4. The solution was filtered and concentrated before being subjected to column chromatography (0% to 5% MeOH in DCM). The fractions were collected and dried to produce the product **22** a slightly yellow solid upon drying (4.11 g, 89%).

^{1}H NMR (400 MHz, DMSO) δ 11.31 (d, *J* = 6.4 Hz, 1H), 7.40 (dd, *J* = 8.9, 1.4 Hz, 1H), 6.01 – 5.61 (m, 1H), 5.37 – 5.04 (m, 2H), 4.59 (d, *J* = 48.3 Hz, 2H), 3.99 (dd, *J* = 39.7, 5.4 Hz, 2H), 3.49 – 3.36 (m, 2H), 2.81 – 2.54 (m, 2H), 2.43 (t, *J* = 8.2 Hz, 1H), 1.76 (d, *J* = 1.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 167.10, 164.92, 151.52, 142.85, 133.81, 117.41, 108.40, 49.73, 48.60, 48.02, 22.49, 21.80, 12.39.

Mass: $C_{31}H_{31}N_3O_3S$ $[M+H]^+$ Calculated: 284.35, Found: 284.21.

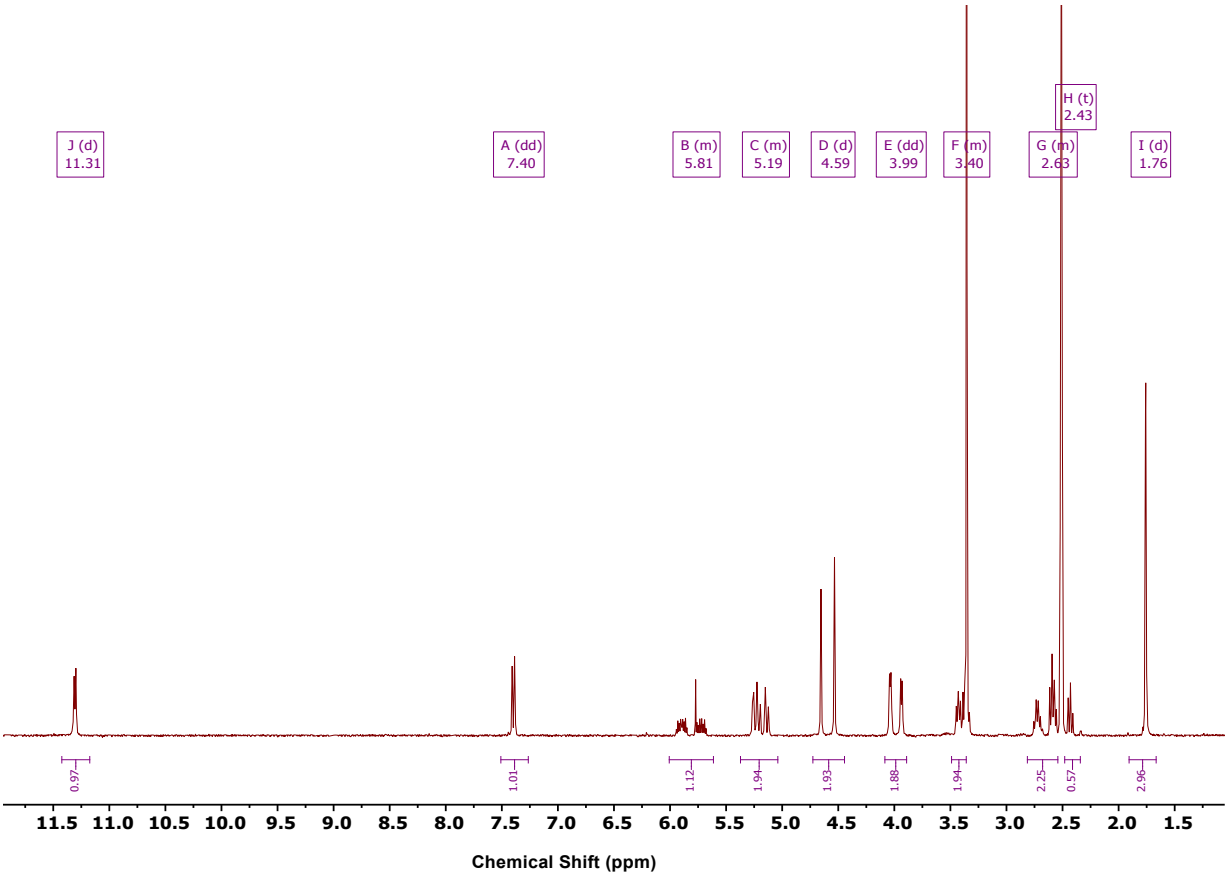


Figure S60. ^{1}H NMR (400 MHz, DMSO) of the Thiol-ene thymine (TE-T) monomer (22).

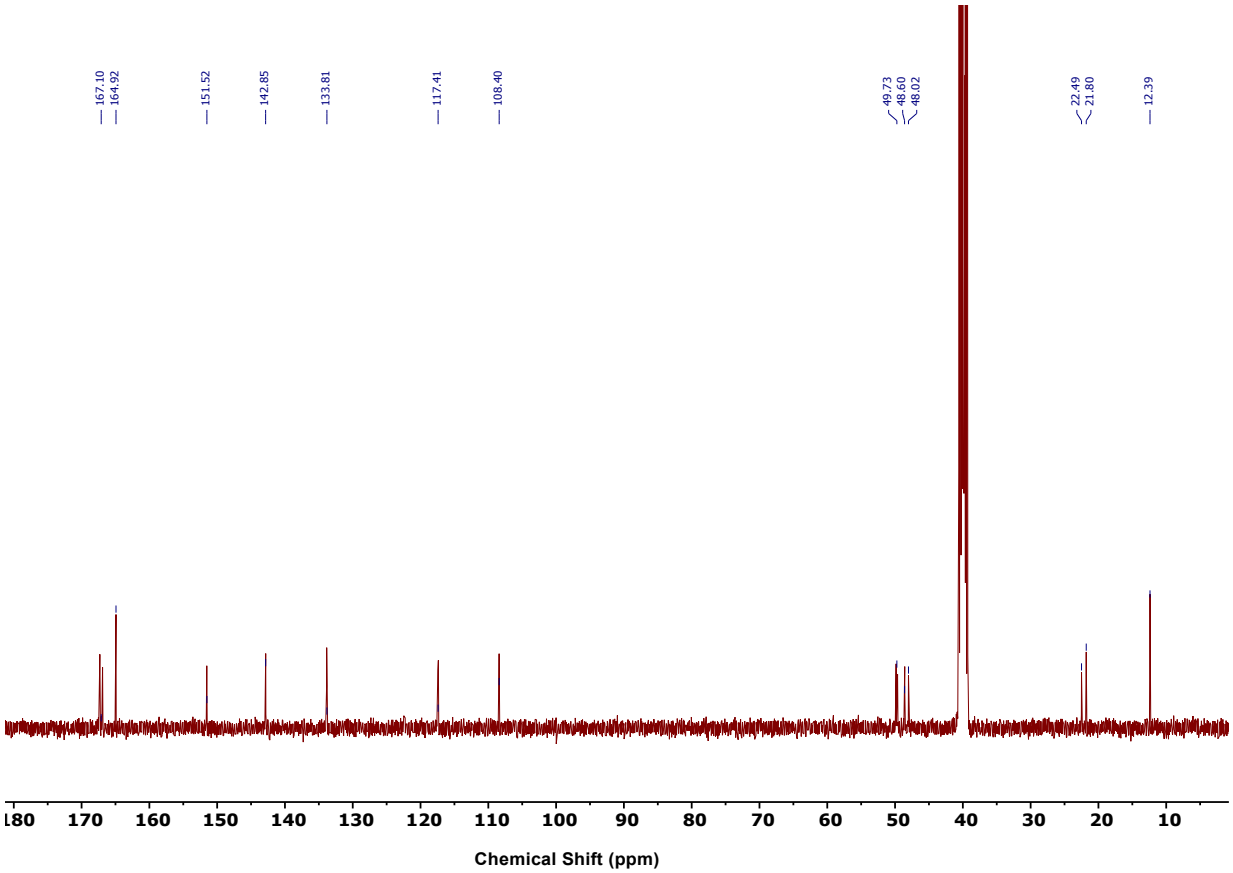


Figure S61. ^{13}C NMR (101 MHz, DMSO) of the Thiol-ene thymine (TE-T) monomer (22).