ChemComm

Oxidation of 8-Thioguanosine Gives Redox-Responsive Hydrogels and Reveals Intermediates in a Desulfurization Pathway

Journal:	*ChemComm*
Manuscript ID	CC-COM-04-2020-002926.R1
Article Type:	Communication

Journal Name

COMMUNICATION

Oxidation of 8-Thioguanosine Gives Redox-Responsive Hydrogels and Reveals Intermediates in a Desulfurization Pathway

Songjun Xiao,* Wes Lee, Fu Chen, Peter Zavalij, Osvaldo Gutierrez* and Jeffery T. Davis*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

A disulfide made by oxidation of 8-thioguanosine is a supergelator. The hydrogels are redox-responsive, as they disassemble upon either reduction or oxidation of the S-S bond. We also identified this disulfide, and 2 other compounds, as intermediates in oxidative desulfurization of 8-thioG to guanosine.

From seabird guano, to GMP nucleotides, to G-quadruplex DNA, guanine's "G" is iconic. We focus here on 8-thioguanosine disulfide **1**, a new compound in the literature, but one that likely exists in Nature. We want to make 2 points in this paper. First, the supergelator disulfide **1** forms redox-responsive hydrogels at low mM concentrations and ambient temperature. Quantum mechanical calculations reveal that disulfide **1** favors a conformation that facilitates formation of a G-quartet mesh. Second, disulfide **1** is likely an intermediate on a natural "desulfurization" pathway that leads from the oxidative metabolite 8-thioG **2** to guanosine **3** (**Fig. 1**).

Supramolecular hydrogels are soft materials, made mostly of water, that typically respond to external stimuli.[1] These gels, held together by non-covalent interactions, are prepared from low-molecular weight gelators, either synthetic compounds or natural products like sugars, peptides and nucleosides.[2] Although hydrogels made from guanosine analogs have long been known,[3] we have witnessed a resurgence in the synthesis of "G"-gels because of their many biomedical, environmental and materials applications.[2,4-6] These "G" gels are often built up from the G-quartet,[7] a H-bonded macrocycle typically templated by cations. The planar G-quartets stack to form G-quadruplex nanofibers that stick to each other to create a matrix that traps water, giving a self-standing gel (**Fig. S1**).

One strategy to drive G-quartet assembly is to use conformationally restricted monomers substituted at the C8 position, which favor *syn* vs. *anti* conformers about the C-N glycosidic bond (**Fig. 2a**). Because the base's "sugar" edge is blocked in the *syn* conformation (**Fig. 2c**), H-bonded ribbon motifs that compete with the G-quartet are inhibited (**Fig. S2-S3**). Sessler found that templating cations were not needed to form G-quartets from a *syn* C8-aryl G.[8] In the realm of soft materials, 8-aryl G analogs form nanogels,[9] and 8-BrG, 8-OMeG and 8-NH_2G,[10,11,5a] all analogs that favor a *syn* conformation, assemble into G-quartet hydrogels. In designing hydrogelators we were drawn to 8-thioG disulfide **1** because: 1) its large C8-substituent should ensure a *syn* conformer; 2) its two G units should enable a cross-linked network, as do other G dimers;[12] and 3) it should be redox-active and respond to reductive or oxidative cleavage of S-S bonds. While redox-responsive hydrogels made from polymers are relatively well-known,[13] there are fewer examples of low molecular weight gelators that give redox-responsive hydrogels.[14] Recently, we used redox chemistry to write, read and erase images on G-quartet hydrogels.[15] Now, we describe another unique and redox-responsive "G" gelator, 8-thioG disulfide **1**.

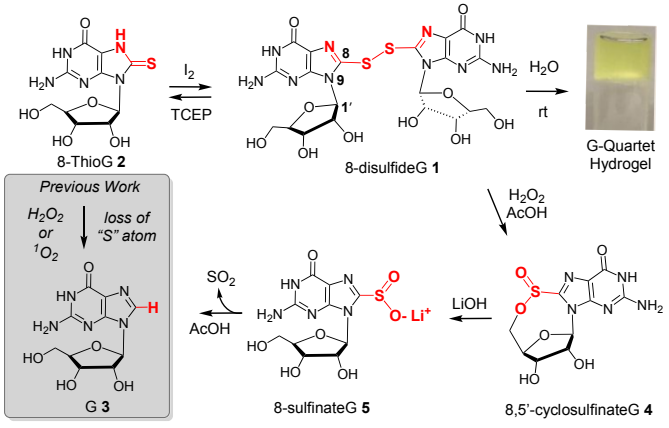

Fig. 1. This study focuses on the properties of 8-disulfideG **1** and its hydrogels.

The first part of this paper describes experimental and computational studies on this new dinucleoside **1** and its redox-responsive hydrogels. The second part of our story deals with further oxidation of 8-disulfideG **1**. Living organisms are

Department of Chemistry & Biochemistry, University of Maryland College Park, MD 20742, USA. E-mail: jdavis@umd.edu, sxiao@terpmail.umd.edu, ogs@umd.edu

Electronic Supplementary Information (ESI) available: DOI: 10.1039/x0xx00000x

constantly bombarded by reactive oxygen species (ROS) that damage DNA.[16] Guanine, the easiest nucleobase to oxidize,[17] reacts with ROS to give a variety of adducts. While 8-oxoguanine is the most well studied oxidation product, 8-thioG **2** is also of biological importance. Thus, Akaike and colleagues discovered that 3',5'-cyclic guanosine monophosphate (cGMP) is oxidized by peroxynitrite to 8-nitro-cGMP, which reacts with endogenous HS^- to form 8-thio-cGMP, a signalling molecule that protects against cardiac damage in mice. The 8-thio-cGMP is subsequently converted back to cGMP via chemical oxidation.[18] Chatgilialoglu also described photo-oxidation of 8-thioG **2** by singlet oxygen to give G **3** (**Fig. 1**).[19] Although C8-disulfides were mentioned as potential oxidation products in both processes, they were not identified and, until now, such species have been elusive. During our studies on hydrogelation we have characterized disulfide **1** as a key intermediate in the oxidative desulfurization of 8-thioG **2** to guanosine **3**.

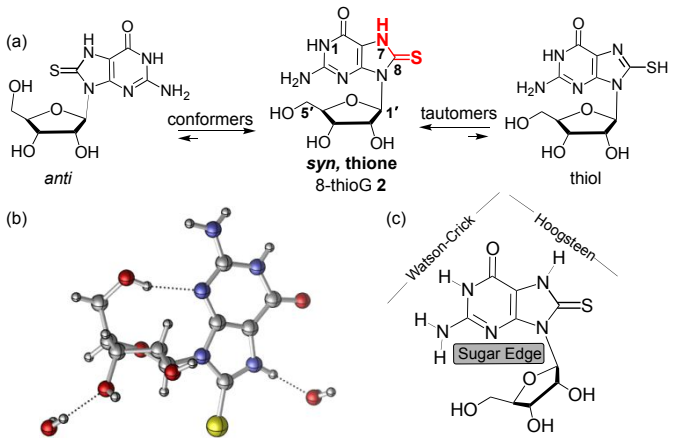

Fig. 2. (a) Conformational and tautomeric isomers for 8-thioG **2**; (b) crystal structure of 8-thioG **2**•2H_2O shows the N7H, C8 thione tautomer, with its C1'-N9 glycosidic bond in the *syn* conformation and a C2'-endo sugar; (c) different hydrogen bonding edges on 8-thioG **2**. The "sugar edge" is blocked when **2** is in a *syn* conformation.

Understanding the structure of 8-thioG **2** is important for unravelling its function in Nature. We used X-ray diffraction to determine that **2** exists as the thione tautomer, with a C=S bond of 1.69 Å (**Fig. 2**). Sulfur's size enforces a *syn* conformation about the C1'-N9 glycosidic bond and an *intra*molecular H-bond (d_{ON}= 2.90 Å) between the 5'-OH group and N3 stabilizes this conformation. The *syn* conformation blocks the "sugar edge" of **2** and orients the nucleobase so that its Watson-Crick and Hoogsteen edges are available for molecular recognition (**Fig. 2c**). Since N7 is protonated, 8-thioG **2** cannot form a G-quartet. We expected that disulfide **1**, with its imino N7 and C8 S-S bond, should form G-quartets, especially if the C1'-N9 conformation is *syn*.

Depending on the oxidant's strength, thioureas can be oxidized to disulfides and further to sulfenic (-SOH), sulfinic (-SO_2H) or sulfonic (-SO_3H) acids.[20] Although disulfide **1** was not known until this study, a similar disulfide had been made by oxidation of 6-thioG with the mild oxidant iodine.[21] Addition of I_2 to a suspension of 8-thioG **2** in MeOH/CH_3CN gave disulfide **1** in 88% yield. MALDI-TOF mass spectrometry showed 3 signals at m/z = 635.019 ($M + Li^+$), 650.925 ($M + Na^+$) and 666.974 ($M + K^+$), corresponding to adducts of **1** with alkali cations. Diagnostic changes occurred in NMR spectra after oxidation of **1**, especially for NH7, N7 and C8. The ^1H NMR spectrum of 8-thioG **2** in DMSO-d_6 has signals for 2 amide protons, N7H and N1H at δ 12.91 and 11.03 ppm. The N7H peak disappeared upon oxidation of 8-thioG **2** to disulfide **1**. Addition of reductant tris(2-carboxyethyl)phosphine (TCEP)[22] to a solution of disulfide **1** regenerated 8-thioG **2** (**Fig. S17**), a welcome finding in our quest for a redox-responsive gelator. Based on an established correlation,[23] the chemical shift of δ 4.99 ppm for H2' indicates the *syn* conformer of **1** dominates (≥ 95%). Solid-state ^{15}N NMR revealed a difference of >100 ppm for N7 in 8-thioG **2** (δ 142) and disulfide **1** (δ 260). Lastly, ^{13}C NMR showed a significant change in the C8 chemical shift (δ 165.7) upon oxidation of 8-thioG **2** to disulfide **1** (δ 139.4).

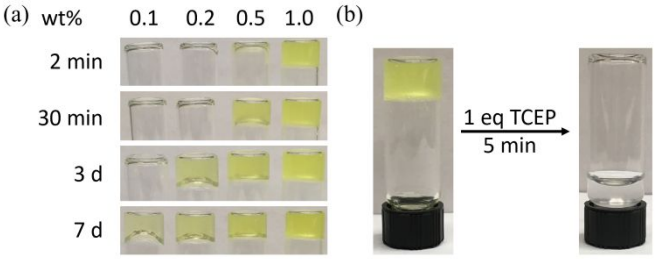

Fig. 3: (a) Inverted vials of 8-disulfideG **1** in water at various concentrations (wt %) and times after sonication. As shown in the lower left, hydrogelation by 8-disulfideG **1** at ambient temperature occurs at concentrations as low as 0.1 wt % (1.6 mM). (b) Addition of 1 eq of an aqueous solution of TCEP to a hydrogel made from 8-disulfideG **1** (0.5 wt%, 8 mM) destroys the gel within minutes, to give a suspension of 8-thioG **2**.

Disulfide **1** is a supergelator, as it forms hydrogels at concentrations ≤ 0.1% (w/v).[24] Sonication of 1.0 wt % of 8-disulfideG **1** (16 mM) in water produced self-supporting hydrogels within 2 minutes at ambient temperature (**Fig. 3a**). Transparent and self-standing hydrogels formed, albeit slowly, even at 0.1 wt% (1.6 mM). Importantly, this disulfide-based hydrogel is redox-responsive. Addition of 1 eq of reductant TCEP destroyed the yellow hydrogel (0.5 wt%, 8 mM) within 5 minutes at ambient temperature to give a colorless suspension of 8-thioG **2** (**Fig. 3b**). This rapid response to TCEP indicates that the gel network requires the covalent S-S bond and a H-bond pattern that allows for G-quartet assembly.[25]

Circular dichroism (CD) spectroscopy is used to probe supramolecular chirality of G-quadruplexes made from DNA and "small-molecule" G-analogs.[26] The CD spectra in **Fig. 4** show that the self-assembled hydrogel made from **1** contains both G-quadruplexes and *axially-chiral* disulfide bonds. The CD spectrum is drastically different when disulfide **1** is incorporated into the gel or when dissolved in DMSO. A solution of disulfide **1** in DMSO (8.0 mM) showed a weak Cotton band (yellow line), indicating little self-association in this solvent. The CD spectrum of the hydrogel (0.5 wt %, 8.0 mM) showed 2 key features that provide insight into the self-association of **1** in water. First, the strong exciton centered at 270 nm (peak at 258 nm and trough at 282 nm) is diagnostic of a chirality axis running down the middle of a stack of G-quartets.[26] Second, the large peak at 360

nm indicates axial chirality about an anisotropic S-S bond in this dinucleoside.[27]

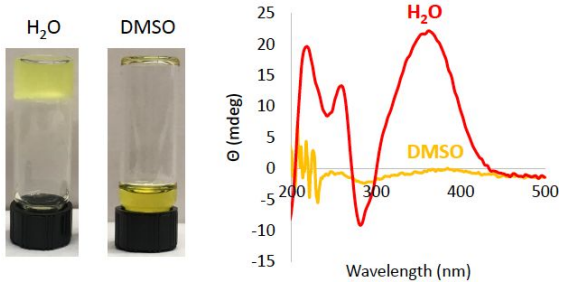

Fig. 4: Photographs of a hydrogel made from 8-disulfideG **1** (0.5 wt%, 8 mM) and a solution of 8-disulfide G **1** (8 mM) in DMSO. The red curve shows CD spectrum of hydrogel made from 8-disulfideG **1** (0.5 wt%, 8 mM) and the yellow curve shows a CD spectrum of a solution of **1** (8 mM) in DMSO.

To further characterize disulfide **1** and explore factors that stabilize specific conformers, especially about the S-S bond, we carried out computational analysis using dispersion-corrected density functional theory. We initiated our studies by performing an extensive conformational search on the C8-S-S-C8 dihedral angles from 0, 90, 180, to -90 degrees (**Fig. S34**). We performed optimizations using B3LYP-D3/6-31G(d)-CPCM(water)[28,29] and carried out single point energy calculations using a larger basis set B3LYP-D3/Def2SVP-CPCM(water)[30] to refine energetics. We found the lowest energy conformer for disulfide **1** to be nearly eclipsed (θ for C8-S-S-C8; **Fig. 5A**). All other conformers were > 7 kcal higher in energy (**Fig. S35**). This eclipsed conformation is unusual for a disulfide, as the C-S-S-C torsion is typically near ± 90°.[31] We reasoned that the nucleobase *and* sugar in disulfide **1** likely influence conformational stability so as to overcome the electronic destabilization that occurs in an eclipsed S-S bond. Inspection of the lowest-energy conformation for **1** revealed that the nucleobases (G^A and G^B in **Fig. 5**) stack one on the other (d =3.5 Å between centroids), in an "anti-parallel" orientation. Along with stabilization via dispersion forces, this "anti-parallel" π-stacking seems favoured by electrostatic interactions between the 6-membered rings, as atoms with positive atomic polar tensor (APT) charges in one ring stack directly over atoms with negative APT charges in the other 6-membered ring (**Fig. S38**). Both G^A and G^B adopt *syn* conformations about their C1'-N9 glycosidic bonds that are stabilized by 5'-OH···N3 H-bonds (d_{HN}= 1.80 Å). Moreover, a G^A-G^B H-bond (d_{HN}= 1.82 Å) between the 2'-OH in one G subunit and N7 in the other subunit, and 1,4 CH-S interactions between H1' and S8 , help rigidify the low-energy structure.[32]

To gain more insight into the role of ribose and the 6-membered ring in stabilizing the "π-stacked" conformation, we conducted calculations on simpler model compounds, including N9-methylguanine disulfide (**MGD**) and N-methylimidazole disulfide (**MTID**) (**Figs. S34-S38**). For **MGD,** we found that the low energy conformation adopted a C-S-S-C torsion of θ=-17.4°, with similar π-π stacking as disulfide **1** (**Fig. 5A**; middle). The importance of π-π stacking interactions in these guanine systems (**1** and **MGD**) is evident from calculations showing that **MTID**, which lacks the purine 6-membered ring and sugar, has a S-S torsion of θ= -64.3, much more typical for a disulfide bond (**Fig. 5A**; right).

The consequence of all these non-covalent forces is that the G^A and G^B units in the low-energy conformation of **1** form a "C-shaped" structure whose exposed H-bonding edges are oriented anti-parallel orientation relative to each other (**Fig. 5B**). As depicted in **Fig. 5C** we hypothesize that this unique π-stacked conformation enables self-assembly to give a dense matrix of cross-linked G-quartets that can trap water to give this redox-sensitive G_4-hydrogel.

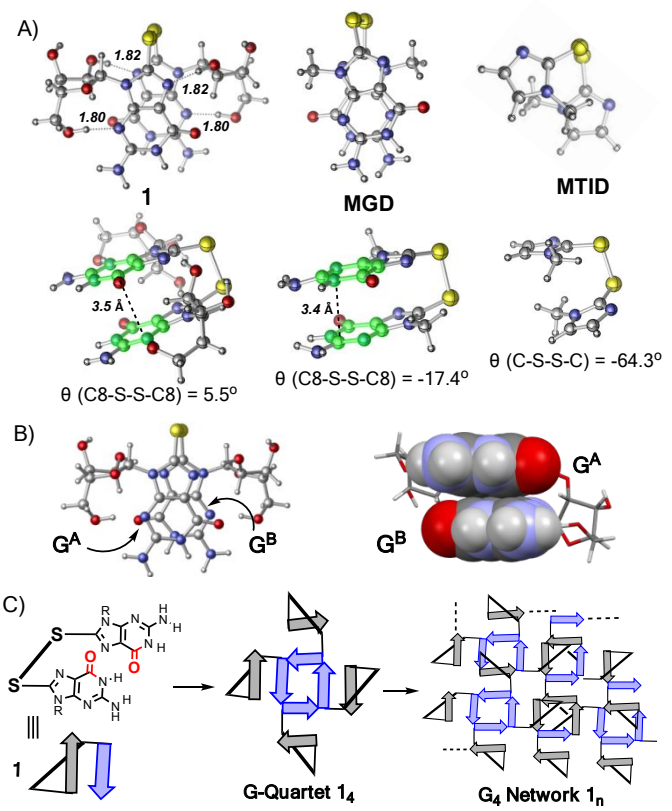

Fig. 5: A) Lowest energy conformations for disulfides **1**, N-methylguanine disulfide (**MGD**) and N-methyl-imidazole disulfide (**MTID**). B) Top view and side view of lowest energy conformation for 8-disulfide G **1**. The purine rings stack directly one over the other in an anti-parallel orientation. C) A cartoon showing how self-assembly of this C-shaped "anti-parallel" conformer would lead to a 3D mesh made up of G-quartets.

Since 8-thioG **2** can be converted to G **3** in Nature,[16,17] we sought more information about that oxidative pathway**.** (**Fig. 1**). We also reasoned that further oxidation to break S-S bonds would disassemble hydrogels made from disulfide **1**. Treatment of **1** with 7 eq of H_2O_2 and acetic acid gave 8,5'-cyclosulfinateG **4** as the only isolated product, with m/z = 330.07 ($M + H^+$). The ^{1}H NMR spectrum revealed features indicating cyclization: 1) signals for diastereotopic H5', H5'' are shifted downfield to δ= 4.74 & 4.40 ppm (δ=3.66 & 3.54 ppm in **1**), consistent with an electron withdrawing substituent at C5'; 2) H5' and H5'' resonances are 0.34 ppm apart, as these atoms are constrained in unique environments by the 8,5'-ring; 3) the 5'-OH signal disappears upon oxidation of **2**. Presumably cyclic nucleoside **4** arises by intramolecular attack of the 5'-oxygen on a protonated C8 sulfinic acid intermediate.

We found that cyclosulfinate **4** could be hydrolysed by base to give 8-sulfinateG **5**. Desulfurization of **5**, with loss of SO_2 to give G **3,** was slow at rt(14 d). Clean formation of G **3** from 8-sulfinateG **5** was accelerated, either by heating to 50 °C or by adding CH_3COOH at rt (**Fig. S32**). This study shows that disulfide **1**, 8,5′-cyclosulfinateG **4** and 8-sulfinateG **5**, new compounds to the literature, are intermediates in oxidation of 8-thioG **1** to G **3**. Importantly, hydrogels made from disulfide **1** respond to oxidation. Thus, addition of disulfide **1** (8 mM, 0.5 wt %) to a solution containing H_2O_2 (112 mM) and acetic acid (51 mM) gave a hydrogel that contains the oxidant that causes its own destruction. This gel disassembled over the course of 10 days at rt, whereas a control without oxidant remained self-standing (**Fig. S33**). So, hydrogels made from disulfide **1** can be dismantled by *either* reduction or oxidation of S-S bonds. This suggests that a biocompatible gel made from disulfide **1** might well protect cells against cytotoxic ROS species such as H_2O_2.[33]

Oxidation of 8-thioG **2**, a bioactive signalling molecule,[16] with I_2 provided 8-disulfideG **1**, a compound we suggest is likely naturally-occurring compound. Disulfide **1** is a supergelator as it forms hydrogels at concentrations as low as 0.1 wt % at rt. This propensity to gel is likely due to the 2 guanosines linked together, since reductive cleavage of the S-S bond destroyed the gel. We hypothesize, based on computations, that disulfide **1** adopts a C-shaped conformation that orients the guanosines in an anti-parallel stacked conformation, which drives formation of a G-quartet network that entrains water. Using stronger oxidation conditions, with excess H_2O_2, we demonstrated that both 8-thioG **2** and its disulfide **1** could be converted to G **3** via oxidative desulfurization and we identified C8-sulfinates that may be metabolic intermediates in this biologically relevant pathway. These hydrogels made from disulfide **1** are also responsive to oxidation by ROS species. Currently, we are trying to control disassembly, under reducing and oxidizing conditions, for release of molecular cargo from this "G" gel.

Acknowledgements

We thank the DOE (DE-FG02-98ER14888 to JD) and NSF (CAREER, 1751568 to OG). We thank the NSF (NSF-1726058) for funding a solid-state NMR spectrometer. We thank the UMaryland computational resources (DeepThought2 and MARCC/BlueCrab HPC clusters) and XSEDE (CHE160082 and CHE160053). We thank Taylor Plank and Gretchen Peters for helpful comments.

Notes and references

1 (a) C. D. Jones and J. W. Steed, *Chem. Soc. Rev.* 2016, **45**, 6546. (b) E. R. Draper and D. J. Adams *Chem*, 2017, **3**, 390. (c) P. R. A. Chivers and D. K. Smith *Nat. Rev. Mater.* 2019, **4**, 463.

2 (a) X. Du, J. Zhou, J. Shi and B. Xu, *Chem. Rev.*, 2015, **115**, 13165. (b) G. M. Peters and J. T. Davis, *Chem. Soc. Rev*. 2016, **45**, 3188. (c) T. Bhattacharyya, P. Saha, J. Dash, *ACS Omega*, 2018, 3, 2230.

3 I. Bang, *Biochem. Z*. 1910, **26**, 293.

4 (a) V. Venkatesh et al. *J. Am. Chem. Soc*. 2017, **139**, 5656. (b) A. Rotaru, A. , G. Pricope, T. N. Plank, L. Clima, L. Ursu, D. Peptanariu, J. T. Davis and M. Barboiu *Chem. Commun*. 2017, **53**, 12668.

5 (a) T. N. Plank, L. P. Skala and J. T. Davis, *Chem. Commun*. 2017, **53**, 6235. (b) S. Xiao and J. T. Davis, *Chem. Commun*. 2018, **54**, 11300.

6 (a) Z. Li, L. E. Buerkle, M. R. Orseno, K. A. Streletzky, S. Seifert, A. M. Jamieson and S. J. Rowan, *Langmuir*, 2010, **26**, 10093. (b) R. Q. Zhong et al. *Adv. Mater*. 2018, **30**, 1706887. (c) F. Carducci, J. S. Yoneda, R. Itri and P. Mariani, *Soft. Matter*, 2018, **14**, 2938-2948.

7 L. Stefan and D. Monchaud *Nature Rev. Chem*. 2019, **3,** 650.

8 J. L. Sessler, M. Sathiosatham, K. Doerr, V. Lynch, K. A. Abboud, A G-*Angew. Chem. Int. Ed*. 2000,**112**, 1356

9 J. E. Betancourt and J. M. Rivera *Langmuir* 2015, **31**, 2095.

10 R. N. Das, Y. P. Kumar, S. Pagoti, A. J. Patil and J. Dash, *Chem. – Eur. J*., 2012, **18**, 6008.

11 L. E. Buerkle, H. A. von Recum, S. J. Rowan, *Chem. Sci*. 2012, **3**, 564.

12 (a) C. Arnal-Herault, A. Banu, M. Michau, D. Cot, E. Petit, and M. Barboiu, Angew. Chem. Int. Ed. 2007, 46, 8409. (b) S. Pieraccini, M. Campitiello, F. Carducci, J. T. Davis, P. Mariani and S. Masiero, *Org.& Biomol. Chem*, 2019, **17**, 2759. (c) Y. He et al. Chem. Sci. 2019,**10**, 4192.

13 X. Sui, X. Feng, M. A. Hempenius and G. J. Vancso, *J. Mater. Chem. B*, 2013, **1**, 1658.

14 (a) M. Ikeda,T. Tanida ,T. Yoshii and I. Hamachi, Adv. Mater. 2011, 23, 2819. (b) J. P. Wojciechowski, A. D. Martin and P. Thordarson *J. Am. Chem. Soc.* 2018, **140**, 2869. (c) D. Navarro-Barreda, C. A. Angulo-Pachón, B. Bedrina, F. Galindo and J. F. Miravet, *Macromol. Chem. Phys*. 2020, **221**, 1900419.

15 S. Xiao, P. Paukstelis, R. D. Ash, P. Y. Zavalij and J. T. Davis, *Angew. Chem. Int. Ed. 2019*, **58**, 18434.

16 Burrows, C. J.; Muller, J. G. *Chem. Rev*. 1998, **98**, 1109.

17 J. Cadet, T. Douki, D. Gasparutto, J. L. Ravanat, *Mol. Mech. Mutagen*. 2003, **531**, 5.

18 M. Nishida et al. *Nat. Chem. Biol*. 2012, **8,** 714.

19 K. Taras-Goslinska, F. Vetica, S. Barata-Vallejo, V. Triantakostanti, B. Marciniak and C. Chatgilialoglu, C. *Molecules* 2019, **21**, 3143.

20 S. Sahua, P. Sahooa, S. Patelb and B.K. Mishraa, *J. Sulfur Chem*. 2011, **32**, 171

21 I. L. Doerr, I. Wempen, D. A. Clarke, and J. J. Fox *J. Org. Chem*. 1961, **26**, 3401-3409

22 J. A. Burns, J. C. Butler, J. Moran, and G. M. Whitesides *J. Org. Chem*. 1991, **56**, 2648-2650.

23 R. Stolarski, L. Dudycz and D. Shugar, *Eur. J. Biochem*. 1980, **108**, 111.

24 (a) J. Liu, P. He, J. Yan, X. Fang, J. Peng, K. Liu and Y. Fang, *Adv. Mater*. 2008, **20**, 2508. (b) K. Murata et al *J. Am. Chem. Soc*. 1994, **116**, 6664. (c) F. M. Menger and K. L. Caran, J. Am. Chem. Soc. 2000, 122, 47, 11679.

25 G-quartets are typically templated by alkali cations, although there are examples of "empty" G-quartets. Here, the hydrogel formed by disulfide **1** does so without addition of metal salts. In fact, addition of salts caused precipitation of **1** from solution without any gel formation (**Fig. S30**). An alternative motif to the G-quartet that might drive gelation, one that we haven't ruled out, is the H-bonded ribbon I shown in Fig. S2.

26 S. Masiero, R. Trotta, S. Pieraccini, S. De Tito, R. Perone, A. Randazzo and G. P. Spada, *Org. Biomol. Chem*. 2010, **8**, 2683.

27 Uridine disulfides also show such optical activity: (a) C. Szantay, M. P. Kotick, E. Shelter, T. J. Bardos, *J. Am. Chem. Soc.* 1967, **89**, 713. (b) S. Irie, Y. Inoue and F. Egami, *J. Biochem*. 1968, **63**, 274.

28 A. D. Becke, *J. Chem. Phys*. 1993, **98**, 5648.

29 A. V, Marenich, C. J. Cramer, D. G. Truhlar, D. G. *J. Phys. Chem. B.* 2009, **113**, 6378.

30 F. Weigend, F. and R. Ahlrichs, *Phys. Chem. Chem. Phys*. 2005, 7, 3297.

31 R. Steudel *Angew Chem Int Ed.* 1975, **14**, 655-664.

32 H. E. van Wart and H. A. Scheraga *Proc. Natl. Acad. Sci. USA*, 1977, **74**,13.

33 B. R. Dollinger, M. K. Gupta, J. R. Martin and C. L. Duvall, *Tissue Engin. Part A*. 2017, **23**, 1120.