TAILORING THE PROPERTIES OF SUPRAMOLECULAR GELS

by

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Figure 4.6. TEM micrographs of (a) 4:1 C₈-DGlu:L₄, (b) 1:1 C₈-DGlu:L₄, and (c) 3:7 C₈-DGlu:L₄. Helical ribbons of approximately 10-20 nm in diameter are observed in all samples in addition to micellar structures that increase in size with increasing L₄ content. The insets are portions of the same images zoomed to show characteristic approximately 10-15 nm wide helical assembly of C₈-DGlu even in the presence of surfactant.

Figure 4.7. 1-D WAXD of a freeze-dried 1 wt % C₈-DGlu gel, C₈-DGlu crystals, and a freeze-dried 1:1 (by weight) C₈-DGlu:L₄ gel (2 wt % total). There exist distinguishable differences between the patterns of the gel and crystalline state. Addition of the surfactant lowers the intensity of the peaks but does not induce any peak shifts. The peaks at approximately 2 degrees (d=3.4-4.0 nm) correlate well with the known bilayer thickness of C₈-DGlu in both gel and crystal form. The largest peak in the crystal form (approximately 5 degrees), however, corresponds to the monolayer length in the crystal (approximately 1.6 nm).

Figure 4.8. Stress sweeps of C₈-DGlu/L₄ gels, where the concentration of C₈-DGlu is fixed at 1.6 wt %, and the L₄ content is adjusted by molar ratio. T = 25 °C and ω = 6.28 rad/s. The modulus and yield stress both decrease with increasing L₄ concentration.
Figure 4.9. DIC optical micrographs of C$_8$-DGlue/L4 gels. (a) 1 wt % 1:0.5 C$_8$-DGlue:L4. (b) 1.6 wt % 1:0.5 C$_8$-DGlue:L4. (c) 1 wt % 1:1 C$_8$-DGlue:L4. (d) 1.6 wt % 1:1 C$_8$-DGlue:L4. The ability of the spherulitic assemblies to percolate and interpenetrate increases with both C$_8$-DGlue and L4 content, suggesting that the L4 may interact with the fibrous assembly of the gel rather than sitting exclusively in micelles in the sol phase.

Figure 4.10. (a) Gels made from 1:1 ratios of C$_8$-DGlue:L4 at various pH values and in salt solutions. The pH was adjusted with citric acid and sodium hydroxide. (b) Stress sweeps of 1:1 C$_8$-DGlue:L4 at various pH values. (c) Stress sweeps of 1:1 C$_8$-DGlue:L4 in aqueous salt solutions. Rheological parameters include $T = 25 \, ^\circ\text{C}$ and $\omega = 6.28 \, \text{rad/s}$.

Figure 4.11. (a) Gels made from 1:1 molar ratios of C$_8$-DGlue:Laureth surfactant, where the C$_8$-DGlue concentration is set at 1 wt %. Left to right: C$_8$-DGlue/L4, C$_8$-DGlue/L7, C$_8$-DGlue/L12, C$_8$-DGlue/L30. (b) Stress sweeps of 1:1 molar ratio C$_8$-DGlue:L$_n$ gels. Rheological parameters include $T = 25 \, ^\circ\text{C}$ and $\omega = 6.28 \, \text{rad/s}$.

Figure 4.12. DIC optical microscopy images of 1:1 (by molar ratio) (a) C$_8$-DGlue:L4, (b) C$_8$-DGlue:L7, and (c) C$_8$-DGlue:L12, and (d) C$_8$-DGlue:L30.

Figure 4.13. TEM images of 1:1 (by molar ratio) (a) C$_8$-DGlue:L4, (b) C$_8$-DGlue:L7, and (c) C$_8$-DGlue:L12, and (d) C$_8$-DGlue:L30.

Figure 4.14. Chemical structures of surfactants (a) Ceteth-20 and (b) Ninol-L5 as well as (c) images of 1:1 weight ratio gels, and (d) a rheological comparison of 1:1 weight ratio gels of C$_8$-DGlue mixed with L4, Ceteth-20, and Ninol-L5.

Figure 4.15. Chemical structures of (a) N-alkyl-D-gluconamides (C$_n$-DGlue, 4.1-4.4), (b) N-alkyl-L-gulonamides (C$_n$-LGul, 4.5-4.8), and (c) N-alkyl-D-ribonamides (C$_n$-DRib, 4.9-4.12), where $n = 4$, 6, 8, or 10. Gels of limited lifetime were formed from a small handful of these molecules and combinations of molecules as outlined in table 4.1.

Figure 4.16. Chemical structure of N-(2-ethylhexyl)-D-gluconamide (2-EHA-DGlue, 4.13). 4.13 was soluble in water to high concentrations. No gelation was observed.
Figure 4.17. (a) Chemical structure of \(N,N'\)-dodecyl-bis-D-gluconamide (DGlu-C\(_{12}\)-DGluc 4.14) and \(N,N'\)-octadecyl-bis-D-gluconamide (DGlu-C\(_{12}\)-DGluc 4.15). Both molecules were insoluble in water. (b) 1,18-diaminododecane (4.21) for the synthesis of 4.15 was synthesized from eicosanedioic acid in 43% yield.

Figure 4.18. (a) Chemical structure of \(N,N'\)-(methylenebis(4,1-phenylene))bis-D-gluconamide (DGlu-MDA-DGluc 4.16). (b) Chemical structure of \(N,N'\)-(1,4-phenylenebis(methylene))bis-D-gluconamide (DGlu-pXD-DGluc 4.17). Both molecules crystallized upon cooling in water. No gelation was observed.

Figure 4.19. Chemical structures of (a) \(N,N'\)-(1,3-phenylenebis(methylene))bis-D-gluconamide (DGlu-mXD-DGluc 4.18), (b) \(N,N'\)-(2-methylpentane-1,5-diyl)bis-D-gluconamide (4.19), and (c) \(N,N'\)-((1R,2R)-cyclohexane-1,2-diyl)bis-D-gluconamide (4.20). All molecules were soluble in water to high concentrations. No gelation was observed.

Figure 4.20. \(N\)-octyl-D-gluconamide (C\(_{8}\)-DGlu 4.1). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.21. \(N\)-hexyl-D-gluconamide (C\(_{6}\)-DGlu 4.2). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.22. \(N\)-decyl-D-gluconamide (C\(_{10}\)-DGlu 4.3). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.23. \(N\)-dodecyl-D-gluconamide (C\(_{12}\)-DGlu 4.4). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.24. \(N\)-hexyl-L-gulonamide (C\(_{6}\)-LGul 4.5). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.25. \(N\)-octyl-L-gulonamide (C\(_{8}\)-LGul 4.6). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.26. \(N\)-decyl-L-gulonamide (C\(_{10}\)-LGul 4.7). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.27. \(N\)-dodecyl-L-gulonamide (C\(_{12}\)-LGul 4.8). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).

Figure 4.28. \(N\)-hexyl-D-ribonamide (C\(_{6}\)-DRib 4.9). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)).
Figure 4.29. *N*-octyl-D-ribonamide (*C*₈-DRib, 4.10). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.30. *N*-decyl-D-ribonamide (*C*₁₀-DRib, 4.11). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.31. *N*-dodecyl-D-ribonamide (*C*₁₂-DRib, 4.12). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.32. *N*-(2-ethylhexyl)-D-gluconamide (2-EHA-DGlu, 4.13). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.33. *N,N*'-dodecyl-bis-D-gluconamide (DGlu-C₁₂-DGlu, 4.14). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.34. *N,N*'-octadecyl-bis-D-gluconamide (DGlu-C₁₈-DGlu, 4.15). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.35. *N,N*'-methylenebis(4,1-phenylene)bis-D-gluconamide (DGlu-MDA-DGlu, 4.16). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.36. *N,N*'-1,4-phenylenebis(methylene)bis-D-gluconamide (DGlu-pXD-DGlu, 4.17). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.37. *N,N*'-1,3-phenylenebis(methylene)bis-D-gluconamide (DGlu-mXD-DGlu, 4.18). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.38. *N,N*'-2-methylpentane-1,5-diyl)bis-D-gluconamide (4.19). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.39. *N,N*'-((1R,2R)-cyclohexane-1,2-diyl)bis-D-gluconamide (4.20). $^{1}$H NMR (600 MHz, DMSO-d₆).

Figure 4.40. 1,18-diaminooctadecane (4.21). $^{1}$H NMR (600 MHz, DMSO-d₆).
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First and foremost, I would like to thank Dr. Stuart Rowan for his gracious employment and endless advice over the past almost seven (geezer, has it really been that long?) years. I’m not sure where I’d be or what I’d even be doing today if it weren’t for him allowing me to work in his lab initially as an undergraduate and then as a graduate student.

I’d also like to thank my parents for supporting me through so many years of education. Comparing this dissertation to my very first science project in 2nd grade makes me realize just how much they’ve given me, and for that, I am truly grateful. This also includes all those years of dance classes, choir, musicals, piano lessons, etc. that have given me the confidence to never be intimidated, which has undoubtedly helped my career immensely!

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Tailoring the Properties of Supramolecular Gels

Abstract

by

LAUREN BUERKLE

Supramolecular gels created from low molecular weight species (gelators) have gathered wide attention over the past few decades on account of their highly ordered assembly and ability to respond to external stimuli. These properties make such gels highly promising candidates for a diverse range of applications including biomaterials, viscosity modifiers, sensors, and liquid crystalline materials.

We have focused on the design and tailoring of guanosine (the ribonucleoside of the nucleobase guanine) hydrogels. It is well known that in an aqueous environment, guanosine forms circular hydrogen-bonded quartets around a monovalent metal ion, most commonly potassium. These quartets then stack to form high-aspect ratio fibers that entangle and branch to form gels.

Despite facile gel formation, crystallization of the guanosine molecules out of the gel is a common occurrence that leads to gel collapse within hours of fabrication. In addition, guanosine and related gelators often require a high potassium concentration or acidic pH to gel, which presents limited practical use in our target application of tissue engineering. We have focused on the modification and analysis of guanosine gels via an additive and/or a change in chemical structure to inhibit crystallization and promote gelation at physiological salt concentrations. Additionally, initial cell culture
experiments suggest that these gel materials show great potential as an easily accessible and inexpensive tissue engineering scaffold.

We also examined the potential for supramolecular gels for use in personal care formulations as electrolyte-resistant rheology modifiers for aqueous systems. Sugar-based gels fit the necessary criteria; however, many of these molecules also crystallize from the gel over time. We achieved lifetime stabilization again via a mixing approach and examined the resulting properties of the stabilized gels.
Chapter 1

Introduction
1.1 Introduction

Supramolecular gels\(^1\) created from low molecular weight species (gelators) have gathered wide attention over the past few decades on account of their highly ordered assembly and ability to respond to external stimuli. The gel characteristics can be controlled or “tailored” to suit a given application by making modifications to the gelling system as a whole (via changes in gelator concentration, gelation medium, etc.) or by modifying the chemistry of the low molecular weight gelators themselves. These properties make such gels highly promising candidates for a diverse range of applications such as biomaterials,\(^2\) stimuli-responsive materials,\(^3\) sensors,\(^4\) liquid crystalline materials,\(^5\) and personal care formulations.\(^6\)

Supramolecular gels are fabricated from a solution of small molecules that, under the proper conditions, can form a gel. Generally, the solution is either cooled or exposed to a poor solvator of the small molecule to promote aggregation. At this stage, the molecules either crystallize out preventing gelation or aggregate further. Since a nano/microscale fibrous assembly is responsible for gelation in most (but not all) systems, the aggregates then begin to bifurcate and entangle (and sometimes crosslink depending on the system) until a fibrous gel structure results (Figure 1.1).
1.2 Preventing Crystallization in Supramolecular Gels

Despite the many discovered applications of supramolecular gels, there remains, however, a drawback to many of these gelators, namely their tendency to crystallize over time, which results in the collapse of the gel therefore limiting its lifetime stability. Crystal formation from a supramolecular gel is based on the fact that the gel is a metastable, kinetically trapped state with gelator molecules contained both in the gel and sol components. Over time, free gelator molecules in the sol state can aggregate to form nucleation points effectively decreasing the concentration of available gelator such that more gelator is released into the sol state and subsequently incorporated into the crystals (Figure 1.2a). Alternatively, a contraction of the solid state can occur to cause crystallization. This is suggested to be a result of surface tension-driven fibril aggregation via a sliding movement, which results in the loss of the gel network (Figure 1.2b). Such processes decrease the lifetime of the gel and therefore its usefulness. Thus,
it is of great interest to develop platforms for preventing the crystallization of small molecule supramolecular gelators.

To this end, original work in designing long-lived supramolecular gels revolved around the design of the gelator itself. Three criterions to achieve supramolecular gelation became apparent in the process – 1. The presence of strong self-complimentary and unidirectional interactions to enforce one-dimensional self-assembly, 2. Control over the fiber-solvent interfacial energy to control solubility and to prevent crystallization, and 3. Some factor to introduce fiber crosslinking for network formation. While simple in theory, these parameters, especially 2 and 3, are sometimes difficult to fulfill within a single small molecule.

Figure 1.2. (a) Illustration of the conversion of sol state gelator to crystallites over time. (b) Illustration of the conversion of a solid gel network to crystallites.
There is a reasonably sized body of work that has furthered criterion 3, where branching is induced via a polymeric additive.\textsuperscript{9,11} Chen, et al. show that the addition of a small amount (as little as 0.004\%) of EVACP (ethylene/vinyl acetate copolymer) into L-DHL (lanosta-8,24-dien-3\-ol:24,25-dihydrolanosterol = 56:44) (Figure 1.3b, (1.1)) in di-(2-ethylhexyl)phthalate results in stable gelation rather than crystallization. The stabilization arises from the fact that EVACP can adsorb onto the surface of certain organic crystals, which disrupts the structural match between new layers and the previously existing crystal surface causing a defect. This defect changes the trajectory and/or size of new growth, which yields a stable, branched system (Figure 1.3a). SEM images show that the resulting self-supporting gel is very different in appearance (Figure 1.3d) from its crystalline precursor (Figure 1.3d). X. Wan, et al. also showed this stabilization effect by mixing PHEMA (poly(2-hydroxyethyl methacrylate) into the usually unstable sugar-based gelator, 4-(4’-ethoxyphenyl)phenyl-\(\beta\)-O-D-glucoside.

The work by Wan, et al. demonstrated the importance of criterion 2, where it was necessary to control the fiber-solvent interfacial energy to increase the lifetime of a gel. It was found that changing the solvent environment, in this case, changing the ratio of water to 1,4-dioxane, allowed tuning of the kinetics of the gel-to-crystal transition that resulted from the solid-state rearrangement shown in Figure 1.2b.
Less explored is the prevention of crystallization with a small molecule additive, and as such, this concept will be addressed in detail in Chapters 2 and 4 of this thesis. One existing literature example, however, involves the mixing of two small molecules that independently form crystallites but gel when mixed. Wang, et al. found that mixing...

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**Figure 1.3.** (a) Illustration of branching caused by crystallographic mismatch from adsorption of an additive on a growing crystal. (b) Structures in L-DHL mixture (1.1). (b) Needle crystals formed from 10 wt % L-DHL/DIOP (Inset – bulk crystal suspension). (c) Branched network formed by adding just 0.004 wt % EVACP to 10 wt % L-DHL/DIOP (Inset – bulk self-supporting gel). Scale bar = 1 µm.\textsuperscript{11}
(1.2) and (1.3) (Figure 1.4a) in water produced stable hydrogels with (1.2):(1.3) ratios of 7:1 to 1:3. In the case of a higher (1.2) content (e.g. 2:1 (1.2):(1.3)), gelation proceeded via the growth of fibrillar assemblies (Figure 1.4b) similar in appearance to (2) crystals. Conversely, changing the ratio of (1.2):(1.3) to 1:2 results in a gelation process started by particular aggregates (Figure 1.4c) similar in appearance to crystals of (1.3). Thus, not only has more stable gelation been achieved, but the gel architecture can be modified by changing the ratio of the two components.

**Figure 1.4.** (a) Structures of compounds (1.2) and (1.3) that crystallize in water separately but form a gel when mixed. (b) When the ratio of (1.2):(1.3) is set at 2:1, a fibrous assembly is seen for the gel, akin to the appearance of (1.2) crystals. (c) When the ratio of (1.2):(1.3) is set at 1:2, a particulate-like assembly is seen for the gel, akin to the appearance of (1.3) crystals.
1.3 Multi-Component Supramolecular Gels

The work by Wang, et al. highlighted in Figure 1.4 introduces to this thesis the concept of multi-component supramolecular gels. Generally speaking, multi-component supramolecular gels contain two or more types of small organic molecules (excluding solvents) within the gel programmed to interact with each other, most commonly via hydrogen bonding, donor-acceptor interactions, or metal-ligand interactions, in such a way as to induce gelation or otherwise impact gel properties. As is illustrated in Figure 1.5, there are three distinct categories within the genre – 1. More than one molecule must be present for a gel to form; 2. Two or more independent gelators can be mixed together to modulate properties by forming a co-gel or a self-sorting gel; and 3. One molecule is the gelator while the other molecule(s) are nongelating and is/are designed to alter the gel’s functional or thermomechanical properties. Whichever the circumstance, multi-component supramolecular gels introduce an additional level of tunability as a result of the tailorable interaction(s) between the components.
1.3.1 Multiple components required to make a gel

The most widely studied area of multi-component supramolecular gels involves the mixing of two or more components that do not gel independently but form gels when combined, most often via hydrogen bonding or donor-acceptor interactions. The first report of this kind was published by H. Shirai, et al. in 1993 and detailed the properties of a mixed system of hydrogen-bonding 5-hexadecyl-2,4,6-triaminopyrimidine and 5,5-didodecylbarbituric acid in organic liquids.\textsuperscript{14} In the decade following, several additional authors also made contributions to the initial literature on the subject.\textsuperscript{15}

The past 8 years have seen several publications by D.K. Smith and collaborators regarding hydrogen bonding two-component systems that contain dendritic peptides and diamine building blocks, which, when combined, yield gels that are tailorable in a number of ways.\textsuperscript{16} The authors first showed that by simply varying the ratio of dendron

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure_15.png}
\caption{Illustration of the three types of multi-component supramolecular gels.}
\end{figure}
(1.4) to the aliphatic diamine, diaminododecane, in toluene (Figure 1.6a), the nanoscale morphology underwent a change from a high-density network of fibers (2:1 dendron:C_{12}-diamine) to entwined and twisted fibers (1:1 dendron: C_{12}-diamine) to ropes (1:2.7 dendron: C_{12}-diamine) and finally to platelets (1:4.5 dendron:C_{12}-diamine) (Figure 1.6b). A change in the gel-sol transition temperature accompanied the morphological changes, where the 2:1 gel was stable up to 37 °C while the transition temperature of the 1:1 gel was dramatically increased to 62 °C. The amine content increase to 1:2.7 showed no thermal transition change; however, upon continuing to increase its content to the final ratio of 1:4.5, gelation was observed only below room temperature.\textsuperscript{16a,c} The authors suggest that initially, the increase in diamine concentration (and subsequent increase in the gel-sol transition temperature) leads to dendron-dendron hydrogen bonds that stabilize the fibrous assembly presumably by alleviating steric crowding effects between the dendritic head groups. The continued increase, however, most likely dilutes the dendron to an extent that it can no longer form a sample-spanning assembly and merely acts as a solubilization medium for the polar diamine in the apolar toluene environment.
The authors also show that the properties of this type of system can be varied by changing the diamine structure. At a concentration of dendron greater than or equal to 15 mM, incorporation of equimolar amounts of $C_{12}$, $C_{11}$, $C_{10}$, $C_9$, and $C_8$ diamines resulted in gels whose gel-sol transition temperatures are 105 °C, 92 °C, 72 °C, 55 °C, and 41 °C.

Figure 1.6. (a) Structure of the (1.4)-diaminododecane complex. (b) Varying the ratio of (1.4) to diamine in toluene resulted in a morphological change from fibers to platelets. A: 2:1 [Dendron] = 9 mM, [C12] = 4.5 mM. B: 1:1 [Dendron] = 9 mM, [C12] = 9 mM. C: 1:2.7 [Dendron] = 9 mM, [C12] = 24.3 mM. D: 1:4.5 [Dendron] = 9 mM, [C12] = 40.5 mM.$^{16a,c}$
respectively.

While the size of diamine can be important in controlling the thermal stability of the gel, the use of aromatic diamines (Figure 1.7a) highlights the importance of molecular geometry in the formation of gels. Mixing dendron (1.4) with either 1,4-diaminobenzene (1,4-BZ), 1,3-diaminobenzene (1,3-BZ), or 1,2-diaminobenzene (1,2-BZ) in toluene leads to very different behavior: 1,4-BZ formed gels, 1,3-BZ formed irreproducible partial gels, while 1,2-BZ did not form gels at all. Interestingly, mixing all four components together (1:1:1:1 mixture at 18 mM) forms a gel in toluene and, from NMR of the sol components, it was shown that the large majority of the diamine incorporated into the gel was 1,4-BZ derivative with much less of the 1,3-BZ and 1,2-BZ derivatives (Figure 1.7b).

![Figure 1.7](image)

**Figure 1.7.** (a) Structures of 1,4/1,3/1,2-diaminobenzene and dendron (1.4). (b) NMR analysis shows that the G2 dendron preferentially incorporates 1,4-diaminobenzene (1,4-BZ) over 1,3 and 1,2-diaminobenzene.\(^{16}\)
The use of the amine/carboxylic acid interaction to generate gels is not limited to dendritic carboxylic acids. S. Bhattacharya, et al. utilized water-insoluble fatty acids with water-soluble amines and found that stearic (C\textsubscript{18}H\textsubscript{37}COOH) and eicosanoic (C\textsubscript{20}H\textsubscript{41}COOH) acids gelled water when combined in approximately a 1:3.5 ratio with a number of linear primary diamines including ethanediamine (EDA), diethylenetriamine (DTA) and its methylated derivative (MDTA), tetraethylenepentamine (TEPA), propanediamine (PDA), 3,3’-iminobis(propylamine) (IBPA) and its methylated derivative (MIPBA), and N,N’-bis(3-aminopropyl)ethylenediamine (BPETA) (Figure 1.8a). Through FTIR, NMR, and crystallographic analysis of both the gel and single crystals, they suggest a lamellar arrangement of the acids where tight ion pairs of protonated amine and carboxylate anions hold the network together. Simultaneously, about half of the primary amines are thought to be unprotonated and form a continuous hydrophilic cavity where the water and excess amine associate to form a dense hydrogen-bonded network leading to the formation of the hydrogel (Figure 1.8b).\textsuperscript{17}
The same authors have also shown that mechanical tailorability can be achieved by mixing lithocholic acid with various amines (BPETA, IBPA, and MDTA), both by changing the ratio of acid to amine as well as by changing the amine itself. Figure 1.9 shows that decreasing the length of the amine chain decreases the moduli and yield stress of the gel.\textsuperscript{18}

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<thead>
<tr>
<th>Amines</th>
<th>Myristic acid</th>
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</table>

\textsuperscript{s} = sol; \textsuperscript{OG} = opaque gel; \textsuperscript{TG} = translucent gel

**Figure 1.8.** (a) Table showing the behavior of various acids and amines in water. (b) Proposed lamellar arrangement of acids and amines (stearic acid and EDA shown) in the gel state.\textsuperscript{17}
As previously mentioned, multi-component gels of this nature have also been created via donor-acceptor interactions.\textsuperscript{19} George, et al. showed that hydrogels could be made by combining a coronene tetracarboxylate tetrapotassium salt (1.5) and a dodecyl-functionalized methyl viologen derivative (1.6) (Figure 1.10a).\textsuperscript{19c} Figure 1.10b shows that (1.6) alone merely forms spherical assemblies in water; however, when (1.5) is added, nanofibers as well as macroscopic gel formation are observed (Figure 1.10c). It is proposed that the charge transfer complex assembles into cylindrical micelles, which continue to grow into nanofibers thus achieving gelation (Figure 1.10d).

\textbf{Figure 1.9.} Mixing various amines with lithocholic acid (LCA) in water results in a variance of the moduli and yield stresses.\textsuperscript{18}
1.3.2 Multiple gelators mixed to tailor properties

The second category of multi-component supramolecular gels involves mixing two or more gelators to tailor the properties of the resulting material. Within this category, the gels fall into two separate groups - co-gels and self-sorting gels. In the case

Figure 1.10. (a) Structures of the donor and acceptor molecules. (b) FE-SEM image of the spherical assemblies formed by (1.6) alone in water (1 x10^{-4} M). (c) AFM of fibers resulting from the combination of (1.5) and (1.6) in a 1:1 ratio. (d) Proposed assembly of the charge transfer complex into nanofibers.19c
of co-gels, two independent gelators combine on the nanoscale to yield a new material with different properties from the original gelators. There are several literature examples of co-gelation of two or more gelators, where the gels are comprised of peptides,\textsuperscript{20} dendrimers,\textsuperscript{21} urethanes,\textsuperscript{22} ureas,\textsuperscript{23} and sugar-based materials,\textsuperscript{24} all of which utilize hydrogen bonding to control the interactions between the gelators.

The peptide-based co-gel system of Žinić, et al. containing an equimolar mixture of (S,S)-bis(LeuOH) oxalamide (1.7) and (S,S)-bis(leucinol) oxalamide (1.8) (Figure 1.11a) was shown to gel up to 7 times larger a volume of p-xylene than either component alone (Figure 1.11b). The co-gel also exhibited greater thermal stability, where the gel-sol transition of a 1:1 mixture was shown to be nearly 20 °C higher than those of either component (Figure 1.11c). This was attributed to the fact that the fibers formed by the interaction of (1.7) and (1.8) bilayers are distereoisomeric, and as such, result in differing gel morphologies to yield the unique properties.\textsuperscript{20a}
(a) Structures of the two gelators \(S,S-1\) (1.7) and \(S,S-2\) (1.8). (b) Values for gelation efficiency expressed as mL of solvent gelled by 1 mg of gelator mixture. The co-gel is significantly more efficient at gelling most solvents than either component alone. (c) Mixtures of the two gelators exhibited significant increases in the gel-sol temperature values when compared to each individual gelator.\(^{20a}\)

Maitra, et al. also utilized chirality in their urethane-based system to yield differing results when transitioning from a single gelator to a co-gel. In their system, mixing the 10% of chiral gelator (1.9) with achiral gelator (1.10) in isooctane (Figure 1.12a) resulted in a change in morphology from intact to broken fibers (Figure 1.12b). This structural change is also reflected in the rheological data, where the 10% addition of
(1.9) to (1.10) in dodecane resulted in the decrease of the storage modulus by over an order of magnitude (Figure 1.12c). The authors argue that the disruptions in morphology and mechanical properties arise from the decrease in packing efficiency introduced by the heterogeneity of the components.

![Figure 1.12](image)

**Figure 1.12.** (a) Structures of the two gelators (1.9) and (1.10). (b) SEM images of xerogels of (1.10) (a) and (1.10) + 10% (1.9) (b) in isooctane. Addition of (1.9) disrupts the ordered fibers of (1.10). (c) The addition of 10% (1.9) to (1.10) also disrupts the mechanical properties of dodecane gels.  

<table>
<thead>
<tr>
<th>Dodecane Gels</th>
<th>G' (Pa)</th>
<th>G'' (Pa)</th>
<th>σ* (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1.10) 30 mM</td>
<td>4600</td>
<td>900</td>
<td>100</td>
</tr>
<tr>
<td>(1.10) (27 mM)/ (1.9) (3 mM)</td>
<td>290</td>
<td>20</td>
<td>25</td>
</tr>
</tbody>
</table>

Finally, F. Lequex, et al. examined mixtures of urea-based gelators, EHUT (1.11) and DMHUT (1.12) (Figure 1.13a) in toluene. IR analysis considers the ratio of 3328
peak (hydrogen-bonded –NH groups) to the 3300 peak (reference) which measures the
shape of the vibration band for hydrogen-bonded NH groups. The high value (ca. 1.25) is
attributed to a thick filament gel state, while the low value (ca. 1.1) is attributed to thinner
filaments in the sol state as elucidated by previous FTIR, rheology and SANS
experiments. Therefore, the transition corresponds to the gel-sol transition temperature,
which is systematically modified from 40 to 65 °C by simply changing the molar ratio of
the two components (Figure 1.13b).

![Figure 1.13. (a) Structures of the two gelators EHUT (1.11) and DMHUT (1.12). (b) IR analysis of the gels shows a tailorable gel-sol transition temperature by simply changing the ratio of the two components.](image)

Figure 1.13. (a) Structures of the two gelators EHUT (1.11) and DMHUT (1.12). (b) IR analysis of the gels shows a tailorable gel-sol transition temperature by simply changing the ratio of the two components. 23
Self-sorting gels are also seen in the literature, although not as frequently as their co-gel counterparts. The sorting is generally based on size, shape, or chirality (Figure 1.14). Smith and co-workers were again able to demonstrate the versatility of their dendrimer-diamine approach by creating self-sorting gels via a change in dendron generation (G1-G3, “size”), amine chain length (C6-C12, “shape”), or dendron chirality (D or L-lysine used as dendron building block, “chirality”).

Fuhrhop and Boettcher examined self-sorting in alkyl aldonaamide gels by varying the type and chirality of the sugar head group as well as the length of the alkyl tail. A combination of chirality and alkyl chain length-based self-sorting was observed when mixing D-Glu 8 (1.13) with L-Glu 12 (1.14) (Figure 1.15 a,b). Thinner P (right-handed) helices were observed for D-Glu 8, while thicker M (left-handed) helices were observed for L-Glu 12 (Figure 1.15c).
Moffat and Smith developed a peptide-based bolaamphiphile system with R-groups at the periphery to lead to an endless number of possible gelator structures. By mixing gelators (1.15) and (1.16), they achieved self-sorting in 9:1 styrene-
Figure 1.16. Chemical structures of (1.15) and (1.16) as well as FEG-SEM images of (1.15) and (1.16) gels and a self-sorting (1.15) (right) + (1.16) (left) gel (bars = 200, 300, and 200 nm).
divinylbenzene (Figure 1.16).\textsuperscript{27} It is suggested that the different functional groups of (1.15) and (1.16) (amide and carbamate, respectively) prefer to hydrogen bond with themselves since the amide-amide bond strength is greater than the amide-carbamate bond strength. It is also mentioned that the difference in size of the R groups may also contribute to the self-sorting behavior.

1.3.3 Mixing a gelator with a non-gelator to tailor properties

The third and significantly less explored type of multi-component supramolecular gels is that of adding a non-gelating molecule to a gel to affect gel properties. As such, this concept is explored extensively throughout this thesis.

Existing literature mostly centers on the addition of non-gelling surfactants to gel systems.\textsuperscript{28} Liu, Strom, and coworkers examined the effect of the surfactants Tween 80 and Span 20 on the organogelator N-lauroyl-L-glutamic acid di-n-butyramide (1.17) (Figure 1.17a). The concept explored in these papers is identical to references 9 and 11 discussed in section 1.2, where the additive is a surface active molecule that can promote bifurcation via crystallographic mismatch, except for in this case, the additive is a small molecule rather than a polymer. Figure 1.17b shows SEM images of (1.17)/Tween 80 gels. More bifurcation is evident in the presence of the Tween 80 surfactant, which is accompanied by an increase in the gel elasticity.\textsuperscript{28b,c}
Steed et al. very recently demonstrated the ability to grow pharmaceutical crystals within a supramolecular gel while simultaneously gaining mechanical tailorability from the crystallization compound. Bisurea gelator (1.18) and crystal-growing CBZ (1.19) are shown in Figure 1.18a. Crystals were grown in 1 wt % of (1.18) in a 1:9 v/v mixture of chloroform:toluene (Figure 1.18b), and the change in mechanical properties with the addition of CBZ is shown in Figure 1.18c. Interestingly, the modulus remains the same upon the addition of CBZ; however, the yield stress increases by an order of magnitude. The authors state that it is not clear if the CBZ contributes to the gel network in any way as it is possible that the reinforcement results from physical support by CBZ crystals.
Figure 1.18. (a) Chemical structures of gelator (1.18) and CBZ (1.19). (b) CBZ crystals being grown in 1 wt % of (1.18) in a 1:9 v/v mixture of chloroform:toluene. (c) Modification of the yield stress of a gel of (1.19) upon addition of the non-gelling CBZ.29
1.4 Chemo-responsive systems: Addition of a component designed to impact the gel properties

An additional genre that could also potentially be considered a part of multi-component supramolecular gels involves addition of an additional component to affect the properties of the gel. There are many examples of this in the literature that include ion,\textsuperscript{30} redox,\textsuperscript{31} and pH\textsuperscript{30i,32} tuning of properties.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure19}
\caption{(a) Chemical structure of gelator (1.20). (b) Gels can be formed in acetone at concentrations > 1.5 wt %. (c) Gelation is reversed on addition of an anion. Re-gelation occurs on addition of a Lewis acid (d) or a stronger anion binder than the gelator (e).\textsuperscript{30b}}
\end{figure}
Itagaki, et al. synthesized the anion-binding urea-based compound (1.20) (Figure 1.19a) that was found to form gels in acetone upon brief sonication (Figure 1.19b). The gels reverted to sols in the presence of easily bound fluoride and chloride anions (Figure 1.19c), which presumably competitively bind to the ureas to disrupt gelation. Most of the solubilization was reversible upon addition of a Lewis acid (BF$_3$OEt$_2$) (Figure 1.19d) or a stronger anion binder such as ZnBr$_2$ (Figure 1.19e).$^{30b}$

![Chemical structure of gelator L1 (1.21)](image)

**(Figure 1.20)**. (a) Chemical structure of gelator L1 (1.21). (b) Silver-complexed gels can be formed in 10:1 toluene:ethanol. Addition of an iodine anion or hydrogen sulfide gas competitively binds the silver to result in dissolution of the gel.$^{30f}$

Similar behavior was seen by Wu. et al., where the addition of anions disrupted the gelation of gelator (1.21), which gels as a silver complex (Figure 1.20 a,b) in 10:1
toluene:ethanol. Addition of anions (Figure 1.20c) or some gases like hydrogen sulfide (Figure 1.20d) removes the silver from the (1.21)-Ag complex and therefore disrupts gelation. TEM images (Figure 1.20e-g) show that the addition hydrogen sulfide or iodide results in destruction of the fibrous assembly into smaller aggregates with the loss of Ag from the gelator-Ag complex.\textsuperscript{30f}

Aida, et al., have developed a gel system whose fluorescence changes upon addition of an extra component. They developed a trinuclear Au(I) pyrazolate complex (1.22) that forms a gel in hexane and whose emission is red. Addition of Ag(I) ions results in the emission being blue-shifted on account of the association between the Au and Ag ions. This shift is reversed upon the addition of the chloride anion since the Ag-Cl interaction is preferred over the Ag-Au interaction (Figure 1.21).\textsuperscript{30g}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.21.png}
\caption{The trinuclear Au(I) pyrazolate complex (1.22) forms gels in hexane that exhibit a blue shift upon the addition of Au-binding Ag. This can be reversed upon the addition of the stronger Ag binder, Cl.}
\end{figure}
Rowan and collaborators showed tailorable mechanical properties by changing the added salt content for gelator E5-BIP (1.23) (Figure 1.22a). Three different gels were made at 8 wt % in acetonitrile - 100% Zn(ClO$_4$)$_2$ (A), 97% Zn(ClO$_4$)$_2$/2% La(NO$_3$)$_3$ (B), and 97% Zn(ClO$_4$)$_2$/2% La(ClO$_4$)$_3$ (C) (Figure 1.22b). Zn(ClO$_4$)$_2$ and La(NO$_3$)$_3$ bind in a 2:1 ratio promoting the formation of linear assemblies, while La(ClO$_4$)$_3$ binds in a 3:1 motif introducing a small amount of crosslinking to the system. The authors suggest that this crosslinking results in a decrease in the crystallinity of the gels, which results in a decrease in both the modulus and the yield stress.$^{30j}$

Finally, there are several examples of pH-modulated tailoring of supramolecular gels as previously mentioned. Li, et al. showed that the viscoelastic properties and morphology of the gelator N,N′N″-tris-(3-pyridyl)trimesic amide (1.24) (Figure 1.23a) in 1:1 H$_2$O:EtOH could be modified by setting the pH at values between 3 and 7 (Figure 1.23d). It is suggested that at pH = 7, there is hydrogen bonding between the amide NH bond and the pyridyl group. Upon decreasing the pH to 5, some of the pyridyl groups are protonated, and as such, only the unprotonated groups remain bound, which leads to a highly bifurcated network resulting in an increasing the mechanical properties (Figure 1.23c). Decreasing the pH from 5 to 3 or below leads to the protonation of the remaining pyridyl groups, and thus a sol is eventually achieved.$^{32a}$
Figure 1.22. (a) Chemical structure gelator E5 (1.23). (b) Gels were formed at 8 wt % in acetonitrile with 100% Zn(ClO₄)₂ (A), 97% Zn(ClO₄)₂/2% La(NO₃)₃ (B), and 97% Zn(ClO₄)₂/2% La(ClO₄)₃ (C). (c) The stress-strain curves were shown to be tailorable with metal content. 

30j
Supramolecular gels are a highly tailorable class of materials that have found use in applications ranging from biomaterials to sensors. As a result of the ability to easily mix one or more small molecules to yield multi-component supramolecular gels, several gel properties, such as viscoelasticity, morphology, thermal stability, and response to the environment, can be easily and systematically tailored. This is typically done by modifying the ratio of the components within the gel or changing some aspect of one or more of the gel components. The largest deficiency of supramolecular gels, crystallization of the gelator over time, has been successfully addressed by several publications (as well as content within this thesis) in attempts to overcome this limitation.

**Figure 1.23.** (a) Chemical structure of the gelating amide compound (1.24). (b) SEM of gel at pH 7. (c) SEM of gel at pH 5. (d) Rheological changes with decreasing pH.
1.6 Thesis Scope

Chapter 2 introduces the concept of guanosine-based supramolecular hydrogels. Guanosine and several of its derivatives are known to form hydrogen-bonded planar quartets in the presence of monovalent metal ions, most commonly potassium, which then stack into columnar assemblies and lead to fiber-based hydrogelation. Unfortunately, guanosine derivatives crystallize quite easily from the gel, and as such, preventing crystallization is the overall goal of this chapter.

Chapter 3 addresses another limitation of guanosine-based supramolecular hydrogels – their reliance on high salt concentrations to template the quartet formation that results in gelation. Since the end goal is to use our guanosine-based gels as tissue engineering scaffolds, we have undertaken studies to identify guanosine-based molecules that gel physiologically relevant salt concentrations.

Chapter 4 addresses supramolecular gels for personal care formulations. It was requested that we develop a synthetically simple, electrolyte-resistant system that exhibited buttery rheology. The challenge of supramolecular gel crystallization was again present in our suggested system and was overcome similarly to what is suggested in Chapter 2.
1.7 References


Chapter 2

Tailoring the properties of guanosine-based supramolecular hydrogels via co-monomer addition
2.1 Introduction

Since the structure of DNA was first elucidated, it has been widely recognized that nucleobases (adenine, cytosine, guanine, thymine, and uracil) have the potential for self-assembly via hydrogen bonding individually or as part of a base pair (Figure 2.1a). Guanine, however, shows the most versatility in this respect; it can independently self-assemble into many different structures, with the most prominent being a circular quartet (upon addition of a monovalent metal ion) and two different linear tapes (Figure 2.1b).¹

The nucleoside of guanine, guanosine, and several of its synthesized derivatives have the additional ability to form three-dimensional stacks of the circular G-quartets in

![Figure 2.1](image-url)
the presence of metal ions (generally $K^+$ or $Na^+$) with the guanine units on the inside and the ribose units on the periphery. This assembly is thought to occur in steps – the guanine units assemble into quartets, then the quartets assemble into octamers, and the octamers assemble into quadraplexes, which when large enough, entangle to form a gel.\(^2\) Alternatively, Guanosine-5’-Monophosphate (5’-GMP) assembles into helical columns, which also then entangle for form a gel (Figure 2.2).\(^5\)

**Figure 2.2.** Self-assembly and gelation of guanosine and guanosine 5’-monophosphate.

Despite the now fairly lengthy history of guanosine-based hydrogels,\(^3\) there remain severe limitations which hinder their potential use in many applications, the most severe of which is the crystallization that occurs upon standing over a couple of hours at room temperature leading to collapse of the gel.\(^4\) Thus, from a materials design point-of-
view and with an eye toward enhancing the potential of these hydrogels for applications, a course of study was launched to find facile methods to not only dramatically enhance the lifetime stability of guanosine-based gels (i.e. hinder crystallization) but to also find ways to ‘tailor’ both the $T_{gel-sol}$ and mechanical properties of these interesting supramolecular materials.

As crystallization from the gel state is a key hurdle that needs to be crossed in order to develop potentially useful guanosine hydrogels, it was hypothesized that mixing guanosine with a non-gelating comonomer that contains the same molecular recognition motif, namely the guanine moiety, could provide enough structural heterogeneity to disfavor crystallization while still allowing enough supramolecular order for gelation to occur. Furthermore, it was expected that additional thermal stability could be imparted to the guanosine hydrogels if the non-gelling comonomer contained hydrophobic groups that encouraged the formation of physical (hydrophobic) crosslinks between the guanosine stacks (Figure 2.3).

Figure 2.3. Illustration of the addition of the hydrophobic effect via hydrophobic groups (pink) attached to the guanosine stacks (blue).
An added advantage of this approach could be the ability to systematically tune the properties of such co-gels by simply varying the ratio of the two comonomers. With these design aspects in mind, a program was initiated aimed at finding a co-monomer that would allow the enhancement and tailoring of the properties of guanosine hydrogels.

During these studies, Yu et al. reported\[^5\] that stable gels can be attained in a KCl/Tris buffer solution by mixing guanosine (G) with the known gelator guanosine 5’-monophosphate (GMP). Studies on the thermal behavior of these hydrogels, which primarily utilized circular dichroism experiments, showed that at high mole fractions (> 0.8) of GMP the gels exhibit a lower critical solution temperature (LCST), i.e. the mixed guanosine derivatives are soluble at room temperature and only gel at elevated temperatures, and that the LCST can be tuned with the ratio of GMP to G. However, no detailed rheological or morphological studies were reported on this system and the long-term stability of these gels was not discussed in detail.

This chapter details the ability to access a series of room temperature stable hydrogels, by simply mixing guanosine with a hydrophobic guanosine derivative.\[^6\] It further shows that the thermomechanical properties of these simple hydrogels can be systematically altered by varying the ratio of the two components and utilizes detailed rheological and morphological studies in an effort to further elucidate the mechanism of this behavior. Finally, the potential of this type of system for use in drug delivery is examined via assembly and drug release experiments with guanosine analogs acyclovir and ganciclovir.
2.2 Results and Discussion

2.2.1 Guanosine/2’,3’,5’-tri-O-acetylguanosine gels

With the goal of targeting “easy-to-access” hydrogel systems, commercially available hydrophobic guanosine derivatives were sought as potential co-monomers for the mixed guanosine hydrogels. Gratifyingly, it was quickly found that 2’,3’,5’-tri-O-acetylguanosine (TAcG, Figure 2.4), which does not gel aqueous potassium chloride on its own, does indeed produce stable hydrogels when mixed with guanosine (G) in varying ratios and is easily synthesized in one step from guanosine.

![Chemical structures of guanosine (G) and 2’,3’,5’-tri-O-acetylguanosine](image1.png)

**Figure 2.4.** (a) Chemical structures of guanosine (G) and 2’,3’,5’-tri-O-acetylguanosine. (b) Synthesis of 2’,3’,5’-tri-O-acetylguanosine.

As in the case of guanosine alone, the gels are fabricated by suspending the two components in aqueous potassium chloride (0.354 M), heating until dissolution is achieved, before allowing them to cool to room temperature. All hydrogels in this chapter
were prepared in this way. Figure 2.5 shows gels having different ratios of $G/T\text{AcG}$ at 2 wt % in aqueous potassium chloride (0.354 M), at (a) 15 minutes and (b) 36 hours after the samples were removed from the heat source. It should be noted that in Figure 2.5b, the 2 wt% samples in the 60/40 to 40/60 $G/T\text{AcG}$ ratio range form self-supporting gels within 30 minutes after being removed from heating; however, Figure 2.5b is shown after 36 hours to illustrate the drastic difference in gel lifetime stability between samples having different compositions. It is thus apparent that after 36 hours, the presence of 2’,3’,5’-tri-$O$-acetylguanosine prevents guanosine crystallization from the gel extremely effectively between 60/40 and 40/60 $G/T\text{AcG}$. In fact, the lifetime stability of these gels is so dramatically enhanced that no crystallization could be observed even after one year standing at room temperature. Encouraged by this behavior it was decided to prepare five different gels with comonomer ratios at 60/40, 55/45, 50/50, 45/55 and 40/60 $G/T\text{AcG}$ and carry out structural, mechanical and thermal studies on these new hydrogels.
Before carrying out detailed studies it was important to know the critical gel concentration of this class of hydrogels. A convenient and non-invasive method to accomplish this is to use light scattering techniques. In particular, dynamic light scattering (DLS) can identify the onset of gelation through the appearance of a slow relaxation mode, which is characteristic of network dynamics. Matsunaga et al. found that the microscopic gel threshold identified by DLS coincides with the macroscopic gel point observed via rheometry. To this end the 50/50 G/TAcG hydrogel was prepared at varying concentrations (0.5 wt % to 1 wt %) and examined using dynamic light scattering. At low concentrations, the normalized time-intensity autocorrelation function

**Figure 2.5.** Visual appearance of 2% wt gels made from varying ratios of 2',3',5'-tri-O-acetylguanosine (TAcG) and guanosine (G) in 0.354 M potassium chloride (a) 15 minutes and (b) 36 hours after being removed from the heat source. Samples were inverted just prior to being photographed. The ratios are listed as G/TAcG content, and the samples shown are visually representative of the entire G/TAcG ratio range listed underneath each sample. Specifically, the samples shown from left to right for both (a) and (b) are 100/0 G/TAcG, 90/10 G/TAcG, 50/50 G/TAcG, 30/70 G/TAcG, and 0/100 G/TAcG.
of light scattered by these samples (Figure 2.6) features only a fast relaxation mode at short timescales whose decay rate slowly decreases with concentration - this is indicative of small aggregates floating around in a sol phase with no higher-ordered gel phase. However, as the concentration increases to approximately 0.8 wt %, a new slow relaxation mode appears at longer times (indicated by “A”), suggesting that 0.8 wt % G/TAcG is the critical gel concentration. In addition, the 0.8 wt % gel appears visually more viscous than the 0.7 wt % sample consistent with the DLS data. As a result, all further studies on this system were carried out at 2% wt. total monomer concentration, i.e. well above the critical gelation concentration for the 50/50 G/TAcG hydrogel.

The fast and slow modes were further analyzed to elucidate the apparent diffusion coefficients (D) and hydrodynamic radii (R_h) of both populations via equations 2.1-2.3. From these equations, the R_h of the fast mode (small aggregates in the sol phase) above the critical gel concentration was found to be approximately 16 nm, while the R_h of the slow mode (larger aggregates comprising the gel network phase) was found to be significantly larger at approximately 1200 nm.

\[
D = \frac{1}{q^2 \tau} \quad (\text{Eq. 2.1})
\]

\[
q = \frac{4 \pi n}{\lambda} \sin \frac{\theta}{2} \quad (\text{Eq. 2.2})
\]

\[
R_h = \frac{k_b T}{6 \pi \eta D} \quad (\text{Eq. 2.3})
\]
It was next investigated how varying the ratio of the two comonomers affects the viscosity of these hydrogels. Oscillatory time and stress sweeps of 2 wt. % samples of the five gels indicate that both the complex viscosity, $\eta^*(\omega)$, and yield stress, $\tau_y$, estimated as the shear stress at which there is a sudden precipitous decrease in the magnitude of $\eta^*(\omega)$, decrease with increasing TAcG content (Figure 2.7a,b). The visual

Figure 2.6. (a) The normalized time-intensity autocorrelation functions of 50/50 G/TAcG at increasing concentrations. The critical gel concentration is at approximately 0.8% as evidenced by the appearance of a second relaxation at longer times (denoted by “A”). Also shown are the apparent diffusion coefficients and hydrodynamic radii of the “fast mode” (b) and the “slow mode” (c).
behavior of the gels backs this up (Figure 2.7c) with the 60/40 G/TAcG gel exhibiting less “creep” upon inversion of the sample vial than the 40/60 G/TAcG gel.

Figure 2.7. (a,b) Oscillatory time and stress sweeps taken after a 30 second pre-shear and 60 minute time sweep of 2 wt % gels containing varying ratios of G/TAcG in 0.354 M aqueous potassium chloride. The deformation frequency $\omega = 10$ rad/sec, and the strain used was 0.2 %. (c) 60/40 and 40/60 G/TAcG inverted samples after allowing gel formation for 30 minutes.

Since the magnitude of $\eta^*(\omega) = \sqrt{G'(\omega)^2 + G''(\omega)^2}/\omega$ is dominated by the value of the storage modulus, $G'(\omega)$, which is substantially larger than the loss modulus, $G''(\omega)$, in the gel state, the results in Figure 2.7 also demonstrate that the gel elasticity diminishes.
with increase in $\text{TAcG}$ content. The difference in molecular weight of the two components means that, while all the gels are at a total of 2 wt. %, changes in their ratio results in a slight change in the gels molar concentration (e.g. total monomer concentration is 62 mM for the 60/40 gel and 58 mM for the 40/60 gel) of these samples. To ensure that this change in molar concentration is not responsible for the decrease in complex viscosity a control experiment was carried out on the 60/40 gel at 58 mM (Figure 2.8) which shows little to no effect in the sample’s rheology with this slight change in concentration.

![Figure 2.8](image)

**Figure 2.8.** Comparison of the complex viscosities a 62 mM 60/40 $\text{G/TAcG}$ gel and a 58 mM 60/40 $\text{G/TAcG}$ gel. The concentration change has little to no effect on the complex viscosity values.

The ability to systematically tune the elasticity and yield stress of these hydrogels by simply altering the comonomer ratio is potentially very useful. Thus, in order to obtain a better understanding of how the addition of $\text{TAcG}$ alters the molecular and nanoscale assembly in these hydrogels, small-angle neutron scattering (SANS) studies of
the hydrogels in KCl/D$_2$O solution (0.354 M KCl) were carried out. In the intermediate q-regime of the scattering profiles, a dependence of the scattered intensity, I(q), on the scattering vector, q, viz. I(q) \sim q^{-1}, was observed, which is characteristic of the presence of rigid rod structures. However, a peak at high q (Figure 2.9) is also observed that could not be accounted for with a simple rigid rod assembly. Gottarelli and coworkers\textsuperscript{8} have reported SANS profiles of gels composed of guanosine-based molecules in chloroform solution containing potassium picrate, which have similar peaks at high q. These self-assembling species have long hydrocarbon tails attached to guanosine, and the scattering is influenced by the fact that guanosine and the hydrocarbon tails have distinctly different scattering length densities. Thus, the authors were able to accurately reproduce the scattering profiles using a core-shell cylinder model with the further assumption of the coexistence of populations of short and long cylinders, with the former in large excess. It was then attempted to simulate the scattering profiles following the analysis of Gottarelli et al.\textsuperscript{8} However, it was not possible to simultaneously reproduce the experimental data at high and low q, because the scattering intensity contribution from the short cylinders was always negligible compared to that from the long cylinders, even when large numbers of short cylinders were assumed. Thus, another possibility was considered - that the core-shell cylinder model itself can reproduce the peaks at high q without the bimodal length distribution assumption. Lehn and coworkers,\textsuperscript{9} in fact, anticipated the existence of a peak at large q arising from the cross-sectional form factor but were not able to observe the peak on account of a relatively high incoherent background.
In D$_2$O, the labile hydrogens in TAcG and G are exchanged with deuterium, which results in reduced contrast between the guanine units (the “core”) and the solvent. The hydrogens on the acetyl groups of the ribose ring do not exchange, however, so contrast is maintained on the periphery of the stacks (the “shell”). Therefore, an appropriate model for our supramolecular structure approximates that of a hollow cylinder (Figure 2.10).

**Figure 2.9.** The experimental SANS data and simulated fit with the core shell cylinder model (Eq. 2.1). Data courtesy of Dr. Zheng Li.
The form factor expression developed by Livesay\textsuperscript{10} for a monodispersed cylinder, having a circular cross-section, with a core-shell scattering length density profile was utilized:

\[
P(q) = \frac{\text{scale}}{V_{\text{shell}}} \int_0^{\pi/2} f^2(q, \alpha) \sin \alpha d\alpha \quad \text{(Eq. 2.4)}
\]

\[
f(q, \alpha) = 2(\rho_{\text{core}} - \rho_{\text{shell}}) \sqrt{\frac{\pi}{V_{\text{core}}} J_0(q r \sin \alpha)} \frac{J_1(q r \sin \alpha)}{(q r \sin \alpha)}
\]

where

\[
+2(\rho_{\text{shell}} - \rho_{\text{solvent}}) \sqrt{\frac{\pi}{V_{\text{shell}}} J_0(q (H + t) \cos \alpha)} \frac{J_1(q (r + t) \sin \alpha)}{[q (r + t) \sin \alpha]} \quad \text{(Eq. 2.5)}
\]

with \(j_0(x) = \sin(x)/x\), \(V_{\text{core}} = \pi r^2 L\), and \(V_{\text{shell}} = \pi (r + t)^2 L_{\text{total}}\), where \(r\) is the radius of the core of the cylinder, \(t\) is the thickness, \(L\) is the length of the core, \(H=L/2\), \(L_{\text{total}}=2H+2t\), and \(J_1(x)\) is the first order bessel function. \(\alpha\) is defined as the angle between the cylinder axis and the scattering vector, \(q\). The integral over \(\alpha\) averages the form factor over all possible orientations of the cylinder. The shell thickness, \(t\), is considered to be uniform.
over the entire surface of the core. The form factor is normalized by the total particle volume such that \( P(q) = \text{scale} \times \langle f^2 \rangle / \text{Vol} + \text{bkg} \), where \( f \) is the scattering amplitude, the \( \langle \cdot \rangle \) denote an average over all possible orientations of the cylinder, and scale and bkg are fitting constants. Utilizing the core-shell model as expressed in Eq. 2.4, an excellent least squares fits to the experimental SANS profiles of the three gels over the entire range of q-vectors can be obtained, as illustrated in Figure 2.9 for the 50/50 G/TAcG gel. In generating these fits the SANS analysis software package v3.0 from the NIST Center for Neutron Research on Igor Pro 6.0 was used, inputting the computed scattering length densities of the core, shell and solvent, and constraining the fit to reproduce the position of the high-q peak in the SANS profile. The latter peak, evident in Figure 2.9 near 0.3 Å\(^{-1}\), arises from the cross-sectional form factor of the rod, and from the position of the peak the cylinder diameter can be estimated. The results of such fits to the experimental scattering profiles of the three gels are summarized in Table 2.1.

<table>
<thead>
<tr>
<th>Gel Content</th>
<th>Core Radius (Å)</th>
<th>Shell Thickness (Å)</th>
<th>Total Radius (Å)</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60/40 G/TAcG</td>
<td>7.1±0.2</td>
<td>7.7±0.1</td>
<td>14.8±0.3</td>
<td>479±38</td>
</tr>
<tr>
<td>50/50 G/TAcG</td>
<td>6.9±0.2</td>
<td>7.8±0.1</td>
<td>14.7±0.3</td>
<td>320±10</td>
</tr>
<tr>
<td>40/60 G/TAcG</td>
<td>7.3±0.2</td>
<td>8.1±0.1</td>
<td>15.4±0.3</td>
<td>302±10</td>
</tr>
</tbody>
</table>

**Table 2.1.** Summary of G/TAcG assembly dimensions based on core-shell cylinder model fitting of the SANS data.

It is evident that the measured total radius appears to increase with TAcG content, which is consistent with the fact that the physical size of TAcG is larger than that of G. The core-shell dimensions obtained from the SANS data match well to the dimensions expected based on comparison with literature values of related structures.\(^{11,12}\) The data also display an indisputable trend of decreasing rod length, L, with increasing TAcG
content. Identifying \( L \) as akin to either the persistence length of the guanosine stacks which comprise the network fibers, or as a measure of the structural order of the fibers, and hence a measure of the fiber rigidity, it can be seen that the decrease correlates well to the decrease in complex modulus and yield stress with increasing \( \text{TAcG} \) content evident in Figure 2.7. Taken together these observations are consistent with the \( \text{TAcG} \) being incorporated, presumably randomly, into the guanosine assembly. The fact that \( \text{TAcG} \) is physically larger than guanosine may hinder the stacking of two G-quartets that each contain multiple \( \text{TAcG} \) molecules, which in turn effectively truncates the growth of the \( \text{TAcG}/\text{G} \) stacks. Therefore, the probability of the stack growth being terminated increases with \( \text{TAcG} \) content, leading to shorter stack length and reduced mechanical properties.

In an attempt to compliment the SANS data and verify a G-quartet-type assembly, wide-angle x-ray diffraction was carried out on freeze-dried gels of varying \( \text{G/TAcG} \) ratio. As can be seen in Figure 2.11, four key peaks emerge. The large peak at the highest angle corresponds to the KCl salt (\( d = 3.15 \) Å) in the gel. The next peak (\( d = 3.4 \) Å) can most likely be attributed to the vertical \( \pi-\pi \) stacking distance between quartets. Continuing to lower angles, the next peak at approximately 22 Å corresponds to the known width of a single G-quartet.\(^{13}\) (It is not surprising that this value is slightly closer to the anticipated value than the values the SANS data produced, since the entire molecular assembly is visible to the WAXD, as opposed to the case of the SANS data.) Like the SANS data, there appears to be a slight trend of increasing quartet width with increasing \( \text{TAcG} \) content. Finally, additional peaks at extremely low angles potentially correspond to higher-ordered structures.
To obtain visual insight into the nature of the assembly of G/TAcG gels, atomic force microscopy was performed on gel samples that were prepared similarly to the previously reported method of Kimizuka et al.\textsuperscript{14} The height images can be seen in Figure 2.12, while 3-D images are shown in Figure 2.13.

\textbf{Figure 2.11.} WAXD of freeze-dried G/TAcG gels. The visible peaks correspond to the KCl salt, π-π stacking between quartets, the width of a single quartet, and dimensions of larger aggregates.

To obtain visual insight into the nature of the assembly of G/TAcG gels, atomic force microscopy was performed on gel samples that were prepared similarly to the previously reported method of Kimizuka et al.\textsuperscript{14} The height images can be seen in Figure 2.12, while 3-D images are shown in Figure 2.13.

\textbf{Figure 2.12.} Height AFM images of the 2 wt % hydrogels comprised of (a) 60/40 G/TAcG and (b) 40/60 G/TAcG.
It is apparent from the images that while both systems contain bundles of individual \( \text{G/TAcG} \) nanofibers, the overall morphology varies when transitioning from 60/40 \( \text{G/TAcG} \) (Figure 2.12a) to 40/60 \( \text{G/TAcG} \) 2 wt % gels. In the 60/40 \( \text{G/TAcG} \) gel (Figure 2.12a), there appears to be a population of relatively uniform, bifurcated fiber bundles, while in the 40/60 \( \text{G/TAcG} \) gel (Figure 2.12b), a more heterogeneous network structure is observed, containing fibers with a larger dispersity in both width and length. This can be further seen in the 3-D height images shown in Figure 2.13, where vast differences in height are also apparent.

![3-D projections of the AFM height data of (a) 60/40 \( \text{G/TAcG} \) and (b) 40/60 \( \text{G/TAcG} \) 2 wt % gels. It is apparent that the fibers of the 40/60 \( \text{G/TAcG} \) sample are much more diverse in height than those of the 60/40 \( \text{G/TAcG} \) sample.](image)
Figure 2.14. Sectioning of populations of 60/40 G/TAcG thick (left) and thin (right) nanofiber bundles from the image in Figure 2.12a in order to probe their approximate dimensions.
Compared to the fibers of the 60/40 G/TAcG gel (Figures 2.12a and 2.14), the fibers of the 40/60 G/TAcG (Figure 2.12b) gel appear predominately shorter and contain wider more irregular bundles (Figure 2.15). While it is possible that some aggregation accompanies the drying of specimens on the mica substrate prior to AFM imaging, these results suggest that, in both gels, the individual fibrillar guanosine stacks probed by the

**Figure 2.15.** Sectioning of populations of 40/60 G/TAcG thick (left) and thin (right) nanofiber bundles from the image in **Figure 2.12b** in order to probe their approximate dimensions.
SANS experiment bundle together to form the fibers which constitute the gel network nano/microstructure.

The observations of decreased fiber length and increased fiber thickness with increasing TAcG content correlate well with the data generated by rheology (decreased elasticity and yield stress with increased TAcG content) and small-angle neutron scattering (increased assembly diameter and decreased length with increased TAcG content). A similar observation - that thicker fibers yield diminished gel stability as a result of weaker interfiber interactions through less effective interpenetration and decreased surface area - has previously been made.\textsuperscript{15} Again, these trends for G/TAcG gels can potentially be explained by the aforementioned hypotheses of physical crosslinking as a result of the hydrophobic effect between guanosine stacks to yield thicker fibers as well as fiber length destabilization imparted by the inability of several TAcG molecules to stack on top of one another as a result of their larger size (Figure 2.16).
In addition to the influence of the G/TAcG composition on the mechanical properties, the effect on thermal properties was also examined. DSC of samples with ratios between 60/40 G/TAcG and 40/60 G/TAcG (Figure 2.17) show that all gels show an enhancement in thermal stability (T_{gel-sol} > 60 °C) compared to the G only hydrogel (T_{gel-sol} ca. 50 °C). Interestingly, a different stability pattern appears from that of the mechanical data (where increasing TAcG yields weaker gels), in that the 50/50 sample appears to have the highest T_{gel-sol} of approximately 63 °C.

Figure 2.16. Illustration of guanosine quartets compared to G/TAcG mixed quartets. The increased size of TAcG potentially destabilizes the stacks by decreasing their stacking efficiency, which results in shorter assemblies.
Intrigued by the DSC results, a variable temperature-NMR experiment was run on a 2 wt % 50/50 G/TAcG gel to determine the extent of gelation by comparing G and TAcG peak integrations to an internal dioxane standard as the sample is cooled from 75 °C to 25 °C (Figure 2.18). Since NMR detects guanosine in the solution phase of the sample, the G peak integrations decrease with respect to the internal standard as the sample is cooled to room temperature, and the guanosine derivatives are incorporated into the gel. As can be seen in, both G and TAcG appear to be incorporated into the gel equally with approximately 90% of each component being incorporated at 25 °C. There is an apparent difference, however, in the temperature at which G and TAcG are incorporated into the gel. G begins to disappear from the solution phase by 65 °C, while TAcG does not enter the gel phase until approximately 55 °C. This is not altogether unexpected, since G is a gelator, while TAcG is not, and may, in part, explain why the SANS data shows that gel assemblies with higher TAcG content appear to be shorter in

**Figure 2.17.** Differential scanning calorimetry of 2 wt % G/TAcG hydrogels at varying ratios in 0.354 M KCl. The 50/50 TAcG/G sample has the highest transition temperature of approximately 63 °C.
length as \text{TAcG} may be more prevalent at the ends of the fibrous assemblies and thus be in a better position to truncate the stacks, as hypothesized above.

\textbf{Figure 2.18.} VT-NMR data of a 2 wt \% 50/50 G/TAcG gel. The integration of the internal standard (dioxane) peak (green arrow) was compared with the integrations of the \text{G} (orange arrow) and \text{TAcG} (purple arrow) C8 proton peaks to determine the percent gelation at a given temperature (\textit{Table 2.2}).
Table 2.2. Peak integration values for the VT-NMR experiment shown in Figure 2.18.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>G Peak Integration</th>
<th>TAcG Peak Integration</th>
<th>Dioxane Peak Integration</th>
<th>Percent Gelation According to G</th>
<th>Percent Gelation According to TAcG</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 °C</td>
<td>0.690</td>
<td>0.451</td>
<td>1</td>
<td>0% (assumed)</td>
<td>0% (assumed)</td>
</tr>
<tr>
<td>65 °C</td>
<td>0.572</td>
<td>0.470</td>
<td>1</td>
<td>17%</td>
<td>0%</td>
</tr>
<tr>
<td>55 °C</td>
<td>0.401</td>
<td>0.333</td>
<td>1</td>
<td>42%</td>
<td>26%</td>
</tr>
<tr>
<td>45 °C</td>
<td>0.249</td>
<td>0.176</td>
<td>1</td>
<td>64%</td>
<td>61%</td>
</tr>
<tr>
<td>35 °C</td>
<td>0.156</td>
<td>0.095</td>
<td>1</td>
<td>77%</td>
<td>79%</td>
</tr>
<tr>
<td>25 °C</td>
<td>0.077</td>
<td>0.046</td>
<td>1</td>
<td>89%</td>
<td>90%</td>
</tr>
</tbody>
</table>

In addition to VT-NMR, temperature sweeps of $G'$ and $G''$, taken at deformation frequency $\omega = 10$ rad/sec, and strain = 0.2 %, were also carried out on the rheometer to probe events in the gel phase. The results from the DSC, VT-NMR, and rheology temperature sweeps overlay nicely and all point toward a final gel melting temperature between 60 and 70 °C (Figure 2.19). Most interesting, however, is the step that occurs in modulus during the rheological temperature sweep between approximately 45 and 65 °C. It is not immediately clear whether this step is a pre-existing elastic mechanism exposed by the disappearance of the low-temperature elasticity or the appearance of a new process, e.g. due to an increased reinforcing influence of the hydrophobic effect with temperature. Upon heating above ca. 65 °C the gel completely fails, presumably on account of breaking the ion-dipole, hydrogen bonding, and $\pi-\pi$ interactions known to stabilize the guanosine stack assemblies.
If the hydrophobic effect is the cause of the step observed in the variable-temperature rheology, it can be expected that a difference in the temperature sweep profile would be evident when varying gel composition between 60/40 G/TAcG and 40/60 G/TAcG, i.e. as the amount of the hydrophobic TAcG increases. This is, in fact, found to be the case, as seen in Figure 2.20. With increased TAcG content, and thus increased hydrophobic content, the onset of the plateau occurs at lower temperatures (indicated in Figure 2.20 by “A”). Additionally, the composition dependence of the moduli switches at ca. 45-50 °C, with the 40/60 G/TAcG sample having the highest modulus, whereas the 60/40 G/TAcG gel has the highest modulus at room temperature. Finally, the composition-dependence of the final gel melting temperatures corresponds to that of the DSC data (indicated in 2.20 by “B”), i.e. the 50/50 G/TAcG gel is the most stable. In summary, this implies that the thermal stability of the gel is a delicate balance between the hydrophobic effect, enhanced by the addition of TAcG and increase of

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**Figure 2.19.** Overlay of variable-temperature rheology, NMR, and DSC experiments for a 2 wt % 50/50 G/TAcG gel in 0.354 M KCl. The percent gelation of guanosine (G) was calculated from the VT-NMR experiment by comparing the integration of the guanosine C8 proton peak to that of an internal dioxane standard.
temperature, versus the loss of ion-dipole, hydrogen bonding, and \( \pi-\pi \) interactions at higher temperatures and the destabilization the bulkier TAcG introduces within the aggregate.

![Figure 2.20](image)

**Figure 2.20.** Temperature-dependent rheological behavior of 2 wt % gels G/TAcG gels of varying ratios in 0.354 M KCl. The onset of the plateau as a result of hydrophobic interactions is denoted by “A”, while the final gel melting, which is within reasonable agreement with the DSC data is denoted by “B”.

Further insight into the origin of the unusual temperature dependence of the storage modulus may be obtained by investigating the frequency dependence of the storage and loss moduli. We thus carried out frequency sweeps of \( G' \) and \( G'' \) on a 2 wt % 50/50 G/TAcG sample in five degree increments from 25 °C to 70 °C (Figure 2.21a). A distinct change in curve profile occurs between the 30 and 35 °C measurements, which roughly corresponds to the temperature at which the initial decreases in \( G' \) and \( G'' \) occur in the temperature sweep (Figure 2.20). At room temperature (25 °C), the frequency profile of the moduli has certain similarities to that of an entangled polymer network,\(^{17}\) with an apparent plateau modulus in \( G' \), and a decay in both \( G' \) and \( G'' \) at low frequencies (Figure 2.21b), where entangled chains can reptate past each other and
disentangle. However, at these low frequencies (the so-called terminal regime of entangled solutions), the typical behavior scales as $G'' \propto \omega^2$ and $G' \propto \omega$, where $G'' > G'$, whereas in the present data, at lowest frequencies, $G' \approx G''$ with similar frequency dependence. This is consistent with the observation that weak gel behavior persists at low deformation frequencies (Figure 2.7). At temperatures above 35 °C, the apparent plateau modulus at intermediate frequencies disappears, and the storage modulus levels off at an equilibrium value of ~10 Pa. (Figure 2.21c). Therefore, the frequency-sweep experiments tell us that the “crosslinking” which contributes to the low temperature modulus at intermediate frequencies (Figure 2.20) is transient in nature, and may reflect stress stored in interfiber entanglements which have a relaxation time of the order of the inverse crossover frequency, $\omega \sim \omega_c^{-1} = 1/2.3 = 0.43$ sec (Figure 2.21b). From Figure 2.21, comparing the NMR data versus the rheological results, it appears that the disappearance of these entanglements on increasing temperature reflects the conversion of the gel into a sol fraction of approximately 50%. Moreover, from Figure 2.21a, it is seen that the low frequency equilibrium modulus, which characterizes the permanent crosslinks that determine the thermal stability of the gel, has a relatively weak temperature dependence, and in fact appears to exhibit an increasing trend between 35 °C and 60 °C, consistent with the idea that the hydrophobic effect makes a significant increasing contribution to the gel stability, partially compensating for the progressive increase of sol fraction with increased temperature.
Since we were able to demonstrate this concept that mixing a non-gelating guanosine derivative with guanosine produced stable, crystallization-free gels, our interests next turned to the potential application of this type of system. Since many guanosine derivatives are utilized as drugs in the treatment of herpes and related conditions, one could imagine being able to incorporate a guanosine-based drug into the

Figure 2.21. Rheological frequency response of a 2 wt % 50/50 G/TAcG hydrogel in 0.354 M KCl. (a) Frequency response of the storage modulus at various temperatures. (b) Frequency response of the storage and loss moduli at 25 °C. (c) Frequency response of the storage and loss moduli at 35 °C.

2.2.2 Drug delivery with mixed guanosine gels

Since we were able to demonstrate this concept that mixing a non-gelating guanosine derivative with guanosine produced stable, crystallization-free gels, our interests next turned to the potential application of this type of system. Since many guanosine derivatives are utilized as drugs in the treatment of herpes and related conditions, one could imagine being able to incorporate a guanosine-based drug into the
G-quartet structure, which could potentially allow for controlled release of the drug since supramolecular interactions would have to be overcome to allow release. The two drugs most structurally similar to guanosine are acyclovir and ganciclovir (Figure 2.22a), and their method of action involves their phosphorylation by the thymidine kinase of herpes-infected cells. Following this, they are then converted to the triphosphate form, which incorporates into the DNA and acts as a premature chain terminator due to the lack of 3’ and 5’ –OH groups.¹⁹

Gratifyingly, it was again found that crystallization-free gels could be accessed by mixing a guanosine derivative (acyclovir or ganciclovir) with guanosine produced crystallization-free gels within a certain ratio of the two components. In this case, the stable gels were made with ratios of 90/10 and 80/20 G/drug. Above that drug content, crystallization, presumably of the drug, occurred (Figure 2.22b).
Before any release experiments could be carried out, it was necessary to confirm that the drug could be incorporated into the gel in the same way as the TAcG of the previous system. To that end, the VT-NMR experiment was repeated with guanosine and acyclovir at 2 wt % 80/20 G/ACV in 0.354 M KCl. Figure 2.23a shows the 1H NMR spectra between 25 and 80 °C, where the dioxane standard is denoted by the blue arrow, the guanosine is denoted by the red arrow, and the acyclovir is denoted by the green arrow. The analysis of the peak integrations (Figure 2.23 b) shows that both guanosine and acyclovir are being incorporated into the gel, although at different amounts. Guanosine is over 90% incorporated into the gel state at 25 °C, while acyclovir is only

![Figure 2.22](image_url)

**Figure 2.22.** (a) Structures of guanosine (G), acyclovir (ACV), and gancyclovir (GCV). (b) 2 wt % gels (0.354 M KCl) made from varying ratios of guanosine and acyclovir after 5 days. No crystallization occurs in gels containing 90/10 and 80/20 G/ACV ratios.
between 60 and 80% incorporated into the gel at 25 °C. Additionally, this incorporation can be shown by both increasing and decreasing the temperature between 25 and 80 °C, which further exhibits the thermoreversibility of a system such as this.

Since successful incorporation of the drug into the gel was easily achieved, the next step was to examine the release of the ACV from the G/ACV gel. To do this, 5 mL of a 2 wt % 80/20 G/ACV gel in 0.354 M KCl was placed in a dialysis bag and put into a gently stirring beaker filled with 500 mL of 0.354 M KCl. The beaker was covered and allowed to stir while hourly aliquots were taken to analyze via HPLC. Figure 2.24a shows the HPLC spectra, where the guanosine peak is denoted by the red arrow, and the acyclovir peak is denoted by the green arrow. It is evident that between 5 and 23 hours, the drug is completely released from the gel, which has also completely dissociated during that period of time since the peaks of both components do not change in size beyond 23 hours. The peak integrations to quantitatively demonstrate this can be seen in Figure 2.24b.
Figure 2.23. (a) VT-NMR spectra of a 2 wt % 80/20 G/ACV gel in 0.354 M KCl. The dioxane standard is denoted by the blue arrow, the guanosine is denoted by the red arrow, and the acyclovir is denoted by the green arrow. (b) Analysis of the peak integrations shows that guanosine is over 90% incorporated into the gel state at 25 °C, while acyclovir is between 60 and 80% incorporated into the gel at 25 °C.
Figure 2.24. (a) HPLC spectra over time of a 2 wt % G/ACV gel in 0.354 M KCl after gentle stirring in a dialysis bag in 500 mL of 0.354 M KCl. No change in peak appearance for either the G or the ACV occurs after 23 hours, suggesting complete dissolution of the gel. (b) Analysis of the peak integrations quantitatively shows that both G and ACV are fully dissociated between 5 and 23 hours.
Based on these results, it was clear that this completely supramolecular system could not tolerate exposure to a large mobile phase due to complete dissolution of the gel by the induced concentration decrease of the gelator. We then postulated that a covalently crosslinked polymer matrix may delay the complete dissociation of the gel from a diffusion standpoint to allow for slower release of the drug that could be potentially more controlled by the breaking of the supramolecular interactions between the drug and the gel rather than the complete dissolution of the gel. We chose the very simple PEGDA system to attempt a proof of concept. The optimal crosslinking conditions for the most robust gel matrix were found to be 20 wt % PEGDA and 0.1 % crosslinker (Irgacure 2959) dissolved in PBS (Figure 2.25a). 1 mL of the solution was then injected into a 1 mm thick cell made from glass slides, and the setup was irradiated with 50 mW/cm² 320-390 nm light for 15 seconds per side to yield a mechanically stable gel film.

To incorporate the guanosine/drug gel, 1 mL of a 50/50 mixture of 40 wt % PEGDA/0.2 wt % Irgacure and 4 wt % 80/20 G/GCV in 0.354 M KCl (the more hydrophilic ganciclovir was used in place of acyclovir to allow for higher drug concentrations) was placed in the cell and irradiated under the previously used conditions. A picture of the resulting gel is shown in Figure 2.25b.
The HPLC release experiment was repeated in a similar manner to the G/ACV system, where 5 mL of gel, or in this case, 5 mL gel films, were placed in a dialysis bag and allowed to gently stir in 500 mL of 0.354 M KCl. A second set of samples was made containing 1 mL of a 50/50 mixture of 40 wt % PEGDA/0.2 wt % Irgacure and 0.8 wt % GCV in 0.354 M KCl to determine if incorporation of the GCV into G-quartets had an effect on the release profile. As can be seen in Figure 2.26a/b (GCV = green arrow, G = red arrow), incorporation of the GCV into G-quartets does delay its release by approximately 1 hour. It also appears that a small amount of G/GCV may remain unreleased after 26 hours when compared to the GCV alone (Figure 2.26c).

Figure 2.25. (a) Chemical structures of PEGDA and the Irgacure 2959 photocrosslinker. (b) Gel made from 1 mL of a 50/50 mixture of 40 wt % PEGDA/0.2 wt % Irgacure and 4 wt % 80/20 G/GCV in 0.354 M KCl after irradiation with 50 mW/cm² 320-390 nm light for 15 seconds per side.
Figure 2.26. (a) G/GCV release from a PEGDA/G/GCV gel. G is denoted by the red arrow, while GCV is denoted by the green arrow. Complete release occurs between 5 and 24 hours. (b) GCV release from a PEGDA/GCV gel. GCV is denoted by the green arrow. The onset of release begins one hour later for the PEGDA/G/GCV gel relative to the PEGDA/GCV gel, but still appears to be complete between 5 and 24 hours. (c) Graph of peak areas for both gels.
However, the G/GCV gel again rapidly dissociates between 5 and 24 hours despite being encapsulated within a covalently crosslinked polymer matrix. These results clearly demonstrate the potential of this system for controlled release, however, future systems would need to have guanosine covalently attached to a covalently crosslinked polymer network such that the release of the drug is completely dictated by the breaking of the supramolecular interactions\(^2\) between the drug and the guanosine as opposed to the concentration-induced breakup of the guanosine gel network.

2.3 Conclusions

We have shown that simple mixing of the commercially available non-gelating 2',3',5'-tri-O-acetylguanosine (\(\text{TAcG}\)) with the gelator guanosine (\(\text{G}\)) results in the formation of hydrogels in potassium chloride solutions that exhibit dramatically enhanced lifetime and thermal stability compared to those of \(\text{G}\) alone. Furthermore, we show that both the mechanical and thermal properties can be tailored by simply altering the ratio of the two components. Our studies suggest that a combination of changes in the fiber morphology (length and diameter) along with hydrophobic effects (enhanced by the addition of the \(\text{TAcG}\)) is critical to the thermomechanical behavior of these gels, consistent with the more hydrophobic \(\text{TAcG}\) being able to be incorporated into the G-stacks. Specifically, at room temperature, the modulus and yield stress, as well as the effective rod length of the cylindrical G quartets each uniformly decrease with increased \(\text{TAcG}\) content. This suggests that incorporation of multiple \(\text{TAcG}\) molecules into adjacent G-quartets can sterically disrupt the non-covalent interactions that stabilize the guanosine stack assemblies. The further destabilization of these interactions with increased temperature appears to be the principal factor determining the progressive...
increase in sol fraction with temperature up to the gel melting point. However, several observations indicate that the loss of these interactions is partially offset by the enhanced hydrophobicity, viz. (i) the unexpected dependence of \( T_{\text{gel-sol}} \) on \( T\text{AcG/G} \) composition, being highest for the gel of 50/50 composition, (ii) the storage modulus \((G'(\omega))\) of the 50/50 gel shows an increasing trend with temperature up to \( T_{\text{gel-sol}} \), and, (iii) the gel modulus at 50 °C indicates an opposite trend with \( G/\text{TAcG} \) composition to that observed at room temperature, i.e. \( G'(\omega) \) increases with increased \( \text{TAcG} \) content. We believe that these results, namely the use of a co-monomer, which can either be a gelator or (as reported herein) a non-gelator that contains the necessary supramolecular motif to allow formation of mixed assemblies, can be used to systematically tailor the properties of low molecular weight hydrogels. In this case, for example, we have shown that the lifetime stability of guanosine-based hydrogels, which has previously limited their usefulness, can be dramatically enhanced by simple incorporation of a guanosine-based non-gelator. As such it points to a facile route to dramatically increase the potential usefulness of such hydrogels in a number of arenas.

We have also shown some preliminary application work with regards to this type of mixed guanosine gel and its application in drug delivery. Stable, crystallization-free gels were achieved by mixing guanosine with the two guanosine-based drugs, acyclovir and ganciclovir. Unfortunately, the breakup of the gel network in the presence of large volumes of water caused nearly complete release over less than 24 hours, rather than having the release dictated by the breaking of the interactions between the guanosine moieties. The release was slightly delayed by mixing the guanosine-based gel into a covalently-crosslinked PEGDA gel; however, future work necessitates the covalent
attachment of guanosine to a crosslinked polymer system to hopefully yield more controlled release.

2.4 Experimental

2.4.1 Materials. Guanosine, 4-dimethylaminopyridine, triethylamine, acetic anhydride, hydroxylammoniumsulfate dihydrate, acyclovir, and ganciclovir were purchased from Sigma-Aldrich and perchloric acid and ammonium hydroxide were purchased from Fisher Scientific. All reagents were used without further purification. Solvents were distilled from suitable drying agents.

2.4.2 Synthesis of 2',3',5'-tri-O-acetylguanosine. 1.91 g guanosine and 86 mg 4-dimethylaminopyridine were placed in a flask and purged with argon. 25 mL of acetonitrile was added, followed by 5.65 mL of triethylamine. The mixture was cooled to 0 °C, and 2 mL of acetic anhydride was added. The reaction was allowed to proceed for 1 hour at 0 °C and 3 hours at room temperature, followed by quenching with 2 mL of methanol, and evaporation of the solvent until the volume was reduced to 10 mL. Approximately 50 mL of diethyl ether was added dropwise over 30 minutes, and the precipitate was collected and dried. The resulting material was stirred in 25 mL of acetone for 5 hours at 50 °C, filtered, and dried. 71% yield. \(^\text{1H NMR (600 MHz, DMSO-d}_6\):} \(\delta 10.70\) (s, 1H, NH), 7.90 (s, 1H, CH), 6.50 (s, br, 2H, NH\(_2\)), 5.95 (d, 1H, CH), 5.76 (t, 1H, CH), 5.46 (t, 1H, CH), 4.33 (m, 1H, CH\(_2\)), 4.28 (m, 1H, CH), 4.22 (m, 1H, CH\(_2\)), 2.08 (s, 3H, CH\(_3\)), 2.01 (d, 6H, CH\(_3\)). \(^{13}\text{C NMR:} \delta 170.74, 170.09, 169.92, 157.40, 154.75, 151.63, 136.25, 117.55, 85.08, 80.08, 72.87, 70.97, 63.74, 21.19, 21.04, 20.85.
Figure 2.27. $^1$H NMR (600 MHz, DMSO-d$_6$) of 2',3',5'-tri-O-acetylguanosine (TAcG).
2.4.3 Gel Preparation. For 2 wt % samples, desired amounts of guanosine and triacetylguanosine were transferred to 1 dram vials to which 1mL of water was added. The suspensions were sonicated until good dispersions were achieved (approximately 30 seconds). 1 mL of 0.708 M KCl was then added to each vial, and the suspensions were heated to 100 °C until they became clear. The resulting solutions were allowed to cool to room temperature upon which gelation was observed over approximately 5 minutes. In the case of fabricating more dilute gels, the KCl concentration was linearly scaled down by weight percent. For all experiments detailed below, fresh gels were made just prior to use.

**Figure 2.28.** $^{13}$C NMR of 2’,3’,5’-tri-O-acetylguanosine (TAcG).
2.4.4 Dynamic Light Scattering. In order to measure the critical gel concentration, dynamic light scattering measurements were completed with a Brookhaven Instruments BI-240 Goniometer and BI-9000 digital correlator. Samples at various concentrations were prepared individually and measurements were taken over 30 minutes at 25 °C at a scattering angle of 90°. The data were normalized and fit with a double exponential decay in Origin to obtain apparent diffusion coefficients and hydrodynamic radii.

2.4.5 Small Angle Neutron Scattering. SANS measurements were conducted on the time-of-flight small angle diffractometer at the Intense Pulsed Neutron Source facility of Argonne National Laboratory, IL. 2 wt % gels containing 40/60 TAcG/G, 50/50 TAcG/G, or 60/40 TAcG/G were prepared in 0.354 M KCl/D₂O solutions. The gels were contained in quartz cells and maintained at a temperature of 25 °C while data was acquired over 6 hours, and the signal of pure D₂O in a quartz cell was subtracted as the background. Microcal Origin™ 8.0 was used to perform linear least-squares curve fitting analysis. Matlab 7.5.0.342 was used to compute the scattering intensity integrated over the entire range of scattering vector. SANS Analysis v3.0 software from NIST Center for Neutron Research was used to fit the scattering curve on Igor Pro 6.0.

2.4.6 Wide-Angle X-Ray Diffraction. Wide angle x-ray diffraction (WAXD) was done on a Rigaku x-ray diffractometer at room temperature with Cu Kα irradiation at a voltage of 30 kV and a current of 30 mA. The spectra were recorded with a sampling width of 0.05 and a scan speed of 0.2 deg/min. Freeze-dried gel powder was crushed and laid into the sample holder, and a glass slide was forcibly run over the sample until a flat surface was achieved.
2.4.7 Atomic Force Microscopy. Atomic force microscopy was carried out on a Veeco di Multimode V instrument in air tapping mode with an antimony (n) doped silicon tip (spring constant 20-80 N/m). 10 μL of a heated gel solution was deposited onto freshly cleaved mica (SPI Supplies) and pressed between the mica and a glass slide to yield a thin layer that was allowed to gel for 30 minutes. The glass slide was subsequently removed, and the gel was allowed to air dry for 10 minutes prior to imaging.

2.4.8 Differential Scanning Calorimetry. DSC measurements were completed on a Mettler Toledo DSC822 instrument in hermetic pans at a heating rate of 3 °C/minute.

2.4.9 Variable-Temperature NMR. A 2 wt % sample of gel was prepared in a 0.354 M KCl/D₂O solution. While in the heated sol state, 20 μL of dioxane was added, and the solution was transferred to an NMR tube. The tube was immediately transferred to the NMR instrument at 75 °C and was allowed 15 minutes to equilibrate. The 75 °C measurement was then taken, after which the temperature was decreased to 65 °C and the sample allowed to equilibrate for 15 minutes. This process was repeated until the final measurement at 25 °C was completed.

2.4.10 Rheological Measurements. Rheological measurements were taken on an Anton Paar Physica MCR 501 rheometer with a 50 mm bottom plate and either a 25 mm parallel plate or a 25 mm cone (1° angle) on the top. The gap was fixed at 200 μm for the parallel plate apparatus and 53 μm for the cone and plate apparatus. To prevent water evaporation, the loaded samples were surrounded by low molecular weight polydimethylsiloxane.
For the oscillatory stress sweep study, the cone and plate apparatus was utilized. The samples were pre-sheared at 500% strain and an angular frequency of 30 rad/s and then were allowed to recover for 60 minutes under 0.2% strain at an angular frequency of 10 rad/s. Following recovery, the samples were subjected to a continuous shear ramp from 1 to 2000 Pa at a frequency of 10 rad/s, or until failure occurred. A pre-shear, as opposed to a thermal conditioning, was utilized to ensure that the concentration of each sample did not change as a result of evaporation after being loaded into the rheometer.

The temperature sweep measurements were completed using the parallel plate apparatus. The samples were subjected to the same pre-shear and recovery conditions as detailed above followed by a continuous temperature ramp from 25 °C to 80 °C at a strain of 0.2%, a frequency of 10 rad/s and a rate of 3 °C/minute.

The frequency sweep experiments were also completed using the parallel plate apparatus, and the samples were again subjected to the same pre-shear and recovery experiments as detailed above followed by a continuous frequency ramp from 0.01 to 100 Hz (need to change to angular) at a strain of 0.2% at a constant temperature between 25 and 70 °C.

2.4.11 HPLC Analysis of Drug Release. 5mL gel samples (0.354M KCl) were made and placed in a dialysis membrane filled with 0.354M KCl. The dialysis membrane was then placed in a beaker filled with 500 mL 0.354M KCl and stirred at room temperature. Aliquots for HPLC analysis were taken at specified time points and run with a mobile phase of 98/2 H₂O (0.1% TFA)/MeCN (0.082% TFA) to 75/25 for all samples.
2.5 Acknowledgements

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2.6 References


Chapter 3

Tailoring the properties of guanosine-based supramolecular hydrogels via chemical structure modification
3.1 Introduction

The use of supramolecular gels as scaffolds in tissue engineering has grown significantly over the past decade as alternatives to the more widely studied covalent polymer hydrogels. The inherent properties of supramolecular gels namely their ease of mechanical and chemical tailorability, implantation (injectability), and similarity in properties to natural tissue make them intriguing candidates for a range of biomedical applications. By and large, these gels are based on the self-assembly of peptide-amphiphile molecules, where the self-assembly depends, in part, on the peptide sequence of each molecule. These gels have proven themselves promising candidates for clinical use as they have shown good biocompatibility and the ability to differentiate stem cells based on the peptide sequence incorporated into the gelator. However, they also have limitations in that the properties of the resulting gels are generally modified with a change in peptide sequence, and synthesis and purification of the peptide-amphiphile molecules can be time-consuming and costly.

As an alternative, the natural occurrence and ease of modification of guanosine and its derivatives make hydrogels of these molecules excellent candidates for physiological applications from both a biocompatibility and a cost standpoint. However, as it has been shown in the previous chapter of this thesis, there have remained two severe limitations which hindered the potential use of guanosine gels in many applications – poor lifetime stability (e.g. with guanosine in aqueous potassium chloride crystallization occurs upon standing over a couple of hours at room temperature, leading to collapse of the gel) and/or the need for specific salt concentrations or pH values.
which are not conducive to \textit{in vivo} applications. For example, the monophosphates of guanosine also form gels, but only at low pH values when the phosphate is uncharged.\textsuperscript{12}

Since we have already addressed the prevention of crystallization of guanosine-based hydrogels, the next key challenge, therefore, was to design a system that gels cell media and/or physiologically relevant saline solutions. In the case of most guanosine systems, including our previous G/TAcG system detailed in Chapter 2, the concentration of the monovalent metal ion (as stated above) required to template the formation of the G-quartet and subsequent gelation is significantly higher than physiological concentrations (\textit{ca.} 5 mM KCl and 100 mM NaCl). To overcome this limitation, we hypothesized that a guanosine moiety that is more predisposed to self-assemble into the G-quartet would reduce the salt concentration required for gelation.

3.2 Results and Discussion

3.2.1 Achievement and Discussion of Physiologically-Relevant Gelation

As mentioned above, most guanosine derivatives require the formation of the G-quartet (Figure 3.1) and its subsequent self-assembly into stacks to result in the formation of hydrogels. An important component is this process is the presence of a templating metal ion (usually monovalent ions such as K\textsuperscript{+}), which stabilizes the formation of the macrocyclic G-quartet. We hypothesized that a guanosine moiety that is more predisposed to self-assemble into the G-quartet would reduce the salt concentration required to form a hydrogel and potentially allow gelation of cell media. It has been proposed that the guanosine moiety, which usually exists predominantly in the \textit{anti}-conformation, adopts the \textit{syn}-conformation (Figure 3.1) when incorporated into the G-
quartet. Thus it can be expected that increasing the population of the syn-conformation via chemical modification should allow for an overall decrease in salt concentration required for gelation. Sessler and co-workers have shown that metal free quartets in chlorinated solvents or the solid state can be formed if a bulky aryl group is placed on the 8-postion of guanosine and the ribose hydroxyl groups are protected as isobutyryl esters, although no gelation is reported for this system. Utilizing this knowledge, we found that placement of a smaller group (methoxy) on the 8-position of guanosine along with smaller esters (acetates) on the ribose hydroxyl groups does indeed result in a guanosine derivative that not only gels salt solutions at physiologically relevant concentrations (i.e. 100mM Na\(^+\)) but also cell media.

![Synthesis of 8-methoxyguanosine](image)

**Figure 3.1.** Guanosine prefers the anti conformation, which promotes the formation of linear tapes. The syn conformation must be achieved to promote quartet formation.

Synthesis of the 8-methoxyguanosine (8OMeG, 3.2) was accomplished in two steps from guanosine (G) via the bromination of the 8-position to yield 8-bromoguanosine (8BrG, 3.1), followed by reaction with sodium methoxide in methanol and DMSO to yield 8OMeG (Figure 3.2).
It is known that guanosine prefers the *anti*-conformation in a ratio of approximately 40% *syn* to 60% *anti*, while the 8-bromoguanosine (*8BrG*) shifts the equilibrium to predominantly the *syn* conformation (90% *syn*:10% *anti*). The ratio of equilibrium values is reflected by a downfield shift of the –CH proton in the 2′ position (Figure 3.3). By comparing the $^1$H NMR spectra of *G*, *8MeOG*, and *8BrG* (Figure 3.3) and extrapolating from the previously reported *syn:*anti data for *G* and *8BrG* we estimated that placement of the methoxy group on the 8-position shifts the *syn:*anti ratio of *8OMeG* to ca. 70:30. Interestingly, *8OMeG* was found to make stable gels at high KCl (354 mM) concentrations and metastable, fast crystallizing gels at more biologically relevant salt concentrations (*ca.* 5 mM KCl and 100 mM NaCl). Thus, while not what we are targeting, *8OMeG* does show a marked improvement in gelation over *G* at these concentrations, which either forms metastable gels that crystallize over a period of hours (354 mM KCl) or no gelation at all (*ca.* 5 mM KCl/100 mM NaCl). This does suggest...
that shifting the *syn:anti* ratio more toward the *syn* conformer does aid the aqueous gelling ability of the guanosine derivatives. It is important to note, however, that this is not the only parameter in play here. **8BrG** which has *syn:anti* ratio of ca. 90:10 only crystallizes/precipitates in either 5 mM KCl/100 mM NaCl or 354 mM KCl, without any gelation suggesting, as one would predict, that solubility of the gelator plays an important role as well.\(^{16}\)

**Figure 3.3.** \(^1\)H NMR of **G**, **8OMeG**, and **8BrG** in DMSO. The peaks from the protons at the 2’-position are highlighted by the dotted lines showing the steady conversion of the anti conformation to the syn conformation.
Given that Sessler has shown that metal-free G-quartets can be formed with an 8-substituted guanosine derivative that has isobutyryl esters on the ribose,\textsuperscript{13b} we prepared 8-methoxy-2’,3’,5’-tri-O-acetylguanosine (\textit{8OMeTAcG}) by acetylation of \textit{8OMeG} (Figure 3.2). Gratifyingly, we found that \textit{8OMeTAcG} was not only able to gel aqueous solutions containing physiologically relevant salt concentrations (100 mM NaCl/5mM KCl) at gelator concentrations as low as 0.5 wt. % but was also able to gel cell media (DMEM) with no crystallization observed over the course of a few months. Similar to the case of \textit{8BrG}, only crystallization/precipitation was observed for \textit{8BrTAcG}. In order to examine the effect of the templating cations on the formation of these gels we prepared a series of samples that vary in concentrations of either NaCl or KCl (Figure 3.4). Interestingly, \textit{8OMeTAcG} was found to behave very differently in NaCl solutions than it does in KCl solutions of the same concentration and actually exhibits a marked preference for gel formation in the NaCl solutions. This is unusual since the size of the quartet cavity generally dictates a preference for K\textsuperscript{+} ions.\textsuperscript{17} As can be seen in Figure 3.4, gels made with 100-400 mM NaCl exhibit sample-spanning gel behavior, while gels made with 100-400 mM KCl contain only small gel chunks that do not hold together but instead break apart upon vial inversion.
The preference of the 8OMeTAcG for NaCl is also shown in quite drastic differences in rheological behavior. The NaCl gels all exhibit measurable, consistent storage moduli (approximately $10^3$-$10^4$ Pa) and distinct yield stresses (approximately $10^1$-$10^2$ Pa) in the cone and plate rheometer (Figure 3.5a), while the KCl gels produce wavering modulus values no greater than $10^2$ Pa and no distinct yield stresses (Figure 3.5b).

**Figure 3.4.** 1 wt % 8OMeTAcG gels in 100-400 mM NaCl or KCl. As can be seen, the gels formed with NaCl are sample-spanning and hold together upon inversion, while the KCl gels do not.
Interestingly, there exists a gel strength dependence on the salt concentration for the NaCl gels, with a maximum in gel strength observed at 200-300mM NaCl. To further probe this behavior, TEM was carried out on 1 wt % 8OMeTAcG gels in 100-400 mM NaCl (Figure 3.6, respectively). The anticipated fibrous morphology exists for all four salt concentrations; however there does appear to be a growing population of larger fibrous aggregates as the NaCl concentration increases. The formation of larger aggregates is also consistent with the increasing opacity of the gels with increasing NaCl concentration. Additionally, a decrease in intensity of the WAXD peak corresponding to the width of a single stack also suggests increased aggregation (Figure 3.7). Thus, since the Na⁺ templates the assembly of 8OMeTAcG, it is within reason that increasing the salt concentration, up to a point, promotes further assembly, resulting in an increase in mechanical properties. However, a continued increase of the salt concentration could result competition for water solvation and thus a “salting out” / aggregation of the

Figure 3.5. (a) Stress sweeps of 1 wt % 8OMeTAcG in NaCl. Distinct modulus and yield stress values exist in addition to modification of their values with a change in NaCl concentration. (b) Stress sweeps of 1 wt % 8OMeTAcG in KCl. Wavering modulus and unclear yield stress values are present in addition to no KCl concentration dependence. T = 25 °C and ω = 6.28 rad/s.
$8\text{OMeTAcG}$ fibers yielding larger fibrous assemblies and subsequently decreased mechanical properties and more opaque gels.

Figure 3.6. TEM images of 1 wt % $8\text{OMeTAcG}$ in (a) 100 mM NaCl, (b) 200 mM NaCl, (c) 300 mM NaCl, and (d) 400 mM NaCl. There appears to be an increase in the population of larger fibrous aggregates as the NaCl concentration increases.
TEM of 1 wt % 8OMeTAcG in 100 and 400 mM KCl (Figure 3.8) on the other hand shows that the nanoscale morphology appears to be sheet-like rather than fibrous in nature. This highlights the dramatically different templating behavior of the Na\(^+\) and K\(^+\) ion with this gelator and is consistent with the weaker mechanical properties observed in the K\(^+\)-templated systems.

**Figure 3.7.** WAXD of 8OMeTAcG in 100 and 400 mM NaCl. The peaks ca. 24 Å correspond to the width of a single 8OMeTAcG stack, while the peaks ca. 3 Å correspond to the vertical distance between quartets. The decrease in intensity of the peak ca. 24 Å with increasing salt concentration suggests the increased presence of aggregates too large to be seen in WAXD.
To further probe the different templating effect of Na\(^+\) and K\(^+\) ions on the assembly of 8OMeTAcG circular dichroism measurements were performed on 1 wt % 8OMeTAcG gels in 100 mM NaCl and 100 mM KCl. The CD spectra are visibly quite different as a consequence of using the two different salts (Figure 3.9). Interestingly, the spectrum of 1 wt % 8OMeTAcG in 100 mM NaCl is exceedingly similar to the known spectrum of guanosine 5’-monophosphate, which has been proposed to form a continuous G-helix (Figure 3.11) rather than distinct G-quartets in aqueous solution. This is evidenced in the CD spectrum by the increased intensity at ca. 290 nm, which indicates a tilting or deformation of the tetrameric planes. The spectrum of 1 wt % 8OMeTAcG in KCl, however, is very similar in appearance to the spectrum of our previous G/TAcG system in 354 mM KCl as well as guanosine or guanosine 3’-monophosphate, which coincides with the expected behavior of a planar quartet system where there are maxima at ca. 240 nm and 260 nm. This then would explain the fiber forming capability of

![TEM images of 1 wt % 8OMeTAcG in (a) 100 mM KCl, (b) 400 mM KCl. Sheet morphology is observed for both samples rather than the nanofibrous morphology observed with NaCl.](image)
8OMeTAcG with NaCl and not KCl. The size of a G-quartet is fixed since it is a planar structure with set hydrogen bond lengths, whereas a helix has the ability to contract horizontally allowing more efficient binding of the smaller Na\(^+\) ions and gelation of NaCl solutions. This horizontal contraction is evident in the WAXD data (Figure 3.10), where peaks consistent with the width of a single guanosine-based fiber are observed at 24.5 Å for the Na\(^+\) system and 26.4 Å for the K\(^+\) system.

**Figure 3.9.** CD spectra of 1 wt % 8OMeTAcG in 100 mM NaCl and 100 mM KCl and 1 wt % 50/50 G/TAcG in 354 mM KCl.
The ribose acetate groups play an important role here as well. We have shown in mixed G/TAcG gels that TAcG acts as a G-stack terminator, with more TAcG resulting in the formation of smaller stacks. This is presumably a consequence of the steric bulk of the acetates hindering the extended growth of the stacks. This would explain why the K+ 

![Figure 3.10](image-url)  
**Figure 3.10.** WAXD of 8OMeTAcG in 100 mM NaCl and 100 mM KCl. The width of the stacks decreases from 26.37 to 24.53 Å when switching from potassium to sodium to allow gelation by the smaller sodium ion.

![Figure 3.11](image-url)  
**Figure 3.11.** Depiction of a G-quartet (orange) and a guanosine-based continuous helical structure (blue). The ability for the gelation of NaCl despite its smaller size could be facilitated by the horizontal contraction of the helix.

The ribose acetate groups play an important role here as well. We have shown in mixed G/TAcG gels that TAcG acts as a G-stack terminator, with more TAcG resulting in the formation of smaller stacks. This is presumably a consequence of the steric bulk of the acetates hindering the extended growth of the stacks. This would explain why the K+
templated G-quartets $8\text{OMeTAcG}$ do not form fibrous assemblies. However, the adjustable nature of a helical assembly allows for more vertical space to accommodate the presence of the acetate units and thus long fibrous assemblies can be formed with the smaller Na$^+$ cation. To examine this in more detail CD experiments were carried out with both $8\text{OMeG}$ and $8\text{OMeTAcG}$ at pregelation concentrations (0.3 wt. %) in 100mM NaCl (CD of the $8\text{OMeG}$ is difficult at higher concentrations due to crystallization). The CD spectrum (Figure 3.12) of $8\text{OMeG}$ is consistent with it forming G-quartet assemblies but the placement of the acetate groups on the ribose results in the dramatically different CD spectrum of $8\text{OMeTAcG}$, which is more consistent with a helical assembly.

![Figure 3.12. CD spectra of 0.3 wt. % $8\text{OMeG}$ and $8\text{OMeTAcG}$ in 100 mM NaCl.](image)

3.2.2 Biological evaluation of $8\text{OMeTAcG}$ gels as potential tissue engineering scaffolds

Since a guanosine derivative able to gel physiologically relevant solutions has now been identified, we examined whether $8\text{OMeTAcG}$ exhibits other desirable
characteristics for tissue engineering, namely a lack of cytotoxicity, the ability to culture cells within the three-dimensional gel network, injectability, and mechanical tailorability. As guanosine-based systems are relatively unexplored with respect to their use as tissue engineering scaffolds, we conducted a colorimetric cell proliferation assay (MTT assay) to ensure that 8OMeTAcG is non-toxic. 5,000 C166 cells suspended in 10 µL of DMEM were injected into 50 µL of various concentrations of 8OMeTAcG/DMEM (between 0 and 2 wt %) gel in a 96-well plate, covered with 100 µL of additional DMEM, and incubated for 48 hours followed by application of the dye solutions and measurement of absorbance at 570 nm. At low gelator concentrations, cell proliferation was statistically comparable to that on control surfaces (Figure 3.13a). At higher gelator concentrations (2%), there appeared to be a decrease in proliferation. One limitation of the MTT assay is that if cells are not able to adhere to a matrix, they will not proliferate, and therefore will have lower absorbance readings, when compared to control surfaces with good cell adhesion. To examine if this was the case we added 1 wt % gelatin to the gelation mixture. As gelatin is not expected to elicit additional survival advantages in the cells we concluded that the observed boost in cell proliferation (close to control levels) was attributed to better cell adhesion in the gelatin containing hydrogel, and that 8OMeTAcG hydrogels themselves had low to no toxicity.

To further evaluate cytotoxicity we conducted an apoptosis assay, evaluating cells grown in the presence of the 8OMeTAcG gel. To this end, 500,000 cells were suspended in 100 µL of DMEM and injected into 1 mL of 2 wt % 8OMeTAcG/DMEM gel. The gels were covered with an additional 2 mL of DMEM and incubated for 48 hours. Positive and negative controls were generated by plating at the same density on ordinary
tissue culture polystyrene, with the positive controls being plated in the presence of camptothecin to induce apoptosis. The cells were then treated with two labels: PE-Annexin V (to screen for early apoptosis) and 7-AAD (to screen for late apoptosis/necrosis); and evaluated by flow cytometry. As can be seen in Figure 3.13b, the fraction of cells undergoing apoptosis only increases about 10% in the presence of 8OMeTAeG gel with or without added gelatin (as compared to a > 60% reduction in viability when in the presence of a known apoptotic agent). Thus, the reduced absorbance signal seen in the MTT assay is more likely to be from reduced proliferation due to low adhesion, rather than a consequence of toxicity of the hydrogel.
Further confirmation of cell viability and gel penetration was done by imaging cells in a hydrogel. 100 µL of 2 wt % 8OMeTAcG + 1 wt % gelatin (to increase adhesion) was placed in a well of a 4-well Permanox chamber slide and covered with an additional 200 µL of DMEM. 10,000 GFP-labeled C166 cells in 10 µL of DMEM were injected into the gel, and the cells were cultured for 24 hours. As can be seen in both

Figure 3.13. (a) MTT assay with C166 cells of various concentrations of 8OMeTAcG/DMEM gel. The apparent decrease in viability is most likely due to a lack of adhesion, not cytotoxicity. (b) Flow cytometry evaluation of apoptosis/necrosis. Only a ~10% decrease in viability occurs when cells are incubated within the gel suggesting little toxicity.
confocal microscopy (Figure 3.14a) and standard fluorescence microscopy (Figure 3.14b), the cells have a healthy spindle-shaped morphology (as opposed to cells grown without gelatin, in Figure 3.14c) and are present within the bulk of the gel, again suggesting that the 8OMeTAcG/gelatin gel is suitable as a tissue engineering scaffold.

Figure 3.14. (a) Confocal microscopy (xy-projection and z-stack) of GFP+ C166 cells in a 2 wt % 8OMeTAcG/DMEM gel (with added gelatin). (b) Fluorescence optical microscopy image of GFP+ C166 cells in a 2 wt % 8OMeTAcG/DMEM gel (with added gelatin). (c) Fluorescence optical microscopy image of GFP+ C166 cells in a 2 wt % 8OMeTAcG/DMEM gel without gelatin.
To ensure that the added gelatin has little to no effect on the mechanical properties or morphology of a 2 wt % $8\text{OMeTAcG}$/DMEM gel, the standard stress sweep experiment (Figure 3.15a) as well as TEM (Figure 3.15b) were carried out on a 2 wt % $8\text{OMeTAcG}$/1 wt % gelatin gel in DMEM. The rheology data shows little difference between the two samples suggesting the gelatin does not affect the gel network, as was expected. Imaging the gel, however, proved to be incredibly difficult as a result of the presence of the various organic and inorganic cell media ingredients. It is clear that the $8\text{OMeTAcG}$ assembly is still fibrous in nature; however, since only large aggregates of material could be found on the grid, the image cannot be directly compared to a sample in a simple salt solution.

![Figure 3.15](image)

**Figure 3.15.** (a) Stress sweep of a 2 wt % $8\text{OMeTAcG}$ gel compared with a 2 wt % $8\text{OMeTAcG}$/1 wt % gelatin gel in DMEM showing the addition of gelatin does not affect the mechanical properties of the gel, where $\omega = 6.28$ rad/s, $T = 25$ °C, and $\gamma = 0.2\%$. (b) TEM of a 2 wt % $8\text{OMeTAcG}$/1 wt % gelatin gel in DMEM.

Since one potential advantage of a supramolecular gel over a standard covalently crosslinked polymer gel is injectability, we next performed a rheological shear recovery
experiment on 2 wt % 8OMeTAcG gels in both a saline solution and DMEM (Figure 3.16). The gels were heated to solutions and pipetted onto the rheometer plate, and the gelation was monitored at a stress of 0.1 Pa. After 30 minutes, a stress of 250 Pa was applied for 30 seconds to force the gels to yield. The gels were then monitored at a stress of 0.1 Pa for 30 minutes to measure their recovery. The data clearly shows that the mechanical integrity of the gels is mostly recovered within seconds highlighting their ability to recover from high shear and thus demonstrating their potential for use as injectable scaffold materials. To further illustrate this, we verified that the gels can be injected through a 26-gauge needle and recover to the gel state within seconds (Figure 3.16 inset).

![Figure 3.16](image)

**Figure 3.16.** Shear recovery of 2% 8OMeTAcG gels in 100 mM NaCl and DMEM. The gels were melted followed by monitoring of their recovery at a stress of 0.1 Pa for 30 minutes. The gels were then subjected to a stress of 250 Pa, which resulted in yielding, and their subsequent recovery was again monitored at 0.1 Pa for 30 minutes. $\omega = 6.28$ rad/s, $T = 25\, ^{\circ}C$. The inset is an image of a droplet of 8OMeTAcG gel being held vertically on a glass slide 30 seconds after being injected through a 26-gauge needle.
Finally, there is a growing body of evidence that supports the theory that the physical nature of the cellular microenvironment can have a profound effect on numerous cell processes including adhesion, cytoskeletal organization and even stem-cell differentiation. Therefore, an important property of an ideal tissue engineering gel is the ability to systematically and easily tune its mechanical properties. We have previously shown that the modulus and yield stress of $G/T\text{AcG}$ hydrogels can be tailored by simply altering the ratios of the two components. Thus we examined if a similar effect could be achieved with co-gels of $8\text{OMeTAcG}$ with the non-gelating $\text{TAcG}$ and found that stable co-gels could be obtained over a wide range of $8\text{OMeTAcG}:\text{TAcG}$ (10:0 to 3:7) in 100mM NaCl, where the total $8\text{OMeTAcG}/\text{TAcG}$ content is set at 2 wt %. Furthermore, shear rheology of these gels (Figure 3.17) shows that this co-gel approach is again effective to modulate both the storage modulus and yield stress over one to two orders of magnitude.

**Figure 3.17.** The storage modulus and yield stress of $8\text{OMeTAcG}$ gels in 100 mM NaCl can be systematically tailored by the addition of varying ratios of $\text{TAcG}$. $\omega = 6.28 \text{rad/s}$, $T = 25 \, ^\circ \text{C}$, $\gamma = 0.2\%$. 

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Presumably, at least part of the decrease in the mechanical properties observed in Figure 3.17 is on account of the decreasing concentration of the gelling component 8OMeTAcG (from 2 wt.% in the 10:0 gel to 0.6 wt.% in the 3:7 gel). Thus to examine if the TAcG is being incorporated into the 8OMeTAcG gel fibers, a further set of experiments were carried out on gel samples that contained a constant concentration of 8OMeTAcG (1 wt. %) with varying amounts TAcG. TEM of 8OMeTAcG:TAcG (1 wt. % : 0.2 wt. %) gel in 100 mM NaCl shows the same fibrous morphology as the 8OMeTAcG gel in 100 mM NaCl (Figure 3.18). Additionally, TAcG is itself not a gelator and cannot form fiber-like structures in 100mM aqueous NaCl, thus if TAcG is being incorporated into the 8OMeTAcG fibers then we might expect to see an effect on the mechanical properties of such a series of gels. Thus, both stress sweep (Figure 3.19a) and temperature sweep (Figure 3.19b) rheological experiments were carried out on a series of 1 wt. % 8OMeTAcG in 100 mM NaCl with 0, 0.2 and 0.5 wt. % TAcG added.

Figure 3.18. TEM image of a 8OMeTAcG:TAcG gel in 100 mM NaCl with 1 wt % 8OMeTAcG and 0.2wt% TAcG. There is no evident morphological difference between this sample and those of 8OMeTAcG alone.
The stress sweep experiments show that a drop in modulus of about an order of magnitude is observed between the 8OMeTAcG only gel and the gel which also contains 0.5 wt% TAcG with only slight differences in the yield point in these three gels being observed. Temperature sweep experiments show an even more dramatic difference and show both the decrease in modulus and a decrease in melting temperature upon addition of increasing amounts of TAcG. Both these observations are consistent with the incorporation of the non-gelating TAcG into the 8OMeTAcG fibers reducing their stability. It is interesting to note that particularly in the pure 8OMeTAcG gel that we observe a drop in modulus with temperature until ca. 50°C where we see a leveling off and even slight increase in modulus with temperature until ca. 55°C. This is an effect we have observed before in mixed G:TAcG gels Error! Bookmark not defined. and has been assigned to an increase in the contribution to the hydrophobic effect from the acetylated ribose moieties on the outside of the assemblies upon an increase in
temperature, which results in stronger fiber-fiber interactions within this temperature range. A further increase in temperature to above 60°C results in an increase in solubility of the gelator and destabilization of the other non-covalent interactions holding the helix together and thus a “melting” (the gel-sol transition) of the gel is observed.

**Figure 3.20.** VT-NMR data of a 2 wt % 50/50 8OMeTAcG/TAcG gel in D₂O. 20 µL of dioxane was added as an internal standard, and thus, by comparing the integrations of the TAcG peaks circled in red and the 8OMeTAcG peaks circled in green with the dioxane, it was established that the TAcG is over 50% incorporated into the gel state at 25 °C, while the 8OMeTAcG is approximately 95% incorporated at 25 °C.
As was done with the G/TAcG system in Chapter 2, variable temperature NMR experiments\textsuperscript{21} on a 2 wt % 50/50 8OMeTAcG/TAcG gel in D\textsubscript{2}O to determine extent of gelation confirmed that over 50% of the available TAcG and 95% of 8OMeTAcG is actually being incorporated into the gel with the rest remaining in the sol (Figures 3.20 and 3.21 and Tables 3.1 and 3.2). The calculations were made by comparing the integrations of both the acetyl peaks (approx. 2 ppm) and the –CH peaks (approx. 6 ppm) to an internal dioxane standard.

![Figure 3.21](image)

**Figure 3.21.** Zoomed VT-NMR data from Figure S14 of a 2 wt % 50/50 8OMeTAcG/TAcG gel in 100mM NaCl D\textsubscript{2}O solution. 20 µL of dioxane was added as an internal standard, and thus, by comparing the integrations of the TAcG peaks circled in red and the 8OMeTAcG peaks circled in green with the dioxane, it was established that the TAcG is over 50% incorporated into the gel state at 25 °C, while the 8OMeTAcG is approximately 95% incorporated at 25 °C.
Table 3.1. Peak integration values for the acetyl peaks (approx. 2 ppm) in the VT-NMR experiment shown in Figures 3.20 and 3.21.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Percent Gelation According to G</th>
<th>Percent Gelation According to TAcG</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 °C</td>
<td>0% (assumed)</td>
<td>0% (assumed)</td>
</tr>
<tr>
<td>65 °C</td>
<td>2.5%</td>
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</tr>
<tr>
<td>45 °C</td>
<td>65.8%</td>
<td>31.4%</td>
</tr>
<tr>
<td>35 °C</td>
<td>83.1%</td>
<td>42.7%</td>
</tr>
<tr>
<td>25 °C</td>
<td>93.1%</td>
<td>51.4%</td>
</tr>
</tbody>
</table>

Table 3.2. Peak integration values for the CH peaks (approx. 6 ppm) in the VT-NMR experiment shown in Figures 3.20 and 3.21.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Percent Gelation According to G</th>
<th>Percent Gelation According to TAcG</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 °C</td>
<td>0% (assumed)</td>
<td>0% (assumed)</td>
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</tr>
<tr>
<td>35 °C</td>
<td>96.5%</td>
<td>54.1%</td>
</tr>
<tr>
<td>25 °C</td>
<td>Negligible Integration</td>
<td>59.6%</td>
</tr>
</tbody>
</table>

3.2.3 Other 8-functionalized guanosine derivatives

Since the major theme of this thesis is the intentional design of supramolecular gelators, we set out to examine the effect of modifying the functional group at the 8-
position as well as the inclusion or exclusion of acetyl groups. As was earlier mentioned, 8BrG and 8BrTAcG do not exhibit gelation but rather crystallization, but 8OMeG (Figure 3.22a, 3.2), which is significantly easier to solubilize in water than 8OMeTAcG, gels 0.354M KCl (like the G/TAcG system) with no observed crystallization, although the formed gel is opaque in appearance (Figure 3.22b). Again, unlike the previous G/TAcG system, 8OMeG was also able to gel a physiologically relevant 109.5 mM NaCl/5.36 mM KCl mixture (Figure 3.22c), however, the resulting gel is unstable, thus exhibiting the importance of the acetyl groups for stable gelation.

![Chemical structure of 8-methoxyguanosine (8OMeG, 3.2).](a) (b) Gel made from 2 wt % 8OMeG in 0.354M KCl. (c) Crystallizing gel made from 2 wt % 8OMeG in a mixture of 109.5 mM NaCl/5.36 mM KCl.

We next evaluated molecules containing the same chain length at the 8-position as the methoxy group, namely 8-vinylguanosine (8VG, Figure 3.23a, 3.5), 8-acetyleneguanosine (8AG, Figure 3.23b, 3.6), and 8-vinyl-2’,3’,5’-tri-O-acetylguanosine
(8VTAcG, Figure 3.23c, 3.7), all of which were graciously provided by the Sessler research group at the University of Texas.

8-vinylguanosine and 8-vinyl-2’,3’,5’-tri-O-acetylguanosine exhibited some solubility upon heating in aqueous salt solution with 8VG being the more soluble of the two, however no gelation was observed in 100 mM NaCl or 0.354M KCl solutions. We were, however, able to make stable, crystallization free gels by mixing the more soluble guanosine with 8-vinylguanosine in different ratios (Figure 3.24) at a total concentration of 2 wt % in 0.354M KCl. Changing the ratio of the two components resulted in a small amount of tailorability of mechanical properties, although this tailorability was significantly reduced compared to the G/TAcG and the 8OMeTAcG/TAcG systems previously discussed. 8-acetyleneguanosine was completely insoluble in water, even with heating.

**Figure 3.23.** Chemical structures of 8-vinylguanosine (8VG, 3.5, a), 8-acetyleneguanosine (8AG, 3.6, b), and 8-vinyl-2’,3’,5’-tri-O-acetylguanosine (8VTAcG, 3.7, c). All three compounds were provided by the Sessler Research Group.
As a result of limited solubility in the absence of a heteroatom in the 8-position, we attempted to move toward the use of larger, heteroatom-containing functional groups to hopefully further lower the salt concentration necessary for gelation via additional steric bulk. To this end, we synthesized 8-ethoxyguanosine (8OEtG, 3.8, Figure 3.25a). 8OEtG was found to be only partially soluble in water, and though it was able to gelate 0.354M KCl without crystallization, its limited solubility resulted in particulate-like gels. Since 8OMeTAcG is significantly less water-soluble than 8OMeG, it is likely that 8-ethoxy-2’,3’;5’-tri-O-acetylguanosine (8OEtTAcG, 3.9, Figure 3.19b) will be difficult to solubilize in water, and as such, it was not synthesized. Along these lines, we attempted the syntheses of ethylene oxide-based guanosine derivatives 8-(2-methoxyethylamino)-guanosine (3.10, Figure 3.20a) and 8-(2-methoxyethoxy)-guanosine (3.11, Figure 3.20b) without success due to limitations in purification methods as detailed in the experimental section.

Figure 3.24. 2 wt % gels made from varying ratios of G:VG and their accompanying mechanical behavior.
3.3 Conclusions

In conclusion, 8OMeTAcG is a new, easy-to-access hydrogelator that shows great potential for use in tissue engineering. Gels can be easily formed at low concentrations (0.5-2 wt %) in both saline solutions and cell media and boast little-to-no cytotoxicity as well as facile injectability, and mechanical tailorability. Both the modulus and yield stress can be easily manipulated allowing these systems to be systematically studied as remoldable tissue scaffolds. Finally, the ability to access co-gels of 8OMeTAcG with
other guanosine moieties (e.g. TAcG) suggests that it may be possible to target co-gels with peptide functionalized guanosine derivatives e.g. with a desired peptide sequence to help promote cell adhesion, migration, proliferation or differentiation, without significant adverse effects on the mechanical integrity of the gel. Combined, these attributes suggest that 8OMeTAcG is a potentially exciting new platform for a universally applicable tissue engineering scaffold.

We also took an in-depth look at the dependence of 8OMeTAcG on its salt environment. It was found that 8OMeTAcG was able to make sample-spanning gels in NaCl with robust mechanical properties and a fibrous morphology on the nanoscale. Conversely, 8OMeTAcG in KCl made gels with little bulk stability and a sheet-like morphology on the nanoscale. The difference of assembly to lead to these results in the bulk is evident in circular dichroism spectra, where 8OMeTAcG appears to form a continuous helix in NaCl, while it appears to form the standard quartets in KCl.

Finally, we looked at the effect of changing the functional group on the 8-position of guanosine as well as the affect of the acetyl groups on the ribose ring. 8OMeG and 8OEtG were found to gel 0.354 M KCl without crystallization; however, they formed crystalline gels in 100 mM NaCl, which suggests the acetyl groups play a key role in stabilizing the assembly structure. Derivatives without heteroatoms (8VG, 8VTAcG, and 8AG) were partially to completely insoluble and did not gel any aqueous solution on their own. 8VG, however, did form stable gels in 0.354 M KCl when mixed with guanosine. Finally, it was shown that more than one heteroatom must be present to ensure solubility if the group at the 8-position is larger than a methoxy group.
3.4 Experimental

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

3.4.2 Synthesis of 8-Bromoguanosine (3.1). In one flask, 3 mL of bromine was added to 300 mL of distilled water and stirred for approximately 15 minutes to dissolve. In another flask, 10 g of guanosine was suspended in 60 mL of distilled water and to it was added the bromine solution in 10-20 mL aliquots at a rate where the reaction was allowed to return to a colorless state between additions. Once the reaction remained a yellow color, the resulting solid was filtered and washed with 60 mL cold water and 30 mL cold acetone. The product was recrystallized from distilled water in 20 mL vials, where approximately ¼ of the vial was filled with product and the rest with water. Quantitative yield. $^1$H NMR (DMSO-D$_6$): $\delta$ 10.78 (s, 1H, NH), 6.47 (s, 2H, NH$_2$), 5.66 (d, 1H, CH), 5.42 (d, 1H, OH), 5.06 (d, 1H, OH), 4.98 (t, 1H, OH), 4.89 (m, 1H, CH), 4.11 (m, 1H, CH), 3.83 (m, 1H, CH), 3.62 (m, 1H, CH$_2$), 3.50 (m, 1H, CH$_2$). $^{13}$C NMR (DMSO-D$_6$): $\delta$ 156.1, 154.2, 152.7, 121.8, 118.2, 90.3, 86.5, 71.2, 71, 62.7.
Figure 3.27. $^1$H NMR of 8-bromoguanosine (3.1) in DMSO.

Figure 3.28. $^{13}$C NMR of 8-bromoguanosine (3.1) in DMSO.
3.4.3 Synthesis of 8-Methoxyguanosine (3.2, 8OMeG). 730 mg 8-bromoguanosine was placed in a flask and purged with argon. 15 mL DMSO was added, and the mixture was stirred until the 8-bromoguanosine dissolved. A suspension of 900 mg sodium methoxide in 8 mL of methanol was added to the reaction, and the reaction was allowed to proceed for 18 hours at 65 °C under argon. The reaction was then cooled to room temperature and neutralized with approximately 1.5 mL of acetic acid. The neutralized solution was added dropwise to 400 mL of diethyl ether, and the resulting precipitate was collected by filtration and washed with cold acetone. After drying, the product was recrystallized from distilled water in 20 mL vials, where approximately ¼ of the vial was filled with product and the rest with water. 67% yield. $^1$H NMR (DMSO-D$_6$): $\delta$ 10.53 (s, 1H, NH), 6.27 (s, 2H, NH$_2$), 5.57 (d, 1H, CH), 5.28 (d, 1H, OH), 4.98 (d, 1H, OH), 4.82 (t, 1H, OH), 4.69 (m, 1H, CH), 4.02 (m, 1H, CH), 3.93 (s, 3H, OCH$_3$), 3.75 (m, 1H, CH), 3.53 (m, 1H, CH$_2$), 3.42 (m, 1H, CH$_2$). $^{13}$C NMR (DMSO-D$_6$): $\delta$ 156.3, 153.7, 152.3, 151, 111.6, 86.7, 85.8, 71.2, 62.7, 57.1.
Figure 3.29. $^1$H NMR of 8-methoxyguanosine (3.2) in DMSO.

Figure 3.30. $^{13}$C NMR of 8-methoxyguanosine (3.2) in DMSO.
3.4.4 Synthesis of 8-Methoxy-2’,3’,5’-Tri-O-Acetylguanosine (3.3, 8OMeTAcG). 23

250 mg 8-methoxyguanosine and 10 mg 4-dimethylaminopyridine were placed in a flask and purged with argon. 10 mL of acetonitrile was added, followed by 668 µL of triethylamine. The mixture was cooled to 0 ºC, and 236 µL of acetic anhydride was added. The reaction was allowed to proceed for 1 hour at 0 ºC and 3 hours at room temperature, followed by quenching with 600 µL of methanol, and evaporation of the solvent. The residue was dried and recrystallized from approximately 10 mL of 2-propanol. 83% yield. 1H NMR (DMSO-D6): δ 10.6 (s, 1H, NH), 6.4 (s, 2H, NH2), 5.86 (t, 1H, CH), 5.78 (d, 1H, CH), 5.46 (t, 1H, CH), 4.32 (m, 1H, CH), 4.23 (m, 1H, CH2), 4.13 (m, 1H, CH2), 3.97 (s, 3H, OCH3), 2.07 (s, 3H, COCH3), 2.03 (s, 3H, COCH3), 1.99 (s, 3H, COCH3). 13C NMR (DMSO-D6): δ 170.7, 170, 156.3, 154, 151.5, 150.6, 112, 84.2, 79.3, 71.5, 70.6, 63.3, 57.6, 21.1, 21, 20.9. MALDI-MS (α-cyano/NaCl): 461.5 [M+Na].
Figure 3.31. $^1$H NMR spectrum of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (3.3) in DMSO.

Figure 3.32. $^{13}$C NMR spectrum of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (3.3) in DMSO.
Figure 3.33. COSY spectrum of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (3.3) in D$_2$O.

Figure 3.34. HMBC spectrum of 8-Methoxy-2',3',5'-Tri-O-Acetylguanosine (3.3) in D$_2$O.
3.4.5 Synthesis of 8-bromo-2′,3′,5′-tri-O-acetylguanosine (3.4). 275 mg 8-bromoguanosine and 10 mg 4-dimethylaminopyridine were placed in a flask and purged with argon. 10 mL of acetonitrile was added, followed by 668 µL of triethylamine. The mixture was cooled to 0 °C, and 236 µL of acetic anhydride was added. The reaction was allowed to proceed for 1 hour at 0 °C and 3 hours at room temperature, followed by quenching with 600 µL of methanol, and evaporation of the solvent. The residue was dried and recrystallized from approximately 10 mL of 2-propanol. 83% yield. $^1$H NMR (DMSO-D$_6$): δ 10.93 (s, 1H, NH), 6.59 (s, 2H, NH$_2$), 5.98 (t, 1H, CH), 5.86 (d, 1H, CH), 5.62 (t, 1H, CH), 4.38 (m, 1H, CH$_2$), 4.30 (m, 1H, CH$_2$), 4.18 (m, 1H, CH$_2$), 2.08 (s, 3H, COCH$_3$), 2.04 (s, 3H, COCH$_3$), 1.96 (s, 3H, COCH$_3$). $^{13}$C NMR (DMSO-D$_6$): δ 170.79, 170.10, 156.09, 154.47, 152.58, 120.78, 117.88, 88.31, 79.95, 71.95, 70.57, 63.41, 21.14, 20.97, 20.91.
Figure 3.36. $^1$H NMR spectrum of 8-Bromo-2',3',5'-Tri-O-Acetylguanosine (3.4) in DMSO.

Figure 3.37. $^{13}$C NMR spectrum of 8-Bromo-2',3',5'-Tri-O-Acetylguanosine (3.4) in DMSO.
3.4.6 8-vinylguanosine, 8-acetyleneguanosine, and 8-vinyl-2’,3’5,’-tri-O-vinylguanosine (3.5-3.7). All three compounds were graciously provided by the Sessler Research Group at the University of Texas.

3.4.7 Synthesis of 8-ethoxyguanosine (3.8, 8OEtG). 730 mg 8-bromoguanosine was placed in a flask and purged with argon. 15 mL DMSO was added, and the mixture was stirred until the 8-bromoguanosine dissolved. A suspension of 1134 mg sodium ethoxide in 8 mL of ethanol was added to the reaction, and the reaction was allowed to proceed for 18 hours at 65 °C under argon. The reaction was then cooled to room temperature and neutralized with approximately 1.5 mL of acetic acid. The neutralized solution was added dropwise to 400 mL of diethyl ether, and the resulting precipitate was collected by filtration and washed with cold acetone. After drying, the product was recrystallized from distilled water in 20 mL vials, where approximately ¼ of the vial was filled with product and the rest with water. 64% yield. $^1$H NMR (DMSO-D$_6$): δ 10.53 (s, 1H, NH), 6.29 (s, 2H, NH$_2$), 5.57 (d, 1H, CH), 5.30 (d, 1H, OH), 4.98 (d, 1H, OH), 4.82 (t, 1H, OH), 4.68 (m, 1H, CH), 4.34 (m, 2H, CH$_2$), 4.01 (m, 1H, CH), 3.74 (m, 1H, CH), 3.53 (m, 1H, CH$_2$), 3.41 (m, 1H, CH$_2$), 1.32 (t, 3H, CH$_3$).
3.4.8 **8-ethoxy-2’,3’,5’-tri-O-acetylguanosine (3.9), 8-(2-methoxyethylamino)-guanosine (3.10), and 8-(2-methoxyethoxy)-guanosine (3.11).** 8-ethoxy-2’,3’,5’-tri-O-acetylguanosine was not synthesized due to the moderate insolubility of 8-ethoxyguanosine, since adding acetyl groups further increases the hydrophobicity of the molecule. The syntheses of 8-(2-methoxyethylamino)-guanosine (3.9) and 8-(2-methoxyethoxy)-guanosine (3.10) were attempted without success due to restrictions on purification methods. Briefly, the synthesis of 3.9 involved refluxing 730 mg of 8BrG in 15 mL of 2-methoxyethylamine for 16 hours under argon. The residual amine was

![Figure 3.38. $^1$H NMR spectrum of 8-ethoxyguanosine (3.8) in DMSO.](image)
evaporated and the residue dried. Analysis of the crude product via NMR showed only approximately 50% conversion. Increasing the reaction time to 3 days and the temperature by 5 degrees resulted in only approximately 70% conversion according to NMR. The converted product could not be separated from the starting material via recrystallization from water or alcohols. Without access to prep-scale HPLC, the synthesis was abandoned.

The synthesis of 3.10 also suffered a similar fate. 5 mL of DMSO and 1.25 mL of 2-methoxyethanol was added to 384 mg of sodium hydride and stirred at 65 °C for 30 minutes. To the mixture was added 730 mg of 8BrG in 5 mL of DMSO, and the mixture was stirred for 16 hours at 65 °C. The reaction was neutralized with approximately 2 mL of acetic acid but could not be precipitated into diethyl ether as usual. It was found that adding 5 mL of methanol to the reaction mixture after quenching allowed for precipitation into 400 mL of diethyl ether. In order to prevent the precipitate from turning into an oil during and after filtration, it was necessary to pour off 350 mL of the ether and stir the resulting sludge in 100 mL of acetone for 15 minutes. The residue could then be collected by filtration. Regrettably, the entirety of the residue was soluble in water and partially soluble in alcohols. Despite that the crude NMR showed >90% conversion, the residue could not be cleaned of the residual salts generated during the reaction. Proceeding to the next reaction with the crude material to put acetyl groups on the material in the same way as the synthesis of 8OMeTAcG was unsuccessful due to insolubility of the crude material in acetonitrile. Again, without access to prep-HPLC, the synthesis was abandoned.
3.4.9 General procedure for testing of gelation ability. 20 mg of a molecule or molecules of interest was/were suspended in 1 mL of distilled water (or aqueous solution) in a 1 Dram vial. The suspension was heated to approximately 100 °C until dissolution was achieved (about one minute maximum). The sample was then allowed to cool to room temperature upon which gelation, and/or crystallization was observed. To make the gels with 1% gelatin for cell culture, a heated solution of 4 wt % 8OMeTAcG in DMEM was mixed with an equal volume of a heated 2 wt % solution of gelatin. The resulting solution was mixed via shaking over heat and allowed to cool.

3.4.10 Rheology. Rheological measurements were taken on a TA AR2000ex rheometer with a Peltier temperature control system and a 20 mm cone (1° angle) with a solvent trap. For the oscillatory stress sweep study, the samples were pre-sheared at 500% strain and an angular frequency of 30 rad/s and then were allowed to recover for 60 minutes under 0.2% strain at an angular frequency of 6.28 rad/s. Following recovery, the samples were subjected to a continuous shear ramp from 0.1 to 1000 Pa at a frequency of 6.28 rad/s. A pre-shear, as opposed to a thermal conditioning, was utilized to ensure that the concentration of each sample did not change as a result of evaporation after being loaded into the rheometer. In the case of the shear recovery experiment, the gels were melted followed by monitoring of their recovery at a stress of 0.1 Pa for 30 minutes. The gels were then subjected to a stress of 250 Pa, which resulted in yielding, and their subsequent recovery was again monitored at 0.1 Pa for 30 minutes. All portions of the experiment were done with \( \omega = 6.28 \text{ rad/s} \) and \( T = 25 ^\circ \text{C} \).
3.4.11 TEM. Transmission Electron Microscope measurements were carried out on a Zeiss Libra 200EF microscope utilizing carbon-coated 200 square mesh copper grids. To prepare the samples for analysis, a piece of gel was placed on the grid and allowed to remain for five minutes. The piece was then gently removed with filter paper and the grid allowed to air dry overnight. The following day, the samples were sputter-coated under vacuum with a palladium layer approx. 1 nm in thickness for visualization purposes.

3.4.12 Circular Dichroism. 1 wt % 8OMeTAcG gels were placed into a 0.1 mm quartz cuvette and analyzed on a Jasco J-810 CD spectrometer from 325-200 nm with 5 scans per sample.

3.4.13. MTT Cell Proliferation Assay. 50 µL of appropriately concentrated 8OMeTAcG/DMEM/(Gelatin) gel (or straight DMEM in the case of the negative controls) was plated into a 96-well plate so that each sample was done in triplicate. The wells on the periphery of all four sides of the plate were not used and instead filled with DMEM or PBS so as to prevent evaporation over the course of the experiment due to the small well size. The plate was allowed to equilibrate at 37 °C for 30 minutes, after which C166 cells were injected at a density of 5,000 cells/well (in a minimal amount (10 µL) of DMEM) into the gel or DMEM, covered with an additional 100 µL of DMEM, and incubated for 48 hours at 37 °C. Next, 15 µL of the dye solution was added to each well (mixing the contents of each well with the pipette during addition), and the plate was incubated for an additional 4 hours at 37 °C. 100 µL of the solubilization/stop mix was
then added to each well, and the plate was wrapped in foil and allowed to incubate in the sterile hood for 1 hour. The contents of each well were then mixed and their absorbance read with a Tecan Safire plate reader (XFLUOR4 Software, COS96ft Plate Definition File, 4 reads/well, 10 flashes, 100 ms between move and read) at a wavelength of 570 nm with a reference wavelength of 650 nm. To convert absorbance values into the normalized absorbance (and hence % viability), the values from each set of 3 wells were averaged and divided by the averaged absorbance value of the negative control.

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

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

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

3.5 Acknowledgements

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3.6 References


Chapter 4

Nonionic surfactant-induced stabilization and tailorability of sugar-amphiphile gels
4.1 Introduction

Beyond the widely studied areas of supramolecular gels in biological applications, such materials are also potentially useful as rheology modifiers in commercial water-based personal care formulations. Historically, covalent polyelectrolytes have been utilized (one example is Carbopol®, a crosslinked polyacrylic acid manufactured by The Lubrizol Corporation), which is limited by the salt conditions under which high viscosity can be maintained. Additionally, nonionic covalent polymers such as PEG-based associative thickeners, cellulose ethers, guar gum and other polysaccharides have also found use, but they tend to be deficient in terms of texture in that they exhibit long (stringy) rheology as opposed to the preferred short rheology (buttery) state.

Supramolecular gelators have the potential to overcome these known deficiencies in that several interactions, most notably hydrogen bonding and the hydrophobic effect, can be achieved regardless of salt conditions. Additionally, since supramolecular gels are not made from entangled covalent polymer chains, shorter rheological behavior can be achieved.

Amphiphilic sugar gelators are one class of materials that fits the parameters listed above. They rely on hydrogen bonding and the hydrophobic effect for assembly, and as such should be robust to salt concentrations as opposed to charged polymers. Additionally, they are typically synthesized from inexpensive, commercially available materials (lactones and amines, Figure 4.1) via simple reactions, and gels can be made at very low monomer concentrations, both of which are important aspects to consider for potential commercial products.
There has been a wide range of sugar-based gelators that have been developed over the years.\textsuperscript{1} The relatively simple and easy-to-access amphiphilic sugar-based small molecule hydrogelator, N-octyl-D-gluconamide ((C\textsubscript{8}-DGlu, 4.1), Figure 4.2), and several related molecules have been extensively studied by Pfannemüller and Welte, et al.\textsuperscript{2-5} and Furhop et al.\textsuperscript{6-11} It has been suggested through TEM and molecular modeling that C\textsubscript{8}-DGlu assembles into quadruple helices of ribbons, which lead to fibrous gel formation around 1 wt %. These ribbon-like assemblies do differ from the recrystallized state, where the C\textsubscript{8}-DGlu is packed into parallel sheets. However, like many small molecule gelators, over the course of a few hours, the molecules rearrange from these ribbons into crystallites, and the gel behavior is lost. Fuhrhop et al. have shown, however, that these gels can be stabilized both in the short term in the presence of phosphotungstic acid (PTA)\textsuperscript{6,12} and for at least five months in the presence of the charged surfactant, sodium dodecylsulfate (SDS).\textsuperscript{9,12} While no mechanism for PTA stabilization is elucidated, the

\textbf{Figure 4.1.} Proposed assembly of sugar-based hydrogelators based on hydrogen bonding and the hydrophobic effect.
authors hypothesize that the micelles formed by the SDS solubilize any C₈-DGlu crystal nuclei that begin to form before they can induce macroscale crystallization. However, the authors also note that for 2 wt. % C₈-DGlu gels can only be formed with a molar ratio of up to 2.5:1 C₈-DGlu:SDS. Any additional SDS added results in lack of gelation, and consequently, they argue that the critical gel concentration for C₈-DGlu gelation had not been reached as a result of the added surfactant.

As part of our ongoing research program aimed at investigating potential methods of systematically tuning the properties of supramolecular gels, it was shown in Chapter 2 of this thesis that the lifetime stability of guanosine-based hydrogels gel can be

![Image of proposed assembly of C8-DGlu in water. TEM and molecular modeling suggest helical ribbons or threads.](image)

**Figure 4.2.** Proposed assembly of C8-DGlu in water. TEM and molecular modeling suggest helical ribbons or threads.
dramatically enhanced by simple addition of a non-gelating additive. In addition, we demonstrated that the gels’ thermomechanical properties can be systematically tailored by simply altering the ratio between the components of the gel. With this knowledge and intrigue with respect to the Fuhrhop work on the C$_8$-DGlu/SDS system, we were interested in further exploring the impact of surfactants on low molecular weight gelators, particularly nonionic surfactants that are structurally more similar to C$_8$-DGlu (Figure 4.3a). The commercially available Laureth-n (Ln, Figure 4.3b) series, which contains alkyl moieties attached to an oligo(ethylene glycol) chain (n being the number of ethylene glycol repeat units), was chosen as we could investigate a series of such surfactants with different hydrophobic/hydrophilic ratios. We also explored a handful of other surfactants as well as several additional amphiphilic sugar gelators in an effort to elucidate the structure-property relationship of this class of gels to further the ability to intentionally design supramolecular gelators.

![Chemical structures of (a) N-octyl-D-gluconamide (C$_8$-DGlu, 4.1), and (b) Laureth-n (Ln).](image)

**Figure 4.3.** Chemical structures of (a) N-octyl-D-gluconamide (C$_8$-DGlu, 4.1), and (b) Laureth-n (Ln).
4.2 Results and Discussion

4.2.1 Characteristics of N-octyl-D-gluconamide/Laureth-4 gels

N-octyl-D-gluconamide (C₈-DGlu, Figure 4.3a) is easily accessed via the reaction of octylamine with D-gluconolactone in quantitative yield. When heated, it readily dissolves in water or aqueous salt solution and gels within a couple of minutes upon cooling. However, almost immediately crystallites start to form within these gels resulting in the expulsion of water and collapse of the gel. To examine the effect of mixing the nonionic commercially available surfactant Laureth-4 with C₈-DGlu, we prepared a series of samples that contained a total concentration of 2 wt % in water and varied the weight ratio of the two components (C₈-DGlu:Laureth-4) ranging from 10:0, 9:1,…1:9, 0:10 (Figure 4.4). The samples were heated until dissolution, and upon cooling to room temperature gel formation was observed when the C₈-DGlu:L₄ weight ratio was greater than or equal to 3:7 (Figure 4.4). The rate of gel formation does, however, appear to depend on the amount of surfactant; it takes up to one hour to form a mechanically stable gel with a C₈-DGlu:L₄ weight ratio of 3:7, whereas gels with lower amounts of surfactant form within minutes. Gratifyingly, gels with a C₈-DGlu:L₄ weight ratio between 4:1 and 3:7 (which equates to molar ratios of approximately 5:1 C₈-DGlu:L₄ to 1:2 C₈-DGlu:L₄) all exhibit significant life-time stabilities (>6 months). All of these gels are translucent to opaque with the translucency slightly increasing with L₄ content. With a C₈-DGlu ratio higher than 4:1 C₈-DGlu:L₄, the gels that are formed crystallize over a period of a few minutes to a few hours.
The enhanced lifetime stability of these two-component gels allowed us to examine their properties in greater detail. The rheological properties of the C₈-DGlu/L₄ gels depend heavily on the ratio of C₈-DGlu:L₄. As shown by oscillatory stress sweeps, as the weight ratio of C₈-DGlu:L₄ decreases, both the storage modulus (G′) and yield stress (defined as the point where there is a sudden decrease in G′) show decreasing trends (Figure 4.5). This can be explained, at least in part, by the fact the concentration of the gelating molecule (C₈-DGlu) is decreasing with increasing L₄ content from 1.6 wt % in the 4:1 C₈-DGlu:L₄ sample to 0.6 wt % in the 3:7 C₈-DGlu:L₄ sample. However, no gelation is observed for C₈-DGlu alone at or below a concentration of 1 wt %, which suggests some contribution by the surfactant to the gelation of this system. Thus by simply changing the ratio of the two components hydrogels can be accessed with storage modulus and yield stress values that span three orders of magnitude.

**Figure 4.4.** 2 wt % Gels and suspensions created from various ratios of C₈-DGlu:L₄. From 10:0 to 9:1 C₈-DGlu:L₄, the resulting gels were unstable and crystallized over time. From 4:1 to 3:7 C₈-DGlu:L₄, gels stable over at least six months were formed. From 1:4 to 0:10 C₈-DGlu:L₄, no gelation was observed.
In an effort to probe how the C8-DGlu:L4 ratio is contributing to the tailorability and lifetime stability of the gels, samples were prepared for TEM analysis (Figure 4.6) at ratios of 4:1 C8-DGlu:L4, 1:1 C8-DGlu:L4, and 3:7 C8-DGlu:L4. Helical ribbons of approximately 10-20 nm in diameter (consistent with C8-DGlu literature) were observed in all of the samples in addition to what appear to be micellar aggregates. Generally speaking, these micellar aggregates appear to grow in size with increasing L4 content.

From the analysis and comparison of these images, the C8-DGlu, as would be expected from the literature, is clearly the key contributor to the fibrous network and L4 contributes to the formation of micellar aggregate species. It is not clear from this data whether the L4 aggregates interact with the C8-DGlu fibers in the gel phase, or if the aggregates are contained solely within the sol phase. In addition, we cannot rule out the possibility that the L4 somehow interacts with the fibers e.g. by forming a thin (possibly monolayer) coating on the C8-DGlu fibers that cannot be resolved in the TEM. It should

Figure 4.5. Stress sweeps of C8-DGlu/L4 gels, where $T = 25 \, ^\circ\text{C}$ and $\omega = 6.28 \, \text{rad/s}$. The data show a general trend of decreasing storage modulus and yield stress with decreasing C8-DGlu content.
be noted, however, that WAXD measurements of C₈-DGlu in the gel state, the crystalline state, and when mixed with L4 support the hypothesis of the C₈-DGlu being the primary contributor to the fibrous network. As can be seen in Figure 4.7, the gel and crystalline forms vary greatly in their peak distributions. The predominant peak in the crystalline form is of monolayer thickness, while the predominant peak in the gel form is the bilayer thickness expected for the helical ribbons. Upon addition of L4 to C₈-DGlu, peak shifts do not occur – instead, peak broadening and decreased intensity is observed. This again suggests that there is no dimensional change to the C₈-DGlu fibers.

Again with respect to the presence of the micellar aggregates, their presence does correlate with the previously mentioned hypothesis of Fuhrhop et al., which states that the surfactants may stabilize C₈-DGlu gels by solubilizing the crystal nuclei of C₈-DGlu within their micelles. Alternatively, mixed micelles of the surfactant and C₈-DGlu could be formed reducing the effective concentration of the C₈-DGlu available for gelation. Therefore, it would seem that a couple of simple conditions must be met to achieve gelation without crystallization for this type of system – the concentration of the gelator (C₈-DGlu) must be high enough to build a fibrous network even in the presence of the surfactant, and/or the concentration of the surfactant must be high enough to create enough micelles in order to solubilize crystal nuclei of the gelator and therefore prevent crystallization.

In the case of the system studied here, the concentration of the surfactant needs to be approximately two to three orders of magnitude larger than the value of the critical micelle concentration of the surfactant (4x10⁻⁵ M for L4) to yield stable gels. It is interesting to note here that in comparison to the previously reported anionic surfactant
system where the C₈-DGlu/SDS gels are only stable from a molar ratio of 10:1 to 2.5:1 C₈-DGlu:SDS, the C₈-DGlu/L4 gels reported here are stable to a much higher ratio of the non-ionic surfactant (from a molar ratio of 5:1 to 1:2 C₈-DGlu:L4).

**Figure 4.6.** TEM micrographs of (a) 4:1 C₈-DGlu:L4, (b) 1:1 C₈-DGlu:L4, and (c) 3:7 C₈-DGlu:L4. Helical ribbons of approximately 10-20 nm in diameter are observed in all samples in addition to micellar structures that increase in size with increasing L4 content. The insets are portions of the same images zoomed to show characteristic approximately 10-15 nm wide helical assembly of C₈-DGlu even in the presence of surfactant.
In an attempt to further probe the mechanism of the effect of L4, we conducted rheology experiments by varying the molar ratios as opposed to the weight ratios with the C₈-DGlu content fixed at either 1 wt % or 1.6 wt %. As can be seen in Figure 4.8, a differing pattern emerges between the two sample sets. When the weight content of C₈-DGlu is fixed at 1 wt %, the 1:1 C₈-DGlu:L4 gel shows the highest storage modulus and yield stress; whereas, at 1.6 wt %, the mechanical properties decrease with increasing L4 content with the 1:0.5 C₈-DGlu:L4 gel exhibiting the best properties. Therefore, this again suggests that the L4 is possibly contributing to the stability of the gel in ways other than just forming micellar aggregates in the sol phase. The decrease in storage modulus and yield stress with increased L4 content could also possibly be explained by a larger micellar aggregate population removing more of the C₈-DGlu from the system to reduce the effective concentration of the gelator available for fiber formation.

Figure 4.7. 1-D WAXD of a freeze-dried 1 wt % C₈-DGlu gel, C₈-DGlu crystals, and a freeze-dried 1:1 (by weight) C₈-DGlu:L4 gel (2 wt % total). There exist distinguishable differences between the patterns of the gel and crystalline state. Addition of the surfactant lowers the intensity of the peaks but does not induce any peak shifts. The peaks at approximately 2 degrees (d=3.4-4.0 nm) correlate well with the known bilayer thickness of C₈-DGlu in both gel and crystal form. The largest peak in the crystal form (approximately 5 degrees), however, corresponds to the monolayer length in the crystal (approximately 1.6 nm).

In an attempt to further probe the mechanism of the effect of L4, we conducted rheology experiments by varying the molar ratios as opposed to the weight ratios with the C₈-DGlu content fixed at either 1 wt % or 1.6 wt %. As can be seen in Figure 4.8, a differing pattern emerges between the two sample sets. When the weight content of C₈-DGlu is fixed at 1 wt %, the 1:1 C₈-DGlu:L4 gel shows the highest storage modulus and yield stress; whereas, at 1.6 wt %, the mechanical properties decrease with increasing L4 content with the 1:0.5 C₈-DGlu:L4 gel exhibiting the best properties. Therefore, this again suggests that the L4 is possibly contributing to the stability of the gel in ways other than just forming micellar aggregates in the sol phase. The decrease in storage modulus and yield stress with increased L4 content could also possibly be explained by a larger micellar aggregate population removing more of the C₈-DGlu from the system to reduce the effective concentration of the gelator available for fiber formation.
The effect of the L4 can be most notably seen in optical micrographs of the gels (Figure 4.9). Clearly, increasing the amount of L4 in the gel while holding the C8-DGlu concentration constant has a profound effect on the gel morphology. At 1 wt % C8-DGlu and 1:0.5 C8-DGlu:L4, there appear to be extremely small spherulitic assemblies that remain mutually exclusive of each other. By simply increasing the ratio to 1:1, much larger assemblies form and begin to percolate and interpenetrate throughout the sample. In contrast, when considering the 1.6 wt % gel, the spherulites have already begun to percolate and interpenetrate at 1:0.5. This explains the difference in the rheological behavior pattern between 1 wt % and 1.6 wt % C8-DGlu molar ratio samples. This necessity for percolation and interpenetration has been previously identified in the literature as a means for increasing gel mechanical properties.15

From the TEM images there appears to be no significant difference in the gel nanofiber structure upon increasing the ratio of L4, with the width and appearance of C8-DGlu fibers qualitatively agreeing with previously published results. However, the optical
microscopy images do show a significant difference in microscale gel appearance by simply increasing the L4 content. Therefore, again, it is possible that either the L4 is getting drawn into the fibrous structure of the C₈-DGlu assembly, or there is a thin (possibly monolayer) coating of L4 on the C₈-DGlu fibers that cannot be resolved in the TEM.

Figure 4.9. DIC optical micrographs of C₈-DGlu/L4 gels. (a) 1 wt % 1:0.5 C₈-DGlu:L4. (b) 1.6 wt % 1:0.5 C₈-DGlu:L4. (c) 1 wt % 1:1 C₈-DGlu:L4. (d) 1.6 wt % 1:1 C₈-DGlu:L4. The ability of the spherulitic assemblies to percolate and interpenetrate increases with both C₈-DGlu and L4 content, suggesting that the L4 may interact with the fibrous assembly of the gel rather than sitting exclusively in micelles in the sol phase.
4.2.2 Effect of pH or salt on N-octyl-D-gluconamide/Laureth-4 gels

In addition to the behavior of C₈-DGlu gels in plain water, it is useful to consider the impact of pH and/or salt concentration on gel formation as many applications, most notably personal care and physiological applications, either require a set pH or salt content, or conversely, require functionality under a myriad of conditions. Given that the predominant driving force for the self-assembly of C₈-DGlu is the hydrophobic effect along with additional stabilization via hydrogen bonding, it may be expected that gels should be able to be formed under a wide variety of circumstances. Figure 4.10 illustrates this, where stable gels can be made over a pH range of 4-10 (pH adjusted with NaOH and citric acid) and varied salts such as 100 mM NaCl and concentrated CaCO₃. The various environmental conditions do have a small effect on the dynamic mechanical behavior of the gels, however, as shown in Figure 4.10b/c.
4.2.3 *N*-octyl-D-gluconamide and Laureth-7, 12 and 30

Following our examination of the C₈-DGlu/L₄ system, we set out to determine the effect of changing the length of the Laureth hydrophilic group, for which we used the commercially available Laureth-7 (L7), Laureth-12 (L12), and Laureth-30 (L30) to compare to the properties of L₄ (all CMC values are on the order of 10⁻⁵ M). Figure 4.11 shows the resulting gels made from C₈-DGlu and various Laureth surfactants in 1:1 molar ratios with the C₈-DGlu content being set constant at 1 wt %. The rheological behavior shows a trend toward lower moduli and yield stresses with increasing ethylene...
oxide chain length (The L30 sample is not shown as its modulus was too low to be measured by cone and plate rheology). This behavior can, in part, be attributed to the fact that the overall weight component of the C₈-DGlu is decreasing as the length of the Laureth ethylene oxide chain increases. It is also feasible that the higher ethylene oxide content surfactants solubilize more of the gelator or that any reinforcement provided by the micelles may become more fluid as the length of the surfactant molecules increase, as the bending modulus of nonionic surfactant monolayers is known to decrease with increasing hydrophilic chain length.¹⁶ It is again, however, a point of interest that the mechanical properties of C₈-DGlu gels can be very easily tailored simply by changing the mixing surfactant.

Figure 4.11. (a) Gels made from 1:1 molar ratios of C₈-DGlu:Laureth surfactant, where the C₈-DGlu concentration is set at 1 wt %. Left to right: C₈-DGlu/L4, C₈-DGlu/L7, C₈-DGlu/L12, C₈-DGlu/L30. (b) Stress sweeps of 1:1 molar ratio C₈-DGlu:Lₙ gels. Rheological parameters include T = 25 °C and ω = 6.28 rad/s.

Additionally, differences in the nano- and microscale morphologies are evident when transitioning to longer ethylene oxide chains again suggesting that the surfactants
play a major role in the gel characteristics. Figure 4.12 shows DIC optical microscopy images of C$_8$-DGlu when mixed with L4, 7, 12, or 30. The size of the spherulitic assemblies decreases vastly with increasing ethylene oxide chain length, which corresponds to the increase in gel transparency with increasing ethylene oxide chain length.

![DIC optical microscopy images](image)

**Figure 4.12.** DIC optical microscopy images of 1:1 (by molar ratio) (a) C$_8$-DGlu:L4, (b) C$_8$-DGlu:L7, and (c) C$_8$-DGlu:L12, and (d) C$_8$-DGlu:L30.

Figure 4.13 shows a comparison of TEM images of C$_8$-DGlu when mixed with L4, 7, 12, or 30. The fibrous assemblies again maintain their appearance with changing the surfactant; however, the micellar aggregates decrease in size with increasing ethylene oxide chain length. This is not entirely unexpected as L7, 12, and 30 are significantly
more water-soluble than L4. In fact, L4 is known to exist in a distribution of lamellar and micellar structures, while any Laureth surfactant with an ethylene oxide chain length greater than 5 exists in micellar form only.\textsuperscript{17}

\textbf{Figure 4.13.} TEM images of 1:1 (by molar ratio) (a) C\textsubscript{8}-DGluc:L4, (b) C\textsubscript{8}-DGluc:L7, and (c) C\textsubscript{8}-DGluc:L12, and (d) C\textsubscript{8}-DGluc:L30.
4.2.4 \textit{N}-octyl-D-gluconamide with other non-ionic surfactants

Finally, to further examine the flexibility of this mixing approach to preventing crystallization, gels were created by mixing C$_8$-DGluc with the nonionic surfactants Ceteth-20 and Ninol-L5 (Figure 4.14). While the gels made from 1:1 weight ratios of C20 or Ninol-L5:C$_8$-DGluc are significantly weaker than the 1:1 C$_8$-DGluc:L4 gel in terms of storage modulus and yield stress (with the C$_8$-DGluc concentration being set at 1 wt %), both gels exhibited similar behavior to that of the C$_8$-DGluc/L4 system, where no crystallization was observed over the course of a few months.

![Chemical structures and images of gels](image)

**Figure 4.14.** Chemical structures of surfactants (a) Ceteth-20 and (b) Ninol-L5 as well as (c) images of 1:1 weight ratio gels, and (d) a rheological comparison of 1:1 weight ratio gels of C$_8$-DGluc mixed with L4, Ceteth-20, and Ninol-L5.

4.2.5 Structure-property evaluation of other amphiphiles

Since stable hydrogelation cannot be achieved with C$_8$-DGluc without adding a stabilizer such as a surfactant, we set out to examine the bulk effect of designed structural changes to potential sugar-amphiphile gelators in an attempt to further the area of intentional design of supramolecular gelators. We first synthesized several
monoamphiphiles similar in structure to \( C_8\)-DGl (4.1-4.12) by utilizing D-gluconic acid, L-gulonic acid, and D-ribonic acid as well as alkyl amines with carbon chain lengths between 6 and 12 (Figure 4.15).

![Chemical structures](image)

**Figure 4.15.** Chemical structures of (a) \( N \)-alkyl-D-gluconamides (\( C_n\)-DGlu, 4.1-4.4), (b) \( N \)-alkyl-L-gulonamides (\( C_n\)-LGul, 4.5-4.8), and (c) \( N \)-alkyl-D-ribonamides (\( C_n\)-DRib, 4.9-4.12), where \( n = 4, 6, 8, \) or 10. Gels of limited lifetime were formed from a small handful of these molecules and combinations of molecules as outlined in table 4.1.

The hydrogelating ability of these monoamphiphiles was tested both independently and when mixed with another monoamphiphile. Table 4.1 summarizes the observed behavior of compounds 4.1-4.12 in distilled water. Gelation was observed in several samples (noted by green boxes with checkmarks); however, crystallization occurred in all of these samples over the course of a few hours. For the rest of the samples, either precipitation or solubilization was observed (noted by red boxes with x marks). Therefore, even when mixed, straight-chained \( N \)-alkyl-D-aldonamides can pack too efficiently in the crystalline state to be stable in the gel state.
Table 4.1. Gelation ability of \( N \)-alkyl-D-gluconamides (C\( _n \)-DGlu, 4.1-4.4), (b) \( N \)-alkyl-L-gulonamides (C\( _n \)-LGul, 4.5-4.8), and (c) \( N \)-alkyl-D-ribonamides (C\( _n \)-DRib, 4.9-4.12) alone and mixed with each other. A green box with a check means a gel of some form was noted. A red box with an x means the molecules stayed in solution or precipitated/crystallized immediately upon cooling.

From these results, we next synthesized an \( N \)-alkyl-D-gluconamide with a branched alkyl chain (4.13, Figure 4.16) in an attempt to introduce a small amount of disorder into the system. Unfortunately, a single ethyl branch caused too much disruption and yielded a completely water-soluble product. These results clearly demonstrate the fine lines that exist between insolubility, crystallization, gelation, and solubilization.
A similar pattern was also seen for bolaamphiphiles. Bolamphiphiles with straight-chain $C_{12}$ and $C_{18}$ amines (Figure 4.17a, compounds 4.14 and 4.15) were found to be insoluble in water, even at high temperatures. Utilizing para-substituted aromatic amines ($4,4'$-methylenedianiline (4.16) or $p$-xylylenediamine (4.17)) instead of alkyl amines resulted in soluble materials that crystallized without any observed gelation (Figure 4.18).

![Figure 4.16. Chemical structure of $N$-(2-ethylhexyl)-$d$-gluconamide (2-EHA-DGlu, 4.13). 4.13 was soluble in water to high concentrations. No gelation was observed.](image)

![Figure 4.17. (a) Chemical structure of $N,N'$-dodecyl-bis-$d$-gluconamide (DGlu-$C_{12}$-DGlu, 4.14) and $N,N'$-octadecyl-bis-$d$-gluconamide (DGlu-$C_{12}$-DGlu, 4.15). Both molecules were insoluble in water. (b) 1,18-diaminododecane (4.21) for the synthesis of 4.15 was synthesized from eicosanedioic acid in 43% yield.](image)
Finally, gluconamide bolaamphiphiles with order-disrupting hydrophobic groups (m-xylylenediamine (4.18), 1,5-diamino-2-methylpentane (4.19), or trans-1,2-diaminocyclohexane (4.20)) were found to be soluble in water to high concentrations without any observed gelation (Figure 4.19).

![Figure 4.18](image-url)  
**Figure 4.18.** (a) Chemical structure of $N,N'-(methylenebis(4,1-phenylene))bis-D$-gluconamide (DGlu-MDA-DGlu, 4.16). (b) Chemical structure of $N,N'-(1,4$-phenylenebis(methylene))bis-D-gluconamide (DGlu-pXD-DGlu, 4.17). Both molecules crystallized upon cooling in water. No gelation was observed.

![Figure 4.19](image-url)  
**Figure 4.19.** Chemical structures of (a) $N,N'-(1,3$-phenylenebis(methylene))bis-D-gluconamide (DGlu-mXD-DGlu, 4.18), (b) $N,N'-(2$-methylpentane-1,5-diyl)bis-D-gluconamide (4.19), and (c) $N,N'-(1R,2R)$-cyclohexane-1,2-diylbis-D-gluconamide (4.20). All molecules were soluble in water to high concentrations. No gelation was observed.
4.3 Conclusions

We have demonstrated that the crystallization of C₈-DGlu hydrogels can be prevented via the addition of nonionic surfactants. Stable gels were formed down to a C₈-DGlu concentration of 0.6 wt %, which is lower than the previously reported 2 wt % with the ionic surfactant, SDS. The enhanced lifetime stability of these gels allowed us to probe the mechanical properties of this class of supramolecular gel for the first time. Tailored mechanical properties can be achieved by simply altering the ratio of C₈-DGlu:surfactant or by changing the size of ethylene oxide segment of the surfactant. We have also shown that stable C₈-DGlu gels can be created regardless of pH or salt additives, although a difference in the gels mechanical properties is observed. Thus, for the C₈-DGlu gelator, the addition of a non-gelling, nonionic surfactant allows a facile approach to not only dramatically enhance the lifetime stability of the gel but to also tailor its mechanical properties opening the door to further investigations of this class of gelators for a range of potential applications including water-based personal care formulations.

Additionally, several other sugar-based mono- and bola-amphiphiles were synthesized in an attempt to further the understanding of intentional design of gelators. Unfortunately, all of the compounds synthesized were either too insoluble, crystalline, or soluble in water, and we were unable to access the gel state through the studied combinations of commercially available lactones and amines.
4.4 Experimental

4.4.1 Materials. All lactones and amines were purchased from Sigma-Aldrich unless otherwise noted. All solvents were purchased from Fisher Scientific. All surfactants were provided by Lubrizol Advanced Materials. All materials were used without further purification. The surfactants consist of a single alkyl chain length and a small distribution for each in the ethylene glycol component. For example, Laureth-4 contains 3-5 ethylene glycol units. C₈-DGlu was prepared using a previous literature procedure.

4.4.2 General procedure for the synthesis of amphiphiles. To the lactone (1 mmol for monoamphiphiles and 2 mmol for bolaamphiphiles) in methanol (10 mL) was added the appropriate amine (2 mmol for monoamphiphiles and 1 mmol for bolaamphiphiles). The solution was refluxed for 6 hours at 75 °C under Ar and then concentrated to approximately 5 mL under reduced pressure. The product was precipitated with diethyl ether, filtered, and dried under vacuum to give a white powder in quantitative yield.
**N-octyl-D-gluconamide (C₈-DGlu, 4.1).** $^1$H NMR (600 MHz, DMSO-$d_6$): δ 7.58 (t, 1H, NH), 5.33 (d, 1H, OH), 4.52 (d, 1H, OH), 4.45 (d, 1H, OH), 4.37 (d, 1H, OH), 4.32 (t, 1H, OH), 3.93 (t, 1H, CH), 3.85 (m, 1H, CH), 3.54 (m, 1H, CH), 3.43 (m, 2H, CH₂), 3.32 (m, 2H, CH), 3.02 (m, 2H, CH₂), 1.36 (t, 2H, CH₂), 1.21 (m, 10H, CH₂), 0.82 (t, 3H, CH₃).

Figure 4.20. *N*-octyl-D-gluconamide (C₈-DGlu, 4.1). $^1$H NMR (600 MHz, DMSO-$d_6$).
**N-hexyl-D-gluconamide (C₆-DGlu, 4.2).** $^1$H NMR (600 MHz, DMSO-d₆): $\delta$ 7.57 (t, 1H, NH), 5.31 (d, 1H, OH), 4.50 (d, 1H, OH), 4.44 (d, 1H, OH), 4.35 (d, 1H, OH), 4.30 (t, 1H, OH), 3.94 (t, 1H, CH), 3.86 (m, 1H, CH), 3.54 (m, 1H, CH), 3.44 (m, 2H, CH₂), 3.34 (m, 2H, CH), 3.03 (m, 2H, CH₂), 1.37 (t, 2H, CH₂), 1.22 (m, 6H, CH₂), 0.83 (t, 3H, CH₃).

**Figure 4.21.** N-hexyl-D-gluconamide (C₆-DGlu, 4.2). $^1$H NMR (600 MHz, DMSO-d₆).
\textbf{N-decyl-D-gluconamide (C_{10}-DGlu, 4.3).} \textsuperscript{1}H NMR (600 MHz, DMSO-d\textsubscript{6}): \textsuperscript{\delta} 7.56 (t, 1H, NH), 5.32 (d, 1H, OH), 4.50 (d, 1H, OH), 4.43 (d, 1H, OH), 4.35 (d, 1H, OH), 4.30 (t, 1H, OH), 3.95 (t, 1H, CH), 3.87 (m, 1H, CH), 3.55 (m, 1H, CH), 3.44 (m, 2H, CH\textsubscript{2}), 3.34 (m, 2H, CH), 3.04 (m, 2H, CH\textsubscript{2}), 1.37 (t, 2H, CH\textsubscript{2}), 1.21 (m, 14H, CH\textsubscript{2}), 0.83 (t, 3H, CH\textsubscript{3}).

\textbf{Figure 4.22.} \textit{N-decyl-D-gluconamide (C_{10}-DGlu, 4.3).} \textsuperscript{1}H NMR (600 MHz, DMSO-d\textsubscript{6}).
\textit{N-dodecyl-D-gluconamide (C\textsubscript{12}-DGl, 4.4).} $^1$H NMR (600 MHz, DMSO-d\textsubscript{6}): \( \delta \) 7.58 (t, 1H, NH), 5.32 (d, 1H, OH), 4.51 (d, 1H, OH), 4.44 (d, 1H, OH), 4.36 (d, 1H, OH), 4.31 (t, 1H, OH), 3.93 (t, 1H, CH), 3.85 (m, 1H, CH), 3.53 (m, 1H, CH), 3.43 (m, 2H, CH\textsubscript{2}), 3.33 (m, 2H, CH), 3.02 (m, 2H, CH\textsubscript{2}), 1.36 (t, 2H, CH\textsubscript{2}), 1.20 (m, 18H, CH\textsubscript{2}), 0.82 (t, 3H, CH\textsubscript{3}).

\textbf{Figure 4.23.} \textit{N-dodecyl-D-gluconamide (C\textsubscript{12}-DGl, 4.4).} $^1$H NMR (600 MHz, DMSO-d\textsubscript{6}).
N-hexyl-L-gulonamide (C6-LGul, 4.5). $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.83 (t, 1H, NH), 5.43 (d, 1H, OH), 4.64 (d, 1H, OH), 4.48 (d, 1H, OH), 4.44 (t, 1H, OH), 4.36 (d, 1H, OH), 3.86 (t, 1H, CH), 3.58 (m, 2H, CH), 3.51 (m, 1H, CH), 3.41 (m, 1H, CH$_2$), 3.30 (m, 1H, CH$_2$), 3.04 (m, 2H, CH$_2$), 1.35 (m, 2H, CH$_2$), 1.21 (m, 6H, CH$_2$), 0.83 (t, 3H, CH$_3$).  

Figure 4.24. N-hexyl-L-gulonamide (C6-LGul, 4.5). $^1$H NMR (600 MHz, DMSO-d$_6$).
$\textbf{N-octyl-L-gulonamide (C}_8\text{-L.Gul, 4.6).}$ \textsuperscript{1}H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.81 (t, 1H, NH), 5.43 (d, 1H, OH), 4.63 (d, 1H, OH), 4.47 (d, 1H, OH), 4.42 (t, 1H, OH), 4.35 (d, 1H, OH), 3.86 (t, 1H, CH), 3.59 (m, 2H, CH), 3.51 (m, 1H, CH), 3.41 (m, 1H, CH$_2$), 3.30 (m, 1H, CH$_2$), 3.03 (m, 2H, CH$_2$), 1.37 (m, 2H, CH$_2$), 1.21 (m, 10H, CH$_2$), 0.82 (t, 3H, CH$_3$).

\textbf{Figure 4.25.} $\textbf{N-octyl-L-gulonamide (C}_8\text{-L.Gul, 4.6).}$ \textsuperscript{1}H NMR (600 MHz, DMSO-d$_6$).
N-decyl-L-gulonamide (C\textsubscript{10}-L.Gul, 4.7). \textsuperscript{1}H NMR (600 MHz, DMSO-d\textsubscript{6}): \( \delta \) 7.82 (t, 1H, NH), 5.44 (d, 1H, OH), 4.63 (d, 1H, OH), 4.48 (d, 1H, OH), 4.43 (t, 1H, OH), 4.35 (d, 1H, OH), 3.86 (t, 1H, CH), 3.58 (m, 2H, CH), 3.51 (m, 1H, CH), 3.41 (m, 1H, CH\textsubscript{2}), 3.30 (m, 1H, CH\textsubscript{2}), 3.03 (m, 2H, CH\textsubscript{2}), 1.35 (m, 2H, CH\textsubscript{2}), 1.21 (m, 14H, CH\textsubscript{2}), 0.81 (t, 3H, CH\textsubscript{3}).

Figure 4.26. N-decyl-L-gulonamide (C\textsubscript{10}-L.Gul, 4.7). \textsuperscript{1}H NMR (600 MHz, DMSO-d\textsubscript{6}).
**N-dodecyl-L-gulonamide (C_{12}-LGul, 4.8).** $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.81 (t, 1H, NH), 5.42 (d, 1H, OH), 4.63 (d, 1H, OH), 4.47 (d, 1H, OH), 4.42 (t, 1H, OH), 4.35 (d, 1H, CH), 3.86 (t, 1H, OH), 3.59 (m, 2H, CH), 3.51 (m, 1H, CH), 3.41 (m, 1H, CH$_2$), 3.30 (m, 1H, CH$_2$), 3.02 (m, 2H, CH$_2$), 1.36 (m, 2H, CH$_2$), 1.20 (m, 18H, CH$_2$), 0.82 (t, 3H, CH$_3$).

![Figure 4.27. N-dodecyl-L-gulonamide (C_{12}-LGul, 4.8). $^1$H NMR (600 MHz, DMSO-d$_6$).]
\( \text{N-hexyl-D-ribonamide (C}_6\text{-DRib, 4.9).} \) \( ^1\text{H NMR (600 MHz, DMSO-} \text{d}_6) \): \( \delta \) 7.55 (t, 1H, NH), 5.47 (d, 1H, OH), 4.73 (d, 1H, OH), 4.51 (d, 1H, OH), 4.32 (d, 1H, OH), 3.98 (d, 1H, CH), 3.59 (m, 1H, CH), 3.52 (m, 2H, \text{CH}_2), 3.35 (m, 1H, CH), 3.04 (m, 2H, \text{CH}_2), 1.37 (m, 2H, \text{CH}_2), 1.22 (m, 6H, \text{CH}_2), 0.83 (t, 3H, \text{CH}_3).

**Figure 4.28.** \( \text{N-hexyl-D-ribonamide (C}_6\text{-DRib, 4.9).} \) \( ^1\text{H NMR (600 MHz, DMSO-} \text{d}_6) \).
N-octyl-D-ribonamide (C₈-DRib, 4.10). $^1$H NMR (600 MHz, DMSO-d₆): δ 7.55 (t, 1H, NH), 5.47 (d, 1H, OH), 4.73 (d, 1H, OH), 4.51 (d, 1H, OH), 4.32 (d, 1H, OH), 3.98 (d, 1H, CH), 3.60 (m, 1H, CH), 3.52 (m, 2H, CH₂), 3.34 (m, 1H, CH), 3.03 (m, 2H, CH₂), 1.37 (m, 2H, CH₂), 1.21 (m, 10H, CH₂), 0.83 (t, 3H, CH₃).

Figure 4.29. N-octyl-D-ribonamide (C₈-DRib, 4.10). $^1$H NMR (600 MHz, DMSO-d₆).
**N-decyl-D-ribonamide (C<sub>10</sub>-DRib, 4.11).** $^1$H NMR (600 MHz, DMSO-$d_6$): δ 7.55 (t, 1H, NH), 5.47 (d, 1H, OH), 4.72 (d, 1H, OH), 4.51 (d, 1H, OH), 4.32 (d, 1H, OH), 3.98 (d, 1H, CH), 3.59 (m, 1H, CH), 3.52 (m, 2H, CH<sub>2</sub>), 3.34 (m, 1H, CH), 3.02 (m, 2H, CH<sub>2</sub>), 1.37 (m, 2H, CH<sub>2</sub>), 1.21 (m, 14H, CH<sub>2</sub>), 0.83 (t, 3H, CH<sub>3</sub>).

**Figure 4.30.** N-decyl-D-ribonamide (C<sub>10</sub>-DRib, 4.11). $^1$H NMR (600 MHz, DMSO-$d_6$).
N-dodecyl-D-ribonamide (C\textsubscript{12}-DRib, 4.12). \textsuperscript{1}H NMR (600 MHz, DMSO-d\textsubscript{6}): \(\delta\) 7.55 (t, 1H, NH), 5.48 (d, 1H, OH), 4.75 (d, 1H, OH), 4.52 (d, 1H, OH), 4.33 (d, 1H, OH), 3.97 (d, 1H, CH), 3.58 (m, 1H, CH), 3.51 (m, 2H, CH\textsubscript{2}), 3.33 (m, 1H, CH), 3.01 (m, 2H, CH\textsubscript{2}), 1.35 (m, 2H, CH\textsubscript{2}), 1.20 (m, 18H, CH\textsubscript{2}), 0.82 (t, 3H, CH\textsubscript{3}).

\textbf{Figure 4.31.} N-dodecyl-D-ribonamide (C\textsubscript{12}-DRib, 4.12). \textsuperscript{1}H NMR (600 MHz, DMSO-d\textsubscript{6}).
**N-(2-ethylhexyl)-D-gluconamide** (2-EHA-DGlu, 4.13). $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.45 (t, 1H, NH), 5.36 (d, 1H, OH), 4.53 (d, 1H, OH), 4.46 (d, 1H, OH), 4.36 (d, 1H, CH), 4.33 (t, 1H, OH), 3.95 (t, 1H, CH), 3.85 (m, 1H, CH), 3.53 (m, 1H, CH), 3.43 (m, 2H, CH$_2$), 3.32 (m, 1H, OH), 2.98 (m, 2H, CH$_2$) 1.38 (m, 1H, CH), 1.21 (m, 8H, CH$_2$), 0.83 (t, 3H, CH$_3$), 0.79 (t, 3H, CH$_3$).

**Figure 4.32.** N-(2-ethylhexyl)-D-gluconamide (2-EHA-DGlu, 4.13). $^1$H NMR (600 MHz, DMSO-d$_6$).
\textit{N,N’-dodecyl-bis-D-gluconamide (DGlue-C_{12}-DGlue, 4.14).} $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.58 (t, 2H, NH), 5.33 (d, 2H, OH), 4.52 (d, 2H, OH), 4.45 (d, 2H, OH), 4.37 (d, 2H, CH), 4.32 (t, 2H, OH), 3.93 (t, 2H, CH), 3.85 (m, 2H, CH), 3.54 (m, 2H, CH), 3.43 (m, 4H, CH$_2$), 3.32 (m, 2H, OH), 3.02 (m, 4H, CH$_2$N), 1.36 (t, 4H, CH$_2$), 1.21 (m, 16H, CH$_2$).

\textbf{Figure 4.33.} \textit{N,N’-dodecyl-bis-D-gluconamide (DGlue-C_{12}-DGlue, 4.14).} $^1$H NMR (600 MHz, DMSO-d$_6$).
N,N'-octadecyl-bis-D-gluconamide (DGlu-C$_{18}$-DGlu, 4.15). $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.58 (t, 2H, NH), 5.33 (d, 2H, OH), 4.52 (d, 2H, OH), 4.45 (d, 2H, OH), 4.37 (d, 2H, CH), 4.32 (t, 2H, OH), 3.93 (t, 2H, CH), 3.85 (m, 2H, CH), 3.54 (m, 2H, CH), 3.43 (m, 4H, CH$_2$), 3.32 (m, 2H, OH), 3.02 (m, 4H, CH$_2$N), 1.36 (t, 4H, CH$_2$), 1.21 (m, 28H, CH$_2$).

Figure 4.34. N,N'-octadecyl-bis-D-gluconamide (DGlu-C$_{18}$-DGlu, 4.15). $^1$H NMR (600 MHz, DMSO-d$_6$).
$N,N'$-(methylenebis(4,1-phenylene))bis-D-gluconamide (DGlu-MDA-DGlu, 4.16).

$^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 9.44 (t, 2H, NH), 7.56 (d, 4H, CH), 7.09 (d, 4H, CH), 5.66 (d, 2H, OH), 4.57 (d, 2H, CH$_2$), 4.50 (m, 4H, OH), 4.34 (m, 4H, OH), 4.10 (t, 2H, CH), 3.95 (m, 2H, CH), 3.73 (d, 2H, CH), 3.54 (m, 2H, CH), 3.46 (m, 4H, CH$_2$).

Figure 4.35. $N,N'$-(methylenebis(4,1-phenylene))bis-D-gluconamide (DGlu-MDA-DGlu, 4.16). $^1$H NMR (600 MHz, DMSO-d$_6$).
$N,N'$-((1,4-phenylenebis(methylene))bis-D-gluconamide (DGlu-pXD-DGlu, 4.17). $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 8.12 (t, 2H, NH), 7.17 (s, 4H, CH), 5.44 (d, 2H, OH), 4.56 (d, 2H, OH), 4.50 (d, 2H, OH), 4.44 (d, 2H, OH), 4.34 (t, 2H, OH), 4.23 (m, 4H, CH$_2$), 4.02 (t, 2H, CH), 3.92 (t, 2H, CH), 3.54 (m, 2H, CH), 3.45 (m, 4H, CH$_2$), 3.30 (m, 2H, CH).

Figure 4.36. $N,N'$-(1,4-phenylenebis(methylene))bis-D-gluconamide (DGlu-pXD-DGlu, 4.17). $^1$H NMR (600 MHz, DMSO-$d_6$).
$N,N' \text{-}(1,3\text{-phenylenebis(methylene)})\text{bis-D-gluconamide} \ (\text{DGlue-mXD-DGlu, 4.18})$. $^1H$ NMR (600 MHz, DMSO-$d_6$): $\delta$ 8.09 (t, 2H, NH), 7.16 (m, 4H, CH), 7.12 (m, 2H, CH), 5.42 (d, 2H, OH), 4.54 (d, 2H, OH), 4.47 (m, 4H, OH), 4.30 (t, 2H, CH), 4.27 (m, 4H, CH$_2$), 3.94 (m, 2H CH), 3.54 (m, 2H, CH), 3.47 (m, 4H, CH$_2$), 3.36 (m, 2H, CH).

Figure 4.37. $N,N' \text{-}(1,3\text{-phenylenebis(methylene)})\text{bis-D-gluconamide} \ (\text{DGlue-mXD-DGlu, 4.18})$. $^1H$ NMR (600 MHz, DMSO-$d_6$).
$N,N'$-(2-methylpentane-1,5-diyl)bis-D-gluconamide (4.19). $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.57 (t, 1H, NH), 7.51 (t, 1H, NH), 5.35 (d, 1H, OH), 5.32 (d, 1H, OH), 4.50 (d, 2H, OH), 4.44 (d, 2H, OH), 4.38 (t, 2H, OH), 4.30 (d, 2H, OH), 3.94 (m, 2H, CH), 3.87 (m, 2H, CH), 3.54 (m, 2H, CH), 3.44 (m, 4H, CH$_2$), 3.35 (m, 2H, CH), 3.02 (m, 2H, CH$_2$), 2.87 (m, 2H CH$_2$), 1.56 (m, 1H, CH$_2$), 1.43 (m, 1H, CH$_2$), 1.35 (m, 1H, CH$_2$), 1.27 (m, 1H, CH$_2$), 1.00 (m, 1H, CH), 0.78 (d, 3H, CH$_3$).

Figure 4.38. $N,N'$-(2-methylpentane-1,5-diyl)bis-D-gluconamide (4.19). $^1$H NMR (600 MHz, DMSO-$d_6$).
$N,N'$-((1R,2R)-cyclohexane-1,2-diyl)bis-D-gluconamide (4.20).  $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.46 (t, 2H, NH), 5.32 (d, 2H, OH), 4.56 (d, 2H, OH), 4.43 (m, 4H, OH), 4.34 (t, 2H, OH), 3.94 (d, 2H, CH), 3.87 (m, 2H, CH), 3.54 (m, 4H, CH), 3.45 (m, 4H, CH$_2$), 3.35 (m, 2H, CH), 1.86 (m, 2H, CH$_2$), 1.60 (m, 2H, CH$_2$), 1.25 (m, 4H, CH$_2$).

Figure 4.39. $N,N'$-((1R,2R)-cyclohexane-1,2-diyl)bis-D-gluconamide (4.20).  $^1$H NMR (600 MHz, DMSO-$d_6$).
4.4.3 Synthesis of 1,18-diaminoctadecane (4.21). 5 g of eicosanedioic acid (342.51 g/mol, 1 eq. 14.6 mmol) was mostly dissolved in 230 mL of chloroform. 62 mL of concentrated sulfuric was added, and the solution was heated to 50 °C. 6.956 g of sodium azide (65.01 g/mol, 7.3 eq. 107 mmol) was added over a period of 2 hours, and the solution was then left to stir for an additional 2 hours at 50 °C. The sulfuric acid portion of the solution was extracted and poured into 60 mL of ice water to obtain a white precipitate, which was stirred at 80 °C with dropwise addition of 10 M sodium hydroxide until the pH reached 11 (approximately 300 mL of sodium hydroxide over 3 hours). The final product was extracted with approximately 100 mL of chloroform and the solvent removed by evaporation. The residue was recrystallized from cold ethanol, filtered, and washed with diethyl ether to give 1.78 g (43% yield) of 1,18-diaminoctadecane. $^1$H NMR (600 MHz, CD$_3$OD): δ 2.64 (t, 4H, CH$_2$N), 1.40 (m, 4H, CH$_2$), 1.24 (m, 28H, CH$_2$).
4.4.4 General procedure for testing of gelation ability. 20 mg of a molecule or molecules of interest was/were suspended in 1 mL of distilled water (or aqueous solution) in a 1 Dram vial. The suspension was heated to approximately 100 °C until dissolution was achieved (about one minute maximum). The sample was then allowed to cool to room temperature upon which gelation, and/or crystallization was observed. In the case of the gel containing calcium carbonate, calcium carbonate was added to water at an initial concentration of 100 mM. With heating, no less than 0.47 mM went into solution.

Figure 4.40. 1,18-diaminoctadecane (4.21). $^1$H NMR (600 MHz, DMSO-$d_6$).
The remaining solid was removed via filtration, and the resulting solution was used to create gels.

4.4.5 Rheology. Rheological measurements were taken on a TA AR2000ex rheometer with a Peltier temperature control system and a 20 mm cone (1° angle) with a solvent trap. For the oscillatory stress sweep study, the samples were pre-sheared at 500% strain and an angular frequency of 30 rad/s and then were allowed to recover for 60 minutes under 0.2% strain at an angular frequency of 6.28 rad/s. Following recovery, the samples were subjected to a continuous shear ramp from 0.1 to 1000 Pa at a frequency of 6.28 rad/s. A pre-shear, as opposed to a thermal conditioning, was utilized to ensure that the concentration of each sample did not change as a result of evaporation after being loaded into the rheometer.

4.4.6 TEM. Transmission Electron Microscope measurements were carried out on a Zeiss Libra 200EF microscope utilizing carbon-coated 200 square mesh copper grids. To prepare the samples for analysis, a piece of gel was placed on the grid and allowed to remain for five minutes. The piece was then gently removed with filter paper and the grid allowed to air dry overnight. The following day, the samples were sputter-coated under vacuum with a palladium layer approx. 1 nm in thickness for visualization purposes.
4.4.7 **Wide-Angle X-Ray Diffraction.** Wide angle x-ray diffraction (WAXD) was done on a Rigaku x-ray diffractometer at room temperature with Cu Kα irradiation at a voltage of 30 kV and a current of 30 mA. The spectra were recorded with a sampling width of 0.05 and a scan speed of 0.2 deg/min. Freeze-dried gel powder was crushed and laid into the sample holder, and a glass slide was forcibly run over the sample until a flat surface was achieved.

4.4.8 **Optical Microscopy.** Optical Microscopy images were taken in DIC mode on a Leica DM6000 microscope by placing the gel solutions in an assembled cell with an interior thickness of approximately 200 µm.

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4.6 **References**


Bibliography


