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Structural Selection in G-Quartet-Based Hydrogels and Controlled Release of Bioactive Molecules

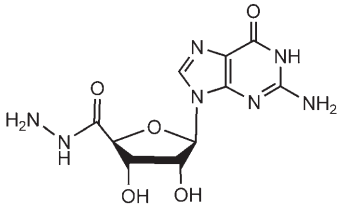
Nampally Sreenivasachary and Jean-Marie Lehn*[a]

Abstract: A guanosine-5′-hydrazide can entrap biologically interesting molecules such as acyclovir, vitamin C, and vancomycin into its hydrogel network. Controlled release of these molecules was monitored by ^{1}H NMR spectroscopy. The hydrazide may potentially form mixed G–G quartets with analogous compounds containing a guanine group. ^{1}H NMR spectroscopy was used to study the inclusion of various guanine derivatives into the hydrogel. The structural selectivity was found to depend strongly on both the shape and the charge of the additive and may arise from the strong cohesion of the supramolecular architecture of the gel and the resulting resistance to perturbation by foreign bodies. Hydrogels thus offer a promising medium for highly selective, controlled release of bioactive substances.

Keywords: bioactive molecules · drug delivery · G-quartets · guanine · hydrogels

Introduction

Drug-formulation and -delivery systems have a strong impact on medical technology[1]. Increasing attention has been focused on methods for the continuous delivery of over prolonged time periods in a controlled fashion. In recent years, there have been numerous developments in drug carriers and controlled-release systems. In addition to cross-linked polymers,[2] hydrogels also have been of interest for drug release because of their hydrophilic character and potential biocompatibility.[3,4] In the course of our investigations into supramolecular hydrogels,[5] we became interested in the controlled release of small molecules, such as flavors, fragrances,[6] and drug molecules that would be either covalently linked through a reversible bond or connected through noncovalent interactions such as hydrogen bonds. Guanosine-5′-hydrazide **1** was found to yield stable hydrogels and to react with aldehydes to form reversible acylhydrazone derivatives in an aqueous medium. The resulting constitutional dynamic system[7] displayed marked component selection driven by evolution towards the formation of the most organized hydrogel-like phase.[5]



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Guanosine derivatives can form tetrameric arrangements through Hoogsten-type hydrogen-bonding, supramolecular G-quartet (G_4) macrocycles, which stack into columnar G_4 assemblies in the presence of cations such as Na^+, K^+, and NH_4^+ with the subsequent formation of hydrogels (Figure 1)[8]. Modified guanosine derivatives have been used to generate a wide variety of new materials.[8–12]

The guanosine-5′-hydrazide **1** may, in principle, incorporate substrate molecules either through hydrogen-bonding supramolecular interactions or through the formation of a reversible covalent acylhydrazone bond with carbonyl compounds. Indeed, **1** was found to yield supramolecular hydrogels in the presence of cations and to react with biologically interesting aldehyde derivatives to form acylhydrazones reversibly, with component selection.[5] ^{1}H NMR spectroscopic studies allowed the determination of the fractions of bound and free component.

[a] Dr. N. Sreenivasachary,[+] Prof. Dr. J.-M. Lehn
Institut de Science et d′Ingénierie Supramoléculaires
Université Louis Pasteur
8 Allée Gaspard Monge
F-67000 Strasbourg (France)
Fax: (+33) 390-245-140
E-mail: lehn@isis.u-strasbg.fr

[+] Present Address:
AC Immune SA, EPFL, PSE-B
Ecublens, Lausanne
CH-1015 (Switzerland)

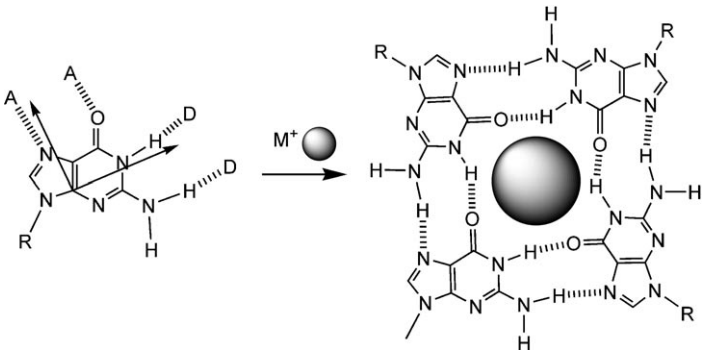


Figure 1. Self-assembly of guanine derivatives into G_4-quartets in the presence of metal ions.

These previous results led to the conclusion that additives in the gels formed by **1** may be present in three different incorporation states involving:

1) reversible covalent connection, through acylhydrazone formation with carbonyl-containing compounds;
2) noncovalent inclusion into the gel structure, with possible insertion into the G-quartets in the case of guanine derivatives;
3) occlusion as free molecules in the scavenged solvent of the gel.

For states 1) and 2), the additives may be expected to be "NMR-silent", their ^{1}H NMR signals being strongly broadened (beyond detection) by motional freezing, whereas they are "NMR-active" in state 3), allowing the determination of the fraction of free molecules.

Abstract in Telugu: గ్వానోసిన్ హైడ్రజిడ్ జలద్రావణంలో జల అర్ధఘనము (hydrogel) గా ఏర్పడుచున్నది. ఈ అర్ధఘనము లోని వలలాంటి నిర్మాణములో జీవసంబంధాసక్తి (biologically interesting) గల అణువులైన అసైక్లోవీర్, వాంకోమైసిన్, మరియు విటమిన్ 'సి' లు చిక్కుబడగలవు. చిక్కుబడిన ఈ అణువుల యొక్క ఆధీనపు విడుదలను ప్రోటాన్ ఎన్ ఎం ఆర్ ('HNMR) వర్ణపటమాపకం ద్వార అనుసరించడమైనది. గ్వానిన్ సంబంధిత వివిధ అణువులతో గ్వానోసిన్ హైడ్రజిడ్ శక్యమైన జి-జి (G-G) మిశిత చతుష్కములను (mixed quartets) ఏర్పరచగలదు. గ్వానిన్ సముదాయము గల వివిధ అణువులు చతుష్కములో ప్రవేశించును. ఈ మిశిత చతుష్కములో సరియైన అణువుల ఎంపిక అనేది, వాటి యొక్క ఆకారము మరియు ఆవేశము రెండింటి పైన ఆధారపడి ఉంటుందని ప్రోటాన్ యన్ యం ఆర్ వర్ణపటమాపకం ద్వారా పరిశీలించడమైనది. ఈ హైడ్రోజెల్ (hydrogel) లోని అణువుల క్రమ అమరిక వల్ల ఏర్పడే బహుళాణునిర్మాణము (supramolecular architecture) భిన్నమైన పదార్ధముల వైకల్య రహిత ఏన్నికకు దోహదము చేయుచున్నది, అందువల్ల జీవసంబంధిత పదార్ధముల ప్రత్యేక, ఆధీనపు విడుదలకు ఈ హైడ్రోజెల్ ను మధ్యస్థ పదార్ధముగా ఉపయోగించుటకు అవకాశము కలదు.

State 1) was studied previously.[5] It appeared that it would be of great interest to investigate states 2) and 3), as such data would provide, on the one hand, information about the selectivity of incorporation or occlusion into the gel, and on the other, a basis for the study of controlled release from the gel.

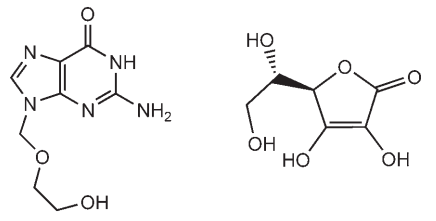
Three release processes may be considered: 1) release from a stable gel unperturbed by the additive(s); 2) release with concomitant leaching of the gel-forming material, depending on the destabilization of the gel by the additives; 3) a combination of 1) and 2). The degree of destabilization of the gel by the additive(s) may be estimated from the decrease in the melting temperatures of the gels.

Results and Discussion

Controlled Release of Bioactive Molecules from the Gel of 1

Guanosine-5′-hydrazide **1** can potentially form mixed G-quartets with compounds that contain a guanine moiety, which thus would be incorporated into its hydrogel; quartet formation controls their release into the surroundings.

Acyclovir (**2**) is an antiviral agent that contains a guanine group and that has been used for the treatment of herpes simplex infections.[13] However, oral treatment is often difficult owing to insufficient drug concentration in saliva when acyclovir is administered in conventional tablet form. As a consequence, several methods have been developed for its controlled release, including encapsulation into biodegradable polymer nanospheres,[14] acyclovir–dextran conjugates obtained by imine bond formation as macromolecular carriers for long-term drug therapy,[15] and liposomes as delivery



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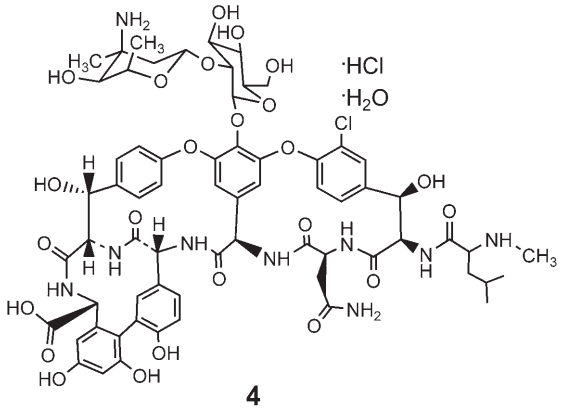

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systems.[16] We became interested in using the hydrogel formed by **1**[5] as a potential method for the controlled release of this active ingredient and related substances in a supramolecular fashion.

The release experiments were conducted in D_2O. The guanosine-5′-hydrazide **1** was dissolved in D_2O together with acyclovir (**2**) and KCl in several glass test tubes. The test tubes were heated gently at 60 °C and cooled to room temperature. Once the hydrogel was formed (approximately 2 h), deuterated buffer solution was added very carefully to the top of the hydrogel. ^{1}H NMR spectra of samples of this top layer were recorded at various time intervals (*tert*-butanol served as an internal reference). The percent of acyclovir released into the supernatant buffer solution was determined by integrating the signals for the acyclovir guanine proton 8-H relative to the signal for *tert*-butanol. The release of **2** reached a plateau at 55 % after 9 h, after which about 7–8 % of hydrazide **1** was also released (Figure 2).

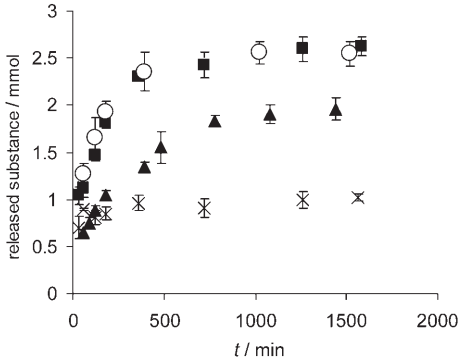

Figure 2. Controlled release profiles from the hydrogel formed by **1** as determined by ^{1}H NMR spectroscopy for (■) Acyclovir **2**, (55 %) (○) Vitamin C **3**, (55 %), (▲) Vancomycin **4**, (39 %) (x) Guanosine-5′-hydrazide **1** (7 %). The experiments were conducted in 0.5 M sodium acetate buffer at pD 6 or D_2O and 20 °C. Each result indicated is the average of three experiments. Error bars represent the standard deviation obtained from three experiments in each case

The controlled release of other biologically interesting molecules, vitamin C (**3**) and vancomycin (**4**) was also studied. In the case of vitamin C, about 55 % was released into the buffer solution within 24 h, whereas for vancomycin only about 39 % was released (Figure 2). The release of acyclovir was found to be appreciably faster than expected when compared to vitamin C and vancomycin. The different levels of release of the additives at saturation may result from the different distributions between the gel and aqueous phases at equilibrium.

We therefore investigated the state of the three compounds in the gel. ^{1}H NMR spectroscopic measurements indicated that about 70 % of the acyclovir was occluded in the solvent of the gel and 30 % was included in the gel. Similarly, it was shown that vitamin C **3** and vancomycin **4** were occluded less (about 15 % and 40 %, respectively).

Next, we considered the nature of the release process (see above). We tested the effect of the addition of a G-analogue on the melting temperature of the hydrogel (Figure 3). The hydrogel of guanosine-5′-hydrazide **1** was prepared in the presence of G-ester **8** in a solution of KCl in D_2O. The melting temperature (T_{gel}) was measured by the test-tube tilting method. The mixed hydrogel melted at 49 °C, whereas the hydrogel of **1** on its own melted at 61 °C under the same conditions. This suggests that the assembly of the hydrogel of **1** is destabilized by incorporation of the foreign G-analogue additive, possibly through formation of mixed G-quartets (see below). In contrast, the melting temperatures tested in the presence of G-analogues **2**, **5**, **6**, and **7** decreased only to about 55 °C, in agreement with the fact that they do not form mixed G-quartets and hence do not participate markedly in the gel structure. The results are summarized in Figure 3.

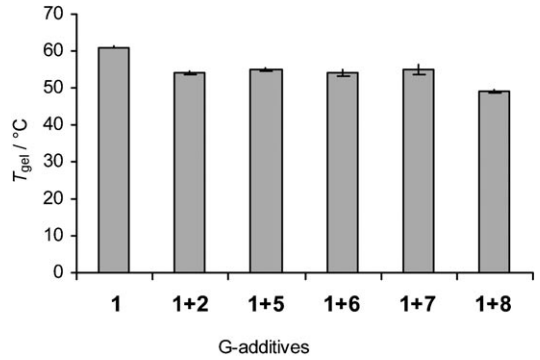

Figure 3. Temperature of gelation T_{gel} determined visually for mixtures of guanosine-5′-hydrazide **1** (15 mM) with G-analogues (5 mM) in D_2O and K^+ (100 mM); T_{gel} values in °C for **1** alone 61 °C; **1**+**2** (54 °C); **1**+**5** (55 °C); **1**+**7** (54 °C), **1**+**6** (55 °C): **1**+**8** (49 °C).

It thus appeared that **1** is strongly selective towards quartet formation. This result prompted us to carry out a ^{1}H NMR spectroscopic investigation into the structural selection relationships of different types of guanosine derivatives and their inclusion in hydrogel networks through eventual incorporation into the G-quartet superstructure.

Selective Incorporation of Guanosine Derivatives into the Hydrogel Formed by 1

Several derivatives **6**–**12** of guanosine **5** were investigated for the selectivity of their incorporation into the hydrogel formed by **1**. Compounds **7**–**12** were synthesized as described in the literature.[17] To our knowledge, there are no reports of the self-assembly of mixed G-quartets[18] and their subsequent hydrogel formation.

Compounds **2** and **5**–**12** bear a G group that can, in principle, be incorporated into the G-quartets formed by **1** and thus into the supramolecular network of its hydrogel. They should, therefore, allow a study of the structural effects on the selectivity of such incorporation. We determined the inclusion of compounds **2** and **5**–**12** into the hydrogel by

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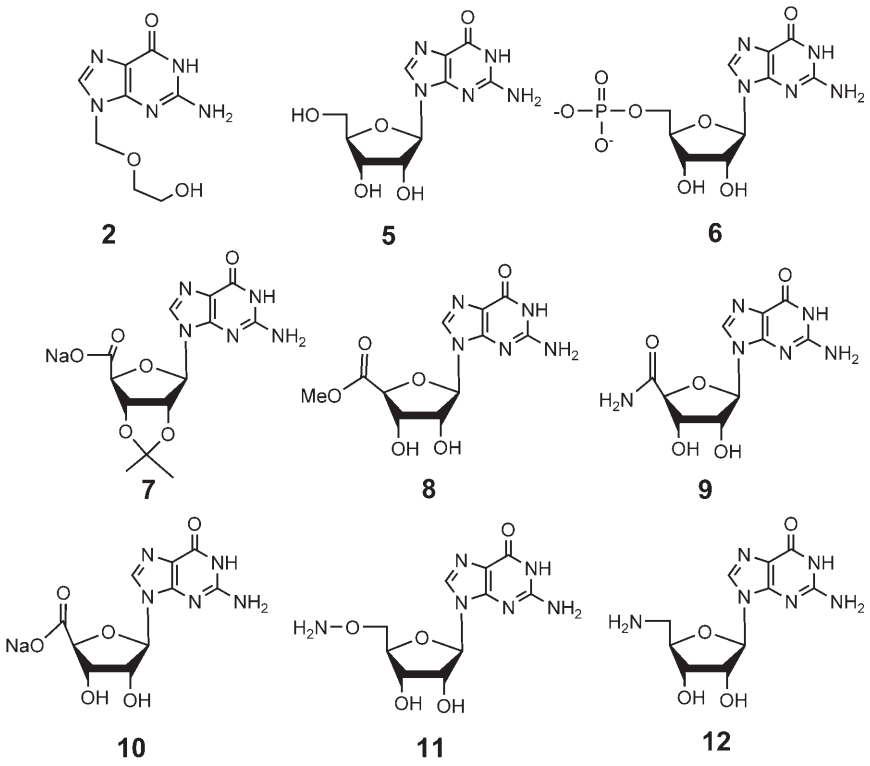

means of ^{1}H NMR spectroscopic studies. The hydrogel was prepared from **1** and a G-derivative (**2**, **5–12**) at concentrations of 15 mM and 5 mM, respectively, in a solution of KCl in D_2O. The ^{1}H NMR spectra were recorded once the sample was fully gelated (see Experimental Section). The percentage of free G-derivative was determined by integrating the observable signal for 8-H of the guanine group with respect to an internal *tert*-butanol reference. The calculated fractions of incorporation of each additive into the gel are summarized in Figure 4.

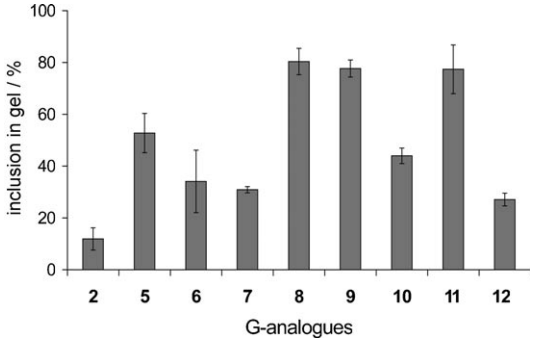

Figure 4. Percentage of incorporation of compounds **2** and **5–12** (5 mM each) into the gel formed by **1** (15 mM), calculated from the percentage of free compound determined by ^{1}H NMR spectroscopy: **2** (12±4%), **5** (53±8%), **6** (34±12%), **7** G-CO_2Na (31±1.2%), **8** G-CO_2Me (81±5%), **9** G-$CONH_2$ (78±3%), **10** G-COOH (44% ±3%), **11** G-CH_2ONH_2 (78±9%), **12** G-CH_2NH_2 (27±3%). Each result indicated is the average of three experiments. Error bars represent the standard deviation, obtained from three experiments in each case. KCl solution in D_2O (100 mM).

From these results we can deduce that:

1) There is a high selectivity of incorporation into the gel despite the presence of a G group in all the additives. Thus, inclusion is very sensitive to small structural modifications. One may surmise that the included molecules are directly incorporated into the G-quartet supramolecular core entity.
2) The highest inclusion is found for compounds **8**, **9**, and **11**, neutral analogues of the hydrogelator **1**, in which the hydrazide group -$CONHNH_2$ of **1** is replaced by the very closely related -$COOCH_3$ and -$CONH_2$ as well as the more different -CH_2ONH_2 groups.
3) The comparatively lower incorporation of **6**, **7**, and **10** may be due to the fact that all three compounds are negatively charged (**7** is expected to be ionized at pD 6) and are thus appreciably more soluble in aqueous media. The same may hold for the positively charged, protonated **12**.
4) Acyclovir (**2**) is least incorporated as it is also the least closely related to **1**, lacking the ribose group.
5) The hydrogel formed by **1** thus presents high discrimination power against the incorporation of foreign additives into the cohesive network of the supramolecular assembly. It displays both shape (see **2**) and charge (see **6**, **7**, **10**, and **12**) selectivity.

Release Experiments with 6 and 8

With the information about the selectivity of incorporation of G-analogues into the hydrogel of **1** in hand, guanosine monophosphate (GMP, **6**) and G-ester **8** were selected for release experiments aimed at analyzing whether there is a relationship between the selection of such G-containing additives and their release, potentially due to the formation of mixed G-quartets.[18] The release experiments were conducted similarly as described above for **2** and **3**.

The guanosine-5′-hydrazide **1** and G-ester **8** were dissolved in a solution of KCl in D_2O, and the sample was gently heated until dissolution took place. Gelation occurred upon cooling down, D_2O was added carefully on top of the gel, and ^{1}H NMR spectra of samples of the top layer was recorded at various time intervals in the presence of an internal reference (*tert*-butanol). The amount of G-ester **8** released into the supernatant was determined by integrating

the signals for 8-H of G-ester **8** relative to the signal for *tert*-butanol. The same experiment was conducted with **6**. It was found that **6** and **8** were released to about 41% and 8%, respectively (Figure 5a). In all the experiments in which **8** (5 mM) was added to **1** (15 mM), a transparent gel was obtained, whereas the gel remained opaque upon addition of **6**. This may be due to the fact that substantial incorporation of **8** into the gel may increase the fraction of mixed G–G quartets, resulting in less of the fibrous gel structure formed by **1**.[5a,b] Release experiments of G-ester **8** for more than 36 h was not possible as the transparent gel dissolved in water after 38 h (Figure 5a).

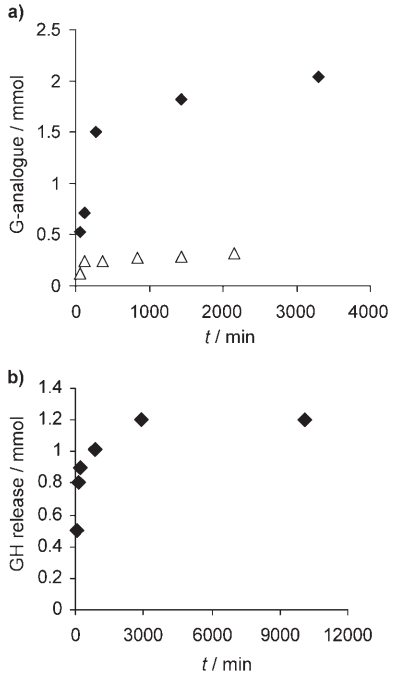

Figure 5. a) Controlled release profile of (◆) **6** (41%) and (△) **8** (8%) from the hydrogel formed by **1** (15 mM) in KCl/D_2O (100 mM) as determined by ^{1}H NMR spectroscopy (20°C); b) Controlled release of (◆) **1** (10%) as a function of time determined by NMR spectroscopy.

As expected, the more highly incorporated G-ester **8** (80%) was released much more slowly than GMP **6** (35% incorporation, Figure 4). This result speaks in favor of a tighter participation of **8** in the G-quartet formation and hence its slower release than that of the GMP **6** derivative. In a control experiment conducted with **1** alone, without the addition of the G-analogue, the release of **1** reached a plateau at 10% after 58 h (Figure 5b). For all release experiments conducted in the presence of G-analogues **2**, **3**, **6**, and **8**, G-hydrazide **1** was also leached/released to about 8–10%. Figure 6 illustrates the process of controlled release of a G-containing compound from a mixed G-quartet.

Conclusions

The present results show that the hydrogel formed by the guanosine hydrazide **1** presents high structural selectivity towards the incorporation of G-containing compounds into the network of the gel. Assuming that such incorporation would occur by entry into the G quartet core formed by **1**, one may surmise that the G_4 supramolecular entity and its stacked assemblies strongly discriminate against foreign substances, owing to the cohesion of the supramolecular network of the gel that resists perturbation. The hydrogel formed by **1** and by extension hydrogels in general[3,4,19] are thus promising media for the controlled release of bioactive molecules, with rates of release depending on the nature of inclusion into the gel, by occlusion or incorporation. Such release would also be expected to depend on the supramolecular architecture of the gel and its dependence on physical triggers and chemical effectors.

Experimental Section

General

Acyclovir, guanosine monophosphate, guanosine, vancomycin, and vitamin C were obtained commercially (Sigma–Aldrich) and used without further purification. NMR spectra were recorded on a Bruker 400 MHz spectrometer. Deuterated buffer solutions for NMR measurements were prepared in D_2O using a desired concentration of CD_3COOD (for pD 4–6) and then the pH (pD) was adjusted by using a solution of NaOD in D_2O. The pD of the solution was monitored with a pH meter and the pD of buffer solution is equal to the pH meter reading + 0.4.

Determination of Controlled Release Profile by ^{1}H NMR Spectroscopy

The fraction of drug molecules diffused from the hydrogel network into the supernatant was determined by ^{1}H NMR spectroscopy by following a standard procedure, as described for acyclovir (**2**). Hydrazide **1** and acyclovir (**2**) were dissolved in several test tubes in sodium acetate buffer

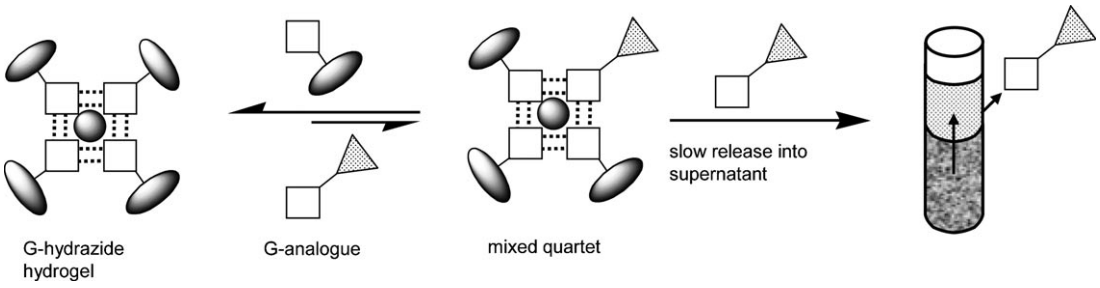

Figure 6. A schematic illustration of the formation of mixed quartets and their subsequent selective controlled release of G-analogues into the supernatant.

(0.5 M, pD 6; 500 μL) to make up concentrations of 15 mM, and 5 mM respectively. The containers were heated until **1** dissolved completely and then cooled to room temperature. When gelation was occurred, sodium acetate buffer (500 μL) was added very carefully on top of the hydrogel. At different time intervals, the supernatant from different test tubes was transferred carefully to an NMR tube by using a syringe. The ^{1}H NMR spectra were recorded with an internal reference *tert*-butanol (3 mM). The percentage of molecule released from the hydrogel was determined by integrating the signals for 8-H of **2** relative to the signal for *tert*-butanol. In a separate NMR tube, **2** and *tert*-butanol were dissolved in D_2O (500 μL) to concentrations of 5 mM and 3 mM, respectively, and the ^{1}H NMR spectrum was recorded. The integration of the signal for 8-H of the completely dissolved acyclovir with respect to the signal for internal *tert*-butanol (3 mM) gave the amount of acyclovir. The difference between the integral for total free acyclovir (**2**) (100% solution) and the integral of the released acyclovir at various time intervals gave the fraction of compound released into the supernatant from the hydrogel. The release profile for vitamin C (**3**) and vancomycin (**4**) were obtained in a similar way.

General Procedure for ^{1}H NMR Determination of the Inclusion of Guanosine Derivatives into the Hydrogel Formed by **1**

The fraction of the G-derivative (**2** in this example) in the hydrogel was determined by ^{1}H NMR spectroscopy. Into an NMR tube were introduced **1** (2.3 mg), a solution of **2** in D_2O (50 mM; 50 μL), D_2O (400 μL), a solution of KCl in D_2O (1 M; 50 μL), and a solution of *tert*-butanol in D_2O (300 mM; 5 μL) to yield a solution of **1**, the G derivative, KCl, and *tert*-butanol with concentrations of 15 mM, 5 mM, 100 mM, and 3 mM, respectively. The NMR tube was heated gently until **1** dissolved and was then cooled to room temperature. The ^{1}H NMR spectra were recorded once the sample was fully gelated. The percentage of free G-derivative was determined by integrating the signal for 8-H with respect to that for the internal reference. The signal for 8-H is sharp for free **2** in solution, whereas that for **2** engaged in the gel it is broadened beyond detection. Integration of the observable signal for 8-H with respect to the internal reference gives the amount of **2** still free in the gelated solution. In a separate NMR tube, **2** and *tert*-butanol were dissolved in D_2O (500 μL) to concentrations of 5 mM and 3 mM, respectively, and the ^{1}H NMR spectrum was recorded. The integration of 8-H of completely dissolved **2** with respect to internal *tert*-butanol (3 mM) gave the amount of acyclovir. The difference between the integral of total free acyclovir (100% solution) and the integral of acyclovir in the hydrogel gave the fraction of free **2** in the hydrogel. Similarly, the percentage of the inclusion of the guanosine derivatives **5**, **6**, **7**, **8**, **9**, **10**, **11**, and **12** were calculated by using ^{1}H NMR spectroscopy.

Acknowledgements

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[1] a) A. S. Hoffman, *Adv. Drug Delivery Rev.* **2002**, *54*, 3–12; b) Y. Qiu, K. Park, *Adv. Drug Delivery Rev.* **2001**, *53*, 321–339; c) I. C. Kwon, Y. H. Bae, S. W. Kim, *Nature* **1991**, *354*, 291–293; d) D. A. LaVan, T. McGuire, R. Langer, *Nat. Biotechnol.* **2003**, *21*, 1184–1191; e) M. Vert, *Prog. Polym. Sci.* **2007**, *32*, 755–761; f) M. Vert, *Biomacromolecules* **2005**, *6*, 538–546.

[2] C.-S. Ha, J. A. Gardella, Jr., *Chem. Rev.* **2005**, *105*, 4205–4232.

[3] See for controlled release: a) F. Hirayama, K. Uekama, *Adv. Drug Delivery Rev.* **1999**, *36*, 125–141; b) P. Gupta, K. Veramani, S, Garg, *Drug Discovery Today* **2002**, *7*, 569–579; c) Y. Qiu, K. Park, *Adv. Drug Delivery Rev.* **2001**, *53*, 321–339; d) A. Friggeri, B. L. Feringa, J. V. Esch, *J. Controlled Release* **2004**, *97*, 241–248; e) H. He, X. Cao, L. J. Lee, *J. Controlled Release* **2004**, *95*, 391–402; f) Y-Y. Liu, X-D. Fan, *Biomaterials* **2005**, *26*, 6367–6374.

[4] a) H. K. Ju, S. Y. Kim, Y. M. Lee, *Polymer* **2001**, *42*, 6851–6857; b) X. Z. Zhang, D. Q. Wu, C. C. Chu, *Biomaterials* **2004**, *25*, 3793–3805; c) F. Quaglia, G. Varricchio, A. Miro, M. I. L. Rotonda, D. Larobina, G. Mensitieri, *J. Controlled Release* **2001**, *71*, 329–337; d) A. Zumbuehl, L. Ferreira, D. Kuhn, A. Astashkina, L. Long, Y. Yeo, T. Laconis, M. Ghannoum, G. R. Fink, R. Langer, D. S. Kohane, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 12994–12998.

[5] a) N. Sreenivasachary, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**. *102*, 5938–5943; for details on the structure of the hydrogel, see: b) E. Buhler, N. Sreenivasachary, S.-J. Candau, J.-M. Lehn, *J. Am. Chem. Soc.* **2007**, *129*, 10058–10059.

[6] a) B. Levrand, Y. Ruff, J.-M. Lehn, A. Herrmann, *Chem. Commun.* **2006**, 2965–2967; b) A. Herrmann, *Angew. Chem.* **2007**, *119*, 5938–5967; *Angew. Chem. Int. Ed.* **2007**, *46*, 5836–5863.

[7] a) J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4763–4768; b) J.-M. Lehn, *Prog. Polym. Sci.* **2005**, *30*, 814–831.

[8] W. Guschlbauer, J. F. Chantot, D. Thiele, *J. Biomol. Struct. Dyn.* **1990**, *8*, 491–511.

[9] G. P. Spada, A. Carcuro, F. P. Colonna, A. Garbesi, G. Gottarelli, *Liq. Cryst.* **1998**, *3*, 651–654.

[10] G. Gottarelli, G. P. Spada, A. Garbesi in *Comprehensive Supramolecular Chemistry, Vol 9* (Eds.: J. L Atwood, J. E. D Davies, D. D. MacNicol, F. M. Vögtle, J.-M. Lehn) **2006**, Pergamon, Oxford, **1996**, pp 483–506.

[11] J. T. Davis, *Angew. Chem.* **2004**, *116*, 684–716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698.

[12] a) A. L. Marlow, E. Messina, G. P. Spada, S. Masiero, J. T. Davis, G. Gottarelli, *J. Org. Chem.* **1999**, *64*, 5116–5123; b) A. L. Marlow, J. T. Davis, *Tetrahedron Lett.* **1999**, *40*, 3539–3542; c) G. Gottarelli, S. Masiero, G. P. Spada, *J. Chem. Soc. Chem. Commun.* **1995**, 2555–2557.

[13] J. J. O'Brien, D. M. Campoli-Richards, *Drugs* **1989**, *37*, 233–309.

[14] M. Fresta, G. Fontana, C. Bucolo, G. Cavallaro, G. Giammona, G. Puglisi, *J. Pharm. Sci.* **2001**, *90*, 288–296.

[15] J. Tu, S. Zhong, P. Li, *Drug Dev. Ind. Pharm.* **2004**, *30*, 959–965.

[16] Z. Pavelic, N. Skalko-Basnetb, J. Filipovic-Grcic, A, Martinaca, I. Jalsenjaka, *J. Controlled Release* **2005**, *106*, 34–43.

[17] a) J. B. Epp. , T. S. Widlanski, *J. Org. Chem.* **1999**, *64*, 293–295; b) M. Perbost, T. Hoshiko, F. Morvan , E. Swayze, R. H. Griffey, Y. S. Sanghvi, *J. Org. Chem.* **1995**, *60*, 5150–5156.

[18] The formation of mixed G-quartets between D- and L- guanosine enantiomers has been reported: X. Shi. , J. C. Fettinger. , J. T. Davis, *J. Am. Chem. Soc.* **2001**, *123*, 6738–6739.

[19] N. A. Peppas, J. Z. Hilt, A. Khademhosseini, R. Langer, *Adv. Mater.* **2006**, *18*, 1345–1360.

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