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Identification by ^{15}N Refocused INADEQUATE MAS NMR of Intermolecular Hydrogen Bonding that Directs the Self-Assembly of Modified DNA Bases

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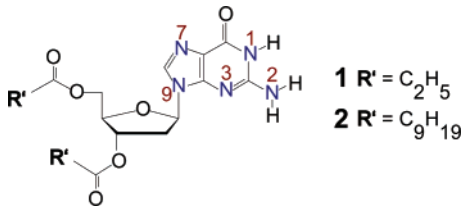
Hydrogen bonding is a key interaction in determining the three-dimensional shape and hence the physiological function of biomacromolecules. For example, hydrogen bonding between complementary CG and AT base pairs plays a central role in the structure of double-helical DNA. Considering the DNA bases, guanine is very versatile in terms of acting as both a hydrogen-bonding donor and acceptor. Indeed, guanine readily self-assembles in the presence of metal ions, such as Na^+, to form G-quartets (Figure 1a) that have been exploited by supramolecular chemistry, for example, in the construction of nanowires and biosensors.[1] Recently, it has been shown by X-ray single-crystal diffraction that the lipophilic deoxyguanosine derivative **1** self-assembles to form an alternative ribbon-like polymeric structure (Figure 1b).[2] Moreover, the longer-chain derivative **2** has been used to fill the gap between nanocontacts obtained by electron beam lithography, so as to produce devices with interesting electrical properties, namely, they are photoconductive,[3] and when the gap between the contacts is smaller than 100 nm, they act as a rectifier.[4,5] The same molecule has also been used for biophotonic applications.[6] To date, it has not been possible to obtain a diffraction crystal structure for **2**. This communication describes the determination by solid-state NMR of the intermolecular hydrogen-bonding arrangement and hence the type of self-assembly in **1** and **2**.

The recent observation of solution-state NMR *J* couplings across hydrogen bonds has generated much interest since their observation and measurement allows the identification of hydrogen-bonded partners, as well as the quantification of hydrogen-bond strengths and geometries.[7,8] In the solid state, the direct detection of an *intramolecular* hydrogen bond via the presence of correlation peaks due to a hydrogen-bond-mediated *J* coupling in a ^{15}N refocused INADEQUATE[9,10] spectrum has recently been reported.[11] Furthermore, ^{15}N spin−echo MAS experiments have allowed the quantitative determination of such intramolecular hydrogen-bond-mediated *J* couplings that are a measure of hydrogen-bonding strength.[12]



Two fully ^{15}N-labeled deoxyguanosine derivatives, **1** and **2**, were synthesized (see Supporting Information). As shown below, this study revealed the existence of two polymorphic forms of **2**. For all samples, it is to be emphasized that no metal ions were present, as shown by mass spectrometry (MS) (see Supporting Information).

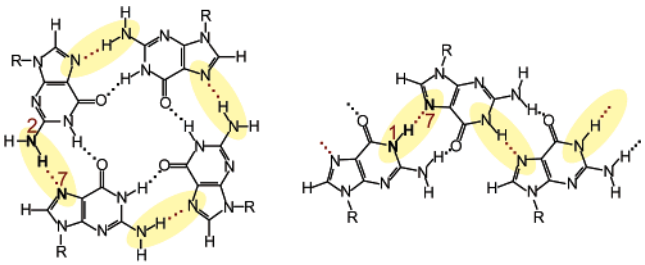


Figure 1. Formation of (a) quartet and (b) ribbon supramolecular structures by guanine, characterized by (a) N2−H···N7 and (b) N1−H···N7 intermolecular hydrogen bonding.

Figure 2a presents a ^{15}N refocused INADEQUATE spectrum of **1**. A doubling of some peaks in the single-quantum (SQ) dimension (notably N7 and N3) is apparent—this is in agreement with the presence of two distinct molecules in the asymmetric unit cell of **1**.[2] Of the four observed pairs of correlation peaks, three can be assigned to intramolecular connectivities, with the following two-bond *J* coupling constants known from solution-state NMR: $^2J_{N1N2} = 2.2$ Hz, $^2J_{N2N3} = 6.0$ Hz, and $^2J_{N3N9} = 3.7$ Hz.[13] The pair labeled in bold italics cannot be due to an intramolecular connectivity since the corresponding intramolecular $^3J_{N1N7}$ coupling is negligible (it was not observed in the solution-state NMR study[13]). Rather, this N1−N7 correlation is due to an *intermolecular* hydrogen-bond-mediated $^{2h}J_{N1-H\cdots N7}$ coupling. The crystal structure of **1** is known,[2] and this observation is consistent with the known ribbon-like structure (see Figure 1b). Note that the peaks due to the intermolecular hydrogen-mediated *J* connectivity are of the same intensity as those due to the stronger of the intramolecular *J* connectivities. This is consistent with the determination by solution-state NMR for C−G hydrogen-bonded DNA base pairs of $^{2h}J_{N-H\cdots N}$ coupling constants of magnitude 6−7 Hz,[7,8] that is, they are of similar magnitude to the intramolecular two-bond coupling constants.

Figure 2 also presents ^{15}N refocused INADEQUATE spectra of two polymorphic forms of **2**, referred to as **2**r (Figure 2b) and **2**q (Figure 2c). As discussed in the Supporting Information, we have found, under standard laboratory conditions, that it is the **2**q polymorph that forms more readily from ethanol. The spectrum of **2**r (Figure 2b) resembles that of **1** (Figure 2a)—note that a doubling of resonances for **2**r is apparent in the ^{15}N cross-polarization (CP) MAS spectrum (see Supporting Information). Specifically, the same N1−N7 correlation due to an *intermolecular* hydrogen-bond-mediated $^{2h}J_{N1-H\cdots N7}$ coupling is observed, hence, indicating that the **2**r polymorph adopts the same ribbon-like structure found in the crystal structure of **1**.[2] By contrast, the spectrum of **2**q (Figure 2c), while sharing the same set of intramolecular correlations, displays a striking difference: the N1−N7 correlation has been

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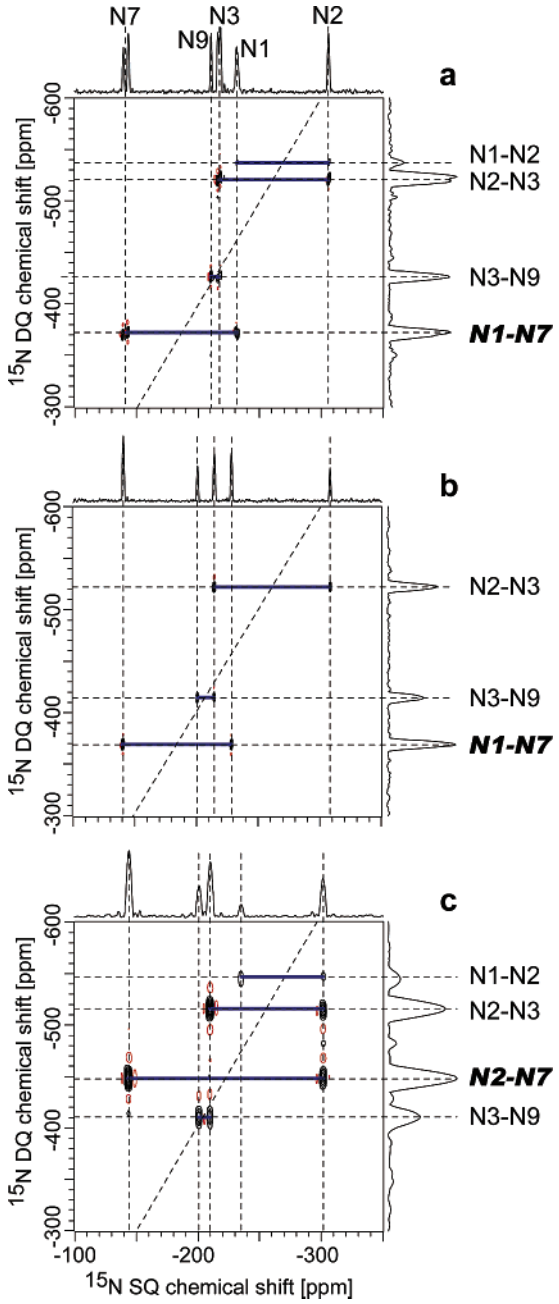

Figure 2. Solid-state NMR ^{15}N refocused INADEQUATE spectra together with skyline projections of (a) **1**, and (b and c) two polymorphic forms of **2**, referred to here as (b) **2**r and (c) **2**q. Experiments were performed on Varian (a and b) Infinity and (c) Infinity+ spectrometers operating at ^{15}N and ^{1}H Larmor frequencies of (a and b) 60.8 and 600.1 MHz and (c) 30.4 and 300.1 MHz, using Varian 3.2 mm double-resonance probes. The MAS frequency was 14.3 kHz. Rotor-synchronized *J* evolution periods of duration (a and b) τ = 9.65 ms and (c) τ = 14.0 ms were used. TPPM[14] ^{1}H decoupling at a nutation frequency of 100 kHz was applied in t_1, t_2, and the *J* evolution periods. For each of 64, t_1 increments of 40 μs (a and b), 128 and (c) 512 transients were co-added. The recycle delay was 7 s. Horizontal bars indicate pairs of double-quantum (DQ) peaks corresponding to pairs of ^{15}N nuclei with a through-bond *J* connectivity. Intermolecular hydrogen-bond-mediated correlations are labeled in bold italics. The $F_1 = 2F_2$ diagonal is indicated as a dotted line. The floor contour level is at 15%. Positive and negative contours are in black and red, respectively.

replaced by a new correlation, N2−N7. Since the intramolecular $^{5}J_{N2N7}$ coupling is negligible, this correlation must be due to an *intermolecular* hydrogen-bond-mediated $^{2h}J_{N2-H\cdots N7}$ connectivity. Noting the different intermolecular hydrogen-bonding arrangements for the quartet and ribbon structures in Figure 1, this observation strongly suggests that **2**q self-assembles to form quartets.

The quartet structure adopted by **2**q in the absence of metal ions is surprising since the accepted dogma is that G-quartet formation requires metal ions.[1] Reported exceptions are rare; a guanosine with a large C8 substituent forms a G-quartet with an empty cavity,[15] while STM images reveal G-quartets for guanine deposited onto Au(111).[16] Kotch et al. have also postulated that a calix[4]arene−guanosine conjugate forms G-quartets with water taking the place of the cation.[17]

We have demonstrated that different N−H···N intermolecular hydrogen-bonding arrangements can be unambiguously identified in ^{15}N refocused INADEQUATE solid-state NMR spectra. The power of the ^{15}N refocused INADEQUATE experiment is that it relies on the *J* coupling—an interaction that requires a chemical bond—to establish correlation between ^{15}N nuclei. This is to be compared with recently presented ^{15}N−^{15}N SQ−SQ correlation spectra where through-space dipolar couplings, either directly between two ^{15}N nuclei[18] or indirectly via proton-driven spin diffusion,[19] were used to identify inter-residue N−H···N hydrogen bonding in RNA. In studying the self-assembly of nucleic acid bases, there is much potential to use a multinuclear solid-state NMR approach, using also, for example, ^{23}Na NMR.[20] Work employing ^{1}H DQ MAS and ^{1}H−^{13}C correlation experiments and chemical shift calculations is ongoing and will be reported elsewhere.

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Supporting Information Available: (i) Synthetic details, (ii) MS analysis, (iii) further NMR experimental details, (iv) ^{15}N CP MAS spectra, and table of ^{15}N chemical shifts. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668.
(2) Giorgi, T.; Grepioni, F.; Manet, I.; Mariani, P.; Masiero, S.; Mezzina, E.; Pieraccini, S.; Saturni, L.; Spada, G. P.; Gottarelli, G. *Chem.—Eur. J.* **2002**, *8*, 2143.
(3) Rinaldi, R.; Branca, E.; Cingolani, R.; Masiero, S.; Spada, G. P.; Gottarelli, G. *Appl. Phys. Lett.* **2001**, *78*, 3541.
(4) Maruccio, G.; Visconti, P.; Arima, V.; D'Amico, S.; Blasco, A.; D'Amone, E.; Cingolani, R.; Rinaldi, R.; Masiero, S.; Giorgi, T.; Gottarelli, G. *Nano Lett.* **2003**, *3*, 479.
(5) Rinaldi, R.; Maruccio, G.; Biasco, A.; Arima, V.; Cingolani, R.; Giorgi, T.; Masiero, S.; Spada, G. P.; Gottarelli, G. *Nanotechnology* **2002**, *13*, 398.
(6) Neogi, A.; Li, J.; Neogi, P. B.; Sarkar, A.; Moroc, H. *Elec. Lett.* **2004**, *40*, 1605.
(7) Dingley, A. J.; Grzesiek, S. *J. Am. Chem. Soc.* **1998**, *120*, 8293.
(8) Dingley, A. J.; Cordier, F.; Grzesiek, S. *Concepts Magn. Reson.* **2001**, *13*, 103.
(9) Lesage, A.; Bardet, M.; Emsley, L. *J. Am. Chem. Soc.* **1999**, *121*, 10987.
(10) Fayon, F.; Massiot, D.; Levitt, M. H.; Titman, J. J.; Gregory, D. H.; Duma, L.; Emsley, L.; Brown, S. P. *J. Chem. Phys.* **2005**, *122*, 194313.
(11) Brown, S. P.; Pérez-Torralba, M.; Sanz, D.; Claramunt, R. M.; Emsley, L. *J. Am. Chem. Soc.* **2002**, *124*, 1152.
(12) Brown, S. P.; Perez-Torralba, M.; Sanz, D.; Claramunt, R. M.; Emsley, L. *Chem. Commun.* **2002**, 1852.
(13) Buchner, P.; Maurer, W.; Ruterjans, H. *J. Magn. Reson.* **1978**, *29*, 45.
(14) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951.
(15) Sessler, J. L.; Sathiosatham, M.; Doerr, K.; Lynch, V.; Abboud, K. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 1356.
(16) Otero, R.; Schock, M.; Molina, L. M.; Laegsgaard, E.; Stensgaard, I.; Hammer, B.; Besenbacher, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 2270.
(17) Kotch, F. W.; Sidorov, V.; Lam, Y.-F.; Kayser, K. J.; Li, H.; Kaucher, M. S.; Davis, J. *J. Am. Chem. Soc.* **2003**, *125*, 15140.
(18) Leppert, J.; Urbinati, C. R.; Hafner, S.; Ohlenschlager, O.; Swanson, M. S.; Gorlach, M.; Ramachandran, R. *Nucleic Acids Res.* **2004**, *32*, 1177.
(19) Riedel, K.; Leppert, J.; Ohlenschlager, O.; Gorlach, M.; Ramachandran, R. *J. Biomol. NMR* **2005**, *31*, 331.
(20) Wong, A.; Wu, G. *J. Am. Chem. Soc.* **2003**, *125*, 13895.

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