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Enhancing Hoogsteen Interactions: A Pyrrole-Containing Purine Nucleoside That Competes with Guanosine Self-Assembly

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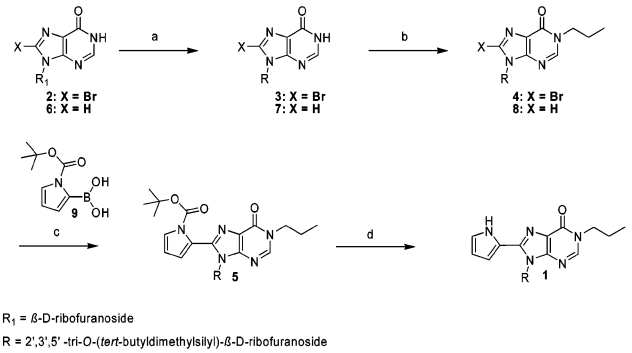
Synthetic nucleobases that allow for two-point Hoogsteen hydrogen bonding interactions[1] have been intensively studied,[2] largely as model systems for a variety of biological recognition events, including DNA triplex and guanine quadruplex formation.[3] The biological roles of these Hoogsteen stabilized structures are of tremendous importance. For example, guanine quadruplexes are connected with telomere stabilization and thus have possible implications in aging and tumorigenesis.[4] More recently, in vitro studies have revealed that the HIV-1 genome contains regions that form stable guanine quadruplexes.[5] Given the importance of the conventional Hoogsteen hydrogen bonding motif, we have been eager to "enhance" the two-point Hoogsteen interaction by the addition of a third hydrogen bond. We show with this communication that this goal may be accomplished by "extending" a purine via the attachement of a pyrrolic subunit. The resulting synthetic nucleoside (**1**) is capable of stabilizing a three-point Hoogsteen-type[6] interaction and binds guanosine in halogenated solvents ($CDCl_3$, CD_2Cl_2) and in the gas phase. This interaction competes with guanosine dimerization and can also disrupt guanosine quadruplex formation. However, removal or protection of the extra hydrogen bonding moiety results in decreased competitiveness.

The synthesis of nucleoside **1** is shown in Scheme 1. The hydroxyl functionalities on 8-bromoinosine **2** were converted to their respective *tert*-butyldimethylsilyl (TBDMS) ethers using a standard protection protocol.[7] The imino NH proton of **3** was subsequently alkylated to give the *N*-propyl nucleoside **4** using 1-bromopropane in the presence of DBU. A palladium (0)-catalyzed Suzuki cross-coupling reaction of **4** with *tert*-butoxycarbonyl (BOC) protected pyrroleboronic acid **9**[8] then yielded precursor **5**. Finally, the pyrrole-appended purine nucleoside target **1** was obtained after selective removal of the BOC protecting group using sodium methoxide in methanol.

Nucleoside **1** was designed to preclude Watson−Crick type interactions (by blocking the imino NH donor site) while favoring Hoogsteen interactions by "extending" this latter hydrogen bonding paradigm to three hydrogen bonds. For this "extension", the pyrrole NH function was chosen due to its H-bonding ability.[9] Incorporating a pyrrole ring at the 8-position of the purine nucleoside results in a donor−acceptor−acceptor (DAA) motif, capable of forming a three-point extended Hoogsteen-type interaction with guanosine **10** (a classic ADD motif) to produce ensemble **I** as shown in Figure 1A. Nucleosides **5** and **8** were made as controls. Compound **8** is expected to bind **10** less effectively than **1** due to the presence of only two hydrogen bonding sites on the Hoogsteen face; likewise, nucleoside **5** is expected to have negligible affinity for **10** due to the presence of the bulky BOC protecting group.

Initial evidence for the formation of ensemble **I** came from ^{1}H NMR spectroscopic studies carried out at 27 °C in CD_2Cl_2. The pyrrole NH proton of **1** resonates at 9.6 ppm, but when mixed with

Scheme 1. Synthesis of Nucleoside **1**[a]



[a] Reaction conditions: (a) TBDMSCl, imidazole DMF; (b) 1-bromopropane, DBU, DMF; (c) $Pd(PPh_3)_4$, Na_2CO_3 (aq), DME, toluene; (d) NaOMe in MeOH, THF.

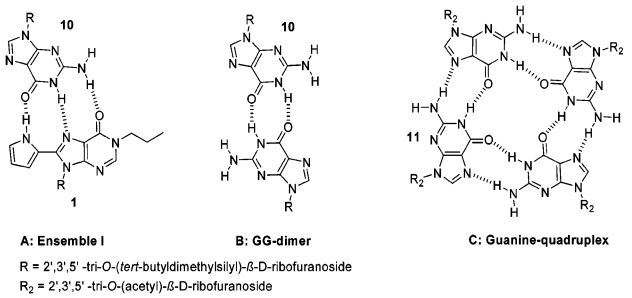


Figure 1. (A) Proposed three-point extended Hoogsteen-type interaction. (B) One of several proposed structures for the guanosine-guanosine dimer.[10] (C) Guanosine quadruplex.

0.6 equiv of guanosine **10**, this resonance shifts downfield to 10.5 ppm, indicative of hydrogen bonding. Under the conditions of the experiment, the imino NH resonance of **10** was also seen to resonate at low field ($\delta = 11.9$ ppm). However, this latter shift could also reflect some guanosine-guanosine (GG) dimer formation (Figure 1B). The formation of GG dimers in chloroform has been previously reported.[10] Schneider and co-workers have quantified the dimerization constant (K_{dimer}) of **10** by ^{1}H NMR spectroscopic titrations, revealing a value of 3×10^2 M^{-1} in $CDCl_3$ when the guanosine amino NH_2 resonance was followed.[10b] Repeating this experiment, a K_{dimer} value of $4.7 \pm 0.8 \times 10^2$ M^{-1} was obtained in this same solvent.[10c]

Due to the complexities arising from GG dimerization, variable-temperature ^{1}H NMR spectroscopic studies were undertaken to detail more precisely the nature of the interactions involved. On cooling to −80 °C two distinct downfield imino NH resonances of **10** were seen at 12.7 and 12.2 ppm, respectively. Low-temperature control experiments, involving guanosine (**10**) dimerization allowed these two resonances to be assigned as the imino NH protons of

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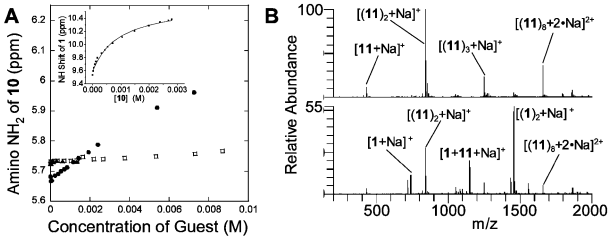

Figure 2. (A) ^{1}H NMR shift of the amino NH_2 resonance of **10** upon titrating **1** (●), **5** (▲),[13] and **8** (□) in $CDCl_3$. (Inset) ^{1}H NMR titration of **1** upon addition of **10** in $CDCl_3$; line shows the curve fit. (B) ESI-mass spectra of **11** (top) showing the doubly charged guanosine octamer and a mixture of **1** and **11** (bottom), revealing a decrease in the octamer peak.

the GG dimer and ensemble **I**, respectively. In addition, the pyrrole NH peak of **1** was identified; at −80 °C it was shifted even further downfield to 11.9 ppm.

With the key resonances assigned, the interaction between **1** and **10** was investigated via ^{1}H NMR spectroscopic titrations at 27 °C in $CDCl_3$. Initial ^{1}H NMR spectroscopic titrations were carried out with **10** as the host and **1**, **5**, and **8** as the guests, respectively. The amino NH_2 resonance of **10** was followed. As seen in Figure 2A, addition of nucleobase **1** induced a clear downfield shift of the amino resonance of **10**. In contrast, controls **5** and **8** induced only slight downfield shifts. Although, these results provide support for the conclusion that **1** binds **10** with higher affinity than either **5** or **8**, a quantitative analysis of binding is precluded due to the strong dimerization of the host **10**.

To circumvent this problem a reverse titration was carried out with nucleobase **1** as the host. The downfield shift of the pyrrole NH was thus followed as the concentration of **10** was increased (see inset, Figure 2A). Here, the initial concentration of **10** was low enough to minimize GG dimerization, and a plot with saturation behavior was observed. After correcting for the free concentration of **10** during the titration, standard curve-fitting procedures were employed,[11] revealing a 1:1 binding stoichiometry and an estimated affinity constant (K_a) of $1.1 \pm 0.1 \times 10^3$ M^{-1}. This value is within the range of association constants for other three-point motifs in $CDCl_3$.[12]

ESI-mass spectrometry studies also support the formation of ensemble **I**. Additionally, the peaks ascribable to the GG dimer as well as higher-order aggregates were also evident. Given this observation and the fact that ESI-MS has been used successfully to study drug interactions with, and the destabilization of, guanine quadruplexes,[14] this technique was used to investigate whether nucleobase **1** can disrupt quadruplex formation (Figure 1C). Here, it was found that the ions corresponding to two stacked 2′,3′,5′-tri-*O*-acetylguanosine **11** (used to overcome mass range limitations**)** quadruplexes[15] as well as the GG dimer and other higher-order aggregates decreased substantially upon the addition of **1** (Figure 2B). Additionally, a complex between **1** and **11** analogous to ensemble **I** was detected. Similar behavior was not observed for control **5** and was less pronounced with control **8**.

In conclusion we have developed a novel nucleoside that binds guanosine through a proposed three-point Hoogsteen-type interaction. The third hydrogen bond is crucial for this increased affinity. This interaction is capable of competing with guanosine self-assembly.

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Supporting Information Available: Synthetic experimental, ^{1}H NMR-variable temperature spectra and -titrations, ESI-MS, and ITC studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) For studies of GG dimers see: (a) ref 2e and (b) Sartorius, J.; Schneider, H.-J. *Chem. Eur. J.* **1996**, *2*, 1446−1452. In the present instance, the imino NH proton signal of **10** (without the addition of **1**) appears at 12.2 ppm, a finding that is consistent with a strongly hydrogen bonded species. The same proton resonates at 10.6 ppm when dissolved in a more competitive solvent, such as DMSO-d_6. (c) Rich and co-workers using IR experiments (Kyogoku, Y.; Lord, R. C.; Rich, A. *Biochim. Biophys. Acta* **1969**, *179*, 10−17) obtained a K_{dimer} value of 10^3−10^4 M^{-1}. This discrepancy possibly stems from higher-order guanosine aggregation that forms under the relatively concentrated conditions used for the ^{1}H NMR spectroscopic study (up to 33 mM). To address this issue, isothermal titration calorimetry (ITC) analyses were conducted under more dilute conditions (ca. 0−1.1 mM), where higher-order aggregation is reduced. A K_{dimer} value of $2.9 \pm 0.2 \times 10^3$ M^{-1} was obtained in $C_2H_4Cl_2$ at 30 °C by this method.
(11) See Supporting Information for details
(12) (a) Zimmerman, S. C.; Corbin, P. S. *Struct. Bond*. **2000**, *96*, 63−94 and references therein. (b) An ITC titration under more dilute conditions was performed in $C_2H_4Cl_2$ at 30 °C resulting in a K_a of $8.5 \pm 0.6 \times 10^3$ M^{-1}. This value is likely a lower limit due to this method being unable to account for the GG dimerization equilibrium.
(13) Here, upon addition of 0.2 equiv of **5** the NH_2 shift of **10** becomes too broad to allow for an accurate peak pick. However, the estimated peak does not shift much from 5.75 ppm, even after 6 equiv of **5** was added.
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(15) Isotope spacing confirms that this ion is consistent with a doubly charged guanine octamer. Similar ions have been observed previously with the use of ESI-MS; see ref 2d.

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