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Kinetic Control in Noncovalent Synthesis: Regioselective Ligand Exchange into a Hydrogen Bonded Assembly

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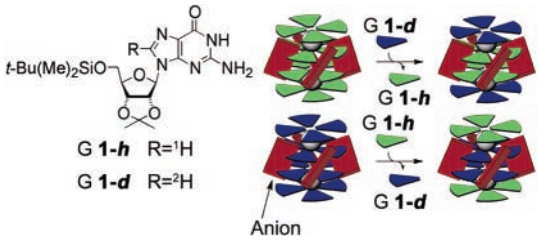
ABSTRACT



This paper illustrates the use of a kinetically controlled exchange reaction to effect regioselective modification of a hydrogen-bonded assembly. Both the bound anion and cation can control the exchange of ligand into the different layers of a synthetic G-quadruplex.

Organic synthesis relies on the proper sequence of reactions, many of them kinetically controlled, to make compounds dense with functionality and stereochemistry. In general, "noncovalent" synthesis of molecular assemblies lacks this same level of kinetic control.[1,2] This limitation arises chiefly because noncovalent assemblies are usually kinetically labile, giving multiple, interconverting structures of similar free energy. This kinetic instability is a problem if the goal is to obtain a single discrete structure.[3] There have been a number of recent advances in trying to gain control over noncovalent synthesis. Crystal engineering,[4] dynamic combinatorial chemistry,[5] and the use of molecular chaperones are some of the methods that have been used to prepare assemblies of defined composition and stereochemistry.[6] Another useful strategy in noncovalent synthesis is to follow a thermodynamic self-

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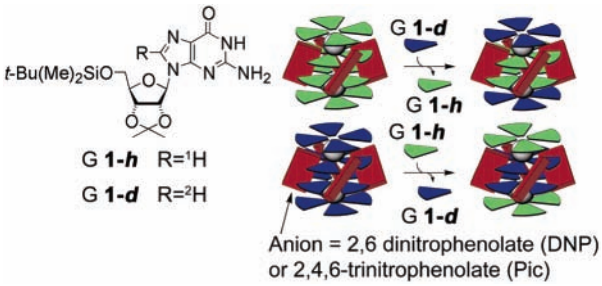
assembly process with a covalent modification that tailors specific locations in the complex.[7,8] In this communication, we use kinetically controlled ligand exchange to selectively modify a hydrogen bonded assembly.[9]

Relative to systems held together by coordination or mechanically locked bonds, there are far fewer hydrogen bonded assemblies that are kinetically stable.[10] We have previously shown that, in the presence of alkali(ine) salts, 16 units of the guanosine derivative **1-*h*** form a stable G-quadruplex.[11] This hexadecamer, containing four stacked G-quartets held together by hydrogen bonds and ion−dipole interactions, also has 4 anion-binding grooves on its surface. In Scheme 1, the spheres represent cations and the blocks

Scheme 1. Regioselective Pseudo-Self-Exchange Reaction of a Guanosine Derivative into a Lipophilic G-Quadruplex



represent anions. Both the cation and the anion influence the thermodynamic and kinetic stability of the quadruplex.[12,13] Below, we demonstrate regioselective control of the pseudo-self-exchange reaction of deuterated ^{2}H8 **1-*d*** with the **1-*h*** subunits in the G-quadruplex. The new observations in this study are that the cation, anion, and solvent can control relative and absolute rates of ligand exchange into different layers of this G-quadruplex.

The regioselectivity of the ligand exchange reaction can be controlled by the bound anion. We first conducted exchange reactions by adding 16 equiv (1 equiv per equiv of **1-*h*** in the hexadecamer) of deuterated nucleoside, **1-*d***,[14] to a CD_2Cl_2 solution of $[\mathbf{1}\text{-}h]_{16}\cdot 2Ba^{2+}\cdot 4DNP^-$ (0.39 mM). A complementary experiment was performed by adding 16 equiv of **1-*h*** to a solution of deuterated G-quadruplex $[\mathbf{1}\text{-}d]_{16}\cdot 2Ba^{2+}\cdot 4DNP^-$. The D_4-symmetric G-quadruplex has distinct "outer" and "inner" G-quartets, and ligand substitution was monitored by ^{1}H NMR spectroscopy. As shown in Figure 1, H8 signals for the outer G-quartet (δ 7.79), inner G-quartet

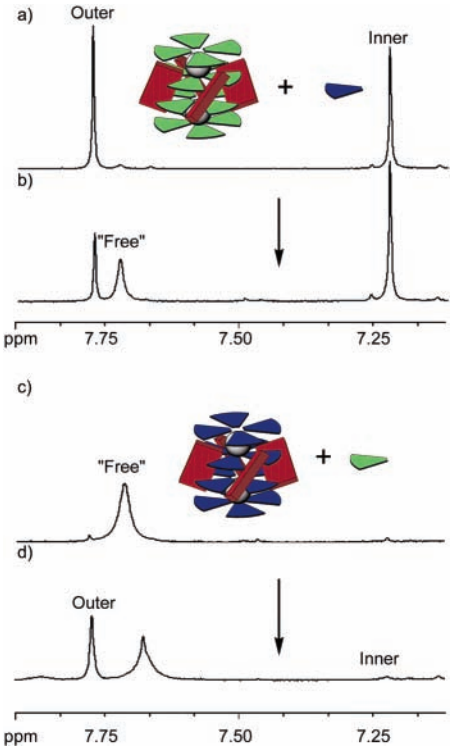


Figure 1. H8 region of ^{1}H NMR spectra in CD_2Cl_2: (a) solution of $[\mathbf{1}\text{-}h]_{16}\cdot 2Ba^{2+}\cdot 4DNP^-$ (0.39 mM) and **1-*d*** (6.2 mM) immediately after mixing; (b) the sample in spectrum a after 4 days at room temperature; (c) solution of **1-*h*** (6.2 mM) and $[\mathbf{1}\text{-}d]_{16}\cdot 2Ba^{2+}\cdot 4DNP^-$ (0.39 mM) immediately after mixing; (d) the sample in spectrum c after 4 days at room temperature.

(δ 7.13), and "free" G **1** (δ 7.73) are in slow exchange on the chemical shift time scale.[15] The upper trace (Figure 1a) was taken immediately after mixing $[\mathbf{1}\text{-}h]_{16}\cdot 2Ba^{2+}\cdot 4DNP^-$ and **1-*d***. The G-quadruplex H8 signals were present in a 1:1

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(12) Cation effects: Kotch, F. W., Fettinger, J. C.; Davis, J. T. *Org. Lett.* **2000**, *2*, 3277−3280.

(13) Anion effects: Shi, X. D.; Mullaugh, K. M.; Fettinger, J. C.; Jiang, Y.; Hofstadler, S. A.; Davis, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 10830−10841.

(14) Synthesis of **1-*d*** involved base-catalyzed H8/D8 exchange of guanosine, followed by routine modification of the ribose. See Supporting Information for experimental details on the synthesis of **1-*d*** and on the preparation and NMR characterization of the completely deuterated G-quadruplex $[\mathbf{1}\text{-}d]_{16}\cdot 2Ba^{2+}\cdot 4DNP^-$.

(15) Free G **1** is a mixture of oligomers, particularly dimers, in CD_2Cl_2. For leading papers on self-association of guanosine derivatives in nonpolar solvents, see: (a) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810−2819. (b) Sartorius, J.; Schneider, H. J. *Chem. Eur. J.* **1996**, *2*, 1446−1452. (c) Gottarelli, G.; Masiero, S.; Mezzina, E.; Spada, G. P.; Mariani, P.; Recanatini, M. *Helv. Chim. Acta* **1998**, *81*, 2078−2092.

ratio and signals for free **1-*h*** were not noticeable. The spectra changed over time as ligand exchange between $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and **1-*d*** proceeded. Figure 1b, taken 4 days after mixing shows a reduced signal for the outer G-quartet (0.35 H) and a concurrent increase in released **1-*h*** (0.60 H). Significantly, there was little change (<3%) in the inner H8 signal, indicating that this layer was protected from exchange with **1-*d***. Indeed, even after extended reaction times, H8 integration for the outer G-quartet, inner G-quartet, and free **1-*h*** was close to the 0.33:1.0:0.67 ratio expected if nucleoside exchange had occurred only with the outer G-quartet.[16] Complementary kinetic experiments with the deuterated isotopomer $[\mathbf{1}\text{-}\boldsymbol{d}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and **1-*h*** confirmed the substitution's high regioselectivity (Figure 1c,d). After 4 days, the inner G-quartet remained completely deuterated. A control experiment, in which a Ba^{2+} G-quadruplex was generated by extraction of $(Ba^{2+})DNP_2$ with a 50−50% mixture of **1-*h*** and **1-*d***, showed equal incorporation of **1-*h*** and **1-*d*** into all layers of the quadruplex, as expected for self-assembly under thermodynamic control.

We reasoned that regioselective substitution of **1-*d*** into the outer G-quartet of $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ was due to kinetic stabilization of the inner G-quartets provided by the hydrogen-bonded DNP anions (2,6-DNP phenol pK_a = 3.96).[17] To confirm this hypothesis, we compared exchange of **1-*d*** into $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^{-}$,[13] a G-quadruplex containing less basic, and more weakly bound, picrate anions (picric acid $pK_a = 0.38$).[18] In the case of the Ba^{2+} picrate assembly, the deuterated nucleoside **1-*d*** was incorporated into both the inner and outer G-quartets at similar rates and without any discernible selectivity. The equilibrium ratio of 0.5:0.5:1.0 for H8 signals of outer G-quartet, inner G-quartet, and free **1-*h*** reflected indiscriminant incorporation of **1-*d*** (16 equiv) into $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^{-}$ (1 equiv). Figure 2 shows a plot of

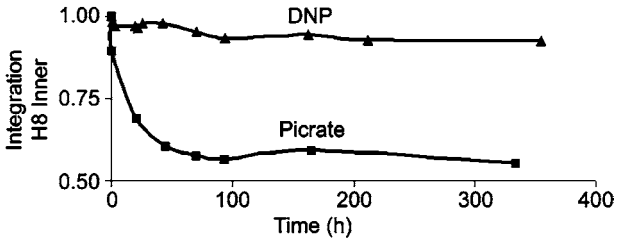

Figure 2. Integration of the H8 NMR signal for the inner G-quartet in the exchange of 16 equiv of **1-*d*** (6.2 mM) with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^{-}$. Exchange was done at room temperature in CD_2Cl_2.

the inner H8 signal integration versus time for the exchange reactions of 16 equiv of **1-*d*** (6.2 mM), with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^{-}$ and with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$. Clearly, the externally bound anions can control the regioselective noncovalent modification of this hydrogen bonded assembly. Under these conditions, the more basic 2,6-DNP anion (relative to picrate) protects the inner G-quartet from ligand exchange with **1-*d***.

The regioselectivity of the ligand exchange reaction can also be controlled by the bound cation. When $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and 16 equiv of **1-*d*** were combined in 50−50% CD_2Cl_2−CD_3CN, equilibrium was attained within 4 h (Figure 3a), as compared to days in CD_2Cl_2. In addition to this

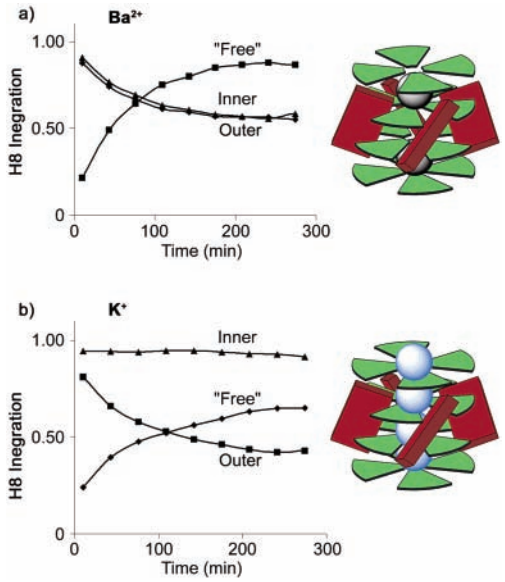

Figure 3. NMR integration of H8 signals in exchange reaction of 16 equiv of **1-*d*** (6.2 mM) with (a) $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and (b) $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 4K^{+}\cdot 4DNP^{-}$. Reactions were done at room temperature in 50−50% CD_3CN−CD_2Cl_2.

increased rate, **1-*d*** was incorporated to the same extent into both the outer and inner G-quartets of $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$, as measured by NMR spectroscopy. Presumably, the nucleobase−DNP hydrogen bond interactions that serve to protect the inner G-quartet from exchange in CD_2Cl_2 are weakened in the more polar mixed solvent, leading to facile dissociation of $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and a consequent loss in the regioselectivity of subunit exchange. To test this idea, we compared exchange reactions for **1-*d*** with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ and $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 4K^{+}\cdot 4DNP^{-}$ in 50−50% CD_2Cl_2−CD_3CN (Figure 3).

The major difference between these two G-quadruplexes is the cation channel's occupancy. Crystal structures show no cation between the inner G-quartets of $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$,[13] whereas an octacoordinate K^{+} is sandwiched between these two G-quartets in $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 4K^{+}\cdot 4DNP^{-}$.[19] We reasoned that this centrally bound K^{+} cation might well

(16) When exchange was carried out at room temperature in CD_2Cl_2 with 160 equiv of **1-*d***, incorporation into the outer G-quartet occurred faster and to a much greater extent (>95% incorporation after 4 days). Again, less pseudo-self-exchange (<20%) was detected in the inner G-quartet after 4 days.

(17) Lagier, C. M.; Oliveieri, A. C.; Harris, R. K. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 1791−1796.

(18) Picrate in $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4Pic^{-}$ exchanges 7-fold faster than a DNP anion in $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$. See EXSY NMR data in Table 2 of ref 13.

(19) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060−4067.

influence ligand exchange into the inner G-quartets. In contrast to the indiscriminant exchange of **1-*d*** with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ (Figure 3a), the reaction between 16 equiv of deuterated nucleoside **1-*d*** and $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 4K^{+}\cdot 4DNP^{-}$ was highly regioselective; the kinetic trace in Figure 3b shows a marked preference for incorporation of **1-*d*** into the outer G-quartet. Apparently, the centrally bound K^+ cation stabilizes interquartet contacts and serves to restrict ligand exchange into the inner G-quartets, even if the DNP anions dissociate much more readily in this more polar solvent.[20] These experiments again illustrate that an assembly's components, in this case the bound cation within the G-quadruplex, can be tuned to enable regioselective noncovalent modification.

This study has shown that regioselective ligand exchange into a synthetic G-quadruplex can be controlled by both the anion and cation that help hold the noncovalent assembly together. By kinetically stabilizing the assembly via stronger ion−ligand interactions, certain regions of this hydrogen-bonded assembly are made resistant to the subsequent subunit exchange reaction. From a synthetic perspective, these experiments demonstrate that noncovalent interactions can be used to stabilize specific regions of large assemblies, allowing for subsequent substitution reactions at other locations to be precisely controlled. With the proof of principle now demonstrated by this pseudo-self-exchange reaction, the next step is to selectively incorporate diverse functionality into these hydrogen bonded assemblies.

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Supporting Information Available: Experimental and other selected spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) In 50:50% CD_2Cl_2−CD_3CN, variable-temperature NMR showed that DNP exchanges faster with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 4K^{+}\cdot 4DNP^{-}$ (coalescence $T = -52$ °C, $\Delta G^{\ddagger} = 10.0$ kcal/mol) than it does with $[\mathbf{1}\text{-}\boldsymbol{h}]_{16}\cdot 2Ba^{2+}\cdot 4DNP^{-}$ (coalescence $T = -8$ °C, $\Delta G^{\ddagger} = 12.0$ kcal/mol). Thus, the ligand exchange regioselectivity seen for the K^+ G-quadruplex is not due to enhanced quadruplex−anion interactions, since DNP anions dissociate more readily from the K^+ G-quadruplex than they do from the Ba^{2+}G-quadruplex.