Molecular recognition in a uradinyl-functionalized stable radical

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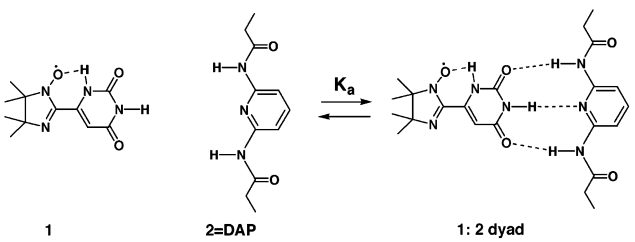
Stable radical 2-(6-uradinyl)-4,4,5,5-tetramethyl-4,5-dihydro-1*H*-imidazole-1-oxyl (1) binds to hydrogen-bonding complement 2,6-di(propylamido)pyridine (DAP) in chloroform with K_a = 220 M^{-1} at 33 °C; ESI-MS shows not only 1:DAP complementary dyad formation, but also 1:(DAP)$_2$ formation at higher concentrations of DAP.

Hydrogen bonds provide some of the strongest noncovalent interactions between molecules.[1] They also tend to be strongly directional, providing a reasonably dependable basis for predicting how a molecule or sets of molecules might assemble in solution or in crystalline arrays. Their use has become a strong theme in both biological molecular recognition work, and in materials science, because they offer a means of controlled supramolecular assembly based on molecular building blocks.[2]

Stable organic radicals have been used extensively in biological chemistry as electron spin resonance (ESR) spin-labels,[3] and recently[4] in selective enhancement of NMR spectral peaks by spin transfer. They also are useful building blocks for molecule-based magnetic materials.[5] Both biological and materials based uses of radicals are enhanced dramatically by designing specific interactions that allow for molecular recognition and supramolecular assembly. In this article, we show that 2-(6-uradinyl)-4,4,5,5-tetramethyl-4,5-dihydro-1*H*-imidazole-1-oxyl, **1**, binds with complementary receptor 2,6-di(propylamido)pyridine, **2** (DAP), opening future prospects for supramolecular assembly strategies where stable radicals are bound through hydrogen-bonding interactions.

Radical **1** was synthesized and purified according to the procedure of Taylor *et al.*[6] It was dissolved in chloroform, and binary solutions of **1** with **2** were formulated by literature procedures,[7] keeping the concentration of **2** constant at 5 mM with the ratio of [**1**] : [**2**] ranging from 0.6 : 5.0 to 14.3 : 5.0. ^{1}H-NMR spectra were obtained for all solutions under the same conditions. Fig. 1 compares two spectra, one for **2** alone and one with [**1**] added.



In previous studies,[7] DAP derivatives have been used as guest molecules in varying concentration, with thymines as hosts. We were unable to use **1** in an analogous manner, because its NMR peaks are not dependably observed due to the paramagnetic effect of the attached radical group. We therefore could not track both host and guest simultaneously by NMR. However, all of the DAP peaks could be observed, with increasing downfield shifts and some peak broadening as the ratio of [**1**] : [**2**] increased. The strongest effect was observed for the DAP NH resonance (see Fig. 1).

The qualitative results are consistent with the behavior expected for binding of a thymine-type host with guest **2**. As [**1**] : [**2**] increases, one expects to shift the binding equilibrium to the right and increase the amount of the **1** : **2** diad. Because of the strong paramagnetic shift in the NH resonance with an increase in bound **1**, this peak shifts downfield as the equilibrium shifts to dyad formation. Following previous practice, we carried out nonlinear least-squares fitting of the NMR data to equations 1,[8] where K_a is the binding constant, δ_{obs} is the observed chemical shift of the propylamido NH of **2**, δ_0 is the extrapolated chemical shift of the N–H of **2** at infinite dilution of **1**, and δ_∞ is the chemical shift of the N–H when the concentration of [**1**] ≫ [**2**]. We found the binding constant $K = 223 \pm 33\ M^{-1}$ with extrapolated $\delta_0 = 7.48 \pm 0.02$ (which matches well with the observed NH chemical shift of 5.0 μM DAP in $CDCl_3$, Fig. 1) and $\delta_\infty = 10.23 \pm 0.12$; all uncertainties are standard deviations. Fig. 2 shows the experimental data and the fitted curve.

$$\delta_{obs} = \delta_0 - \Delta\frac{P-(P^2-4\times[\mathbf{1}][\mathbf{2}])^{0.5}}{2\times[\mathbf{1}]} \qquad \Delta = \delta_\infty - \delta_0 \qquad P = [\mathbf{1}] + [\mathbf{2}] + \frac{1}{K_a} \tag{1}$$

DAP is a known receptor for molecular recognition with thymines, due to formation of a 3-point hydrogen bond. Similar dyad 3-point hydrogen-bonding interactions are energetically worth about 3-6 kcal mol^{-1}.[7c] Based on the observed equilibrium constant for the **1**·**2** complexation, ΔG(binding) ≅ 3.3 kcal mol^{-1} at 33 °C. This association constant is consistent with the net

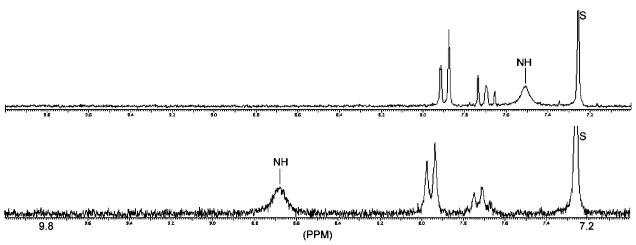


Fig. 1 ^{1}H-NMR spectrum of mixtures of DAP **2** in $CDCl_3$ with [**1**] : [**2**] = 0.0 : 5.0 mM (above), and [**1**] : [**2**] = 7.5 : 5.0 mM (below). Note the shift of the NH peak. S = solvent.

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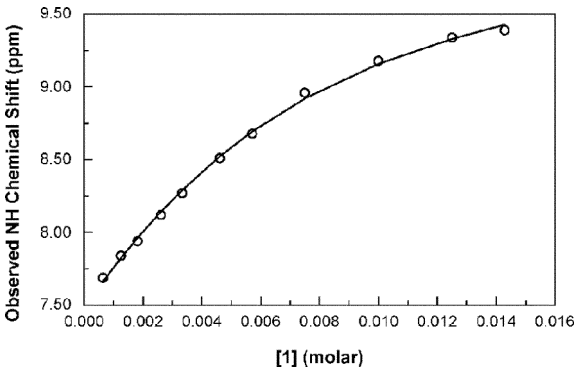
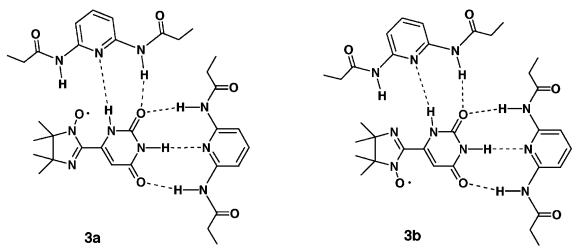


Fig. 2 Chemical shift of NH peak in ppm for DAP **2** as a function of [**1**] in mM in the presence of 5.0 mM [**2**] held as constant. The line shows the best fit of equation 1 to the data (see main text).

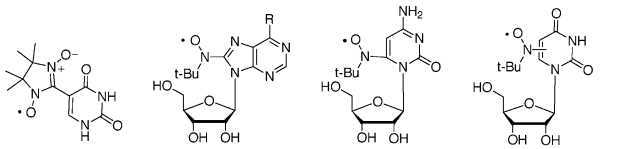
electron withdrawing nature for the iminoyl nitroxide group on the thymine, judging by association constant trends for related species.[7c]

We checked for possible effects of binding upon the ESR spectrum of **1** at room temperature in $CHCl_3$ by adding **2** up to a two-fold excess, but observed no changes in the hyperfine splitting. Previous reports of hyperfine changes found in hydrogen-bonded complexes have occurred in systems where significant spin density is found on the atoms *at* the site being bound.[7] In the **1**·**2** complex, all significant spin density is on the iminoyl nitroxide ring, quite distant from the binding uradinyl site. Therefore, the lack of change in the hyperfine splitting for **1** upon binding is not surprising.



We also checked by electrospray ionization mass spectrometry (ESI-MS) for the formation of complexes of **1** with **2** in solution. The negative ion mode spectrum of **1**:**2** in methanol (equimolar, 4 μM) showed major peaks at *m*/*z* 220, 250, 471. The *m*/*z* 220 and 250 peaks come from **2** and **1**, respectively. The *m*/*z* 471 peak is from the **1**:**2** dyad, and at 63% peak intensity relative to the peak for **1** is quite strong. Interestingly, a solution with double the concentration of **2** relative to **1** showed not only the dyad peak at *m*/*z* 471, but also a very strong peak at *m*/*z* 692 (5.3-fold larger), corresponding to a **1**:(**2**)$_2$ triad. Under these conditions, we did not observe peaks for higher aggregates of **2** with **1**.

While formation of nonspecific aggregates is possible, we feel that the intensity of the *m*/*z* 692 peak found only when DAP is present in high amounts relative to **1** gives support to a secondary binding interaction between the two species, especially given the lack of significant peaks for higher degrees of aggregation. Structures **3a–b** would be consistent with the observed triad peak formation. Molecular mechanics computations suggest that a bifurcated hydrogen bond involving the 2-carbonyl with two NH moieties as in **3a–b** can form, but the second DAP molecule does not form more than a two-center hydrogen-bonding interaction, as represented in the structural diagrams. Although such proposed structures of the triad are speculative given the available information, the fact that uradinyl-based molecules can form crystallographic ribbons[9] shows that uradinyl can engage in similar complementary hydrogen-bonding with multiple species.



Although supramolecular assembly of radicals with hydrogen bonds has been a theme of molecular magnetic design,[5] little has been done in this area using biomolecular recognition motifs. Veciana and coworkers[10] synthesized **4**, an isomer of **1**, reporting its solution phase hydrogen bonding, solid-state crystallography and magnetic behavior. To our knowledge, **4** has not been tested with hydrogen bonding complements. Compound **4** forms hydrogen-bonded ribbons in the solid state, and shows UV-vis evidence for homomolecular complexation in solution. Similarly, Taylor *et al.* found by ESI-MS that **1** forms homomolecular aggregates up to at least the hexamer level in solvents of limited polarity, although its solid state crystal structure is dimeric.[6] That result complements this present study showing complementary hydrogen bond formation of DAP with **1**. In one other related case, Aso *et al.* functionalized[11] a set of ribonucleosides **5**–**7** with *tert*-butyl nitroxide units but these radicals were difficult to purify, and to our knowledge have not been tested for complementary or homomolecular bimolecular association.

Overall, we find that formation of molecular-recognition inspired hydrogen-bonding dyads is not compromised by attachment of a stable – though somewhat bulky – iminoyl nitroxide radical at the 6-position of a uradinyl ring. The binding constant obtained is consistent with the electron-withdrawing nature of the radical substituent. Given the success in this simple model system, one can readily imagine similar stratagems to yield localized high concentrations of radicals bound to specific sites on nucleic acids, or to assemble organic radicals of different types into specific dyads in solution or in crystallized mixed solids.

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