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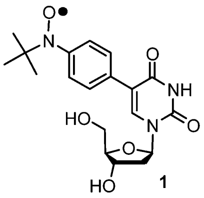
Synthesis and Structure of a Nucleoside with π-Conjugated Nitroxide Spin Label Forming a One-Dimensional Ferromagnetic Chain

Kausik Das,[†] Maren Pink,[‡] Suchada Rajca,[†] and Andrzej Rajca*,[†]

Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588-0304, and
IUMSC, Department of Chemistry, Indiana University, Bloomington, Indiana 47405

Received February 4, 2006; E-mail: arajca1@unl.edu

Spin labeling is a powerful technique used in the study of biological molecules, including oligonucleotides.[1,2] Recently, nucleosides with nitroxide radicals directly attached to the nucleobases have been reported, though efforts to isolate and adequately characterize them were not successful.[3] Synthesis of such nucleosides, in which spin density is delocalized into the nucleobase, could lead to new probes for structure and dynamics of oligonucleotides. Furthermore, hydrogen bonding driven crystal packing of nucleosides may provide a new approach to control of crystal packing of nitroxide radicals and thus to control of exchange coupling, especially to attain ferromagnetic exchange coupling, in the solid state.[4,5]



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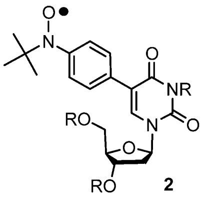

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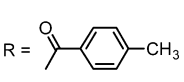


Herein we describe the synthesis, isolation, and characterization of spin labeled nucleosides **1** and **2**, including the structure and magnetism of crystalline **1**. In **1** and **2**, nitroxide radicals are connected with 2′-deoxyuridine moieties via a π-conjugated *p*-phenylene linker, providing stability at ambient conditions and significant delocalization of spin density into the uracyl moiety.

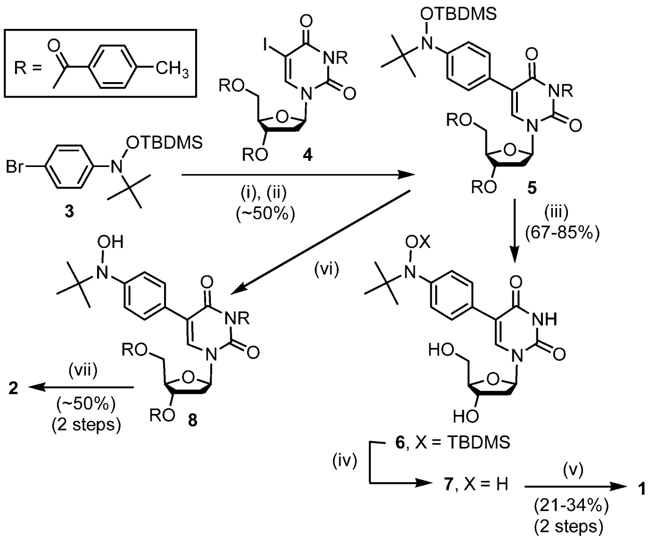
The synthetic route to **1** and **2** is based upon Negishi cross-coupling between the TBDMS-protected hydroxylamine **3** and tritoluyl-protected 5-iodo-2′-deoxyuridine **4**,[6] to provide **5**. Sequential deprotection of **5**, followed by oxidation of hydroxylamine **7**, gives nitroxide radical **1**. Analogous transformations provide nitroxide radical **2** (Scheme 1).

Both **1** and **2** are stable at ambient conditions, especially in the solid state, and are purified by normal phase (and reversed phase for **1**) chromatography. The content of diamagnetic impurities is negligible, as determined by both magnetic measurements and ^{1}H NMR spectroscopy (Figures S17, S22).

The structure of spin labeled nucleoside **1** was confirmed by X-ray crystallography (Figure 1A). The π-systems of nitroxide, *p*-phenylene, and uracyl are nearly coplanar, facilitating delocalization of spin density into the base.[7]

^{1}H NMR spectra of 6 mM **1** in acetone-d_6 and 10 mM **2** in chloroform-*d* do not show resonances for either the *p*-phenylene linker or, notably, vinylic hydrogen at C6 (Figure 1A) of the uracyl moiety, as expected for nuclei with significant spin density. In the spectrum of **1**, the most broadened and most shifted singlet at $\delta \approx -5$ ppm (~9 H) is assigned to the hydrogens of the *tert*-butyl group.

Scheme 1. Synthesis of Nitroxide Radicals **1** and **2**[a]



[a] (i) *t*-BuLi (2.5 equiv), THF, −78 °C, 2 h, $ZnCl_2$ (12 equiv), rt; (ii) $Pd(OAc)_2$ (0.3 equiv), $P(t\text{-}Bu)_3$ (0.6 equiv), **4** (0.6 equiv), −40 °C, 5 h; (iii) KOH (5.0 equiv), MeOH, THF, rt, 3 h; (iv) HF (2.5 M in THF/H_2O, 3.0 equiv), rt, 1.5 h; (v) Ag_2O (1.0 equiv based on **6**), acetone, 0 °C, 3 h; (vi) HF (5.0 M in THF/H_2O, 13.0 equiv), THF, rt, 1.5 h; (vii) Ag_2O (7.0 equiv), $CHCl_3$, rt, 2.5 h.

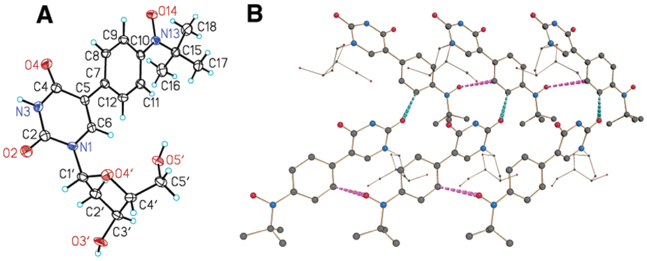


Figure 1. (A) Molecular structure and conformation of nucleoside **1**. Carbon, oxygen, and nitrogen atoms are depicted with thermal ellipsoids set at the 50% probability level. (B) Three unit cells showing molecular packing of **1** into chains along the crystallographic *a*-axis (drawn using Schakal).[12] Intercepted lines correspond to the selected short intermolecular distances shown in magenta (O14−C12) and turquoise (O2−C11).

EPR spectra of 3 mM **1** in acetone and 3 mM **2** in chloroform show the expected coupling pattern for a 4-substituted phenylnitroxide radical and an additional doublet splitting, $a_H \approx 0.045-0.046$ mT, which is assigned to the vinylic hydrogen at C6 of the uracyl moiety (Figure 1A and 2); the ^{1}H-hyperfine splitting from the hydrogens of the *tert*-butyl group is too small to be resolved. Thus, both ^{1}H NMR and EPR spectra indicate significant spin delocalization into the uracyl moiety in **1** and **2**.[8]

Magnetic studies for ~0.007 M **1** in methanol and ~0.07 M **2** in chloroform reveal the expected spin-$^1/_2$ paramagnetic behavior

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[†] University of Nebraska.
[‡] Indiana University.

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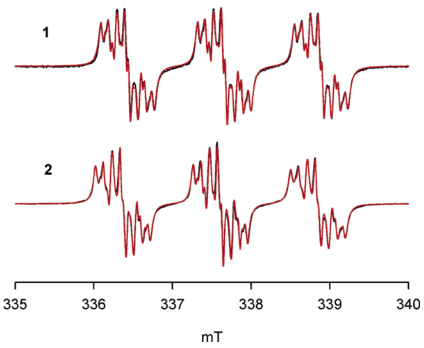

Figure 2. EPR spectra of 3 mM **1** in acetone and 3 mM **2** in chloroform. Black and red traces correspond to the experimental and simulated spectra, respectively. The horizontal axes of the spectra are offset, due to different microwave frequencies. Parameters for the simulations are shown in Figure S2 (Supporting Information).

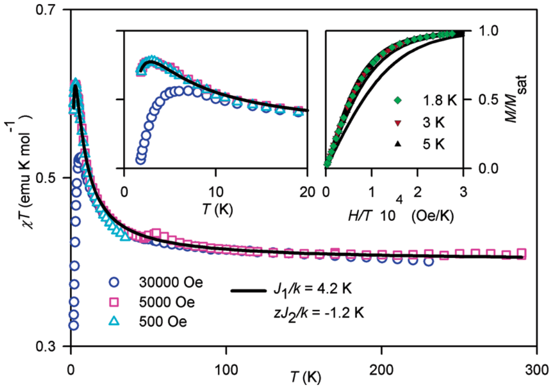

Figure 3. Main and left inset plots: χT vs T and numerical fit to eq 1. Parameter dependencies < 0.87 are found. Right inset plot: M/M_{sat} vs H/T and Brillouin curves for $S = {}^{1}/_{2}$, 1, and ${}^{3}/_{2}$.

for both magnetization (M) vs magnetic field (H) and magnetic susceptibility (χ) vs temperature (T); however, small intermolecular antiferromagnetic coupling, as measured by mean-field parameters, $\theta \approx -0.8$ K and $\theta \approx -0.2$ K, are observed for **1** and **2**, respectively.[9]

For polycrystalline **1**, the χT vs T plot shows an upward turn from a spin-${}^{1}/_{2}$ paramagnetic value of $\chi T \approx 0.38$ emu K mol^{-1} at room temperature to $\chi T \approx 0.6$ emu K mol^{-1} at 3 K; below 3 K, the χT vs T plot shows a small downward turn (Figure 3). The M/M_{sat} vs H/T plots at 5, 3, and 1.8 K are bracketed by Brillouin curves with $S = 1$ and $S = {}^{3}/_{2}$, suggesting the presence of intermolecular ferromagnetic exchange coupling between more than two molecules of **1**. The χT vs T data provide an excellent fit ($R^2 = 0.998$) to a model for a 1-D spin-${}^{1}/_{2}$ Heisenberg chain with the following parameters: intrachain ferromagnetic coupling, $J_1/k = 4.2$ K, mean-field correction for an interchain antiferromagnetic coupling, $zJ_2/k = -1.2$ K, and weight factor, $w = 1.07$ (eq 1, Figure 3).[10,11] Analogous three-parameter fits to alternative models, such as a spin-${}^{1}/_{2}$ pair (eq S2, Supporting Information), are decisively inferior ($R^2 = 0.96$). Further support for a 1-D ferromagnetic chain is provided by the crystal structure of **1**.

Molecules of **1** pack into one-dimensional chains extending along the a-axis (Figure 1B). Both intrachain (O5′−H- - -O3′) and interchain (N3−H- - -O5′, O3′−H- - -O14) classical hydrogen bonds are found (Figure S1, Supporting Information) similar to those in 5-hydroxymethyl-2′-deoxyuridine.[13] While such hydrogen bonds are important for the crystal structure of **1**, other short contacts, in which both heavy atoms possess relatively large spin densities, may be relevant to the observed exchange coupling in the solid state. The closest intrachain contact between the neighboring molecules, O14−C12 of 3.37 Å, may be viewed approximately as a $\pi-\pi$ overlap between the positive spin density of the nitroxide and negative spin density at the *meta*-position of the benzene ring. Based upon the McConnell model, such interaction should be ferromagnetic.[14] Short interchain contacts, such as O2−C11 of 3.16 Å along the b-axis, are also found (Figure 1B); however, the O2 of the uracyl moiety is expected to possess a rather small spin density.[8] These two contacts may be responsible for the stronger intrachain ferromagnetic coupling and the weaker interchain antiferromagnetic coupling, as observed in the solid-state magnetic data of **1**.

In summary, spin labeled 2′-deoxyuridine, in which a significant fraction of the spin density is delocalized from a nitroxide radical to the DNA base residue, was prepared as a crystalline solid, stable at ambient conditions. The crystal packing of **1**, which includes multiple hydrogen bonds, leads to one-dimensional chains of molecules with predominant intrachain ferromagnetic coupling and weaker interchain antiferromagnetic coupling.

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Supporting Information Available: Experimental section, X-ray crystallographic files in CIF format, and complete ref 8b. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) The angles between the least-squares planes of a nitroxide moiety and *p*-phenylene, and *p*-phenylene and uracyl are 6.8(0.4)° and 28.5(0.1)°, respectively.
(8) (a) Using a model compound **9** (Figures S6−S9, Supporting Information), in which the deoxyribose moiety is replaced with a methyl group, spin densities of 0.04, −0.03, and 0.005 at the atoms corresponding to C6, C5, and O2 in **1** are calculated at the UB3LYP/6-31(d) level, respectively. (b) Frisch, M. J.; et al. *Gaussian 03*; Gaussian, Inc.: Wallingford CT, 2004.
(9) For relatively well-soluble **2**, nearly quantitative values of magnetization at saturation, $M_{\mathrm{sat}} = 0.92\ \mu_{\mathrm{B}}$, and $\chi T = 0.36$ emu K mol^{-1} are obtained.
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(11) Equation 1: $\chi T = 0.5w\chi_{\mathrm{1D}}T/[1 - (2zJ_2/3k)\chi_{\mathrm{1D}}]\chi_{\mathrm{1D}} = ({}^{3}/_{4}kT)[(1 + a_1 \cdot K + a_2 \cdot K^2 + a_3 \cdot K^3 + a_4 \cdot K^4 + a_5 \cdot K^5)/(1 + b_1 \cdot K + b_2 \cdot K^2 + b_3 \cdot K^3 + b_4 \cdot K^4)]^{2/3}$; $a_1 = 5.797\,991\,6$, $a_2 = 16.902\,653$, $a_3 = 29.376\,885$, $a_4 = 29.832\,959$, $a_5 = 14.036\,918$, $b_1 = 2.797\,991\,6$, $b_2 = 7.008\,678\,0$, $b_3 = 8.653\,864\,4$, $b_4 = 4.574\,311\,4$, $K = J_1/2kT$.
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