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Vanadium Complex of 2-(2′-Pyridyl)-4,5-dicyanoimidazole Showing Spermicidal and Cytotoxic Properties

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Two complexes having the formulas $VO(DCIPy)_2(H_2O)\cdot 1.5H_2O$ and $VO(DCIPy)_2(H_2O)\cdot 2MeOH$ have been synthesized and characterized (DCIPy = 2-(2′-pyridyl)-4,5-dicyanoimidazolato). The methanol solvated species has been studied by X-ray diffraction, and single crystals form in the space group *P*2(1)/*n*. The hydrated species was studied by electron paramagnetic resonance spectroscopy in both the solid state and in a frozen solution, and the values of $A_{||}$ examined using the additivity relationship. The hydrated species was shown to exhibit both spermicidal and cytoxic properties.

Introduction

Interest in the coordination chemistry of vanadium has been growing rapidly over the past several years, with review articles[1−4] and special journal issues[5] being dedicated entirely to its study. The heightened interest in vanadium coordination compounds stems from its importance in biological systems, which is only now beginning to be appreciated. Fundamental vanadium coordination chemistry is supplying novel complexes whose data provide valuable insight toward the understanding of more complex biological systems. Among the different systems in which vanadium occurs are *Ascidiaceae* (sea squirts), *Ascophyllum nodosum* (knobbed wrack), *Curvularia inaequalis* (a mold), *Azotobacter*, and certain analogues of the *Nitrogenase*.

Recently, bis(bipyridine) vanadyl sulfate, among other oxovanadium species, has been recognized as a pharmacologically active compound.[6−8] Kordowiak, Dąbroś, and Kajda investigated these species as diabetes drugs in rats,[6] finding positive effects on enzyme levels and the liver Golgi complex morphology. These results were further corroborated by Krosniak and co-workers,[7] who also found that aquadichloro-bis-(2-hydroxy-1-methyl-1-cyclopenten-3-on) oxovanadium(IV) caused a significant decrease in the total cholesterol concentration in laboratory animals. The work of D'Cruz, Dong, and Uckun led to the patenting[9,10] and

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subsequent publication[8] of a series of vanadyl compounds shown to have significant spermicidal activity. In addition to being effective spermicides, some of these compounds may also have favorable anti-HIV properties.[11] D'Cruz and co-workers also patented a series of vanadium compounds of this class as possible cancer treatment drug therapies.[12] D'Cruz and co-workers observed that, while 1,10-phenanthroline-based compounds were most effective at killing sperm, the neutral bis-5′-bromo-2′-hydroxyacetophenone vanadyl species had a much more rapid response time (seconds rather than minutes) with similar efficacy.[8]

Recently, we have reported the first use of 2-(2′-pyridyl)-4,5-dicyanoimidazole (HDCIPy) as a ligand.[13] Capitalizing on unique properties of HDCIPy, aqua-bis(2-(2′-pyridyl)-4,5-dicyanoimidazolato) vanadyl hydrate, was synthesized to combine the best properties of two spermicidal vanadium compounds presented by D'Cruz.

While the exact mechanism by which vanadium compounds immobilize sperm is speculative, it is thought to take place through redox generation of reactive oxygen species. According to D'Cruz,[8] "Human sperm are exquisitely sensitive to oxidative stress. This is due to the high content of polyunsaturated fatty acids in their cell membranes, the low levels of cytoplasmic enzymes for scavenging [reactive oxygen species], which initiate lipid peroxidation, and the reduced activity of repair enzymes to recover from oxidative damage." By optimizing the properties of the complex, it is hoped that a fast acting, efficient spermicide suitable for pharmaceutical use may be made.

This compound also adds to the growing body of knowledge of vanadium coordination sites for use in modeling of more complex biological systems. The additivity relations proposed by Wüthrich and further developed by Chasteen and, later, Cornman,[14−16] while not providing any data on axially coordinated ligands, does provide insight on the number and type of donor(s) located cis to the vanadyl center. This is done by calculating the $A_{||}$ hyperfine coupling constants. Until a paper in 2000 by Smith et al.,[17] there were only three commonly used "aromatic imine" donor values. Only one of these corresponded to imidazole, and as Smith demonstrated, the orientation of the imidazole ring relative to the V═O axis influences the value of $A_{||}$ observed. Smith was able to expand the number of well-characterized vanadyl−imidazole complexes by five, each having a different imidazole−vanadyl orientation, and applied this knowledge toward elucidating the structure of vanadium bromoperoxidase.

Experimental Section

VO(DCIPy)$_2$(H$_2$O)·2MeOH (1). A 10 mL methanolic solution of 2-pyridyl-4,5-dicyanoimidazole (57 mg, 0.26 mmol) was added, with stirring, to a 10 mL methanolic solution of vanadyl sulfate trihydrate (150 mg, 0.77 mmol). The dark yellow solution was gravity filtered and set aside for slow evaporation. After 4 days, dark blue crystals suitable for single-crystal X-ray diffraction had grown. These crystals were rinsed twice with acetone, and several were selected for diffraction and EPR studies. The remaining product was dried in vacuo overnight prior to submission for elemental analysis. fw (calcd) 505.34 g/mol. Anal. Calcd for $C_{21}H_{14}N_{10}O_3V$ [VO(DCIPy)$_2$(H$_2$O)·2MeOH]: C, 49.91; H, 2.79; N, 27.72. Found: C, 49.77; H, 3.02; N, 27.50. IR (cm^{-1}): 2232, 1613, 1443, 992.

VO(DCIPy)$_2$(H$_2$O)·1.5H$_2$O (2). The adverse biological effects of methanol (in compound **1**) necessitated the production of a purely hydrated analogue, which may eventually find use in pharmaceutical applications. To form this product, a 10 mL aqueous solution of vanadyl sulfate hydrate (88 mg, 0.54 mmol) was added to a 50 mL aqueous suspension of 2-pyridyl-4,5-dicyanoimidazole (207 mg, 1 mmol). The reaction turned green in <1 min, after which the unreacted ligand was removed by suction filtration. The green solution was allowed to sit until the product precipitated. The green powder was collected by suction filtration and rinsed with water then dried overnight in vacuo before submission for elemental analysis. fw (calcd) 500.33 g/mol. Anal. Calcd for $C_{40}N_{20}H_{26}V_2O_7$ [VO(DCIPy)$_2$(H$_2$O)·1.5H$_2$O]: C, 48.01; H, 2.62; N, 28.00. Found: C, 47.94; H, 2.59; N, 28.34. IR (cm^{-1}): 3548, 2867, 2239, 1608, 1441, 991.

Structural Determination of VO(DCIPy)$_2$(H$_2$O)·2MeOH. Dark blue needles of the title compound were grown from methanol at room temperature. A single crystal of dimensions 0.45 × 0.12 × 0.09 mm^3 was coated in Paratone oil, mounted on a glass loop, and mounted on a Bruker SMART APEX CCD diffractometer equipped with a normal-focus Mo-target X-ray tube (λ = 0.71073 Å) operated at 2000 W power (50 kV, 40 mA). The X-ray intensities were measured at 153(2) K with a detector distance of 6.056 cm from the crystal. A total of 1818 frames were collected with a scan width of 0.3° in ω and a exposure time of 20 s/frame. The range of θ observed for this sample was 2.07−27.56°. The frames were integrated with the SAINT software package[18] using a narrow frame algorithm. The integration of the data yielded a total of 20 548 reflections of which 5569 were independent and 4832 were greater than 2σ(I). The final cell constants were based on the *xyz* centroids of 8780 reflections. Analysis of the data showed negligible decay during data collection. The structure was solved by direct methods and subsequent difference Fourier synthesis and refined with the SHELXL software package,[19] using the monoclinic space group $P2(1)/n$ with Z = 4. All non-hydrogen atoms of the model were refined anisotropically, while hydrogens were placed and calculated using the geometric riding model. Final full matrix least-squares refinement on F^2 converged to R1 = 0.0545 ($I > 2\sigma(I)$) and wR2 = 0.1191 (all data) with GOF = 1.134.

Sperm Motility and Viability in the Presence of VO(DCIPy)$_2$(H$_2$O)·1.5H$_2$O. Semen samples were obtained from men undergoing evaluation for infertility after a minimum of 3 days of abstinence. After liquefaction, standard semen analyses were performed by a single individual. These analyses consisted of assessment of semen volume, pH, viscosity, liquefaction, sperm

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count, sperm motility, sperm agglutination, strict sperm morphology, and cell contamination. Normozoospermic samples containing sperm with forward progression were selected for experimental use. Approval for utilizing semen samples was obtained from the University of Michigan Institutional Review Board.

For 24 and 48 h survival, semen samples were prepared by density gradient separation using Isolate (commercially available from Irvine Scientific, Santa Ana, CA). The supernatant was removed after centrifugation at 300*g* for 20 min and resuspended in 1.0 mL of Hepes-buffered human tubal fluid with 0.2% BSA (Processing media; PM). Initial sperm motility and viability were assessed in each sample ($N = 13$) by evaluating the percent of 200 isolated sperm demonstrating progressive motility and the percent of 200 sperm demonstrating viability by dye exclusion.[20] From each sample, six aliquots of 100 μL were pipetted into Eppendorf tubes for treatment. Treatment groups consisted of 0, 0.1, 1.0, 10, 100, and 1000 μM vanadyl complex. After treatment, samples were incubated at 37 °C at atmospheric conditions for 48 h. Sperm (200) from each group were assessed for sperm motility and viability at 24 and 48 h of culture.

Statistical analysis was carried out using repeated measures mixed models. These data were used to assess the motility and viability by dose over time. The models included time, dose, and the interaction of time and dose. Analyses were performed using SAS software version 9.1.2 with a confidence level $P < 0.05$ considered significant.

Cytoxicity and Antiviral Activity of $VO(DCIPy)_2(H_2O)\cdot 1.5H_2O$. The cytoxicity and antiviral activity of **2** were determined using cell culture methods described recently.[21]

Discussion

The vanadyl complex, **1**, was readily formed by the addition of HDCIPy to a methanolic solution of vanadyl sulfate trihydrate. Slow evaporation afforded deep blue crystals suitable for single-crystal X-ray diffraction. The asymmetric unit of these crystals is presented below in Figure 1. The complex forms in the monoclinic space group $P2_1/n$ ($a = 11.6017(9)$ Å, $b = 17.944(1)$ Å, $c = 12.0775(9)$ Å, and $\beta = 102.743(1)°$). Each metal center has two solvent molecules hydrogen bound to the ligating water. To allow the compound to be tested for biological activity, it was important to remove methanol from the synthesis. This was done by reaction of an aqueous vanadyl sulfate solution with a suspension of the ligand in water, affording a simple analogue with water molecules as solvates instead of methanol.

Structurally, bis(2,2′-bipyridine) vanadyl hydroxo tetrafluoroborate and bis(1,10-phenanthroline) vanadyl hydroxo tetrafluoroborate[22] are very similar to the compound aqua-bis(2-(2′-pyridyl)-4,5-dicyanoimidazolato) vanadyl solvate. The three species form distorted octahedra with the oxo moiety located cis to the oxygen of the bound water or hydroxo. Described in Table 1, the ligand metal bond lengths

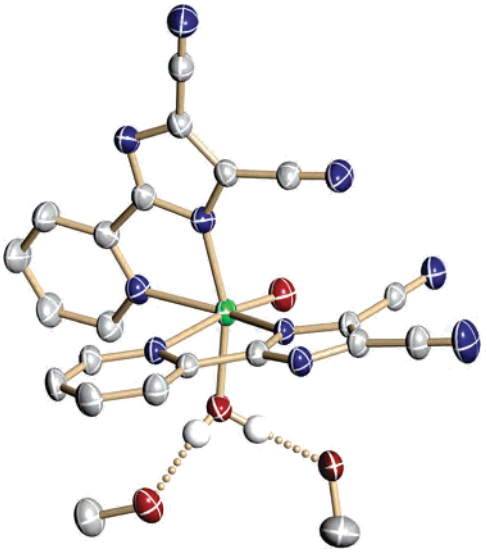

Figure 1. $(DCIPy)_2VO(H_2O)\cdot 2MeOH$ with thermal ellipsoids shown at their 50% probability levels. All hydrogens except those of the coordinated water have been omitted for clarity.

are, on average, shorter in the DCIPy complex than in the charged analogues. Distinguishing the $N_{pyridine}$ moieties in the two complexes as trans or cis to the V=O, the bond lengths of the three cis/cis $N_{pyridine}$ bonds in the bpy and phen structures are shorter than the cis/cis $N_{pyridine}$ bond observed in the DCIPy molecule. The remaining three nitrogen−vanadium bonds in **1** are shorter than those observed in the charged species. In all three molecules, the longest metal−ligand bond is located trans from the oxo moiety, with the DCIPy species being longer by 0.128 Å.

The electron paramagnetic resonance (EPR) spectrum was obtained from a THF solution with 2,2-diphenyl-1-picryl-hydrazyl free radical (DPPH) as an internal standard for *g*. It was assumed that the water ligand was not displaced during the spectroscopic experiment. The width of the signal, approximately 1000 G, allows for direct observation of the characteristic eight line spectrum of the nuclear spin $I = 7/2$ vanadium center. No nitrogen or hydrogen hyperfine coupling is observed. The spectrum was collected at 9.43 GHz. The spectrum, centered at 3390 G, with a proportionality factor, *g*, of 1.988, shows the characteristic line broadening which is indicative of the second-order effects that are commonly observed in vanadium EPR. The hyperfine coupling constant, *a*, was calculated to have a mean value of 96.341×10^{-4} cm^{-1} using equations found in the literature.[23,24]

The EPR of a frozen THF solution of the species is shown in Figure 2. The bottom spectrum (red) is the experimental data. The top spectrum (blue) is the theoretical fitting obtained using a custom fitting program developed and applied by Dr. Bruce McGarvey.[25] The program uses a refined second-order perturbation calculation of the energies to make a fit. From the simulated pattern, the fitting

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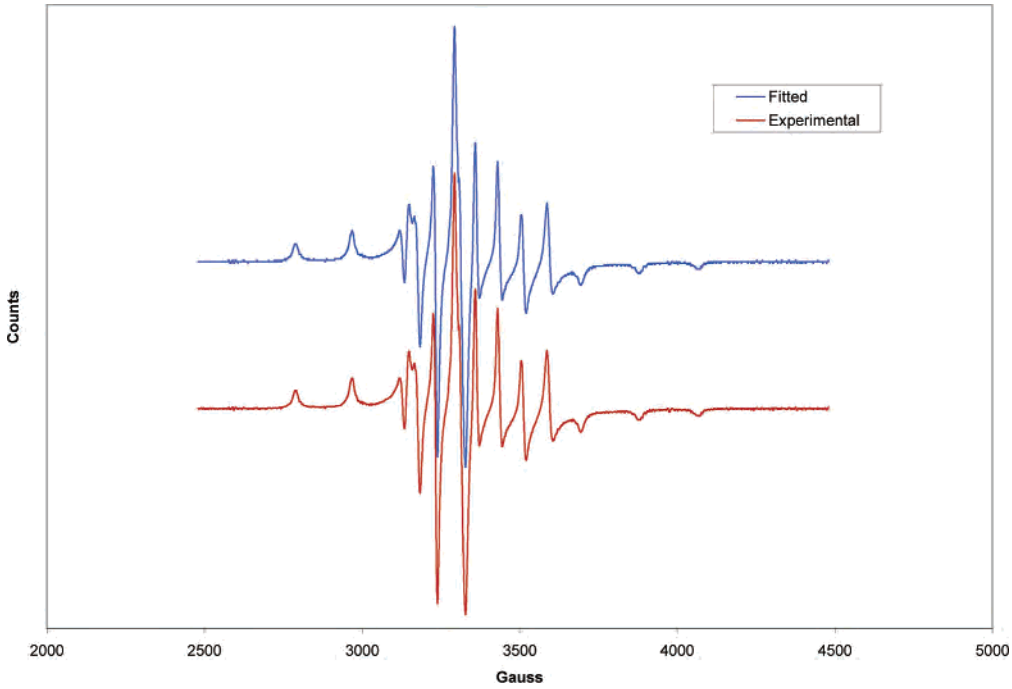

Figure 2. EPR spectrum of $(H_2O)VO(DCIPy)_2 \cdot 2MeOH$. The experimental spectrum, collected in a THF glass, is shown in red versus the blue fitted spectrum.

Table 1. Selected Bond Lengths from Various Vanadyl Species

	bond length (Å)		
species	V−N trans V=O_{oxo} cis V−O_{aqua}	V−N cis V=O_{oxo} cis V−O_{aqua}	V−N cis V=O_{oxo} trans V−O_{aqua}
$[(OH)(phen)_2VO]^+$	2.247	2.112/2.113	2.173
$[(bpy)_2(OH)VO]^+$	2.247	2.113/2.114	2.175
$(H_2O)(DCIPy)_2VO$	2.375	2.075/2.127	2.071

parameters are $g_{||} = 1.945$, $g_{\perp} = 1.980$, $A_{||} = 170 \times 10^{-4}$ cm^{-1}, and $A_{\perp} = 61.9 \times 10^{-4}$ cm^{-1}.

Determination of the coupling constants for $(H_2O)VO(DCIPy)_2$ adds to the work on hyperfine coupling constants as a function of dihedral angle started by Smith et al.[17] θ, the dihedral angle, is defined as the angle created between the planes defined by the vanadyl oxygen, the vanadium, the binding nitrogen of the imidazole ring, and the 2-position carbon. The vanadyl complex studied here contains two imidazole donor ligands—one with $\theta = -110.74°$ and the other $\theta = 175.66°$—making an absolute assignment of $A_{||}$ difficult. Nevertheless, it was possible to determine that the total contribution from both imidazoles to the $A_{||}$ of the complex was 83.6×10^{-4} cm^{-1}. This value is in line with the previous work, which shows that a parallel aligned imidazole ring will contribute approximately 40×10^{-4} cm^{-1} and a perpendicular ring will contribute approximately 45×10^{-4} cm^{-1}. Rings aligned between 0° and 90° will contribute an intermediate value.

2-(2′-Pyridyl)-4,5-dicyanoimidazole vanadyl hydrate has many characteristics in common with the previously studied nitrogen-donor chelate vanadyl species; however, it is charge neutral and may have more favorable solubility properties. Samples of aqua-bis-(2-(2′-pyridyl)-4,5-dicyanoimidazolato) vanadyl hydrate (**2**) prepared in water were tested for spermicidal activity versus normal semen samples from 13 volunteers. Initial results indicate that compound **2** effects both viability (the ability for the cell to live) and motility (the ability of the cell to move) of sperm with dose response actions which are summarized in Figures 3 and 4.

These results indicate that **2** is 100% effective at neutralizing sperm at a 100 μM concentration. The standard spermicide used today in over-the-counter products is nonoxynol-9, (N9) a nonionic surfactant, as a 2−5% suspension.[26] Assuming a density of 1.0 g/mL for these suspensions, the concentrations are in the 32−81 mM range. Aqua-bis-(2-(2′-pyridyl)-4,5-dicyanoimidazolato) vanadyl hydrate is effective at concentrations nearly an order of magnitude less than those seen for the standard N9 product. It should be noted, however, that this is a time-independent comparison. This finding is important given that N9 has recently been found to increase the risk of genital ulceration.[27] Thus, additional tests of the vanadyl species are called for, especially the rate and safety of its effects on humans.

Samples of **2** were also tested for antiviral activity and cytotxicity. The antiviral effects were measured against one pox virus (vaccinia, VV) and two herpes viruses (herpes simplex virus type 1, HSV-1; human cytomegalovirus, HCMV). The cell toxicity measurements were made on

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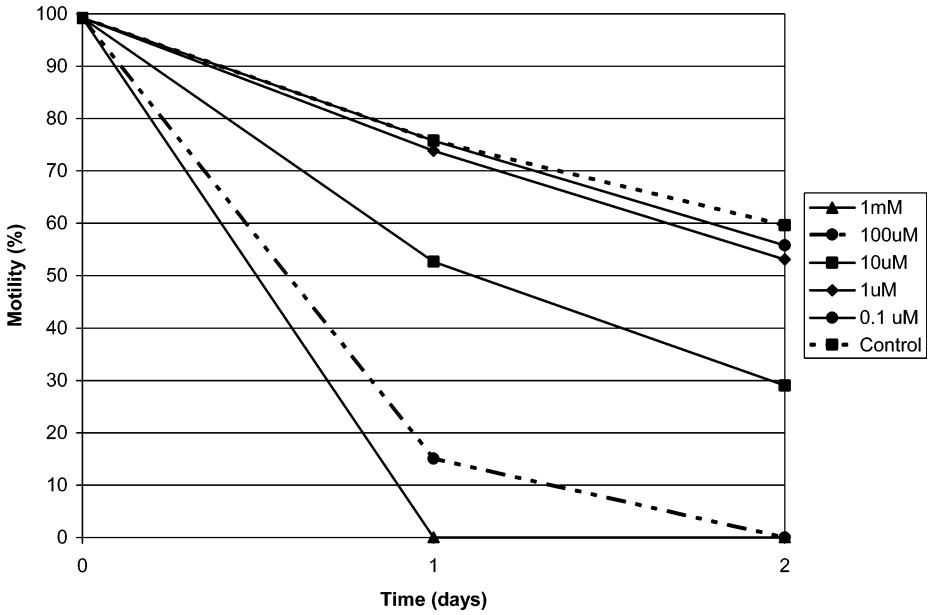

Figure 3. Motility measurements of sperm in the presence of **2** as a function of time. A clear dose−response can be observed.

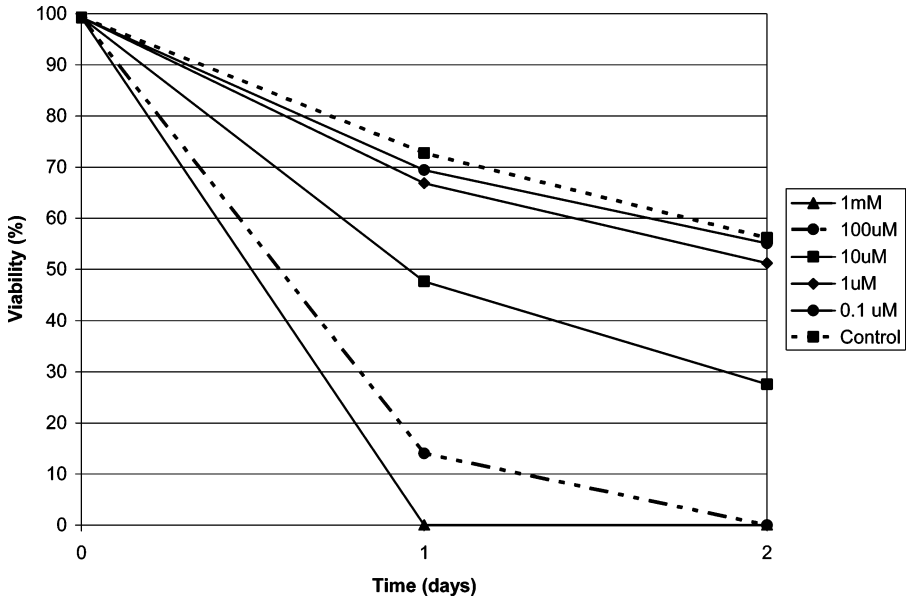

Figure 4. Viability measurements of sperm in the presence of **2** as a function of time. A clear dose−response can be observed.

Table 2. Summary of the Antiviral and Cytoxic Properties of **2**[a]

virus	IC_{50} (μM)
vaccinia	3
human cytomegalovirus	2
herpes simplex type 1	4
cell toxicity	**IC_{50} (μM)**
normal human diploid cells (foreskin fibroblasts)	3
human cancer cells (KB)	5.5[b]

[a] The values, in micromolar concentrations, indicate the 50% inhibitory concentrations. [b] Average of two experiments.

confluent, stationary, human foreskin fibroblasts, and logarithmically growing human cancer cells (KB). The results, summarized in Table 2, are given as the micromolar concentration at which 50% growth inhibition occurred. For compounds to be considered as a candidate for antiviral pharmaceutical development, it is best to see a 100-fold lower concentration for antiviral activity than cytotoxicity. Unfortunately, with our materials the antiviral and cell toxicity concentrations are very similar. This is an indication that the antiviral properties are most likely attributable to toxic effects of **2** on the host cell.

Conclusion

Two novel vanadium complexes have been synthesized using 2-(2′-pyridyl)-4,5-dicyanoimidazole to yield analogous compounds, one as a methanol solvate and the other as a hydrate. The recently reported HDCIPy[13] binds as the anion to the vanadyl moiety to give the neutral species VO-$(DCIPy)_2(H_2O)\cdot 2MeOH$. The crystals of this molecule were fully characterized by single-crystal X-ray diffraction, elemental anaylsis, EPR, and FTIR spectroscopies. Combined, these techniques further support the work of Smith,[17] showing

that the orientation of individual imidazole rings relative to the normal of the vanadium−oxygen bond directly influences $A_{||}$ in the frozen solution spectrum of the paramagnetic species. Furthermore, structural determination allowed for comparison of bond lengths with comparable nitrogen-donor ligand complexes of vanadium. These data illustrate that the anionic ligand produced shortened bond lengths in only the imidazolato portion, with the pyridyl−vanadium bond length being elongated relative to neutral pyridyl-type ligands.

A sample of the compound, prepared as the hydrate, was tested as a spermicidal and antiviral agent. The initial results show that the spermicidal efficacy of this compound is greater than that of the current market standard N9 at significantly lower doses, working to halt both sperm motility and viability. Further studies are called for to measure the rate at which **2** effects the sperm. In a more general cytotoxicity study, it was seen that at 3−4 μM concentrations growth of human foreskin and cancer cells was impeded. Unfortunately, a similar concentration caused growth impedance of two different uninfected cell lines, indicating that this particular derivative is not a good candidate for antiviral use.

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Supporting Information Available: Crystallographic data in cif format. This material is available free of charge via the Internet at http://pubs.acs.org.

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