Volume 34
Number 7
March 29, 1995

Inorganic Chemistry

Communications

Luminescent Labels for Purine Nucleobases: Electronic Properties of Guanine Bound to Rhenium(I)

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Received December 9, 1994

The development of novel DNA sequencing methods and hybridization assays often depends on luminescent labels for mono- and polynucleotides.[1,2] In sequencing technology, the emission properties of the labels must be base-specific.[3,4] We report here the luminescent complex Re(bpy)$(CO)_3$(EtG)$^+$ (**1**), which is to our knowledge the first structurally characterized Re–nucleobase complex (EtG = 9-ethylguanine). The emission of this complex is strongly dependent on the electronic properties of the nucleobase ligand. Analysis of the emission in terms of the energy-gap law provides a sensitive characterization of the electronic nature of the coordinated nucleobase,[5] which affords an intellectual framework for designing luminescent probes for nucleobase ligands and for understanding in general the coordination abilities of DNA bases. In addition to applications in DNA sequencing and diagnostics, rhenium–nucleobase complexes may also be useful in radioimmunotherapy strategies involving DNA–DNA pretargeting.[6]

Complex **1** was prepared via the reaction of Re(bpy)$(CO)_3$(CH_3CN)$^+$ (**2**) with 9-EtG in THF.[7] The proton NMR spectrum of **1** is significantly altered compared to that of free EtG. The CO stretching frequency for the EtG ligand shifts from 1688 cm^{-1} for the free ligand to 1606 cm^{-1} for **1**.[8] The stretching frequencies of the carbonyl ligands are also diagnostic of binding of EtG, with three CO bands observed for **1** (ν(CO) = 2035, 1937, 1919 cm^{-1}) and only two bands observed for **2** (ν(CO) = 2032, 1942 cm^{-1}). This observation suggests that binding of EtG breaks the plane of symmetry in the complex and renders all of the CO ligands inequivalent. This result was surprising because, on the basis of the steric demands of the EtG ligand,[9] we initially expected a symmetric complex, as discussed below.

Diffraction-quality crystals of **1**(PF_6) were grown from acetone and hexanes.[10] The X-ray structure of **1** (Figure 1) shows that the guanine ligand is coordinated via N7, as observed for platinum, ruthenium, and most other metal complexes of purines.[11-16] The Re(bpy)$(CO)_3$ fragment is similar to those

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(7) *fac*-[Re(bpy)$(CO)_3$(EtG)](PF_6) was prepared by a modification of a procedure by Meyer.[5] [Re(bpy)$(CO)_3$(CH_3CN)](PF_6) (400 mg, 0.66 mmol) and 9-ethylguanine (115 mg, 0.64 mmol) were heated at reflux in THF (75 mL) under an argon atmosphere for 3 h. After cooling to room temperature, the solution was filtered through charcoal and Celite to remove any unreacted 9-EtG. The volume was reduced and pentane was added dropwise to precipitate fluorescent canary yellow crystals in quantitative yield. The product was isolated and dried *in vacuo.* Anal. Calcd for [Re(bpy)$(CO)_3$(9-EtG)](PF_6): C, 32.01; H, 2.28; N, 13.06. Found: C, 31.72; H, 2.46; N, 13.03. FAB-MS: *m/z* 606. ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H), 9.30 (d, 2H), 8.60 (d, 2H), 8.30 (t, 2H), 7.85 (s, 1H), 7.80 (t, 2H), 6.70 (s, 2H), 3.80 (q, 2H), 1.05 (t, 3H). UV–vis: λ_{max}(MLCT) = 374 nm (CH_2Cl_2).

(8) IR spectra were recorded on a Bomem Michelson 120 spectrophotometer in CH_2Cl_2 solution. UV–visible spectra were recorded on an OLIS-Modified Cary 14 UV–vis/near-IR spectrophotometer in CH_2Cl_2 solution. Cyclic voltammetry measurements were carried out at a platinum disk electrode in acetonitrile solution with 0.1 M tetraethylammonium perchlorate as supporting electrolyte using a PAR 273A potentiostat. Emission lifetimes were determined using a N_2 laser system as described previously.[5] Samples for lifetime measurements were prepared in deoxygenated (N_2 bubbling for 15 min) CH_2Cl_2 solution. Emission spectra were recorded on a SPEX Industries FluoroMax spectrofluorometer in CH_2Cl_2 solution at an excitation wavelength of 374 nm and were corrected for detector sensitivity. Radiative quantum yields, Φ_r, were measured in deoxygenated methylene chloride solution by numerical integration of the corrected emission spectra relative to [Ru$(bpy)_3$]$(PF_6)_2$ in H_2O as a standard ($\Phi_r = 0.042$ at 25 °C). Corrections were made for differing refractive indices of the solvents by the relationship $\Phi_r = \Phi_r^{obsd}(\eta^2/\eta_{H_2O}^2)$ where the Φ_r^{obsd} is the uncorrected emission quantum yield, η is the refractive index of methylene chloride, and η_{H_2O} is the refractive index of water.

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(10) Crystallographic data for **1**: $C_{23}H_{23}N_7O_5PF_6Re$, fw 808.64, orthorhombic space group *Pcan* with $a = 14.8862(16)$ Å, $b = 16.838(3)$ Å, $c = 23.8570(19)$ Å, $V = 5979.7(13)$ Å^3, and $D_c = 1.796$ g/cm^3 for $Z = 8$. The structure was solved by direct methods and refined by a full-matrix least-squares procedure using 4080 data to an R value of 0.047 ($R_w = 0.049$).

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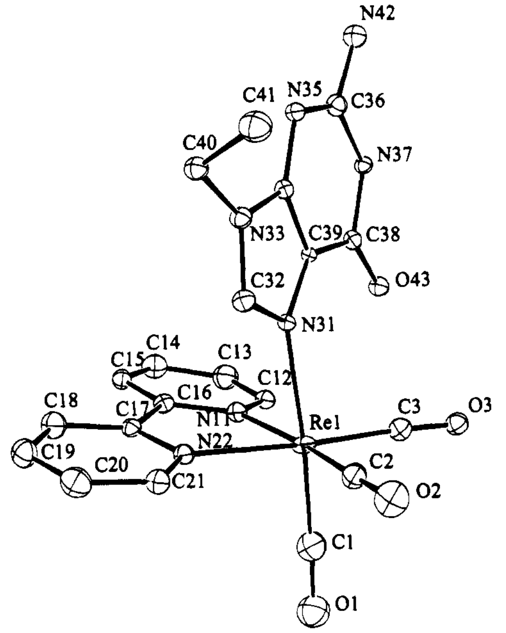

Figure 1. ORTEP diagram of **1**. Selected bond distances (Å): Re–C1, 1.875(17); Re–C2, 1.896(15); Re–C3, 1.919(14); Re–N11, 2.190(11); Re–N22, 2.165(11); Re–N31, 2.220(10). Selected bond angles (deg): C1–Re–C2, 87.0(7); C1–Re–C3, 87.3(7); C1–Re–N11, 94.1(6); C1–Re–N22, 94.5(6); C1–Re–N31, 176.1(6); C2–Re–C3, 89.4(6); C2–Re–N11, 172.7(5); C2–Re–N22, 98.9(5); C2–Re–N31, 93.8(5); C3–Re–N11, 97.8(5); C3–Re–N22, 171.5(5); C3–Re–N31, 96.5(5); N11–Re–N22, 73.8(4); N11–Re–N31, 84.6(4); N22–Re–N31, 81.6(4).

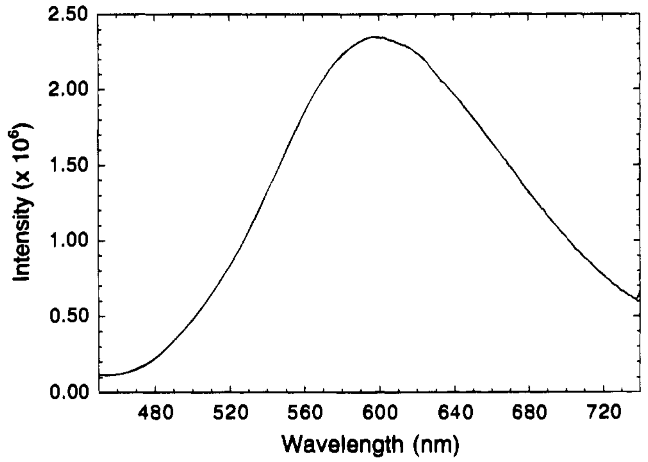

Figure 2. Corrected emission spectrum of **1** (80 μM) in CH_2Cl_2 solution.

Table 1. Emission Properties of $Re(bpy)(CO)_3(L)^+$ Complexes

L	E_{em} (cm^{-1})[a]	ϕ_r	k_{nr} (s^{-1})
Cl^-	16 080	0.005	2.0×10^7
4-NMe_2-py	16 390	0.017	1.0×10^7
4-NH_2-py	16 750	0.052	7.3×10^6
EtG[b]	16 670	0.054	5.6×10^6
N-Me-Im	16 980	0.058	5.8×10^6
4-Et-py	17 640	0.18	1.4×10^6
py	17 920	0.16	1.3×10^6
$P(CH_3)_3$	18 380	0.27	6.2×10^5
CH_3CN	18 660	0.41	4.9×10^5

[a] All data taken from ref 5, except those for L = EtG. [b] This work.

observed in other structurally characterized complexes.[17–20] Recently, van Vliet et al. pointed out that the carbonyl oxygen of coordinated guanine is sterically demanding in octahedral complexes.[9] In the complex $Ru(bpy)_2(EtG)Cl^+$ (**3**), the carbonyl oxygen is accommodated between the two bpy ligands[9] and may explain why the head-to-head conformation of lopsided ligands is relatively rare.[21] We originally suspected on the basis of models that the carbonyl oxygen from guanine in **1** would be situated between the two CO ligands; however, Figure 1 reveals that the carbonyl oxygen is actually accommodated between one bpy ring and an adjacent CO ligand. So in both **1** and **3**, the guanine carbonyl is adjacent to the bpy ligand.

The electronic properties of the complex are exquisitely sensitive to coordination of EtG. For example, the MLCT absorption red-shifts 31 nm (2400 cm^{-1}) upon coordination of EtG. The emission maximum shifts dramatically, moving from 535 nm for **2** to 600 nm for **1** (Figure 2), a red shift of 2030 cm^{-1}. The excited state of **1** is long-lived (τ = 170 ns), with an emission quantum yield of $\phi = 0.054 \pm 0.017$. These properties allow for a very sensitive assessment of the electronic properties of the guanine ligand using the energy-gap law.[5] The energy-gap law is based on a golden-rule analysis of excited-state decay and requires that as the energy gap between the excited and ground states increases, the rate of nonradiative decay (k_{nr}) decreases. Operationally, the energy-gap law states that the there should be a linear relationship between ln k_{nr} and the emission energy (E_{em}). The relevant rates can be calculated as

$$\tau = (k_r + k_{nr})^{-1} \qquad \phi = k_r\tau$$

where k_r is the radiative decay rate. This analysis gives $k_r = 3.2 \times 10^5$ s^{-1} and $k_{nr} = 5.6 \times 10^6$ s^{-1} for **1**.

Caspar and Meyer have examined the emission of $Re(bpy)(CO)_3L^+$ complexes in detail in terms of the energy-gap law. Their data are shown in Table 1 along with the emission properties of **1**. The values for **1** fit on a linear plot of ln k_{nr} versus E_{em} for all of the data in Table 1. This analysis shows that EtG is similar electronically to electron-rich pyridines, such as 4-NMe_2-py and 4-NH_2-py, and clearly is a much better π-donor ligand than py, PMe_3, and CH_3CN. This observation is consistent with our recent report that $Ru(bpy)_2(EtG)OH_2{}^{2+}$ and $Ru(bpy)_2(4\text{-}NMe_2\text{-}py)OH_2{}^{2+}$ have identical redox potentials and DNA oxidation selectivities.[22] It therefore appears that EtG is a relatively good π-donor (although not as good as Cl^-), which is consistent with the shift in the stretching frequency of the EtG carbonyl upon coordination and the red shift of the MLCT absorption.

The quantitative analysis of the electronic properties of guanine ligands provides new principles for designing metal complexes that target guanine residues. For example, oxidation states that bind with high affinity to 4-NMe_2-py, such as Re(V) and Ru(IV), will bind effectively to guanine, suggesting that

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high-valent complexes of EtG should be quite stable. Indeed, we have observed the formation of Ru(bpy)$_2$(EtG)O^{2+}.[22,23] Related considerations can be applied to understanding the binding of Pt(II) and other metals. In terms of designing luminescent labels and probes, we can now use the information in Table 1 to analyze a variety of chromophores bound to EtG. The Re(bpy)(CO)$_3^+$ chromophore is attractive because the quantum yields are generally about an order of magnitude higher than those of related Ru(II) excited states; however, the energy-gap law is somewhat of a two-edged sword, because better ligands have lower quantum yields.[5] Nonetheless, the shifts in emission maximum should be large due to the unusual electronic properties of EtG.

Acknowledgment. We thank Dr. Darryl S. Williams and Sheila R. Smith for assistance with the emission lifetime measurements. H.H.T. thanks the NSF for a Presidential Young Investigator Award and the David and Lucile Packard Foundation for a Fellowship.

Supplementary Material Available: An ORTEP diagram showing the complete atomic labeling scheme and tables giving details of crystal data and refinement, tables of bond lengths and angles, fractional coordinates, thermal parameters, and H atom coordinates (14 pages).

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