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Effects of Nucleobase Metalation on Frontier Molecular Orbitals: Potential Implications for π-Stacking Interactions with Tryptophan

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Biochemical recognition processes mediated through π-stacking interactions are a potential target for rational drug synthesis. A combination of electrostatic, hydrophobic, solvation, charge-transfer, induction, and dispersion interactions has been used to account for the three-dimensional arrangements observed in such motifs. A principal example involves the interaction of purine and pyrimidine rings of nucleic acids with aromatic amino-acid residues such as tryptophan, phenylalanine, and tyrosine. Protonation, alkylation, or coordination of a metal ion such as Pd(II) or Pt(II) to a nucleobase strengthens this interaction by lowering the energy of the lowest unoccupied molecular orbital (LUMO) of the modified nucleobase and improving overlap with the highest occupied molecular orbital (HOMO) in N-acetyl tryptophan. The relative energy difference between the frontier orbitals of isolated molecules, obtained using Density Functional Theory (DFT), is explored as a predictive tool for the strength of the π-stacking interaction of the nucleobase/tryptophan pair. From the optimized structures of these species, evaluation of the donor−acceptor HOMO−LUMO gap ($\Delta\varepsilon_{d\rightarrow a}$) suggests that this parameter is a promising predictor of π-stacking strength for the donor−acceptor pairs presented in this study. The analysis correlates well with experimental association constants, measured by fluorescence spectroscopy, of metallated and alkylated nucleobases with tryptophan in comparison to free nucleobases.

Introduction

Non-covalent π-π stacking interactions between parallel aromatic rings are important for molecular recognition processes relevant to DNA transcription, protein folding, and gene regulation.[1−3] Protein translation mechanisms in eukaryotes and viruses involve interactions between tryptophan (Trp) and methylated nucleobases, as in mRNAcap - eukaryotic initiation factor (eIF) 4E interaction[4−8] and the viral cap-binding protein in vaccinia VP39.[9,10] High mobility group (HMG) proteins recognize platinated DNA by intercalation of a phenylalanine residue into the lesion site.[11,12] π-stacking interactions are also potentially important determinants of effectiveness in the formation, and potential chemotherapeutic use, of DNA-intercalator complexes.[13−15]

Metal coordination strengthens π-stacking interactions between nucleobases and aromatic amino acids by decreasing the π-electron density in aromatic nitrogen heterocycles.[16−18]

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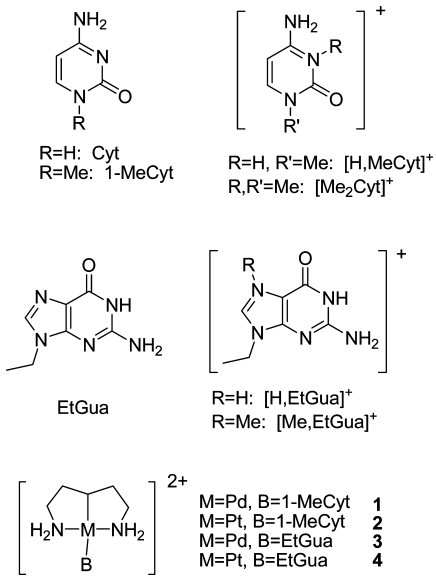

Figure 1. Structures of N−Ac Tryptophan, cytosine, and guanine derivatives and their palladium and platinum metal complexes.

Recent fluorescence experiments have shown enhancement of the association constant between Trp and metallated nucleobases/nucleotides over that for the corresponding free nucleobases/nucleotides, See Figure 1 for structures.[19,20] The enhancement is dependent on the metal ion and nucleobase, favoring Pt(II) over Pd(II) and cytosine over guanine, respectively. Non-covalent interactions between tryptophan and metallated nucleobases have been studied in more complex systems such as the C-terminal zinc finger (F2) from the HIV nucleocapsid protein (NCp7).[21] The complexes $[Pt(dien)(9\text{-EtGua})]^{2+}$ and *cis*-$[Pt(NH_3)_2(Guo)_2]^{2+}$ (9-EtGua = 9-ethylguanine, Guo = guanosine) presumably form π-stacking interactions with the aromatic residues of the F2 zinc finger in solution. The formation of 1:1 adducts was detected by electrospray ionization mass spectrometry (ESI-MS) and confirmed through MS-MS experiments. An understanding of the effect of metalation on π-stacking could lead to the design of chemotherapeutic agents which target specific recognition sites of DNA/RNA−protein and protein−protein interactions. π-stacking interactions between aromatic residues in proteins and nucleic acids, as well as metal complexes of aromatic heterocycles,[22,23] are found in the slipped or parallel displaced π-stacking conformation, Figure 2A.[24,25] A combination of electrostatic, hydrophobic, solvation, charge-transfer, induction, and dispersion interactions has been used to account for the three-dimensional arrangements observed in such motifs.[22] The Hunter−Sanders rules, based on electrostatic considerations, have been suggested as a qualitative explanation of these interactions. According to these rules, a separation is made between a formally positive σ framework and the π-electrons in an aromatic system, such that the interaction can be evaluated in terms of π-π repulsions and π-σ attractions.[22,26] However, this model ignores important contributions to π-stacking interactions, such as induction or short-range repulsions. Morokuma-type decomposition schemes partition the interaction energy into several terms (eq 1):[27]

$$\Delta E_{int} = \Delta E_{electrostat} + \Delta E_{Pauli} + \Delta E_{orb} \quad (1)$$

The $\Delta E_{electrostat}$ term includes the classical Coulombic attraction as well as multipole-multipole interactions. The second term ΔE_{Pauli} is a repulsive interaction between occupied MOs. The third term ΔE_{orb} includes contributions resulting from orbital interactions, including donor−acceptor interactions, polarization effects, and bonding. The electrostatic contribution has been shown to be dominant for interactions of π-stacked nucleobases at the minimum energy conformation.[28] However, while the attractive contribution of the orbital interaction term ΔE_{orb} is smaller than that of $\Delta E_{electrostat}$ in these cases, it still represents a significant portion of the total interaction energy. For example, the value of ΔE_{orb} is -5.12 kcal mol^{-1} whereas ΔE_{int} is -8.61 kcal mol^{-1} for the C−C dimer.[28]

To accurately quantify the strengths of interactions based upon eq 1, the conformation of the π-complex must be known, yet a full conformation analysis, especially for metallated nucleobases, is time-consuming and impractical for rational drug design. The high-level ab initio methods and large basis sets required for proper recovery of the dispersion forces in the π-stacking are also prohibitively expensive for these applications.[29] Development of a screening process to estimate π-stacking strengths based upon calculations of isolated molecules has the advantage of rapidly generating a broad range of possible targets. Although the donor−acceptor contribution to ΔE_{orb} is not the dominant term in ΔE_{int}, it is the only term which may be easily estimated without knowledge of the actual conformation of the π-complex. This is because, if a π-complex is assumed to form via a donor−acceptor interaction, then the strength of the interaction may be estimated by comparison of the energies of the HOMO of the donor system and the LUMO

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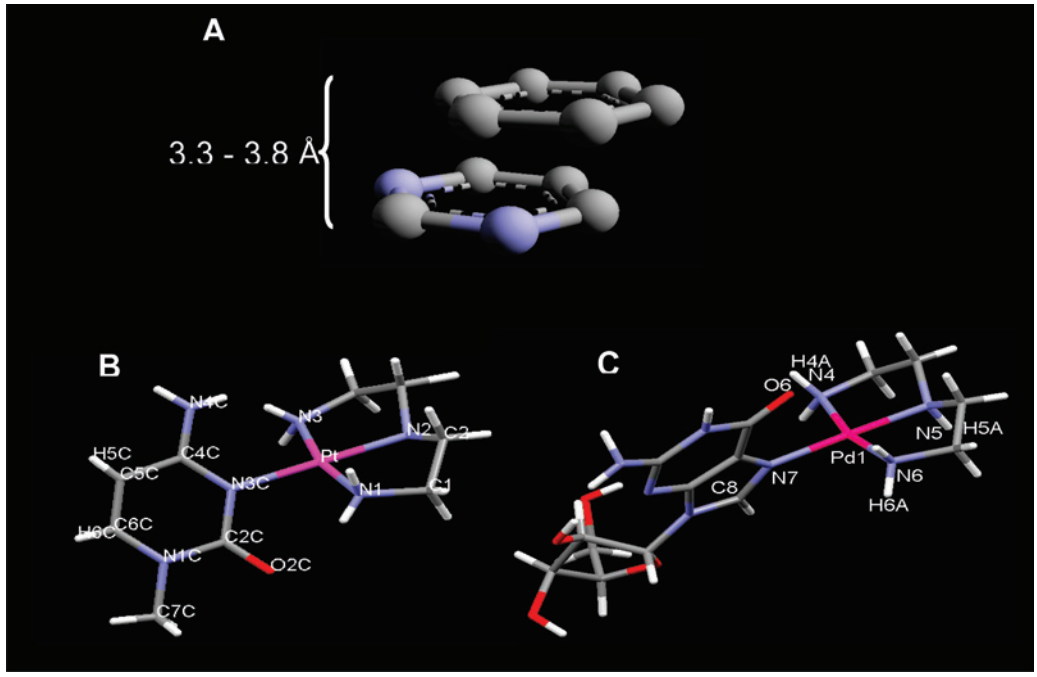

Figure 2. Schematic representation of a parallel displaced or off-centered π-stacking interaction, showing the common interplanar distance (A) and the crystal structure of $[Pt(dien)(1\text{-MeCyt})]^{2+}$ (B) and $[Pd(dien)(9\text{-EtGuo})]^{2+}$ (C). The numbering scheme for $[M(dien)(1\text{-MeCyt})]^{2+}$ and $[M(dien)(9\text{-EtGua})]^{2+}$ complexes is shown.

of the acceptor.[30] The approach of comparing frontier orbital energies has been used to explain π-stacking interactions between alkylated nucleobases with tryptophan.[31,32] Overlap with the HOMO of the electronic-rich indole moiety in tryptophan is enhanced by a decrease in LUMO energy in the nucleobase upon N7 alkylation. Several experimental studies regarding structural characterization of this binary system in the solid state, as well as in solution, have been performed.[33−37] Ishida et al.'s discussion, using semiempirical methods (MNDO), of π-stacking of nucleobases with indole rings focused on the lowering of the nucleobase LUMO upon quaternization.[33] Alternatively, Nakatani and Saito explain the selective alkylation of DNA by pluramycin antibacterial agents as a HOMO-controlled process due to the preferential intercalation into GG sequences because of the high energy of the GG HOMO relative to other stacked base sequences.[38,39]

There is a formal analogy between protonation, alkylation, and metalation of nucleic acids and their biological effects.[19,40] Especially for the effects of alkylation and metalation, both involve an electrophilic interaction of the alkylating or metallating agent on an electron-rich purine or pyrimidine site. Given the previous applications of frontier orbital energies of isolated donor and acceptor molecules in the analysis of π-stacking interactions involving alkylated nucleobases,[30−36] we have estimated the strength of the HOMO/LUMO interactions between metallated nucleobases and tryptophan. We considered this approach valid to understanding the effect of nucleic acid base metalation on π-stacking interactions, given the stated analogy between alkylation and metalation. The long-term aim is to develop a rapid screening process for synthesis and identification of optimal derivatives with enhanced π-stacking interactions for applications in biochemical recognition processes. The systems studied include N3- and N7-protonated and methylated 1-methylcytosine (1-MeCyt) and 9-ethylguanine (9-EtGua), respectively, for comparison to the analogous metallated (Pt and Pd) compounds (Figures 2B and 2C). From the optimized structures of these species, the donor−acceptor HOMO−LUMO gap ($\Delta\varepsilon_{d\rightarrow a}$) was determined as a first approximation of the strength of the π-stacking interaction. Although $\Delta\varepsilon_{d\rightarrow a}$ is primarily an estimate of the charge-transfer contribution to the interaction energy (eq 1), some estimate of the electrostatic contribution is incorporated because of the lowering of the molecular orbital energies for charged species. Correlation of $\Delta\varepsilon_{d\rightarrow a}$ to experimental equilibrium constants suggests that this ap-

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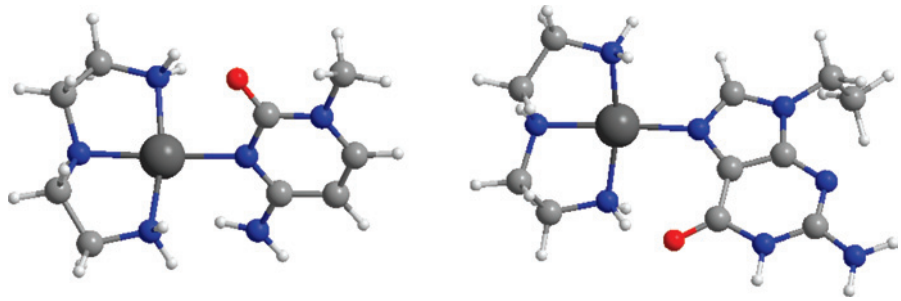

Figure 3. View of the lowest energy rotamers for **2** (left) and **4** (right) showing the hydrogen bond formed by the exocyclic oxygen of 1-MeCytosine (in red) and dien. The "stingray" conformation of the dien ligand is also shown.

proach may constitute a practical means of estimating potential strengths of π-stacking interactions.

Computational Methods

Calculations were carried out at the DFT/mPW1PW91 level[41] with the Gaussian 03 suite of programs.[42] Palladium and platinum were represented by the Ermler−Christiansen relativistic effective core potential basis set[43,44] modified with the Couty−Hall contraction of the (n+1)p functions.[45] Basis sets for nitrogen and oxygen were split valence triple-ζ quality augmented with polarization functions.[46] Carbon and hydrogen were represented by Dunning double-ζ basis sets;[47] polarization functions were added to carbon. Optimized geometries were characterized as minima through calculation of their vibrational frequencies.

Results and Discussion

I. Structure Optimization. Prior to examination of the HOMO/LUMO energies of the alkylated and metallated nucleobases, we confirmed that the structures derived from computation were comparable to those observed by spectroscopic and diffraction methods.[20,48,49]

Ia. Metal-Nucleobases. Optimized geometries for [M(dien)(1-MeCyt)]$^{2+}$ (M = Pd (**1**), Pt (**2**)) and [Pt(dien)(9-EtGua)]$^{2+}$ (M = Pd (**3**), Pt (**4**)) were consistent with known crystal structures.[20,49] The calculated lowest energy rotamers are shown in Figure 3. The calculated bond distances and angles did not differ significantly from those derived from the crystal structures for 1-MeCyt and 9-EtGua complexes. Deviations in bond distances for the first coordination sphere were less than 0.1 Å (see Supporting Information, Tables SI and Tables SII, respectively). The expected "stingray" structure of the dien ligand was obtained where the methylene groups C2 and C3 are symmetrically above the plane of the metal (Figure 3). For **3** and **4**, rotation of the ethyl moiety gave a difference of only 0.5−0.6 kJ/mol between conformers suggesting free rotation in solution.

The asymmetry of the dien ligand produces rotamers in the nucleobase complexes, that have been observed in solution, because of hindered rotation about the M-N3C(N7) axis.[20,50] The reported crystal structure has the exocyclic amine group of 1-MeCyt on the opposite side (exo) of N(2)H for both **1** and **2**. The same-side rotamer (endo) conformation is observed within the Pt(dien)(Cyt) fragments in the X-ray structure of the trinuclear cation *trans*-[$(CH_3NH_2)_2$Pt{(N3−C-*N1*)Pt(dien)}$_2$}]$^{4+}$.[50] Our calculations showed that the endo structure was more stable than the exo (8.2 kJ/mol for **1** and 7.6 kJ/mol for **2**). These results are consistent with a recent computational study which reported a greater energy difference (14.8 kJ/mol) between the rotamers and attributed this discrepancy to crystal packing effects.[51] For **3** and **4**, the exo conformation is more stable by 4.5 kcal/mol in agreement with the X-ray structure of [Pd(dien)(guanosine)]$^{2+}$.[49] Only slight differences in bond distances and angles were observed between the rotamers (Supporting Information, Tables SI).

The coordination geometry of the reported crystal structures are not, in themselves, remarkable. Although the Cyt ring is perpendicular to M(dien)$^{2+}$ in the X-ray structures of **1** and **2** because of interactions with co-crystallized water molecules, hydrogen-bonding between the nucleobase carbonyl and the dien amine protons in the optimized structures produces a tilt in the nucleobase relative to the dien

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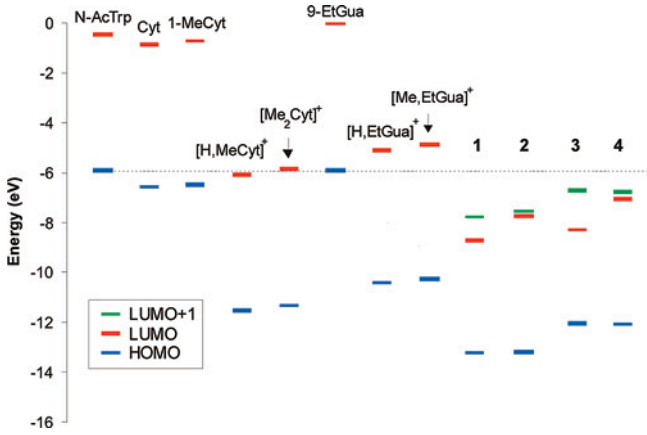

Figure 4. Comparison of HOMO\LUMO energies found for the different nucleobases and metal complexes studied. The energy of the HOMO in N-AcTrp (dashed line) was used to calculate $\Delta\varepsilon_{d\rightarrow a}$, using the LUMO values of the various nucleobases and modified (protonated, alkylated, and metallated) nucleobases as shown. **1**, $[Pd(dien)(1\text{-MeCyt})]^{2+}$; **2**, $[Pt(dien)(1\text{-MeCyt})]^{2+}$; **3**, $[Pd(dien)(9\text{-EtGua})]^{2+}$; **4**, $[Pt(dien)(9\text{-EtGua})]^{2+}$.

plane.[48,52] The torsion angle for the tilt of the nucleobase is larger for Pd versus Pt resulting in longer intramolecular longer O2C···H2N1 distances (Supporting Information, Tables SI and SII). The crystal structure of $[Pd(dien)(guanosine)]^{2+}$ exhibits a long intramolecular H-bond (2.479 Å) between the oxygen atom of the guanosine carbonyl and the proton of the *cis* amine of the dien ligand (H6B); moreover, a stronger intermolecular H-bond is exhibited with the exocyclic amine from another guanosine moiety (2.169 Å, not shown). For the calculated complexes **3** and **4**, the intramolecular H-bond is stronger because of the absence of additional complexes in the extended array. This H-bond (O6···H6B) was consistently longer for Pd compared to Pt complexes (1.826 vs 1.803 Å, **3** and **4,** respectively) in agreement with the results for 1-MeCyt (**1**, **2**).

II. HOMO/LUMO Analysis. Given the good correlation between observed and calculated structures of the M-nucleobase compounds, we examined the properties of the HOMOs and LUMOs of N-AcTrp and the quaternized nucleobases to obtain a qualitative measure of the strength of a potential $\pi-$stacking interaction with N-AcTrp upon protonation, methylation, or metalation. By perturbation theory, the strength of these interactions is inversely proportional to the absolute value of the difference of ε_{LUMO} of the nucleobase and ε_{HOMO} of the N-AcTrp ($\Delta\varepsilon_{d\rightarrow a} = |\varepsilon_{HOMO,N\text{-}AcTrp} - \varepsilon_{LUMO,nucleobase}|$). For pairs of interacting species with similar overlap character, the more similar the energies of these frontier orbitals the smaller is the value of $\Delta\varepsilon_{d\rightarrow a}$, and the stronger the interaction should be between the two rings. The comparison of the HOMO/LUMO energies found for the various nucleobases and metal-nucleobases is shown schematically in Figure 4. These data are tabulated, along with values for $\Delta\varepsilon_{d\rightarrow a}$, in Table 1.

IIa. Free Neutral Nucleobases. HOMO/LUMO energies calculated for the optimized structures of the free neutral nucleobases (Cyt, 1-MeCyt, and 9-EtGua) and *N*-acetyl tryptophan are shown in Table 1. Methylation of Cyt shifts both the HOMO and the LUMO of the nucleobase to slightly higher energies but does not significantly reduce the HOMO/LUMO gap. 9-EtGua as a representative purine exhibited higher energy values for HOMO and LUMO, compared to 1-MeCyt, and a slightly larger HOMO/LUMO gap (5.901 eV). The energies for the frontier orbitals in N-AcTrp were close to those values obtained for the free nucleobases: LUMO, −0.560 eV and HOMO, −6.067 eV. This latter energy was used for calculation of $\Delta\varepsilon_{d\rightarrow a}$ for the different nucleobase-tryptophan systems. The calculated values of the frontier orbital energies resulted in similar, but large, $\Delta\varepsilon_{d\rightarrow a}$ values for the potential $\pi-$stacking interactions between the neutral nucleobases and N-AcTrp (Table 1). The donor−acceptor interaction is predicted to be less favorable ($\Delta\varepsilon_{d\rightarrow a}$ = 5.907 eV) for 9-EtGua than for 1-MeCyt given its higher energy LUMO. These $\Delta\varepsilon_{d\rightarrow a}$ values are consistent with experimentally determined association constants (K_π) which indicate a stronger interaction for 1-MeCyt/N-AcTrp over 9-EtGua/N-AcTrp.[19,20]

IIb. Protonated/Methylated Nucleobases. Protonation or methylation at the N3 position for 1-MeCyt and at the N7 position for 9-EtGua reduced the frontier orbital energies by about 4−5 eV. The decrease in energy was consistently larger for protonated versus methylated nucleobases by approximately 0.25 eV, and larger for 1-MeCyt versus 9-EtGua systems (0.28 eV). The HOMO/LUMO gap for the quaternized systems decreased by approximately 0.3−0.7 eV compared to neutral nucleobases. These results are consistent with the shift to lower wavelengths observed in the electronic spectrum of N3-protonated 1-MeCyt and N7-protonated 9-EtGua compared to the neutral nucleobase (data not shown). Interestingly, protonation, and methylation of 1-MeCyt does not produce a significant change in the HOMO/LUMO energy gap within the modified nucleobases, in contrast to metalation which results in significantly reduced HOMO/LUMO energy gaps by 1−3 eV for compounds **1**−**4** in comparison to the protonated and methylated cases.

The lower LUMO energies of modified nucleobases resulted in more favorable $\Delta\varepsilon_{d\rightarrow a}$ values in comparison to the free neutral nucleobases (Table 1) suggesting a potentially stronger interaction between Trp and the quaternized nucleobases. Visualization of the frontier orbitals for the interacting species showed that the HOMO (N-AcTrp) and LUMOs (quaternized nucleobases) were π-type localized in the aromatic rings (Figure 5). The prediction of a stronger interaction is consistent with recent calculations at the MP2 level which show an increase in π-stacking interaction between methylated adenine and aromatic aminoacids compared with those for the free nucleobase.[53]

IIc. Metallated Nucleobases. Coordination of the nucleobases to $M(dien)^{2+}$ produces complexes with higher net charge compared to methylation (+2 vs +1). HOMO and LUMO frontier orbitals in Pt(II)- and Pd(II)-nucleobase complexes exhibited a larger decrease in energy, in the range

(52) The tilt of the nucleobase is diminished when H-bonding does not occur between dien and the nucleobase. (e.g., $[Pd(dien)(adenosine)]^{2+}$: Arpalahti, J.; Klika, K. D.; Sillanpää, R.; Kivekäs, R. *J. Chem. Soc., Dalton Trans.* **1998**, *8*, 1397–1402.).

(53) Rutledge, L. R.; Campbell-Verduyn, L. S.; Hunter, K. C.; Wetmore, S. D. *J. Phys. Chem. B* **2006**, *110*, 19652–19663.

Table 1. Summary of ($\Delta\varepsilon_{d\rightarrow a}$) Values Found between Tryptophan and Protonated, Methylated, and Metallated Nucleobases Calculated in This Study[a]

		neutral	H^+	Me^+	$(dien)Pd^{2+}$	$(dien)Pt^{2+}$
1-MeCyt	$\Delta\varepsilon_{HOMO-LUMO}$, eV	5.739[b]	5.485	5.526	4.503 (exo)	5.438 (exo)
					4.500 (endo)	5.480 (endo)
	$\Delta\varepsilon_{d\rightarrow a}$, eV	5.232[c]	0.154	0.099	2.777 (exo)	1.838 (exo)
					2.768 (endo)	1.786 (endo)
9-EtGua	$\Delta\varepsilon_{HOMO-LUMO}$, eV	5.901	5.208	5.356	3.786	4.965
	$\Delta\varepsilon_{d\rightarrow a}$, eV	5.907	1.307	1.716	2.366	1.187

[a] $\Delta\varepsilon_{d\rightarrow a} = |\varepsilon_{HOMO,N\text{-}AcTrp} - \varepsilon_{LUMO,nucleobase}|$. The lower the $\Delta\varepsilon_{d\rightarrow a}$, the greater should be the overlap. Note value for $\Delta\varepsilon_{HOMO-LUMO}$ is N-AcTrp: 5.507 eV. [b] Cyt: 5.797 eV. [c] Cyt: 5.134 eV.

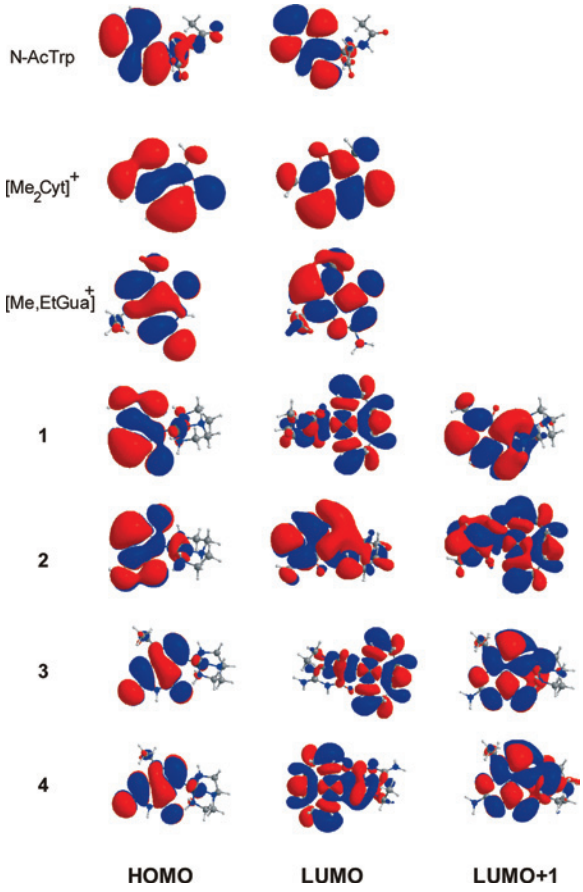

Figure 5. Plot of the HOMO, LUMO, and LUMO+1 for N-AcTrp, and the methylated and metallated nucleobases. **1**, $[Pd(dien)(1\text{-MeCyt})]^{2+}$; **2**, $[Pt(dien)(1\text{-MeCyt})]^{2+}$; **3**, $[Pd(dien)(9\text{-EtGua})]^{2+}$; **4**, $[Pt(dien)(9\text{-EtGua})]^{2+}$.

of 7−8 eV, than for methylated/protonated nucleobases when compared to the corresponding neutral nucleobase. This decrease was consistently larger (ca. 1 eV) for LUMOs in comparison to HOMOs in all metallated cases. The 9-EtGua complexes exhibited higher frontier orbital energy values compared to 1-MeCyt complexes in agreement with results found for neutral and protonated/methylated nucleobases (see Figure 4). Moreover, the LUMO energy for Pt(II)-nucleobase complexes was consistently higher than the Pd(II)-nucleobase analogue by about 1 eV (see **2** and **4** vs **1** and **3** in Figure 4). Interestingly, LUMO and LUMO+1 values for the Pt complexes are very similar, whereas there is a greater difference in the case of Pd, with the LUMO (of **1** and **3**) now being ∼1 eV lower than LUMO+1. These relative differences may have implications for the chemical nature of the overlap in both cases (see below).

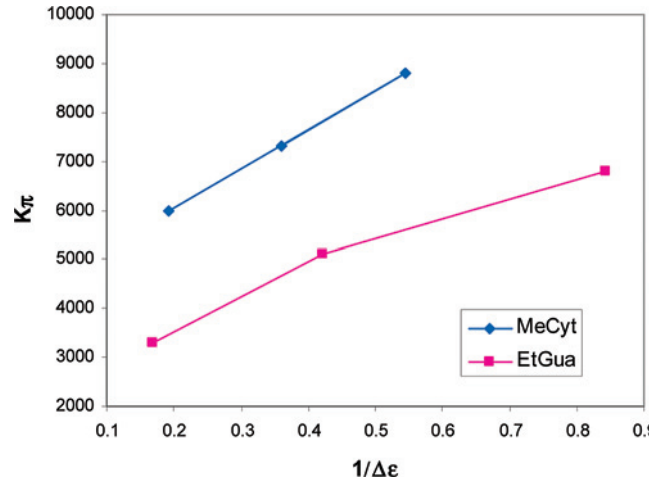

Figure 6. Correlation between association constants, determined for free 1-MeCyt and Pd/Pt-1-MeCyt complexes, with the $\Delta\varepsilon_{d\rightarrow a}$ value.

A plot of the correlation between the frontier orbital data and available experimental K_π values for 1-MeCyt, 9-EtGua and their metallated analogues is shown in Figure 6. Comparison of data for the metal-nucleobase complexes shows that $\Delta\varepsilon_{d\rightarrow a}$ values are smaller for Pt(II) versus Pd(II), consistent with fluorescence experiments where a higher K_π was observed for Pt(II)- versus Pd(II)-nucleobase complexes.[19,20] However, the $\Delta\varepsilon_{d\rightarrow a}$ value predicted a more favorable interaction for 9-EtGua versus 1-MeCyt complexes which indicates a limitation in the application of simple HOMO−LUMO donor−acceptor interactions. The strength of the interaction by perturbation theory is directly proportional to the overlap between the donor and acceptor MOs. Trends in interaction strength will be limited to donor−acceptor interactions of the same type assuming that the orientation of the interaction will be similar for stacking pairs. Thus, grouping of the $\Delta\varepsilon_{d\rightarrow a}$ values by nucleobase shows an inverse correlation with the experimental K_π as expected (Figure 6).

The character of the LUMOs in the complexes varied depending upon the nature of the metal and the nucleobase. For the Pt(II)-MeCyt complex **2** the LUMO was localized in π-type fragment orbitals of the nucleobase ring, whereas those of the Pd(II) complex **1** were localized on the metal-dien fragment (Figure 5). Visualization of the LUMOs in the M(dien)-9-EtGua complexes (**3**, **4**) showed that these were also localized in the metal-dien fragment (Figure 5). For the species in which the LUMO is metal-centered, the next highest virtual orbital (LUMO+1) is localized in the nucleobase. Although LUMO and LUMO+1 are similar in energy for the Pt complexes regardless of their character, the metal-centered LUMO for the Pd complexes is ∼1 eV

lower than LUMO+1 which is consistent with the preference of Pd for d^{10} configurations. The LUMO character may have implications in the chemical nature of the π-stacking interactions. The metal-centered LUMO may indicate that an electrostatic interaction between the metal fragment and Trp is favored, particularly in the case of the Pd complexes. The localization of LUMO in the metal fragment for the Pd complexes could also explain the observed chemistry of the system, where ligand substitution, where the amino-acid displaces the nucleobase, is observed under basic conditions.[20] The similar energies of the two lower unoccupied orbitals for the Pt complexes may suggest that the π-stacking competes with an electrostatic M-Trp interaction. Additional experimental and theoretical work is in progress to clarify the nature of the interactions and their implications.

Conclusions

The understanding of non-covalent π-stacking interactions for metal complexes with biological ligands is in an early stage. The $\Delta\varepsilon_{d\rightarrow a}$ values based upon frontier orbital energies of isolated molecules are a promising predictor of π-stacking strength for the donor−acceptor pairs presented in this study. The results obtained here strengthen the analogy between alkylation and metalation of nucleobases and the chemical and biological consequences. Lowering of ε_{LUMO} for the nucleobases by methylation or metalation allows for greater mixing with the HOMO of the Trp donor through a donor−acceptor interaction. The results presented suggest that the contribution of charge transfer to the π-π stacking energies predicts the experimental order observed as evidenced by comparison of a series of compounds with variation of M and nucleobase. However, the correlation of $\Delta\varepsilon_{d\rightarrow a}$ values with experimental K_π for the nucleobase-Trp pairs demonstrates that comparison of frontier orbital energies by this method is most useful for stacking pairs with the same orbital overlap; thus, derivatives of Gua may be compared, but Gua derivatives should not be compared to Cyt derivatives. Variation in the character of the LUMO constitutes an additional variable to take into account to predict interaction trends. In several cases, especially for Pd complexes, analysis showed that the LUMO was metal-centered rather than nucleobase-centered. If π-stacking is assumed through interaction with LUMO+1 in these cases, we note that the significant difference in the association constant of the Pd and Pt nucleobases does not correspond to their similar LUMO+1 energies. The lower K_π for Pd may also result from competition between π-stacking and ligand substitution, observed in solution under basic conditions. Given these results, it is possible that the interaction in vivo is too complex to be determined based upon $\Delta\varepsilon_{d\rightarrow a}$ values alone. Steric interactions with the bulky metal complex and rotation around the M−N axis may also affect π-stacking. Nevertheless, a reasonable first approximation to the stacking energy may be obtained by comparison of the frontier orbitals in structurally similar donor−acceptor complexes. These results can then be used to direct appropriate synthesis of nucleic acid bases or planar ligands with appropriate substituents to enhance the overall stacking interaction in more complicated biological systems.

Supporting Information Available: Summary of bond distances and angles for the crystal and calculated structures of metal complexes of 1-MeCytosine (Table S1) and guanine derivatives (Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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