# *N*-1- and $\eta^2$-Pyrimidine Linkage Isomers in Complexes of $[Ru^{II}(hedta)]^-$

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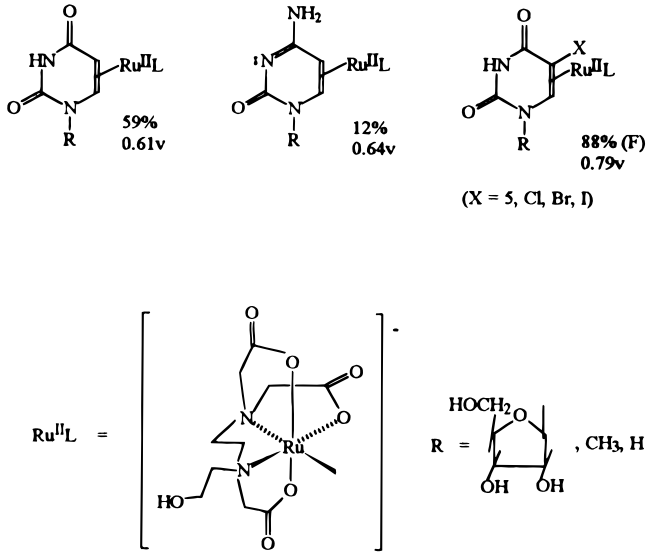
$[Ru^{II}(hedta)(H_2O)]^-$, $hedta^{3-}$ = *N*-hydroxyethylethylenediaminetriacetate, forms N-1-bound pyrimidine complexes *via* a kinetically controlled substitution at N-1 with pyrimidine (pym), 4-methylpyrimidine ($4CH_3$pym), and 4,6-dimethylpyrimidine ($Me_2$pym); $k = 31\ M^{-1}\ s^{-1}$ for pym. Subsequent to N-1 coordination, an intramolecular redistribution of $Ru^{II}$−pyrimidine linkage isomers occurs with the formation of $\eta^2$ attachments, reaching equilibrium with $t_{1/2}$'s of 28.8 (pym), 24 ($4CH_3$pym), and 1 h for $Me_2$pym. The N-1 forms exhibit normal $Ru^{II/III}$ waves at 0.14 (pym), 0.10 ($4CH_3$pym), and 0.16 V ($Me_2$pym) whereas the $\eta^2$ forms shift to more positive values indicative of better $\pi$-acceptor attachments: 0.50 (pym) and 0.44 V ($4CH_3$pym). The ratio of isomers was determined to be as follows by $^1$H NMR and $^{13}$C NMR methods: (pym) $\eta^2(1,2):\eta^2(5,6):\eta^2(1,6)$:N-1 of 43:22:33:2; ($4CH_3$pym) $\eta^2(1,2):\eta^2(5,6)$:N-1 of 10:33:57; ($Me_2$pym) $\eta^2(1,2):\eta^2(5,6)$:N-1 of 6:26:68. $^1$H NMR assignments have been made for all the observed N-1, $\eta^2(1,2)$, $\eta^2(5,6)$, and $\eta^2(1,6)$ isomers. $^{13}$C NMR shifts have been identified for the major isomers of pym and $4CH_3$pym and confirmed HH COSY and HC COSY methods. Several important conclusions are drawn: (1) $\eta^2$ isomers of the $\eta^2(1,2)$ type exhibit significant downfield shifts of H2 of ca 1.20 ppm; (2) $^{13}$C NMR shifts of carbon centers in $\eta^2$-bound diazine rings are downfield of the free ligand by up to +9 ppm and not 40−80 ppm upfield as for $\eta^2$-olefinic complexes; (3) $\eta^2$ protons of the $\eta^2(5,6)$ type shift significantly less upfield than those for $\eta^2$-olefin complexes (ca. 0.4−0.8 ppm *vs* 1.0−2.0 ppm). It was established that the formation of $\eta^2$-bound pyrimidines of $[Ru^{II}(hedta)]^-$ occurs concomitantly with the dissociation of a carboxylate donor of the $hedta^{3-}$ ligand. $^{13}$C NMR spectra reveal the predicted weighted percentage of freed carboxylates based on the isomer distribution between N-1 with all three glycinato arms of $hedta^{3-}$ bound to $Ru^{II}$ ($^{13}$C resonance at 188 ppm) *vs* $\eta^2$ forms with two bound glycinato groups and one free glycinato group of $hedta^{3-}$ (resonating near 174 ppm).

## Introduction

There has been considerable attention to the development of small molecules that form covalent attachments to DNA[1−7] as tools for biotechnology or as chemotherapeutic agents.[1,2,8−18] Stable covalent attachments of metallodrugs and coordination complexes with DNA nucleobases have almost always involved Pt(II), Pd(II), Ru(II/III), and Rh(III) with bonding via the N-7 sites of guanosine bases, and to a lesser extent the N-7 sites of adenosine units.[1,2,5,7,16,17,19,20] Using small nucleosides and related pyrimidines, we discovered that a ruthenium polyaminopolycarboxylate ($Ru^{II}$pac, $[Ru^{II}(hedta)]^-$), will bind at either the normal N-3 coordination position of bases related to cytidine and uridine, or as an $\eta^2$ attachment via the C-5−C-6 olefinic site of pyrimidine as shown here:[21−23]




Since the same C-5−C-6 portion of C and T base residues of

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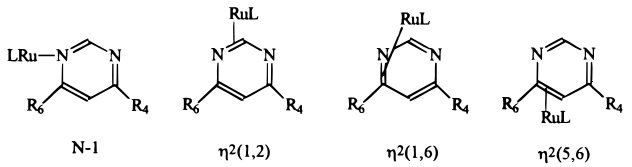
DNA project into the major groove, as do the N-7 purine positions, this opens an additional possible mode of DNA metalation and antitumor control if $\eta^2$ complexes of sufficiently robust nature can be prepared.

There are only a few others reports of $\eta^2$ coordination rather than $\sigma$ lone pair binding of metal centers and N-heterocycles. These include $(NH_3)_5Os^{II}$ complexes of 2,6-lutidine[24] and pyrrole[25] and $(NH_3)_5Ru^{II}$ complexes with dimethyluracil.[26,27] Other $\eta^2$ species with $(NH_3)_5Ru^{II}$ have been proposed as intermediates during the flash photolysis of $[(NH_3)_5Ru(py)]^{2+}$ [28] and during the electrochemically-induced linkage isomerism of $[(NH_3)_5Ru^{II}(\text{isonicotinamide})]^{2+}$.[29] The thermodynamic factors for a migration from an N-bound Ru$^{II}$ nitrogen heterocyclic complex to an $\eta^2$ site have been discussed elsewhere.[30] Substitution reactions between metal centers and N-heterocycles in aqueous solution are more rapid than the subsequent isomerism steps which exchange metal-ligand bonds for the lone pair of N-heterocycles. Kinetically, N-bound complexes form first. The N to $\eta^2$ reorganization costs energy losses in the rupture of the Ru$^{II}$−N $\sigma$ bond and additional activation energy due to partial dearomatization of the N-heterocycle. This barrier is high for pyridines (estimated to be $\geq$36.5 kcal/mol[30]) but anticipated to be lower for pyrimidines—largely due to a 13 kcal/mol lower resonance energy for pyrimidines.[30,31] We have made a preliminary report of the migration of [Ru$^{II}$(hedta)]$^-$ from N-1 of simple pyrimidine (a site corresponding to N-3 of pyrimidine nucleobases due to customary ring numbering procedures of the nucleobase systems) to $\eta^2$ positions about the ring.[30] In the present report we present the detailed analysis of $^1$H and $^{13}$C data, in concert with electrochemical data, which assigns the [Ru$^{II}$(hedta)L] species which are produced with pyrimidine ligands as N-1, $\eta^2$(1,2), $\eta^2$(1,6), and $\eta^2$(5,6) species. The numbering system is chosen to preserve the assignment of ring substituents of the free ligands pyrimidines. The systems studied to date include substituents $R_4$ and $R_6$, which can be either H or $CH_3$.




## Experimental Section

**Reagents.** The ligands pyrimidine (pym), 4-methylpyrimidine (4$CH_3$pym), and 4,6-dimethylpyrimidine ($Me_2$pym) were obtained from Aldrich and used as supplied. $^1$H NMR spectra of the free ligands confirmed the high purity. Ar and $N_2$ gases were passed through Cr(II) scrubbing towers followed by a deionized water rinse tower to remove traces of $O_2$ from the inert gas stream and to saturate the blanketing gases with $H_2O$ vapor to avoid dehydration of aqueous samples. When samples were prepared in $D_2O$, the tank Ar gas was used as delivered from Air Products. This avoids water vapor enriching the $D_2O$ solvent in unwanted amounts of HOD.

Na[Ru$^{II}$(hedta)($H_2O$)]·4$H_2O$ was prepared as described previously.[32−34] The starting ruthenium complex in $D_2O$ was treated with Zn/Hg in Ar-purged 10 mL glass round bottom flasks, sealed with rubber septa. Stirring was maintained with rice-sized magnetic stir bars. Reduced solutions were transferred via syringe needles and syringe tubing into a separate 10 mL flask along the Ar stream. The reaction flask contained weighed amounts of the ligand to achieve desired 0.50:1.00, 0.80:1.00, 1.00:1.00, or 2.00:1.00 ligand:[Ru$^{II}$(hedta)]$^-$ stoichimetries. For $^1$H NMR experiments the solvent was $D_2O$, initially adjusted to pD ~3 with DCl solution. After being mixed and reacted for an appropriate time, the Ru$^{II}$(hedta)−pyrimidine solution was further transferred by syringe tubing into an Ar-purged NMR tube. After being filled with a solution, the septum-sealed NMR tubes were further wrapped with Parafilm to provide an additional barrier against $O_2$. The pD of samples is known to rise to ca. pD 6 over Zn/Hg. For samples at lower pD values, adjustment was made by adding Ar-purged 1.00 M DCl via a syringe to the NMR tube, calculating the pD value from the volume dilution with the sample.

**Instrumentation.** $^1$H and $^{13}$C NMR spectra were obtained as a function of time on Bruker AC300 and AM500 NMR spectromers following standard procedures reported previously.[21−23,33,34] $^1$H NMR spectra were obtained in $D_2O$ referenced against HOD (4.80 ppm) and DSS (0.00 ppm). $^{13}$C spectra were referenced against 1,4-dioxane (69.1 ppm). HH COSY and HC COSY techniques were performed at 500 MHz for $^1$H and 125 MHz for $^{13}$C. $^{13}$C spectra were obtained in proton-coupled and the standard proton-decoupled modes to assist identification of the particular ring carbons under examination.

Electrochemical studies were performed on ca. $5.0 \times 10^{-3}$ M solutions of Ar or $N_2$ purged 0.100 M NaCl plus the desired complex at 22 °C. Measurements were made using an IBM 225 electrochemical analyzer for cyclic voltammetry and differential-pulse polarography modes. The standard three-electrode configuration was utilized with a glassy-carbon working electrode, a Pt-wire auxiliary, and a saturated sodium chloride calomel electrode (SSCE) reference.[22,35] The sweep rates were 50 mV/s for CV and 40 mV/s for DPP, the latter with a 50 mV stepping voltage. Confirmation of the percentages of the various N-1 and $\eta^2$ forms (see text) were made by means of comparison of the ratios of DPP areas and the respective integration ratios of $^1$H NMR lines, and the $^{13}$C spectral amplitudes and integrations for the several species present. A flowing $N_2$ stream was maintained above the solution for electrochemistry to protect the samples against air oxidation for samples maintained for up to 24 h. For longer times, individual samples were prepared in Ar-purged glass bulbs sealed with septa as described above. Aliquots of Ar-protected samples were taken at convenient times and diluted in Ar-purged 0.100 M NaCl for electroanalytical procedures on freshly transferred samples.

## Results and Discussion

**N-1 Bound Complexes.** When [Ru$^{II}$(hedta)($D_2O$)]$^-$ is mixed with pyrimidine (pym), 4-methylpyrimidine (4$CH_3$pym), or 4,6-dimethylpyrimidine ($Me_2$pym) there is an immediate reaction in which the N-heterocycles displaces $D_2O$ ($k$ = 31 M$^{-1}$ s$^{-1}$ for pym). These are rates comparable to the 15 to 30 M$^{-1}$ s$^{-1}$ observed for N-1 attachments of pyridines and pyrazines with [Ru$^{II}$(edta)($H_2O$)]$^{2-}$ and other Ru$^{II}$ (pacs).[36,37] When pyrimidine and [Ru$^{II}$(hedta)($H_2O$)]$^-$ combine, there is a growth in an

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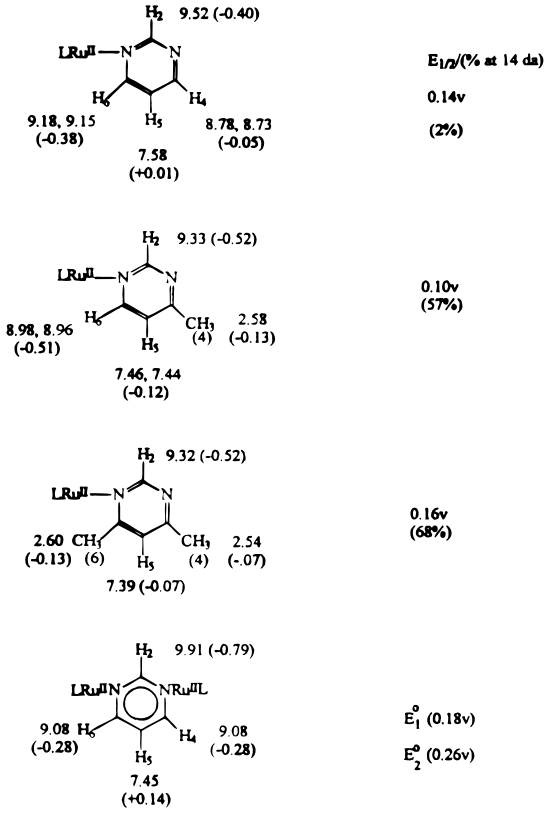
**Chart 1.** Pyrimidine N-1 Forms




orange-brown color compared to the very pale yellow of $[Ru(hedta)(H_2O)]^-$. Reaction is complete in ca. 2.1 min for $2.6 \times 10^{-4}$ M reactants in a 1:1 ratio. The original N-1 complex color then fades slowly to pale yellow with subsequent N-1 to $\eta^2$ isomerization steps which are described below. The initial species exhibit $^1$H NMR patterns with chemical shifts ($\delta$) in ppm and $\Delta\delta$ values, shifts in ppm relative to the free ligand in $D_2O$, as shown in Chart 1 for all N-1 complexes. The assignments for pym and $4CH_3$pym were confirmed by HH COSY and HC COSY spectra. The spectrum for $Me_2$pym was assigned by the known pattern of downfield shifts of the α-positions for N-heterocycles coordinated to anionic metal centers[38] and the agreement with shift patterns for the other N-bound pyrimidines. Since the N-bound isomer of [Ru(hedta)(pym)]$^-$ progressively changes into $\eta^2$ isomers, detectable as overlapping $^1$H NMR features even within a short period after mixing, the $^1$H NMR shift values for the N-bound pyrimidine were also confirmed by a method in which the pyrimidine bridged binuclear complex was prepared using 2:1 $[Ru(hedta)(D_2O)]^-$:[pym] initially. Most of the initial product is the $\{[Ru(hedta)_2](pym)\}^{2-}$ species along with some of the monomer $[Ru(hedta)(pym)]^-$. As the monomer is depleted in forming $\eta^2$ species, the [monomer] is restored by dissociation of the binuclear pool (eq 1). Therefore, both the $^1$H NMR shifts for

$$\{[Ru(hedta)]_2(pym)\}^{2-} + D_2O \rightleftharpoons [Ru(hedta)(D_2O)]^- + [Ru(hedta)(pym)]^- \quad (1)$$

the N-bound bridged binuclear ion and its monomer were easily assigned due to symmetry for the bridged species. The chemical shifts are presented in Chart 1. The original spectrum is presented in Figure 1S of Supporting Information, wherein the pyrimidine protons are labeled 2B through 6B for the bridged complex and 2M through 6M for the monomer for the respective H2 through H6 protons. Comparing the $^1$H NMR results for the various N-1 bound species (Chart 1), it is clear that the H2 proton shifts downfield ca. −0.50 ppm (−0.40 for pym, −0.52 for $4CH_3$pym, and −0.52 for $Me_2$pym). If two metal centers are present, the inductive influence is nearly doubled ($\Delta\delta$ = −0.79 ppm). A greater downfield shift is experienced at the H6 proton or 6-methyl group than at the more remote H4 or $4CH_3$ position as anticipated for a $\sigma$-withdrawing influence of the positive metal center that decreases with distance. The shift of proton 5H, which is meta to the $Ru^{II}$ attachment at N-1, exhibits the predicted upfield shifts[38,39] in the pyrimidine monomer (+0.01 ppm). Again the effect is enhanced to + 0.14 ppm when two $Ru^{II}$ centers influence this position. The presence of $CH_3$ groups in the ring opposes the influence of the metal center upon the 5H position, as downfield shifts of 5H are observed for the $4CH_3$pym and $Me_2$pym complexes.

The DPP and CV data also assist assignment of the N-1 bound complexes. The $[Ru(hedta)(py)]^-$ complex has its $Ru^{II/III}$ wave at glassy carbon at 0.13V *vs* NHE[21] and other $[Ru^{II}(hedta)L]^-$ complexes with various pyridine and pyrazine N-bound complexes have $Ru^{II/III}$ waves between 0.14 and 0.39V *vs* NHE.[40] The monomer pyrimidine N-bound species, which form initially with $[Ru^{II}(hedta)(H_2O)]^-$, have $Ru^{II/III}$ DPP waves at 0.14 (pym), 0.10 ($4CH_3$pym), and 0.16 V ($Me_2$pym). In the case of the $\{[Ru(hedta)]_2(pym)\}^{2-}$ species two waves at 0.18 and 0.26 V are observed for the (II,II) → (II,III) and (II,III) → (III,III) oxidations, respectively.[40] A related pyrimidine-bridged $[Ru_2(ttha)(pym)]^{2-}$ complex has also been described in detail.[40] The $[Ru_2(ttha)(pym)]^{2-}$ complex exhibits these waves at 0.26 and 0.33 V. These data imply little coupling between the Ru centers and small comproportionation constants of 5.7 and 22.7 $M^{-1}$ for the $ttha^{6-}$ and $hedta^{3-}$ binuclear complexes, respectively.[40] The importance of the electrochemical data for the present study is that the normal potentials anticipated for N-bound N-heterocycles are observed for the first detectable species formed as a pyrimidine or methylpyrimidine substitutes on $[Ru(hedta)(H_2O)]^-$. In all cases, except for the (N-1, N-3) blocked binuclear species, changes occur with time for the N-1 monomer-substituted species. These changes proceed with $t_{1/2} \sim 28.8$ h for pym, 24 h for $4CH_3$pym and ca. 1 h for $Me_2$pym. The identities of these product isomers are the important new observations in regards to $\eta^2$ complexation of simple N-heterocycles. Significantly, the amount of the retained N-1 isomers after 14 d, allotted for equilibrium, increase with electron donating $CH_3$ groups on the pyrimidine ring. At 14 d the percentage of the N-1 isomers decrease to 2% (pym), 57% ($4CH_3$pym), and 68% ($Me_2$pym). For this reason detailed studies were carried out for pym and $4CH_3$pym which allow the greatest use of $^1H-^{13}C$ coupling information to assist identification of the products. Ring methylation raises the $pK_a$ of the N-1 lone pair.[31] Therefore increasing the basicity at N-1 retards the subsequent isomerism process by increasing the barrier to rearrangement and stabilizing the N-1 bound reactant.[30]

**Pyrimidine $\eta^2$ Complexes.** When the DPP spectrum of the mixture of $[Ru(hedta)(pym)]^-$ and $[Ru(hedta)(4CH_3pym)]^-$ are observed at mixing and then after 4 days, the amplitudes of the N-1 $Ru^{II/III}$ waves at 0.14 V for the pym complex and 0.10 V for $4CH_3$pym have decreased. The effect is most dramatic for pym. The CV and DPP voltammograms have been presented previously elsewhere,[30] but are also presented in Supporting

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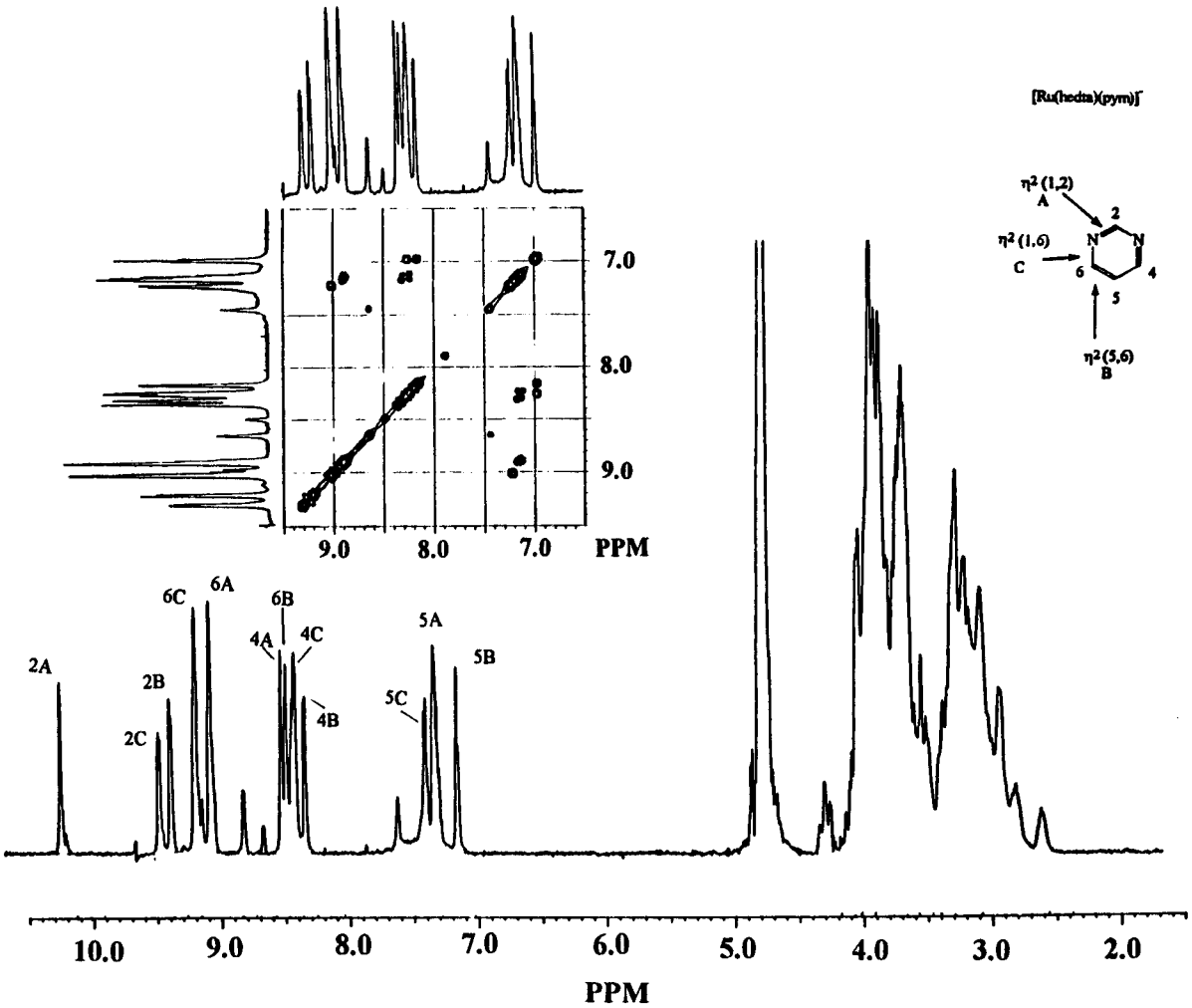


**Figure 1.** 500 MHz $^1$H NMR spectrum for the equilibrium distribution of $\eta^2$ isomers of [Ru(hedta)(pym)]$^-$ (a) Trace species were identified as 2% of the residual N-1 isomer or as traces of the binuclear complex. All resonances have been identified. The [Ru(hedta)] region is highly overlapped and has not been separated for assignments. $[Ru^{II}]_{tot} = 2.61 \times 10^{-3}$M, $D_2O$, 25 °C. (b) Inset in the HH COSY data obtained at 35 °C. The lines all shift slightly upfield due to temperature and salt effects on the HOD reference.

Information (Figure 2S) for convenience. With time, another wave becomes prominant at 0.50 V for pym and at 0.44 V for 4$CH_3$pym. These values for Ru$^{II/III}$ oxidations are similar to those of [Ru$^{II}$(hedta)] stabilized by $\pi$-acceptor olefins which exhibit a range of +0.46 to +0.80 V for this wave, depending on substituents adjacent to the $\eta^2$-bonded olefin position.[23,33]

Although only one electrochemical wave is observed at 0.50 V, the 500 MHz $^1$H NMR spectrum (Figure 1) at 25 °C, the differing growth rates of various signals over 7d, and the 500 MHz HH COSY spectrum (Figure 1 insert at 35 °C) confirms the presence of three new species. The interrelationships resonances for each species was assigned by means of the HH COSY spectrum.

The nature of the three new species, all with redox waves closely overlapped near +0.50 V, might be explained by several origins: (1) stereochemical isomers of N-1 bound [Ru(hedta)(pym)]$^-$; (2) bis complexes of the type [Ru(hedta)(pym)$_2$]$^-$, as these also have more positive waves in the 0.5 V *vs* NHE range[37]; (3) $\eta^2$ complexes of [Ru(hedta)(pym)]$^-$. To assist and simplify the discussion, we will rule out possibilities 1 and 2. There are three possible stereochemical isomers of [Ru(hedta)L]$^-$ complexes: cis-equatorial, trans-equatorial, and cis-polar.[41] $^{13}$C spectra of the CO and py complexes of [Ru(hedta)]$^-$ exhibit only one isomer, presumably the sterically favored cis-equatorial one.[37,41] Were the [Ru(hedta)(pym)]$^-$ complex to simply redistribute, there could only be two new products and the original (cis-equatorial) should be one of the resulting isomers. It should not nearly vanish (the observed situation for pym) as cis-equatorial is the sterically preferred one. Also, there would be no explanation for a change to a Ru$^{II/III}$ wave at +0.50 V produced from the normal 0.10−0.20 V for N-1 complexes. If bis complexes were to form by a redistribution of [Ru(hedta)(pym)]$^-$ into [Ru(hedta)(pym$_2$)]$^-$ and [Ru(hedta)($H_2O$)]$^-$, the latter species would be detected at 0.04V *vs* NHE at equal amplitude to the "bis" wave at 0.50 V. No such Ru$^{II/III}$ wave of [Ru(hedta)($H_2O$)]$^-$ grows with time. Furthermore, in separate studies to be reported elsewhere, we have prepared the authentic bis [Ru(hedta)(pym$_2$)]$^-$ complex.[42] Its $^1$H NMR spectrum is different from any of those species observed in Figure 1. Additionally, upon acidification the bis complex converts to just one (identified in the related paper as $\eta^2$(1,2)[42]) not the mixture found by rearrangement of the N-1 complex at pH ∼ 6. Therefore, the explanation that the 0.50 V wave for the products produced from the rearrangement of the initial N-1 bound [Ru(hedta)(pym)]$^-$ is due to bis complexes is invalid.

The explanation of $\eta^2$ complexes as accounting for the new species are consistent with the facts. There are three $\eta^2$ possibilities, $\eta^2$(1,2), $\eta^2$(1,6), and $\eta^2$(5,6), which are not inclusive of the N-1 coordination as per the stereochemical explanation. The

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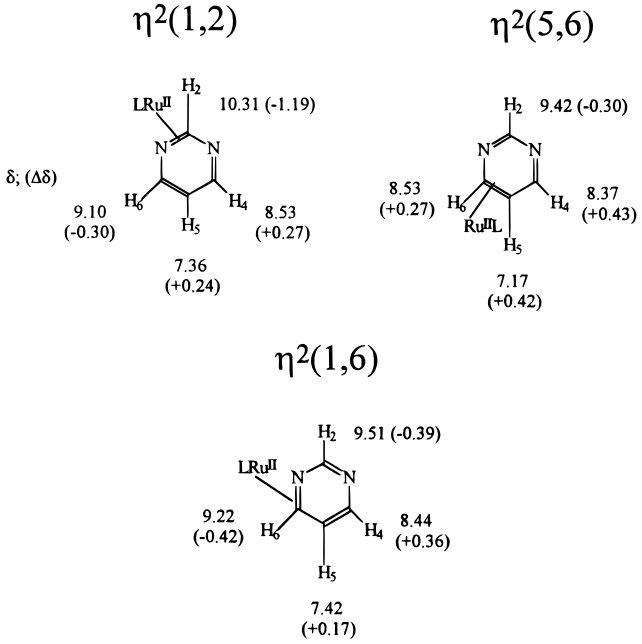
**Chart 2**




incorporation of a $\pi$-accepting $\eta^2$ complex explains the positive $E_{1/2}$ shift for the $Ru^{II/III}$ wave and the labelization of an in-plane carboxylate of $[Ru(hedta)]^-$ as explained below, a process not observed for $[Ru^{II}(hedta)(pyridines)]^-$ which do not migrate or exhibit an altering DPP spectrum with time.

The species assigned as the $\eta^2(5,6)$ isomer is based on the upfield shifts by 0.42 ppm for H5 and 0.21 ppm for H6 for this species. It is known that $\eta^2$ coordination by $Ru^{II}$ or $Os^{II}$ to olefinic groups usually causes 1.0−2.0 ppm upfield $^1$H NMR shifts relative to free olefin ligands.[43−47,33,27] However, a recent paper from our laboratory has shown that these shifts are diminished to less than 0.80 ppm when the olefinic site is alpha to a heterocyclic ring nitrogen.[48] Therefore, the $\eta^2(5,6)$ assignment is consistent with this isomer. One of the isomers experiences a very large −1.19 downfield shift for pym. Related species in the $[Ru(hedta)]^-$ complexes of $4CH_3$pym and $Me_2$pym have corresponding chemical shifts of −1.16 and −1.38, respectively. Therefore from the pattern of all three pyrimidines, including ones having steric hindrance at the $\eta^2$-(1,6) and $\eta^2(5,6)$ positions and still retaining a $^1$H NMR shift of H2 of ca. −1.20 ppm downfield, we conclude that these isomers have a strong interaction between the $Ru^{II}$ center that implies close proximity rather than an "across-the-ring" influence. Therefore these are assigned as $\eta^2(1,2)$ isomers for the species having characteristically large downfield shifts of H2. The remaining isomer is then $\eta^2(1,6)$ for pym and its H6 is also downfield, but less so because $\eta^2(1,6)$ carbons have carbon neighbors whereas $\eta^2(1,2)$ has another N neighbor. The three pym $\eta^2$ isomers exhibit $^1$H NMR shifts as shown in Chart 2. The absence of an isomer corresponding to $\eta^2(1,6)$ for $Me_2$-pym also supports the $\eta^2(1,6)$ assignment for the pym complex since $CH_3$ substitution would hinder $\eta^2(1,6)$ for $Me_2$pym. On

**Chart 3.** $\eta^2$ (1,2) Forms

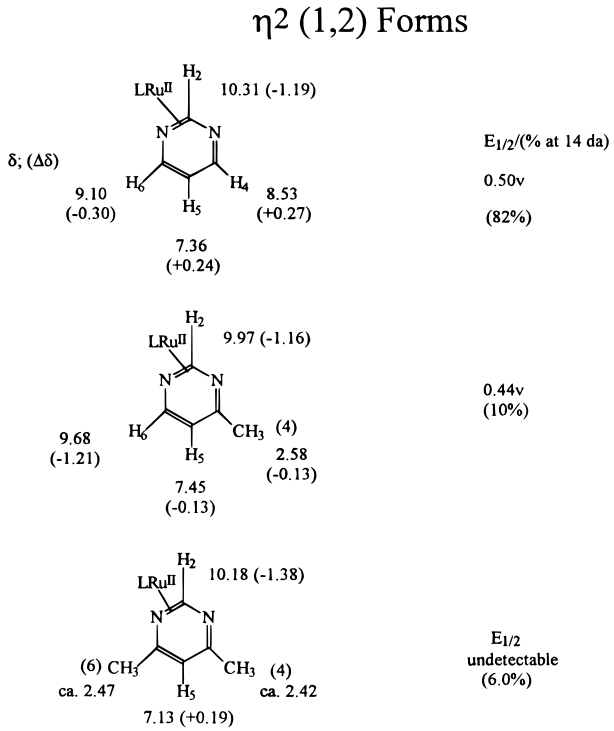
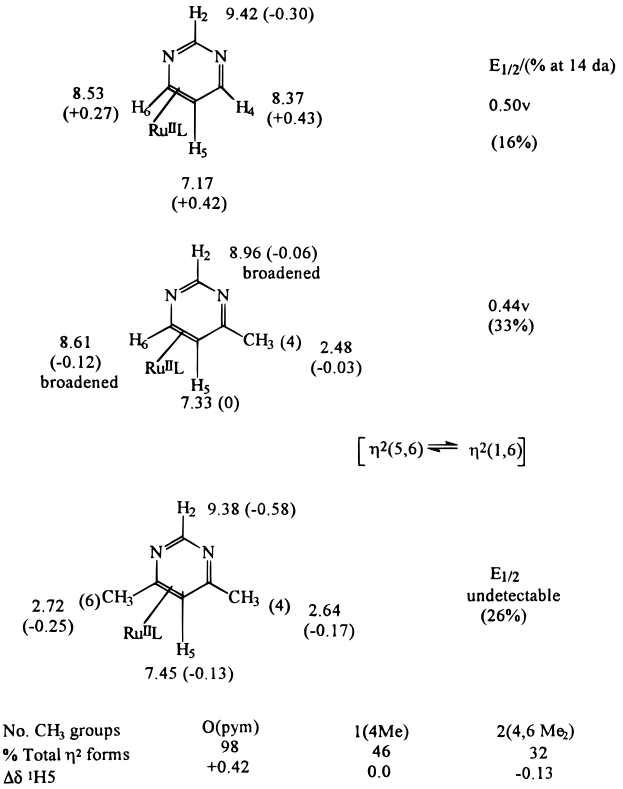



**Chart 4.** $\eta^2$ (5,6) Forms




the basis of the chemical shift trends for H2 and the H5/H6 sets, we have correlated the $\eta^2(1,2)$ and $\eta^2(5,6)$ species for $4CH_3$-pym and $Me_2$pym to those of the pym analogues as shown in Charts 3 and 4. For convenience in labeling spectral lines we will use the letter notation A for $\eta^2(1,2)$, B for $\eta^2(5,6)$, C for $\eta^2(1,6)$ and D for N-1 forms. Form C is only observed for the parent unsubstituted, sterically-unhindered pyrimidine.

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(48) Chen, Y.; Shepherd, R. E. *Inorg. Chim Acta*, in press.

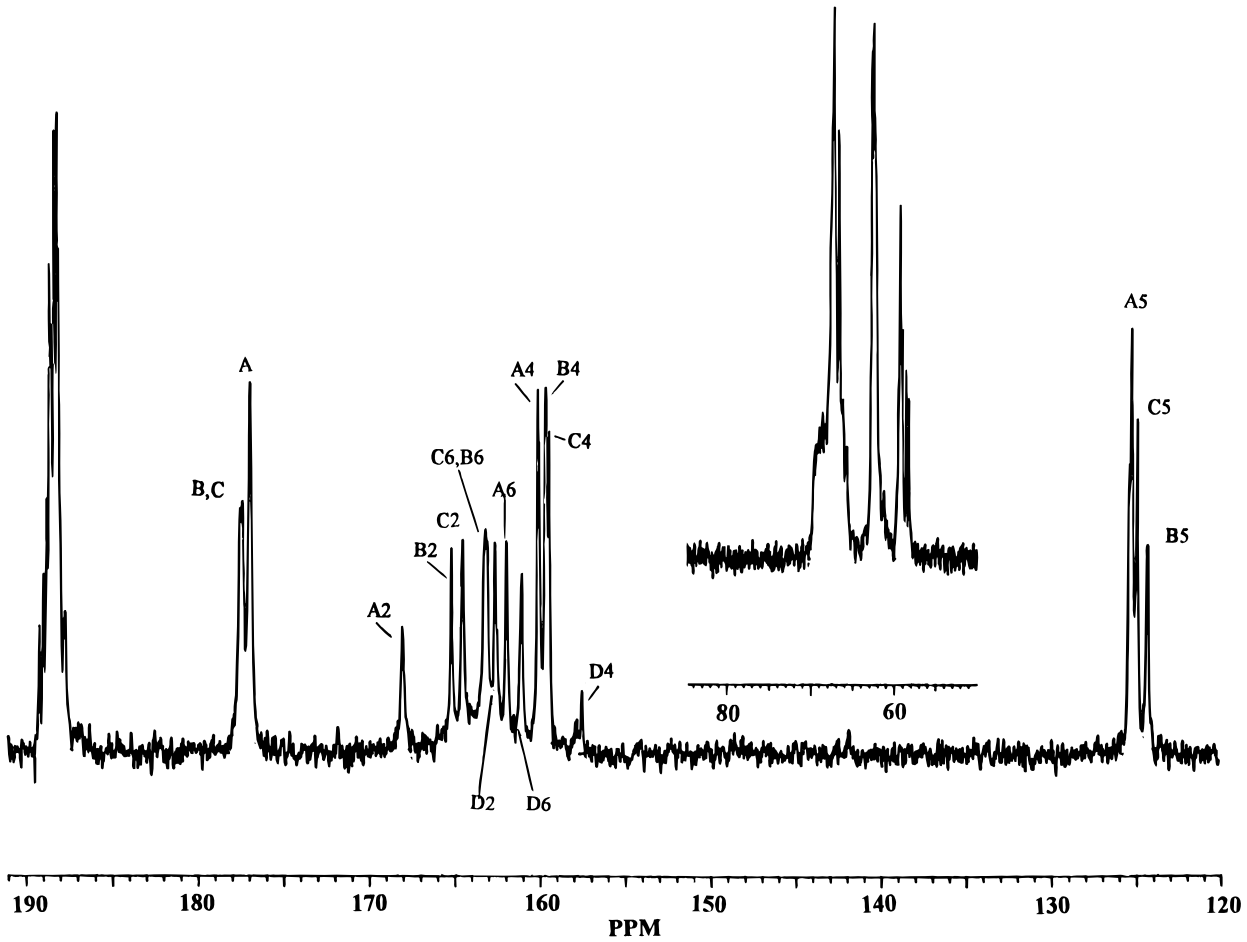
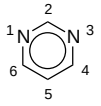



**Figure 2.** 125 MHz $^{13}$C NMR spectrum of the equilibrium distribution of $\eta^2$ isomers of [Ru(hedta)(pym)]$^-$. Letter labels refer to the isomers described in the text. The inset shows the "Ru(hedta)" carbon region where 18 of an allowed 21 different carbons are detected. [Ru$^{II}$]tot = 2.61 × $10^{-2}$ M; [pym]; = 2.61 × $10^{-2}$ M. All spectra were run in $D_2O$ at 25 °C.

**Table 1.** $^{13}$C Shifts in ppm for $\eta^2$ Isomers of [Ru(hedta)(pym)]$^-$ in $D_2O$[a]

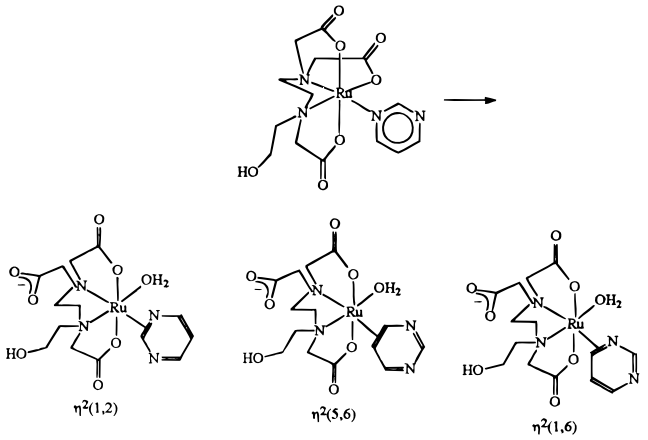


| carbon | free ligand | $\eta^2$(1,2) A | $\eta^2$(5,6) B | $\eta^2$(1,6) C |
|---|---|---|---|---|
| C2 | 159.8 | 167.95 (−8.15) | 165.05 (−5.15) | 164.40 (−4.60) |
| C4 | 159.7 | 160.00 (−0.30) | 159.35 (+0.15) | 159.31 (−0.39) |
| C5 | 124.9 | 125.13 (−0.23) | 124.15 (+0.85) | 124.78 (−0.12) |
| C6 | 159.7 | 161.83 (−2.13) | 162.52 (−2.82) | 163.08 (−3.30) |

[a] $\Delta\delta$ values are in parentheses; a positive shift is an upfield shift, a negative shift is a downfield shift.

On the basis of $^1$H NMR integrations, an equilibrium percentage distributions of $\eta^2$(1,2):$\eta^2$(5,6):$\eta^2$(1,6):N-1 (A:B:C:D) for pym of 41:16:41:2[30] may be calculated. The $^{13}$C NMR spectrum of the [Ru(hedta)(pym)]$^-$ product solution after 15 days is shown in Figure 2 for the hedta-carboxylate carbons (174−190 ppm) and pyrimidine ring carbons (120−168 ppm). The identities of the ring carbons were made in comparison to the free ligand $^{13}$C NMR spectrum and HC COSY spectra (Supporting Information, Figure 3S). The C5 carbon is particularly diagnostic, exhibiting three distinct resonances together with a tiny shoulder on the main resonance. The major C5 peaks of the $\eta^2$ isomers are in the ratio of A:B:C:D of 43:22:33:2 (the percentage of D was evaluated, based on C4) in good agreement with $^1$H NMR integration data.

Also important is the observation that a carboxylate group is displaced while forming the $\eta^2$ species as in eq 2. The ratio of




the areas for bound carboxylates near 188 ppm to that of freed carboxylates at 174 ppm:(C2 + C4 + C6 ring carbons):C5 carbons is 2.0:1.0:3.0:1.1. Therefore the changes responsible for formation of the species giving rise to the +0.50 V wave and three new $^1$H NMR related complexes also occurs with the loss of coordinated carboxylate. This process does not occur for pyridine. The [Ru(hedta)(py)]$^-$ complex has only coordinated carboxylates near 189 ppm after 14 d.[42] With three main species there should be seven types of coordinated carboxylates near 189 ppm; seven are detected. The hedta region is very overlapped; 18 of 21 possible resonances are observed. For

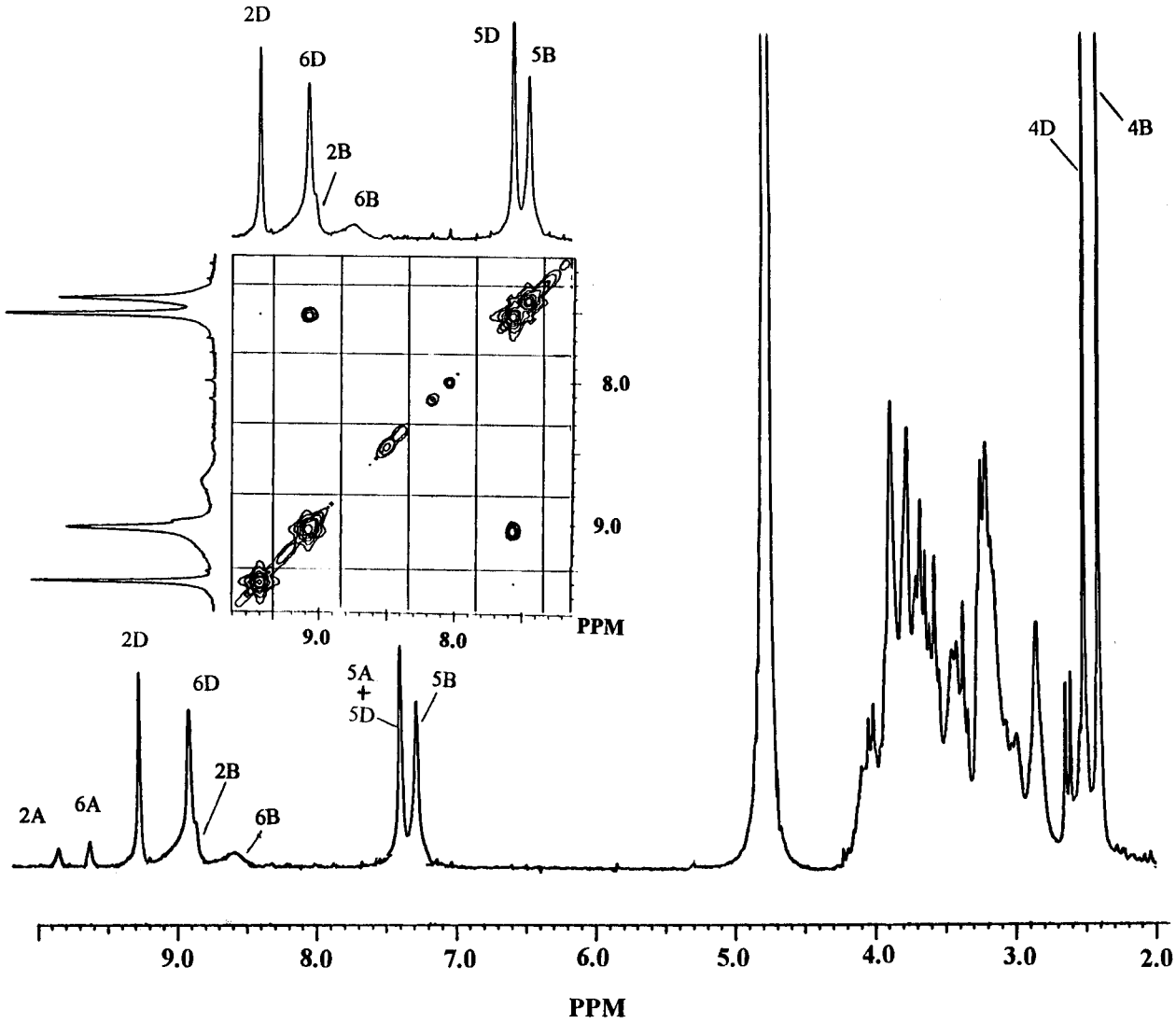
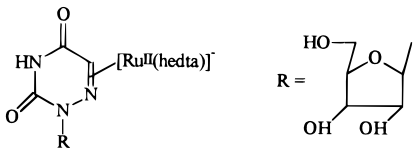



**Figure 3.** 500 MHz $^1$H NMR of 4-Methylpyrimidine N-1 and $\eta^2$ Complexes of $[Ru^{II}(hedta)]^-$ after 10 days. Letter labels refer to isomers described in the text. Insert shows the HH COSY spectrum of the N-1 and $\eta^2$(5,6) isomers $[Ru^{II}]_{tot} = 2.61 \times 10^{-2}$ M; [pym] $= 2.61 \times 10^{-2}$ M; all spectra were run in $D_2O$ at 25 °C.

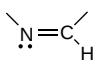
all three species it is likely that the carbons next to OH of the alcohol arms all have the same $^{13}$C shift. The 18 of 21 which are observed are consistent with this analysis.

The HC COSY spectrum allowed us to assign the observed $^{13}$C NMR shifts given in Table 1 along with data for the free pyrimidine ligand.

The striking observation is that the carbons associated with $\eta^2$ coordination, C2 for A, C5/C6 for B, and C6 for C, exhibit no major upfield shift normally associated with $\eta^2$ complexes of olefins. $\eta^2$ attachment to olefinic groups usually exhibit 40 to 80 ppm $^{13}$C upfield shifts for $\eta^2$ coordination. This feature would be disconcerting except that we have recently described the $\eta^2$ complex of 6-azauridine:[48]




The $\eta^2$ attachment in 6-azauridine is the




fragment. Normally metal centers prefer the lone pair of N, but by control of pH we successfully converted all of the N-6 form to the $\eta^2$(C-5−N-6) species.[48] The $^{13}$C spectrum of the 6-azauridine complex exhibits a 0.0 ppm shift for C5 and small downfield shifts at C2 and C4 and C3 of 7 ppm. Therefore, the specified range (40−80 ppm) using $\eta^2$(C═C) complexes as a model for chemical shifts for $\eta^2$(C═N) species falls far off the mark as a reliable estimate. Rather the $\eta^2$ pyrimidine species observed in this report experience very slight downfield shifts at C2 and C6 for $\eta^2$(5,6). When more than one nitrogen replaces C in six-membered aromatic rings, the extent of delocalization, as mirrored by the resonance energy, drops from 36 in benzene to 27 in pyridine and to 14 in pyrimidine.[31] The presence of electronegative N centers in the ring clearly alters the capacity for attached $Ru^{II}$ centers to cause significant redistribution of electron density at the C centers. However, the N centers can tolerate an increase in charge. Therefore $\pi$-back-donation is still operative, stabilizing $Ru^{II}$ and causing a shift in $E_{1/2}$ upon N-1 to $\eta^2$ migration. The implication is that the change in aromaticity for the $\eta^2$ pyrimidine is small and that electron density donated by $Ru^{II}$ is spread among all the ring atoms, but mostly localized at the nitrogens.

**4-Methylpyrimidine $\eta^2$ Complexes**. The $4CH_3$pym case serves as a meaningful test of the conclusions drawn for pym as a ligand. First, there is a substantial amount of the N-1

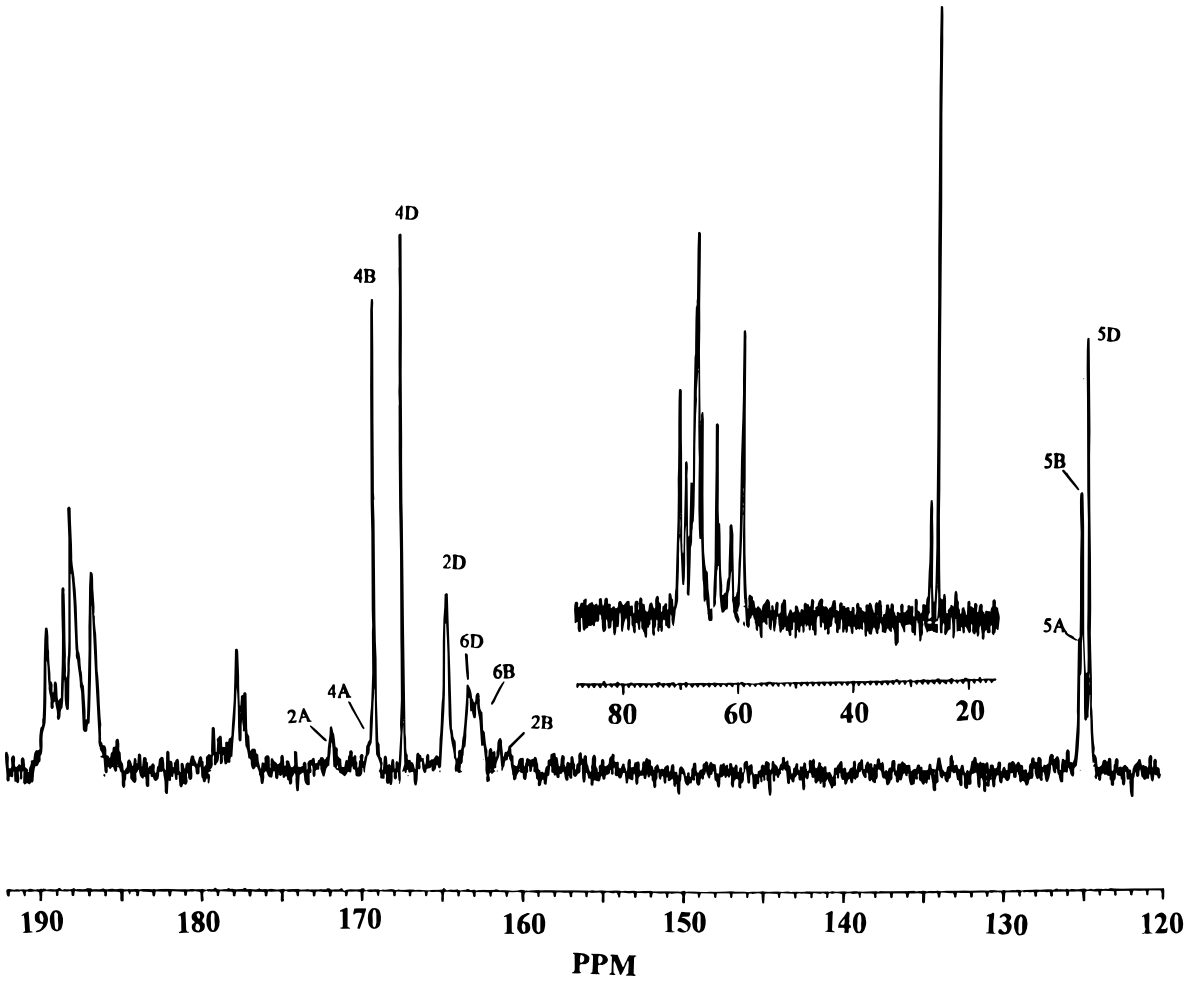



**Figure 4.** 125 MHz proton-decoupled $^{13}$C NMR spectrum of the equilibrium distribution of N-1 and $\eta^2$ isomers of [Ru(hedta)(4CH$_3$pym)]$^-$. Letter designations are as described in text; shifts are in ppm. Insert shows the 20−80 ppm region. [Ru$^{II}$]$_{tot}$ = 2.61 × 10$^{-2}$ M; [pym] = 2.61 × 10$^{-2}$ M at 25 °C.

complex at equilibrium. The observed $^1$H NMR ratios obtained from separate experiments at 300 and 500 MHz are for $\eta^2$(1,2):$\eta^2$(5,6):$\eta^2$(1,6):N-1 ≤10:33:0:57. A 300 MHz $^1$H NMR spectrum of the 4CH$_3$pym complexes as a function of time (not shown) and the final $^1$H NMR and HH COSY spectrum at 500 MHz (Figure 3) permitted the correlation of protons for the N-1 and $\eta^2$(5,6) species as assigned in Charts 3 and 4. At room temperature there is substantial broadening of the H2 and H6 resonances that suggests a dynamic motion, most probably an $\eta^2$(5,6) ⇌ $\eta^2$(1,6) interconversion, which averages the chemical shifts of these protons. For convenience we have discussed this species as the $\eta^2$(5,6) isomer, recognizing the actual complication of fluxionality and indicating this problem in Chart 4. The $^{13}$C 125 MHz NMR spectrum for the carboxylate-pyrimidine ring regions (125−190 ppm) were again diagnostic of the equilibrium mixture between N-1, $\eta^2$(5,6), and $\eta^2$(1,2) species. In Figure 4 the C5 carbons of 4CH$_3$pym bound to [Ru$^{II}$(hedta)]$^-$ show the major N-1 bound complex, lesser $\eta^2$(5,6) species, and minor $\eta^2$(1,2) species in a ratio of 51.5:37.1:11.3 in good agreement with the $^1$H NMR-derived distribution. Assignment of the ring carbons was assisted by the proton-coupled $^{13}$C NMR spectrum in comparison to the decoupled spectrum. The 4CH$_3$ carbon of the N-1 isomer was easily identified by the quartet pattern that appears as a singlet at 25.22 ppm in the decoupled spectrum, whereas the lesser $\eta^2$(5,6) isomer appears at 25.33 ppm in the decoupled spectrum and as a quartet in the coupled spectrum. The minor $\eta^2$(1,2) species resonates at 26.49 ppm and is hardly detectable. The presence of two main species (N-1 and $\eta^2$(5,6) isomers) is also supported by 14 of 14 distinguishable hedta-based resonances between 59.00 and 70.14 ppm. Each hedta ethylene carbon and three glycinato carbons, plus two from the alcohol arm, yield seven unique carbons per isomer. The intensity of the $\eta^2$(1,2) species is too low or its features remain buried under signals for N-1 and $\eta^2$(5,6) resonances in this region. The assignment of C5 carbons near 125 ppm, C6 near 163 ppm, and C4 near 168 ppm, which appear in sets of two major and a very minor component (Figure 4S, of Supporting Information) is readily made because C5 and C6 appear as doublets in the proton-coupled $^{13}$C spectrum whereas C4, attached to CH$_3$, has no additional coupling.

There is also the distribution of bound carboxylato groups with resonances near 188 ppm and labelized carboxylates near 178 ppm. Using the average of the distribution of isomers found via $^1$H and $^{13}$C NMR, *e.g.* 53.3% N-1, 35.5% $\eta^2$(5,6), and 10.8% $\eta^2$(1,2), one can calculate the predicted ratio of bound *vs* free carboxylates *if it is assumed that only the* $\eta^2$ forms labelize carboxylates. The N-1 forms contribute 0.533 × (3) whereas the $\eta^2$ species contribute 0.463 × (2) to the integration near 188 ppm. The $\eta^2$ forms contribute 0.463 × (1) to the area near 178 ppm. The predicted percentage of coordinated carboxylates is then 1.992/2.455 or 81.1%. The experimental ratio was 99.6/133.1 or 80.9% for the areas under the 189 ppm region divided by the areas of both 189 ppm and 178 ppm regions. This vindicates the assumption that only the $\eta^2$-bound species cause the loss of a coordinated glycinato arm of the hedta ligand. Furthermore, it unambiguously removes stereochemical isomers as a viable explanation to the chemical changes now assigned to the N-1 to $\eta^2$ migration. Carbon number assignments in

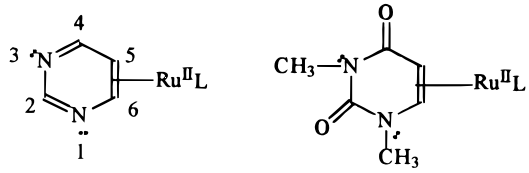
**Table 2.** $^1$H and $^{13}$C NMR Shifts of N-1 and $\eta^2$ Complexes of $[Ru^{II}(hedta)]^-$ and 4-Methylpyrimidine in $D_2O$ at 25 °C (Shifts in ppm)[a]

| N-1 | | | | $\eta^2$(5,6) | | | | $\eta^2$(1,2) | |
|---|---|---|---|---|---|---|---|---|---|
| H2 | 9.52 | C2 | 164.52 (+6.07) | H2 | 8.96 | C2 | 161.00 (+9.59) | C2 | |
| | | C4 | 167.26 (−9.02) | | | C4 | 169.04 (−10.8) | C4 | 169.04 (−10.8) |
| H5 | 7.44 | C5 | 124.55 (−0.13) | H5 | 7.33 | C5 | 124.75 (−0.33) | C5 | 124.99 (−0.51) |
| H6 | 8.96 | C6 | 164.52 (−5.29) | H6 | 8.61 | C6 | 163.24 (−4.01) | C6 | |
| | | $CH_3$ | 25.22 (+0.08) | | | $CH_3$ | 26.33 (−1.03) | $CH_3$ | 26.49 (−1.19) |

[a] $\Delta\delta$ values in parentheses.

Figure 4 were also supported by HC COSY spectra (not shown). The line intensities of the $^1$H and $^{13}$C NMR spectra and the HC COSY spectrum has allowed the assignment of the following resonances in Table 2 for the $[Ru(hedta)(4CH_3pym)]^-$ isomers.

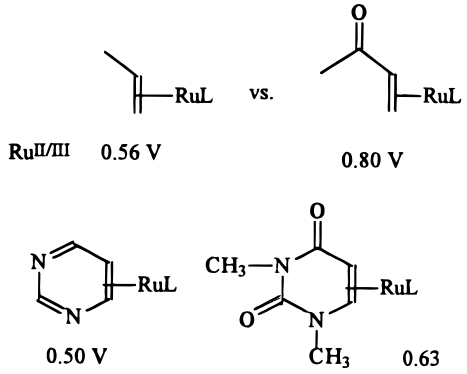
Again no reliable correlation exists between upfield shifts and the site of $\eta^2$ attachment. The observed shifts are small and may be upfield even for C2 in the N-1 isomer. Thus $^{13}$C NMR shifts are not reliable indicators of $\eta^2$ coordination. A rationale behind the $^{13}$C NMR shift insensitivity resides on pyrimidine being a less aromatic ring by about one-third than pyridine[31] and the resistance to changes in orbital coefficients of the ground state wave function, brought on by two nitrogen centers which by their electronegativity enforce asymmetric electronic distributions. There are then fewer ways to accommodate electrons without greatly increasing repulsion energies. Additionally, wave functions for such rings have large coefficients for the N centers, minimizing the influence of carbons. Compare pyrimidine as a ligand to 1,3-dimethyluracil for the $\eta^2$(5,6) bonding as shown here:




| | Δδ | | Δδ |
|---|---|---|---|
| C5 | +0.85 ppm | C5 | +47.0 ppm |
| C6 | -2.82 | C6 | 41.7 |
| H5 | +0.42 | H5 | 0.84 |
| H6 | +0.27 | H6 | -0.29 |

If the bonding is much more localized in the dimethyluracil (DMU) than for the simple pyrimidine (an effect due to the exo keto groups), one anticipates a more direct influence *via* back-bonding on the localized system. The $^{13}$C data supports the effect of localization on the C-5−C-6 bond with large $^{13}$C shifts and modest $^1$H shifts for DMU. A more even and attenuated influence at all positions exists for the $\eta^2$ pym complex. If one assumes less than one-sixth of any ruthenium electron density resides on carbons of the pym ring (greater amounts transferred being to nitrogen atoms) one would predict less than $\leq$+7 ppm upfield for the $\eta^2$ carbons of pym. The influence of an α nitrogen in the ring produces more downfield shifts for both C6 and H6 in both cases as compared to the extent of upfield shifting experienced by C5 and H5, an effect already noted in a prior publication concerning related ring structures.[48]

It has been shown previously that the presence of a carbonyl group alpha to a double bond improves the stabilization of $[Ru^{II}(hedta)]^-$ in the $Ru^{II/III}$ wave. For example, compare propylene *vs* methyl vinyl ketone and pyrimidine *vs* 1,3-dimethyluracil:




The $E_{1/2}$ of these representative $\eta^2$ complexes, each pair with a parent derivative and one having an alpha carbonyl are given in the drawing. Simple olefins having an α carbonyl increase $Ru^{II}$ stabilization by ca. 0.24 V. The situation with N-heterocycles as $\eta^2$ acceptors is somewhat attenuated to 0.13 V, indicative of some withdrawing effect by the presence of nitrogen centers reducing what the α carbonyl functionality normally contributes to $\pi$-back-donation stabilization. Two things are clear from this comparison. The wave at 0.50 V is in a reasonable electrochemical window for $\eta^2$ species of pyrimidine bound to $[Ru^{II}(hedta)]^-$. Second, the exo carbonyls of the pyrimidine nucleobases enhance the stability of $Ru^{II}$ pacs as $\eta^2$ complexes more than the simple pyrimidines. This offers hope for the development of $Ru^{II}$ reagents which exploit $\eta^2$ coordination along DNA strands.

**Acknowledgment.** We gratefully acknowledge support for the laboratory from the Research Corp. and the Donors of the Petroleum Research Fund, administered by the American Chemical Society.

**Supporting Information Available:** Figure 1S, $^1$H NMR spectra of $[Ru^{II}(hedta)^-]$ pyrimidine-bridged binuclear ion and N-1 monomer, Figure 2S, CV and DPP voltammograms of $[Ru(hedta)(pym)]^-$ and $[Ru(hedta)(4CH_3pym)]^-$ after 4 days of isomerism, Figure 3, HC COSY spectrum of the $\eta^2$ isomers of $[Ru(hedta)(pym)]^-$ at 35 °C, and Figure 4, $^{13}$C proton-decoupled and proton-coupled spectra of $[Ru(hedta)(4CH_3pym)]^-$ complexes (3 pages). Ordering information is given on any current masthead page.

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