distances are observed at ≈3.4 Å. It is noted that the monodentate μ-carboxylate bridges forming a Cu_2O_2 core are asymmetric with a short Cu-O_b and a long Cu···O_b bond. This is due to the fact that the Cu atoms are in a square based pyramidal environment and the bridging carboxylate oxygen atom is coordinated to the basal plane of one Cu center (short Cu–O) and to an apical position (long Cu–O) of the second Cu ion and vice versa. This represents the usual electronically determined configuration of a d^9 Cu(II) center. Zn(II) possesses a d^{10} electron configuration, and as is well documented in complex **3**, the two basal Zn–N distances are slightly *longer* than the corresponding apical distance (Δ(Zn–N) = 0.08 Å). This is in contrast to the isostructural copper complex $[L'_2Cu_2(\mu\text{-}OH)_2](ClO_4)_2$, where the basal Cu–N distances are shorter by 0.16 Å than the apical Cu–N bond.

If we now envisage the above bis(μ-carboxylato)dicopper(II) core structure with zinc(II) instead of copper(II), it is quite reasonable to assume that the Zn–O_b distances are equivalent, forming two symmetrical monodentate carboxylate bridges. This would bring the zinc atoms much closer together, and a Zn···Zn distance at ≈3.0 Å is not unreasonable.

In conclusion, we propose that the active site in leucine aminopeptidase may contain the unique structure B shown in Chart II. This proposal takes into account all the structural details available at 2.7-Å resolution and the known coordination chemistry of zinc(II). Finally, we suggest that the loosely bound zinc ion has an additional coordinated water molecule which may be activated via hydrogen bonding to the carbonyl oxygen of Asp-273 in a fashion similar to that in complex **2**.

Acknowledgment. We thank the Fonds der Chemischen Industrie for financial support.

Registry No. **1**, 139240-64-5; **2**, 139168-41-5; **3**, 139168-43-7; **4**, 139168-46-0; $[L_2Zn](ClO_4)_2$, 139240-65-6; phospholipase C, 9001-86-9; leucine aminopeptidase, 9001-61-0.

Supplementary Material Available: For complexes **1–4**, listings of crystallographic data, calculated positions of hydrogen atoms, bond lengths and angles, and anisotropic thermal parameters (24 pages); tables of observed and calculated structure amplitudes (27 pages). Ordering information is given on any current masthead page.

(32) (a) Brown, J. N.; Trefonas, L. M. *Inorg. Chem.* **1973**, *12*, 1730. (b) Chiari, B.; Hatfield, W. E.; Piovesana, O.; Tarantelli, T.; ter Haar, L. W.; Zanazzi, P. F. *Inorg. Chem.* **1983**, *22*, 1468. (c) Greenaway, A. M.; O'Connor, C. J.; Overman, J. W.; Sinn, E. *Inorg. Chem.* **1981**, *20*, 1508. (d) Ciari, B.; Helms, J. H.; Piovesana, O.; Tarantelli, T.; Zanazzi, P. F. *Inorg. Chem.* **1986**, *25*, 870; **1986**, *25*, 2408.

Contribution from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Influence of the 5-Substituent of Uracils and Uridines on η^2 Coordination of $Ru^{II}(hedta)^-$ at the C-5–C-6 Bonds

Rex E. Shepherd,* Songsheng Zhang, Fu-Tyan Lin, and Richard A. Kortes

Received May 8, 1991

The coordination of $Ru^{II}(hedta)^-$ with 5-substituted uracils and uridines (X = F, Cl, Br, I, and COOH as 5-substituents) has been studied by ^1H and ^{19}F NMR spectroscopy in combination with electrochemical methods (CV, DPP). Characteristic CV/DPP waves for the η^2-type coordination for Ru(hedta)$^-$, found previously for uridine- and cytidine-related ligands, has been observed in all cases except for X = COOH, which is bound at N-3. $E_{1/2}$ values in the range 0.61–0.79 V vs NHE are recorded for the η^2 isomers; the fluorouracil and fluorouridine complexes have the most positive $Ru^{III/II}$ waves at 0.76 and 0.79 V, respectively. The ^{19}F resonance is shifted upfield upon coordination of Ru(hedta)$^-$ with 5-fluorouracil (5-FU); the η^2-bound isomer is 21.5 ppm upfield of the free ligand; N-3-bound 5-FU exhibits a 26.7 ppm upfield shift. The H-6 position may be shifted either upfield or downfield of the free ligand on coordination. The largest upfield shifts occur for iodo derivatives, ca. 0.88 ppm in 5-iodouridine. The percentage of the η^2-bound isomer is a sensitive function of the Hammett substituent constant, σ_p. η^2 form of $Ru^{II}(hedta)L^-$ occurs at 0% for X = CH_3 and 59% for X = H and reaches a maximum of 85% for X = Cl. The 80% η^2-bound/20% N-3-bound distribution for X = Br shows that the absence of the η^2 form for X = CH_3 (nucleobase T) is not due to steric hinderance but rather due to the σ-electron donation of CH_3 compared to the withdrawing influence of X = F, Cl, Br, and I. The latter three give η^2/N-3 ratios of ca. 5.7. With 5-fluorocytosine the η^2/N-3 ratio is 0.39, favoring N-3 complexes 2.6:1. Cytosines and cytidines were previously observed to favor N-3 modes by 7.3:1.0. Thus, the influence of a 5-fluoro group assists η^2 binding even for a cytosine ring.

Introduction

$Ru^{II}(hedta)^-$, where $hedta^{3-}$ = *N*-(hydroxyethyl)ethylenediaminetriacetato, was recently observed to adopt an η^2-coordination mode at the C-5–C-6 bonds of cytidine (C) and uridine (U).[1] This coordination mode for $Ru^{II}(hedta)^-$ is unique among the other 725 reported metal complexes of pyrimidine nucleobases and nucleosides.[2] The association constants for η^2 binding of pyrimidine nucleobases by ML_n = $Ru^{II}(hedta)^-$ is about 400-fold higher than $Ru^{II}(NH_3)_5^{2+}$ and 90-fold better than $Os(NH_3)_5^{2+}$.[1,3] The η^2 coordination of these three ML_n units occurs competitively with coordination at the N-3 sites of C and U; the N-3 metalation is the normal binding position of pyrimidine nucleobases.[4-11] Somewhat surprisingly, the DNA nucleobase thymidine (T) does not exhibit η^2 coordination at the C-5–C-6 bond with $Ru^{II}(hedta)^-$.[1] At the time of the original discovery of this η^2-coordination mode for C and U, and its absence for T, we speculated that the

(1) Zhang, S.; Holl, L. A.; Shepherd, R. E. *Inorg. Chem.* **1990**, *29*, 1012.
(2) Lusty, J. R., Ed. *CRC Handbook of Nucleobase Complexes: Transition Metal Complexes of Naturally Occurring Nucleobases and Their Derivatives*; CRC Press: Boca Raton, FL, 1990; Vol. I, pp 9–99.
(3) (a) Zhang, S.; Shepherd, R. E. *Inorg. Chim. Acta* **1989**, *163*, 237. (b) Zhang, S.; Shepherd, R. E. To be submitted to *Inorg. Chim. Acta*.
(4) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Speranzini, R. A. *J. Am. Chem. Soc.* **1981**, *103*, 1111.
(5) Beyerle-Pfnur, R.; Schollhorn, H.; Thewalt, U.; Lippert, B. *J. Chem. Soc., Chem. Commun.* **1985**, 1510.
(6) Goodgame, M.; Jakubovic, D. A. *Coord. Chem. Rev.* **1987**, *79*, 97.
(7) Ghosh, P.; Mukhopadhyay, T. K.; Sarkar, A. R. *Transition Met. Chem.* **1984**, *9*, 46.
(8) Mascharak, P. K.; Williams, J. D.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 6428.
(9) (a) Faggiani, R.; Lippert, B.; Lock, C. J. L. *Inorg. Chem.* **1980**, *19*, 295. (b) Lippert, B. *Inorg. Chim. Acta* **1981**, *55*, 5. (c) Lippert, B.; Schollhorn, H.; Thewalt, U. *J. Am. Chem. Soc.* **1986**, *108*, 6616.
(10) Cartwright, B. A.; Goodgame, M.; Johns, K. W.; Shapski, A. C. *Biochem. J.* **1978**, *175*, 337.
(11) (a) Lippard, S. J.; Barton, J. K. In *Nucleic Acid-Metal Interactions*; Spiro, T. G., Ed.; Metal Ions in Biology, Vol. 1; Wiley-Interscience: New York, 1980; Chapter 2. (b) Lippert, B.; Arpalahti, J.; Krizanovic, O.; Micklitz, W.; Schwartz, R.; Trotscher, G. In *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*; Nicolini, M., Ed.; Martinus Nijhoff: Boston, MA, 1987; pp 563–581.

0020-1669/92/1331-1457$03.00/0

crowding provided by three substituents on the C-5–C-6 "olefinic" region of T might be the source of the inhibition of η^2 coordination with T. No olefin with three substituents adjacent to the double bond has been previously coordinated to $Ru(NH_3)_5^{2+}$ or Ru(hedta)$^-$.[12] In a separate manuscript we will present evidence from 16 ligands which adopt η^2 coordination with Ru(hedta)$^-$ that the stability constant of the Ru^{II}(hedta)(olefin)$^-$ complex is a sensitive function of the electron-withdrawing or electron-releasing character of the substituents surrounding the double bond.[13] K_f varied from 7.51 with 3-deazauracil to 2.06×10^6 with methyl vinyl ketone. The sensitivity places Ru^{II}(hedta)$^-$ midrange on Tolman's scale in its capacity to serve as a π-donating metal center to olefins ($Ni^0 > Pt^0 > Rh^I > Ru^{II}$ [13] $> Pt^{II} > Cu^I > Ag^I$).[31,32] In all of the 16 cases studied with Ru^{II}(hedta)(olefin)$^-$ complexation,[13] each complex contained only one or two neighboring substituents as part of the olefin structure or as part of a pyrimidine ring. The case of a third substituent has been examined in this present work with 5-substituted uracils and uridines. A key member of this series is 5-fluorouracil. The ruthenium polyamino polycarboxylates (Ru–pac's) are of interest as potential antitumor agents since some tumors concentrate Ru–pac's.[14] Ruthenium complexes have exhibited antitumor activity,[15] and in one case, *trans*-$Ru^{III}Cl_4$(imidazole)$_2^-$ serves as a prodrug with more activity toward P388 leukemia than cisplatin.[16] 5-Fluorouracil, as a key compound in the current binding study of 5-substituted uracils and uridines with Ru^{II}(hedta)$^-$, is an important antitumor drug in its own right for a wide spectrum of cancers and leukemias.[17–23] 5-FU prevents formation of base T needed for DNA synthesis and replication of tumor cells.[24]

In this paper we describe the η^2 coordination of Ru^{II}(hedta)$^-$ to 5-FU and other 5-substituted uracils and uridines including X = Cl, Br, I, and H and the absence of η^2 binding with X = CH_3 and COOH. It is clear from this work that η^2 coordination to olefinic units is not prevented by the presence of three substituents adjacent to the double bond as long as one or more of the substituents is electron withdrawing.

Table I. $E_{1/2}$ Values vs NHE of Ru(hedta)$^-$ Complexes with 5-Halogen-Substituted Uracils, Uridines, and Other Ligands at η^2-Olefinic Bonds

L	$E_{1/2}$, V	L	$E_{1/2}$, V
5-fluorouracil	0.76	5-fluorouridine	0.79
5-chlorouracil	0.62	5-bromouridine	0.64
5-bromouracil	0.61	5-iodouridine	0.70
5-iodouracil	0.71	uridine	0.61
5-carboxyuracil	0.07		
uracil	0.62		

Table II. ^{1}H NMR Data for Ru^{II}(hedta)$^-$ Complexes with 5-Halogen-Substituted Uracils and Uridines[a]

	δ(H-6(olefin)), ppm	δ(H-6(N-position)), ppm
	L = 5-Fluorouracil	
free L	7.66, 7.64	
complex	7.98, 7.94	7.36, 7.32
Δ	0.33, 0.30	0.30, 0.32
	L = 5-Chlorouracil	
free L	7.77	
complex	7.65	7.54, 7.05
Δ	0.12	0.24, 0.73
	L = 5-Bromouracil	
free L	7.86	
complex	7.65	7.54, 7.05
Δ	0.21	0.32, 0.81
	L = 5-Iodouracil	
free L	7.97	
complex	7.65	7.53, 7.05
Δ	0.32	0.44, 0.93
	L = 5-Fluorouridine	
free L	8.11, 8.09	
complex	8.24, 8.22; 8.02, 7.99; 7.54, 7.52	8.06, 8.05; 7.25, 7.22
Δ	−0.13, −0.13; 0.10, 0.07; 0.57, 0.57	0.05, 0.04; 0.86, 0.87
	L = 5-Bromouridine	
free L	8.36	
complex	7.81; 7.56	7.32; 7.09
Δ	0.54; 0.80	1.04; 1.27
	L = 5-Iodouridine	
free L	8.44	
complex	7.82; 7.56	7.88; 7.10
Δ	0.63; 0.88	0.57; 1.35

[a] A positive ΔS is an upfield shift.

Experimental Section

Reagents. Na[Ru^{II}(hedta)(H_2O)]·$4H_2O$ (**1**) was prepared and characterized previously.[25] The complex was used from the same source. It was later found useful to prepare K[Ru^{III}(hedta)Cl] (**2**) as an alternate precursor by van Eldik's procedure.[26] In either case the starting ruthenium complex was treated with Zn/Hg in Ar-purged solutions to remove any trace of Ru^{III} from **1** and to reduce **2**, forming Ru^{II}(hedta)(H_2O)$^-$ in solution by aquation of the Ru^{II} complex. These samples were manipulated by gastight syringe methods under Ar as reported previously.[1,3] 5-Fluorouracil, 5-chlorouracil, 5-bromouracil, 5-iodouracil, 5-fluorouridine, 5-bromouridine, 5-fluorocytosine, 2,4-dihydropyrimidine, and 2,4-dihydroxypyrimidine-5-carboxylic acid were obtained from Aldrich and used without further purification.

Instrumentation. Electrochemical studies were performed on solutions under an Ar blanketing using an IMB 225 electrochemical analyzer in the cyclic voltammetry and differential-pulse polarography modes. The sweep rates were 50 mV/s for CV and 40 mV/s for DPP. The DPP stepping voltage was 50 mV. Standardization procedures have been reported elsewhere.[27] A glassy-carbon working electrode, a saturated

(12) (a) Elliott, M. G.; Zhang, S.; Shepherd, R. E. *Inorg. Chem.* **1989**, *28*, 3036. (b) Elliott, M. G.; Shepherd, R. E. *Inorg. Chem.* **1988**, *27*, 3332.
(13) (a) Zhang, S. Ph.D. Thesis, University of Pittsburgh, 1991. (b) Zhang, S.; Shepherd, R. E. *Inorg. Chim. Acta* **1992**, *191*, 271.
(14) (a) Dwyer, R. P.; Mayhew, E.; Roe, E. M. F.; Shulman, A. *Brit. J. Cancer* **1965**, *19*, 195. (b) Clarke, M. J. In *Metal Ions in Biological Systems*; Wiley-Interscience: New York, 1980; Vol. 11, p 231.
(15) (a) Clarke, M. J. In *Chemistry and Biochemistry of Platinum, Gold, and Other Chemotherapeutic Agents*; ACS Symposium Series 209; Lippard, S. J., Ed.; American Chemical Society: Washington, DC, 1983; p 235. (b) Margalit, R.; Gray, H. B.; Podbielski, L.; Clarke, M. *J. Chem.-Biol. Interact.* **1986**, *59*, 231.
(16) (a) Keppler, B. K.; Wehe, D.; Endres, H.; Rupp, W. *Inorg. Chem.* **1987**, *26*, 844. (b) Keppler, B. K.; Rupp, W. J. *J. Cancer Res. Clin. Oncol.* **1986**, *111*, 166.
(17) (a) Pratt, W. B.; Ruddon, R. W. In *The Anticancer Drugs*; Oxford University Press: New York, 1979; pp 122–131. (b) Kinura, K.; Fuji, S.; Ogawa, M.; Bodey, G. P.; Alberto, P. In *Fluoropyrimidines in Cancer Therapy*; Elsiever: Amsterdam, 1983.
(18) (a) Bonadonara, G.; Brusomolino, P.; Valagussa, A.; Rossi, L.; Brugnatelli, C.; Brambilla, M.; DeLena, G.; Tancini, G.; Bajetta, E.; Musrimeci, R.; Veronesi, U. *New Engl. J. Med.* **1976**, *249*, 405. (b) Bondonna, G.; Rossi, A.; Valagussa, P.; Bonfi, A.; Veronesi, U. *Cancer* **1977**, *39*, 2904.
(19) Carter, S. K., Ed. *Proceedings of the Conference on the Chemotherapy of Solid Tumors: Appraisal of 5-Fluorouracil and BCNU*; Cancer Evaluation Branch, National Cancer Institute: Bethesda, MD, 1970.
(20) Fugita, M.; Fujita, F.; Nakano, Y.; Taguchi, T. Experimental Chemotherapy with Fluoropyrimidine Compounds on Human Gastrointestinal and Breast Cancers Xenografted to Athymic Nude Mice. In *Fluoropyrimidines in Cancer Therapy*; Kimura, K., Fujii, S., Ogawa, M., Bodey, G. P., Alberto, P., Eds.; Excerpta Medica: Amsterdam, 1984; pp 121–132 and references therein.
(21) Williams, A. C.; Klein, E. *Cancer* **1970**, *25*, 454.
(22) (a) *Handbook on Drugs of Choice*. Medical Letters, Inc.: New Rochelle, NY, 1977. (b) See ref 17, pp 49–54, for uses of 5-FU as the preferred antineoplastic drug.
(23) Heidelberger, C. In *Antineoplastic and Immunosuppressive Agents Part II*; Sartorelli, A. C., Johns, D. G., Eds.; Springer Verlag: Berlin, 1975; pp 193–231.
(24) Heidelberger, C. Fluorinated Pyrimidines. In *Progress in Nucleic Acid Research*; Davidson, J. N., Cohen, W. E., Eds.; Academic: New York, 1965; Vol. 14, pp 1–50.
(25) Zhang, S.; Shepherd, R. E. *Inorg. Chem.* **1988**, *27*, 4712.
(26) Bajaj, H. C.; van Eldik, R. *Inorg. Chem.* **1989**, *28*, 1980.
(27) Shepherd, R. E.; Zhang, S.; Dowd, P.; Choi, G.; Wilk, B.; Choi, S.-C. *Inorg. Chim. Acta* **1990**, *174*, 249.
(28) Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley: New York, 1972; p 146.
(29) Zhang, S.; Myer, T. K.; Shepherd, R. E. *Transition Met. Chem.*, in press.

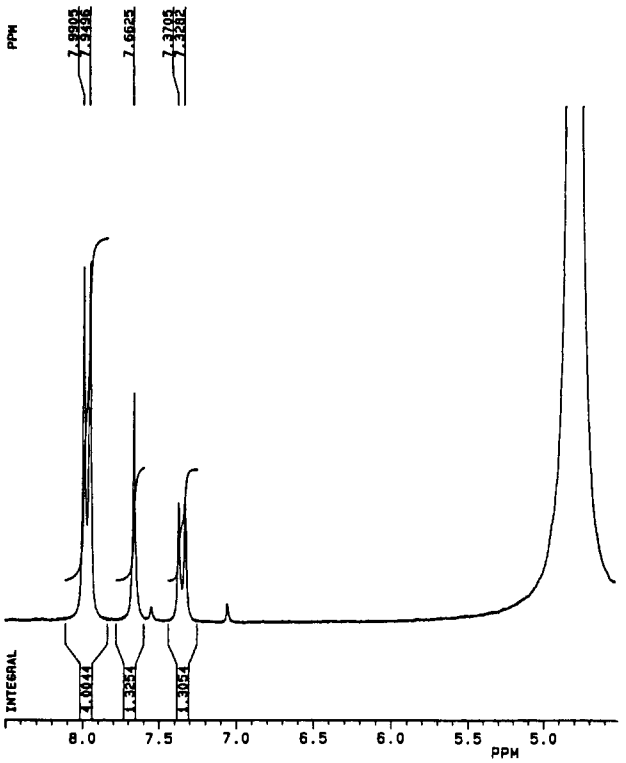


Figure 1. 300-MHz ^1H NMR spectrum of $Ru^{II}(hedta)(5\text{-FU})^-$ in the pyrimidine ring region.

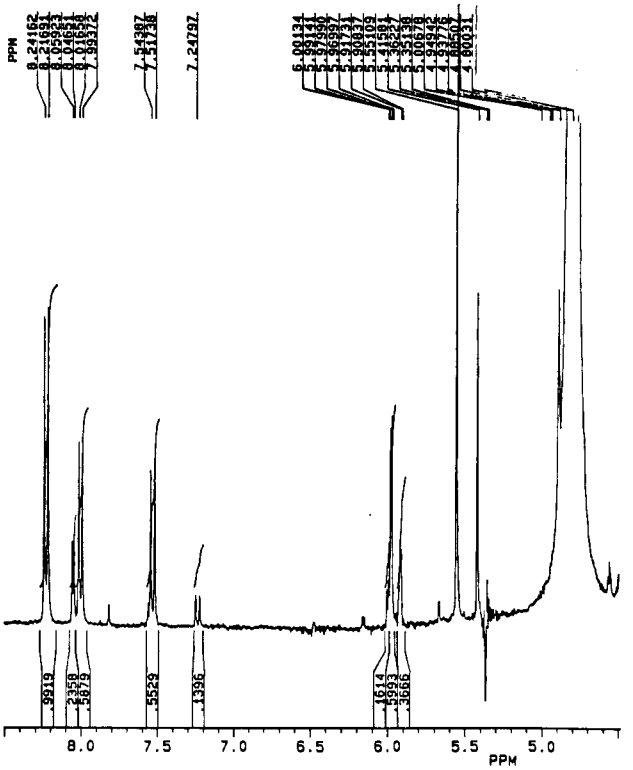


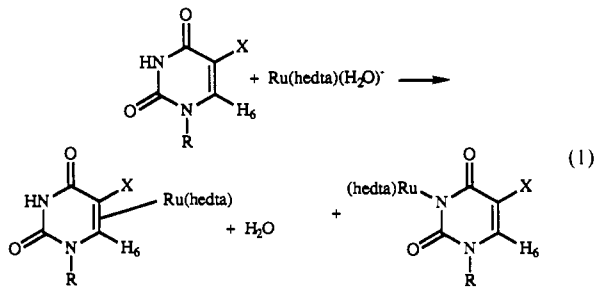
Figure 2. 500-MHz ^1H NMR spectrum of $Ru^{II}(hedta)(5\text{-fluorouridine})^-$ in the pyrimidine ring and ribose sugar region.

sodium chloride calomel electrode (SSCE) reference, and a Pt-wire auxiliary electrode were used in the conventional three-electrode cell assembly. A 0.10 M NaCl solution served as the supporting electrolyte at 22 °C. Amounts of materials bound in the olefinic and N-3 modes were evaluated from areas of the DPP curves. Confirmation of the percentages of η^2 and N-3 modes of coordination were obtained from the integrations of the observed coordinated species of $[Ru(hedta)L]^-$ by ^1H NMR spectroscopy. ^1H and ^{19}F nuclear magnetic resonances were recorded on Bruker AF300 and AF500 NMR instruments at a 70.46 and 117.44 kG, respectively. ^1H frequencies employed were 300.13 and 500.13 MHz. A frequency of 470.5 MHz was used for ^{19}F spectra at the 117.44-kG field. All spectra were recorded in D_2O as the solvent, and HOD (4.80 ppm) or a free ligand resonance served as the internal standard for ^1H spectra; $FCCl_3$ (0.00 ppm) was used as the reference for ^{19}F spectra. Assignments for the ^1H spectra were accomplished with standard decoupling procedures.

Equilibrium was achieved by reaction of the Ru^{II} complex under Ar with the desired ligand at 1:1 stoichiometry for greater than 18 h. Samples were contained in Ar-purged, septum-sealed NMR tubes; filling was achieved by transferring solutions through Teflon tubing under Ar pressure.

Results

When 5-substituted uracils and uridines were mixed with $Ru(hedta)(H_2O)^-$, complexation occurs as in eq 1 for the η^2 and



(1)

N-3 binding modes. CV/DPP waves for the η^2-bound complexes R = H, X = F, Cl, Br, and I and R = ribose, X = F, Br, and I are observed between 0.61 and 0.79 V vs NHE. The related uracil and uridine η^2 complexes have DPP waves at 0.62 and 0.61 V.[1] These data for the olefinic coordination modes of the 5-halouracils and 5-halouridines are presented in Table I.

Supporting evidence for η^2 and N-3 coordination is shown by the chemical shifts of ring H-6 proton. Coupling exists between the H-6 proton and the 5-F substituent for the 5-fluorouracil and 5-fluorouridine cases. The ^1H NMR data for the chemical shifts of η^2-bound isomer, N-3-bound isomer, and free ligand values are presented in Table II.

The case of 5-FU is representative of the results for all of the halouracils. The ^1H 300-MHz NMR spectrum of Ru(hedta)-(5-FU)$^-$ is shown in Figure 1 for the H-6 region of the spectrum. ^1H NMR shift data are collected in Table II for ligands of this report. Splitting of the H-6 proton by the fluorine ($I = {}^1/_2$) nucleus causes doublets to appear for the coordinated complexes. Proton resonances for H-6 reveal the η^2 isomer at δ 7.99, 7.95, the free ligand (5-FU) at δ 7.55, and the N-bound (N-3) isomer at δ 7.37, 7.33. The percentage of η^2 isomer is greater (75.4%). An electrochemical method, integration of the DPP waves for the aquo, η^2, and N-3 species, determined a value of 72.6% for the η^2 isomer in good agreement with the ^1H NMR integrations.

The ^1H 500-MHz NMR spectra for the 5-fluorouridine complexes are shown in the ring (H-6) region and for the region of the ribose sugar protons in Figure 2. Several isomers are detected as noted in Table II. Again the dominant mode of coordination is via the C-5–C-6 olefinic bond. Three isomers are assigned as η^2 related, δ 8.24, 8.22 (39.6%), δ 8.02, 7.99 (23.4%), and δ 7.54, 7.52 (22.1%), on the basis of the total yield of η^2 forms (88.2%) as determined by the DPP method. The remaining 11.8% is assigned to the lesser ^1H resonances for N-3-bound isomers (δ 8.06, 8.05, 9.4%; δ 7.25, 7.22, 5.6%). The integration of the major H-6 resonance peaks accounts for 85.0% η^2 forms in agreement with 88.2% by DPP integration. The ribose region reveals three types of ribose sugar fragments, one at δ 6.00 accounting for 14.3% in reasonable agreement with 15.0% of N-3 forms by integration of the H-6 region's N-3 isomers and multiplet shifts at δ 5.97 and 5.92, which combine to 85.7%, matching the percentage integrations provided by η^2 forms. It is of interest that the ribose unit is sensitive to the position of $Ru(hedta)^-$ coordination and that two main types of η^2-coordinated species are observed for 5-fluorouridine.

The resultant shifts in the H-6 proton for η^2 coordination relative to the free ligand is given in Table III for various 5-substituted

Table III. ^{1}H NMR and $E_{1/2}$ Data for the Ru^{II}(hedta)$^-$ Complexes at Olefinic Bonds

X	Δ compared to free L, ppm: uracil	Δ compared to free L, ppm: uridine	$E_{1/2}$, V vs NHE: uracil	$E_{1/2}$, V vs NHE: uridine
F	−0.32	−0.13 0.10	0.76	0.79
H	−0.23	0.31	0.62	0.61
Cl	0.12		0.62	
Br	0.20	0.54 0.80	0.61	0.64
I	0.32	0.63 0.88	0.71	0.70
−COOH	*a*			
$-CH_3$	*a*			

[a] No formation at olefin bond.

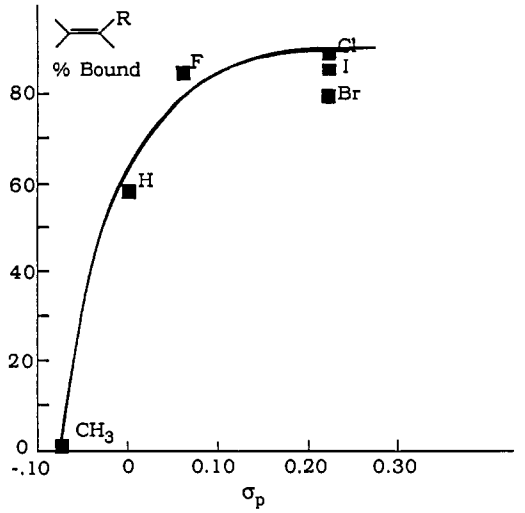


Figure 3. Amount of η^2 isomer of Ru^{II}(hedta)(5-substituted uracil)$^-$ as a function of the substituent's Hammett constant.

complexes. The H-6 proton is known to be influenced by coordination of Ru^{II}(hedta)$^-$ in the C-5–C-6 region of a pyrimidine ring.[1] However, the shift of H-6 may be either upfield or downfield upon coordination. The H-5 resonance is always shifted upfield for η^2 coordination at derivatives related to C and U.[1] However, this position is derivatized by X in the current group of ligands. The characteristic effect of upfield shifts for η^2 coordination cannot be indicated from the H-5 proton because of its absence in these structures. The electrochemical waves (CV/DPP) with the observed $E_{1/2}$ values for the [$Ru^{III/II}$(hedta)$L^{0/-}$] couples are clearly diagnostic of η^2 binding, however.

The 5-fluorouracil derivative was studied by ^{19}F NMR spectroscopy. The data which were obtained showed that 21.5 and 26.7 ppm upfield shifts of ^{19}F singlets occur when Ru^{II}(hedta)$^-$ is coordinated at the η^2 and N-3 positions, respectively. Assignments of η^2 vs N-3 coordination were made on the correlation of the areas for the ^{19}F NMR spectrum of the two isomers and the areas for olefinic coordination and N-3 coordination as deduced by CV/DPP studies and ^{1}H NMR spectroscopy on the same solution. It is known from prior work[1,2] that η^2 complexes of uracils and uridines exhibit $E_{1/2}$ values near 0.62 V vs NHE while N-bound complexes have CV/DPP waves between −0.11 and 0.08 V vs NHE. The same pattern is observed for the 5-substituted uracils and uridines: η^2 complexes, $0.61 \le E_{1/2} \le 0.79$ V; N-3-bound complexes, $-0.11 \le E_{1/2} \le 0.07$ V.

The percentage of η^2-bound vs N-3-bound isomers were evaluated by integration of the DPP waves of the electrochemical methods and the integration of the H-6 resonances of the coordinated complexes by ^{1}H NMR spectroscopy with good agreement (within 3%). The average of the two evaluations for the percentage of the η^2 complex of each Ru^{II}(hedta)L^- is presented in Figure 3 as a function of the Hammett substituent constant, σ_p. Also

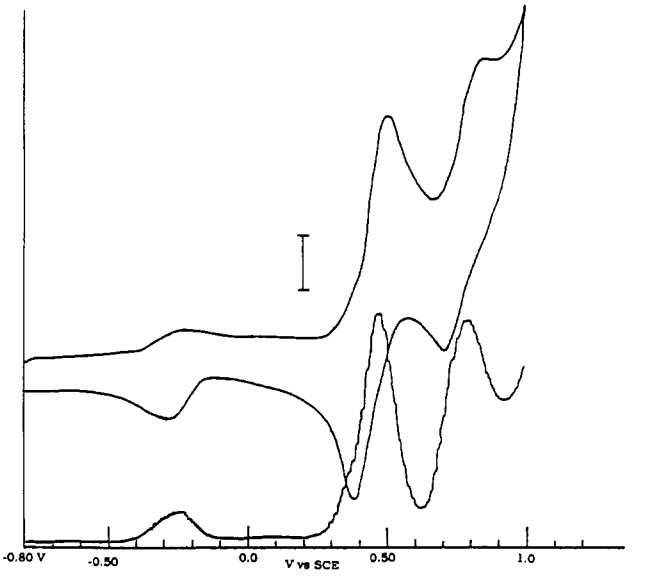


Figure 4. Reversibility of the CV wave for Ru^{II}(hedta)(5-iodouracil)$^-$ (pH = 6.27; [Ru^{II} complex)] = 3.31×10^{-4} M). The current axis indicator is 50 μA for CV and DPP.

included in the figure are the data from prior studies with substituents X = H and CH_3.[1]

The CV waves of the Ru(hedta)L^- complexes usually exhibit irreversible character in the wave attributed to η^2-bound complexes. This is due to aquation of the Ru^{III}–olefin complexes.[1,12] In the current study this pattern was maintained for all of the halouracil and halouridine complexes except for the iodouracil case where reversible behavior in the $Ru^{II/III}$ wave for η^2 coordination is observed (Figure 4).

In the case of uracil-5-carboxylic acid no CV/DPP wave attributable to η^2 coordination is observed. Two overlapped reversible waves occur at 0.00 and 0.07 V vs NHE. These are assigned to the $Ru^{III/II}$(hedta)$(H_2O)^{0/-}$ wave and the N-3-coordinated isomer.

5-Fluorocytosine (5-FC) was also studied as a ligand. The binding constant for cytosines is ca. 10^2 lower than that for uracils.[13] When Ru^{II}(hedta)$(H_2O)^-$ was mixed with 5-fluorocytosine, three types of complexes were detected by DPP: coordination via the exo amine NH_2 ($E_{1/2}$ = 0.10 V vs NHE; 47.4%), an N-3-coordinated form ($E_{1/2}$ = 0.33 V; 24.6%), and an olefin (η^2) bound form ($E_{1/2}$ = 0.77 V; 27.9%). A confirmation of the different coordinated species of 5-FC was obtained by ^{1}H NMR spectroscopy. These results will be published elsewhere.[30] However, it was observed that the integration of doublet resonances at δ 7.59 (45.0%), δ 7.49 and 7.20 (30.2%), and δ 6.97 (24.5%) is in good agreement with the species attributed to the exo-NH_2, η^2, and N-3 complexes, respectively. The data were collected at pD = 2.0. The species attributed to coordination at the amino group underwent slower aquation at pH 5.98 compared to 2.04 in the CV/DPP study. This suggests that the exo amine is deprotonated upon coordination of Ru^{II}(hedta)$^-$. Protonation would greatly reduce the basicity of exo amine, but the area of the N-3-coordinated species did not grow substantially as the species attributed to the exo-NH_2-coordinated complex was lost, nor is there a facile isomerism to the N-3 site as known for the $Ru^{II}(NH_3)_5$(cytosine) case from Clarke's studies.[37] Again cytosines

(30) Zhang, S.; Shepherd, R. E. Manuscript in preparation.
(31) (a) Tolman, C. A. *J. Am. Chem. Soc.* **1974**, *96*, 2780. (b) Tolman, C. A.; Seidel, W. C. *J. Am. Chem. Soc.* **1974**, *96*, 2774. (c) Tolman, C. A.; Gerlach, D. H. *J. Am. Chem. Soc.* **1972**, *94*, 2669.
(32) Munakata, M.; Kitagawa, S.; Kosome, S.; Asahara, A. *Inorg. Chem.* **1986**, *25*, 2622.
(33) Diamantis, A. A.; Dubrawski, J. V. *Inorg. Chem.* **1981**, *20*, 1142.
(34) Diamantis, A. A.; Dubrawski, J. V. *Inorg. Chem.* **1983**, *22*, 1934.
(35) Stritar, J. A.; Taube, H. *Inorg. Chem.* **1969**, *8*, 2281.
(36) Matsubara, T.; Creutz, C. *Inorg. Chem.* **1979**, *18*, 1956.

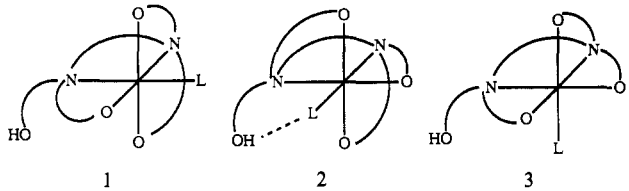
are observed to favor N-3 coordination with less η^2 form present. The $-NH_2$-bound and N-3-bound species were observed to aquate with a half-life of about 15 min at pH 2.04.

Discussion

Complexation of 5-substituted uracils and uridines is observed to occur by both η^2 and N-3 modes in all cases except for substituents X = CH_3 and COOH. A large increase in the relative isomer distribution occurs as a function of the withdrawing character of X, reaching a maximum value for X = Cl with 85% η^2 coordination. It is known that the combined influence of σ withdrawal and π resonance makes Cl a stronger withdrawing substituent than F in organic chemistry. A slight decrease in the amount of η^2 isomer occurs for the larger Br- and I-substituted ligands, indicative of a modest steric effect, since all three X = Cl, Br, and I have nearly identical σ_p values. The most positive $E_{1/2}$ values for the η^2 isomers of $Ru(hedta)L^-$ complexes (L = 5-substituted uracils and uridines) occur for 5-fluoro derivatives ($E_{1/2}$ = 0.76 and 0.79 V for 5-fluorouracil and 5-fluorouridine, respectively). The $E_{1/2}$ value follows the order for X substituents of F > I > Cl ~ Br, but the reason for this is not obvious.

Coordination of the 5-fluoro derivatives produces a large upfield shift of the ^{19}F resonance for both the η^2- and N-3-bound isomers. Apparently the shift of electronic density in the ring upon coordination of the $Ru^{II}(hedta)^-$ π-base is so strong that the carbon near the 5-fluoro group is greatly compensated compared to the free ligand whether Ru^{II} center is within one bond (η^2) distance or three bonds away (N-3).

In the case of 5-fluorouridine there is evidence for multiple isomers of both the η^2-coordinated and N-3-coordinated complexes (see Table II). The olefinic type of complex shows three species, while those of the N-3-bound form distinguish only two less abundant isomers. In the prior study with 3-methyluridine two isomers were observed.[1] We offered the explanation that this might reflect the position of the $Ru^{II}(hedta)$ moiety relative to the position of the ribose sugar, either on opposite sides as established by the olefin unit and N-1 atom or on the same side. The shift difference of the H-6 relative to the free ligand was 0.03 and 0.28 ppm for 3-methyluridine complexes in the η^2 form. Current work[13] suggests this interpretation oversimplifies the complexity for 3-methyluridine of the species in solution. This prior explanation could accommodate the two isomers with pairwise shifts at 8.24, 8.22 ppm (39.6%) and 8.02, 7.99 ppm (23.4%), but it does not explain the third species (7.54, 7.52 ppm; 22.1%) nor the reason for two isomers of the N-3-bound type (8.06, 8.05 and 7.25, 7.22 ppm). When models of $Ru^{II}(hedta)^-$ are constructed, it is observed that three isomers of the $Ru^{II}(hedta)^-$ moiety are feasible. These are shown with L representing the bound ligand in either η^2 or N-3 coordination:



1 2 3

Diamantis and Dubrawski have shown previously that ruthenium(II) polyamino polycarboxylates rearrange the positions of carboxylate donors.[33,34] This is observed as a broadening of the glycinato resonances in the ^1H NMR spectra. The rearrangement processes are much faster than the 18-h period given to achieve substitution and isomerization equilibria for an entering pyrimidine base. Isomer 3 may be formed from isomer 2 by a simple shift of one glycinato functionality from out of the plane containing the two nitrogen donors into the plane. Isomer 1 is formed by a return of a glycinato donor of isomer 3 into the axial position vacated in forming isomer 3. The rearrangements of these glycinato chelate rings offer little steric hinderance as seen easily from models. Therefore isomers 1–3 can readily interconvert in response to the steric, recognition, or solvation requirements of the entering ligand.

In a separate study we have observed that pyrimidine and 4-methylpyrimidine undergo isomerism between N-3 coordination and the η^2 olefinic coordination within the ≥18-h reaction time afforded for complexation in this present study.[30] Substitution is initially favored at N-3 followed by linkage isomerism to give the equilibrium amount of the η^2 form which is sensitive to the ring substituent. Samples of the complexes in this report were similarly treated. Sealed samples of F and I derivatives were examined on several successive days with no apparent change in the isomer distribution between η^2 and N-3 forms with time after the initial 18-h period.

Isomers 1 and 2, which differ by the arrangement of coordinated glycinato arms, place the bound ligand trans to nitrogen donors. The third structure places L trans to a carboxylate donor. Experience with $Ru^{II}(Me_2edda)$, which has been synthesized in our laboratory and characterized as to its ability to discriminate on the coordination of olefins on the basis of structure and branching,[13a,30] has shown that isomer 3 is too crowded to easily allow η^2 coordination of pyrimidines. All three stereochemical isomers have been observed to form complexes with the smaller olefin, methyl vinyl ketone.[13] The $Ru(hedta)(MVK)^-$ complexes of isomers 1–3 exhibit distinctly resolvable ^1H NMR spectra of which isomers 1 and 2 are nearly equally abundant (44.1 and 38.6%) while isomer 3 is only 17.4% abundant.[13]

The three η^2 isomers for 5-fluorouridine may represent contributions from each of the three structures with the pair of shifts at 7.54 and 7.52 ppm indicative of a strained, longer interaction for isomer 3. But the relative abundances of the observed η^2 isomers (39.6, 23.4, and 22.1%) argue against this. Additionally, it is unreasonable that an olefin complex for isomer 3 with a longer bond should be shifted more upfield than those with better olefin π^*–dπ overlap. The models show that structure 2 places the *N*-hydroxyethyl group near the ribose sugar in excellent proximity of H-bonding to the sugar ring. This influence is absent for structure 1. It is our view that this interaction most favors structure 2 with only one orientation of the ribose away from the $Ru^{II}(hedta)^-$ center in order to maximize this H-bonding shown by the dotted interaction in isomer 2. This would be the dominant species which is 39.6% abundant. The other isomers are from structure 1 for the η^2 complexes. These represent another 45.4% of $Ru(hedta)(5\text{-fluorouridine})^-$ species. In this arrangement the H-bonding to the *N*-hydroxyethyl group is lost. Two isomers of nearly equal abundance (23.4 and 22.1%) are then attributed to isomers via structure 1 in which the ribose projects away from the *N*-hydroxyethyl group in two orientations, both of low H-bonding accessibility. These two forms are the same as ones described earlier for 3-methyluridine.[1] However, it is now understood that both structures 1 and 2 should contribute to coordination of 3-methyluridine and not just structure 2 as was considered previously. It seems likely that isomer described previously as having the *N*-hydroxyethyl arm further away includes both forms of isomer 1 for the 3-methyluridine complex. The other species described as nearer to the *N*-hydroxyethyl arm is the H-bonded entity from isomer 2. The differentiation of two isomers for coordinated 5-fluorouridine when bound to structure 1 and the lack of differentiation for 3-methyluridine is probably connected to the large perturbation of atomic orbital contributions to the MO's of the bound ligands by the presence of a ring fluorine instead of hydrogen. We point out that these differences are very subtle effects, given that no multiplicity of detectable isomers by either ^1H or ^{13}C NMR spectroscopy are observed for the η^2 forms when uracil, 1-methyluracil, 1,3-dimethyluracil, or uridine coordinates to $Ru^{II}(hedta)^-$. Clearly both isomers 1 and 2 can contribute to the coordination of these ligands as well; yet no difference in chemical shift is observed. These effects seem to be manifest only when the coordinated uracil system is altered

(37) (a) Clark, M. J.; Buchbinder, M.; Kelman, A. D. *Inorg. Chim. Acta* **1978**, *27*, L27. (b) Clarke, M. J. *J. Am. Chem. Soc.* **1978**, *100*, 5068. (c) Clarke, M. J. The Potential of Ruthenium in Anticancer Pharmaceuticals. In *Inorganic Chemistry in Biology and Medicine*; Martell, A. E., Ed.; American Chemical Society; Washington, DC, 1980; Chapter 10.

significantly by bulky substituents at both N-1 and N-3 or by the presence of the bulky ribose at N-1 plus the electron-withdrawing F substituent at C-5. The extent of ring puckering upon η^2 coordination should be a function of the presence of bulkier substituents or the percentage of s character in the bonds at C-5 and C-6. Since the chemical shift is a function of the percentage of s character in the C–H bond, the differentiation of isomers may be a sensitive function of the number of ring substituents and their electronic properties. Having the ribose functionality at N-1 to provide sufficient steric bulk seems to be a necessary but insufficient criteria to differentiate the spacially different η^2-coordination isomers.

The N-3-bound ring places the C-5–C-6 bond remote from the Ru^{II} center and away into the solvent. Coordination at either structures 1 or 2 could be sensed at H-6 as nearly the same for N-3 coordination and contribute to the 9.4% abundant isomer of N-3 type. The coordination via N-3 to Ru^{II} in structure 3 is the most different environment in terms of hedta-ligand proximity, crowding and solvation leading to a low abundance (5.6%); additionally the trans donor and in-plane donors are most different. It appears that 5-fluorouridine is sensitive at H-6 and the ribose region to these geometrical factors, just as 3-methyluridine detected coordination differences with Ru^{II}(hedta)$^-$ in the prior study. A comparison with the data obtained with 5-fluorouracil (5-FU) shows that all the shifts relative to the free ligands are dissimilar from $\Delta\delta$ for 5-FU. This does not assist in the confirmation of the distribution between the various η^2 forms for our isomer assignments. However, the summations of the integrations for ^{1}H NMR spectra of the η^2 forms and N-3 forms agree well with the areas for η^2 and N-3 coordination determined electrochemically by integration of the DPP waves.

Since the size of the 5-substituents is ca. F $\simeq$ H and Br $\simeq$ CH_3, it is clear that the absence of η^2 coordination by Ru^{II}(hedta)$^-$ for the T nucleobase (X = CH_3) is of electronic, and not steric, origin. The absence of an η^2 isomer for X = COOH appears to be connected to a steric problem. For X = CO_2^- and COOH substituents σ_p values of 0.0 and 0.45 are reported.[28] The ionized form which should predominate at pH $\simeq$ 7 should have as much of the η^2 isomer as X = H (uracil or uridine). We have no other example for a substituent with as large a σ_p value as the protonated carboxylate, but there is no obvious electronic reason why less of the η^2 form would be induced, given the fact that keto groups α to a double bond raise the association constant of Ru^{II}(hedta)$^-$ for the η^2 mode by a factor of 10^2–10^3.[13] Models show that a carboxylate group in the 5-position sterically hinders the interaction of the Ru^{II} center and the olefinic bond. The anionic carboxylate would also be placed very near the ligand carboxylate donors. This could produce an electrostatic repulsion that disfavors η^2 binding relative to a less repulsive interaction of the anionic carboxylate when the ligand binds in the N-3 position. A reviewer has suggested coordination of the carboxylate as a prohibitive mode for η^2 coordination. This seems unlikely given the lability of Ru^{II} for carboxylate donors[33,35] and the low binding constant anticipitated for an acetate chromophore on the basis of the studies of Creutz with Ru^{II}(edta)$^{2-}$[36] or the aquation of Cl^- from Ru^{II}(hedta)Cl^{2-}.[33] However, a transitory interaction of the carboxylate brings the N-3 moiety nearly as close as the C-5–C-6 (η^2) site. One cannot rule out the preassociation of the acetato unit as a catalytic route for N-3 binding.

It has also been observed on the basis of the ability of Ru$(NH_3)_5^{2+}$ and Ru(hedta)$^-$ to undergo shifts in the MLCT spectra of coordinated pyrazines and bipyridines that Ru(hedta)$^-$ is a better π-base toward N-heterocyclic rings when coordinated by the ring nitrogen donors.[29] The donation to the better π-acceptors of the protonated N-heterocyclic ligands occurs with only a small enhancement of donation from Ru–pac's compared to Ru$(NH_3)_5^{2+}$. It appears that the stronger π-acceptor ligands such as olefins maximize the ability of Ru–pac's to π-donate. That is, the olefinic coordination allows a Ru–pac to participate in π-donation more strongly since the Ru^{II}-pac is compensated by the carboxylate functionality as it donates to an olefin. Hence *the preferential binding at the η^2 position of pyrimidine nucleobases can be controlled by the secondary ligation of the ruthenium(II) center.*

These observations suggest that a ruthenium polyamino polycarboxylate complex which is a better π-donor than Ru^{II}(hedta)$^-$ might induce even the nucleobase T to adopt an η^2-coordination mode. This would be of great importance for the use of those complexes in labeling DNA nucleobases within a DNA chain. Efforts in this regard are currently in progress in our laboratories.

Acknowledgment. We gratefully acknowledge support for this research from National Science Foundation Grant CHE-8417751.

Registry No. 1, 118170-03-9; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-fluorouracil), 139461-04-4; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-chlorouracil), 139461-05-5; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-bromouracil), 139461-07-7; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-iodouracil), 139461-09-9; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-fluorouridine), 139461-11-3; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-bromouridine), 139461-13-5; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-iodouridine), 139461-15-7; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = uracil), 125137-61-3; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = uridine), 125137-63-5; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 5-fluorocytocine), 139461-20-4; [Ru^{II}(hedta)(η^2-L)]$^-$ (L = 3-methyluridine), 125137-64-6; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-fluorouracil), 139493-21-3; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-chlorouracil), 139461-06-6; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-bromouracil), 139461-08-8; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-iodouracil), 139461-10-2; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-fluorouridine), 139461-12-4; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-bromouridine), 139461-14-6; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-iodouridine), 139461-16-8; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-carboxyuracil), 139461-17-9; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = uracil), 125137-78-2; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = uridine), 125137-81-7; [Ru^{II}(hedta)(N-3-L)]$^-$ (L = 5-fluorocytocine), 139461-19-1; [Ru^{II}(hedta)(exo-NH_2-L)]$^-$ (L = 5-fluorocytocine), 139461-18-0; [Ru^{III}(hedta)(η^2-L)] (L = 5-iodouracil), 139461-21-5; [Ru^{III}(hedta)(N-3-L)] (L = 5-carboxyuracil), 139493-22-4.