pK_a Estimation of Ruthenium(II)−Arene PTA Complexes and their Hydrolysis Products via a DFT/Continuum Electrostatics Approach

Christian Gossens, Antoine Dorcier, Paul J. Dyson,* and Ursula Rothlisberger*

Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

Received April 13, 2007

A combined density functional theory/continuum electrostatics approach has been used to estimate the pK_a values of a series of metal-based compounds and their hydrolysis products. Specifically, the protonation states and absolute pK_a values of the complexes $[M(\eta^6\text{-arene})(X)(Y)(pta)]^{n+}$ (M = Ru or Os; arene = benzene, *p*-cymene, 1,3,5-trifluorobenzene, benzene-1,3,5-triamine; X, Y = halide, H_2O, OH, guanine; pta = 1,3,5-triaza-7-phosphaadamantane) have been investigated by this approach and supplemented with experimental pK_a determinations using ^{31}P NMR spectroscopy. Compounds of this type have been recently used as anticancer agents and also to catalyze CO_2 reduction. Our calculations show that pta binding to a ruthenium center significantly reduces the basicity of the ligand. The experimentally observed pH-dependent DNA binding is rationalized by the hydroxo/aqua ligand equilibrium in $[Ru(\eta^6\text{-benzene})Cl(OH_2)(pta)]^+$. The applied computational scheme predicts that pK_a tuning can be done most effectively by modifications of the arene ligand.

Introduction

The hydrophilic phosphine ligand 1,3,5-triaza-7-phosphaadamantane (pta) has proven to be a versatile ligand in medicinal chemistry and in aqueous-phase catalysis, and the domain of transition metal−pta chemistry has recently been comprehensively described in an excellent review.[1] In general, the aforementioned applications involve an aquatic environment, and the presence of aqua ligand(s) in conjunction with pta ligand(s) is frequently observed. Since pH plays a critical role in both medicinal and aqueous catalytic applications, involving both activation and potential compound deactivation, it is necessary to be able to determine pK_a values of the N-sites on the pta ligand and of the coordinated water molecules. Such information is not always easy to ascertain experimentally, and in this sense a reliable and affordable computational method would be extremely useful to help clarify experimental data or to predict the pK_a values of, as yet, unsynthesized compounds, in order to evaluate if their synthesis is warranted.

In this paper we calculate absolute pK_a values of organometallic ruthenium complexes with a combined DFT/continuum solvation method. We show that such a computational approach may be used (i) to rationalize the aqueous chemistry of these complexes and (ii) to rapidly screen the effect of structural and electronic changes on a given transition metal compound to predict such properties. The investigated series of ruthenium(II)−arene complexes of the general type $[Ru(\eta^6\text{-arene})(X)_2(pta)]$ (RAPTA; pta = 1,3,5-triaza-7-phosphaadamantane) have been used as potential anticancer compounds[2−5] and as precursors in the reduction of CO_2 in aqueous solution.[6,7] Since they undergo hydrolysis in aqueous solutions (in the absence of chloride or at low chloride concentration), rationalization of the pK_a of both the pta and aqua ligand is important with respect to understanding their mode of activity.[4,8]

Experimental Section

Theoretical Methods. The pK_a value of a compound indicates the relative concentrations of the protonated and deprotonated forms in a solution of given pH. For weak acids, the Henderson−Hasselbach equation holds

$$\text{pH} = \text{p}K_a + \log([\text{deprotonated}]/[\text{protonated}]) \quad (1)$$

While the experimental determination of this physical property is routine for well-defined molecular systems, the accurate computation of pK_a values is still challenging. This is due to the fact that the definition of a Brönsted acid in aqueous solution is defined as

$$BH^+_{aq} \rightarrow B_{aq} + H^+_{aq} \quad (2)$$

which includes not only the free energy change due to the protonation reaction but also the changes in the solvation energies of all species at finite temperature. In principle, *ab initio* molecular dynamics simulations of an explicit solvent system can provide a general solution to this problem, however, at a very high computational cost.[9] In recent years, implicit

* Corresponding authors. E-mail: ursula.roethlisberger@epfl.ch.

(1) Phillips, A. D.; Gonsalvi, L.; Romerosa, A.; Vizza, F.; Peruzzini, M. *Coord. Chem. Rev.* **2004**, *248*, 955−993.
(2) Allardyce, C. S.; Dyson, P. J.; Ellis, D. J.; Heath, S. L. *Chem. Commun.* **2001**, 1396−1397.
(3) Dorcier, A.; Dyson, P. J.; Gossens, C.; Rothlisberger, U.; Scopelliti, R.; Tavernelli, I. *Organometallics* **2005**, *24*, 2114−2123.
(4) Scolaro, C.; Bergamo, A.; Brescacin, L.; Delfino, R.; Cocchietto, M.; Laurenczy, G.; Geldbach, T. J.; Sava, G.; Dyson, P. J. *J. Med. Chem.* **2005**, *48*, 4161−4171.
(5) Scolaro, C.; Geldbach, T. J.; Rochat, S.; Dorcier, A.; Gossens, C.; Bergamo, A.; Cocchietto, M.; Tavernelli, I.; Sava, G.; Rothlisberger, U.; Dyson, P. J. *Organometallics* **2006**, *25*, 756−765.
(6) Horvath, H.; Laurenczy, G.; Katho, A. *J. Organomet. Chem.* **2004**, *689*, 1036−1045.
(7) Geldbach, T. J.; Laurenczy, G.; Scopelliti, R.; Dyson, P. J. *Organometallics* **2006**, *25*, 733−742.
(8) Dorcier, A.; Ang, W. H.; Bolano, S.; Gonsalvi, L.; Juillerat-Jeannerat, L.; Laurenczy, G.; Peruzzini, M.; Phillips, A. D.; Zanobini, F.; Dyson, P. J. *Organometallics* **2006**, *25*, 4090−4096.
(9) Sprik, M. *Chem. Phys.* **2000**, *258*, 139−150.

10.1021/om700364s CCC: $37.00
Publication on Web 06/27/2007

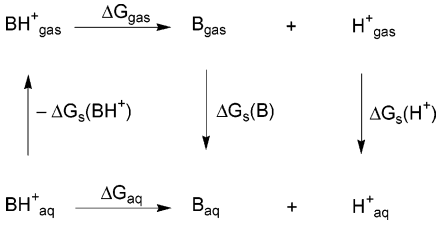
solvent methods have evolved to a level that yields pK_a values accurate up to less than half a pK_a unit for organic compounds, such as phenols.[10] Here, we have applied a similar method to determine the pK_a of transition metal complexes, to test if the approach is also suitable for this type of systems though probably with less accuracy.

Thermodynamics correlates the pK_a value of a compound to the free energy difference (ΔG_{aq}) between its protonated and unprotonated form in solution:

$$pK_a = -\log(K_a) \quad (3)$$

$$\Delta G_{aq} = -2.303RT \log(K_a) \quad (4)$$

Quantum chemical calculations routinely yield free energy estimates for gas-phase systems, whereas solvated species pose more of a challenge. Therefore a thermodynamic cycle



is generally employed to decompose ΔG_{aq} into gas-phase (ΔG_{gas}) and solvation ($\Delta\Delta G_s$) contributions,

$$\Delta G_{aq} = \Delta G_{gas} + \Delta\Delta G_s \quad (5)$$

The free energy of solvation can be calculated using a continuum solvation model that treats water as a medium with a dielectric constant.[11,12] This approximation constitutes the most critical step in this approach and results in the largest error in predicted pK_a values.

Using this concept, pK_a values can be calculated as

$$pK_a = [G(H^+)_{gas} + \Delta G_s(H^+) + G(B)_{gas} + \Delta G_s(B) - G(BH^+)_{gas} - \Delta G_s(BH^+)]/[2.303RT] \quad (6)$$

Experimental values for $G(H^+)_{gas} = -6.28$ and $\Delta G_s(H^+) = -264.61$ kcal/mol have been used as suggested in ref 13, while all other terms in eq 6 have been calculated using DFT/C-PCM as described below. Although we report absolute pK_a values, the main focus of this study is on their relative differences. All pK_a values are assigned to the conjugate base B of the corresponding acid BH^+ (i.e., we associate a pK_a value to pta for the system pta/pta-{*N*-H}$^+$). It should be noted that pK_a values can be calculated in this way even for hypothetical species that do not exist in a real aqueous environment. Highly positive or negative computed "pK_a" values corresponding to such hypothetic conditions should be interpreted as an *indication* for the relative acidity or basicity of the corresponding compound rather than as a *measurable* quantity.

Computational Details

All calculations in this study were performed at the DFT level of theory employing the B3LYP[14,15] functional as implemented in the Gaussian03 package.[16] A mixed basis set using the quasirelativistic Stuttgart/Dresden semicore SDD-ECP[17] with a (8s7p6d)/[6s5p3d]-GTO triple-ζ valence basis set on ruthenium and 6-31+G-(d) on the remaining atoms was used. As shown in previous studies, this method is able to yield accurate structures and energies.[3,5,18−21] The zero-point energies, thermal corrections, and entropies obtained from an analytical frequency analysis have been used to convert the internal energies to Gibbs energies at 298.15 K and 1 atm. ΔG_s was computed using Barone and Cossi's polarizable conductor model (C-PCM).[22,23] The C-PCM calculations were performed with default parameters as single points on the gas-phase geometries since this has been shown to give better results than reoptimization.[24] Water was modeled with a dielectric constant of $\epsilon = 78.39$. The united atom topological model applied on atomic radii of the UFF force field (UA0) was used, and the area of the tesserae was set at 0.2 Å^2. The different reference states of gas-phase (1 atm, 24.46 L at 298.15 K) and C-PCM solvation calculations (1 M) were converted via $\Delta G_{gas}(1\ M) = \Delta G_{gas}(1\ atm) + RT \ln(24.46)$ as suggested in refs 10 and 13. In addition, to visualize acidic/basic parts of the compounds, the electrostatic potential (ESP) was mapped onto the electron density surface at an isovalue of 0.004 au. It is colored around the midpoint of −0.05/0.05 (neutral compounds), 0.1/0.205 (charged +1), and 0.1/0.2 au (charged +2) in blue (positive) and red (negative).

Synthetic Details. The starting materials 1,3,5-triaza-7-phosphaadamantane (pta)[25,26] and ruthenium−arene precursors[27] were prepared as described previously. NMR spectra were recorded on a Bruker 400 MHz Ultrashield spectrometer. Electrospray ionization mass spectra (ESI-MS) were obtained on a Thermofinigan LCQ Deca XP Plus quadrupole ion trap instrument set in positive mode using a literature method.[28]

Experimental pK_a Determinations. The pH values of NMR samples in D_2O were measured at 298 K, directly in a NMR tube, using a 713 pH meter (Metrohm) equipped with an electrode calibrated with buffer solutions at pH values of 4, 7, and 9. The pH values were adjusted with dilute CF_3SO_3D and NaOD (ca. 0.65 M). The pH titration curves were fitted to the Henderson−Hasselbach equation using the program Micromath Scientist (Micromath Scientific Software Inc.) with the assumption that the observed chemical shifts are weighted averages according to the populations of the protonated and deprotonated species. The resonance frequencies change smoothly with pH between the chemical shifts of the charged form HA^+, stable in acidic solution, and those of the neutral, deprotonated form A, which is present at

(10) Liptak Matthew, D.; Gross Kevin, C.; Seybold Paul, G.; Feldgus, S.; Shields George, C. *J. Am. Chem. Soc.* **2002**, *124*, 6421−7.
(11) Tomasi, J.; Mennucci, B.; Cammi, R. *Chem. Rev.* **2005**, *105*, 2999−3093.
(12) Cramer, C. J.; Truhlar, D. G. *Chem. Rev.* **1999**, *99*, 2161−2200.
(13) Liptak, M. D.; Shields, G. C. *J. Am. Chem. Soc.* **2001**, *123*, 7314−7319.
(14) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−52.
(15) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098−100.
(16) *Gaussian 03*, Revision B.03; Gaussian, Inc.: Wallingford, CT, 2004.
(17) Andrae, D.; Haeussermann, U.; Dolg, M.; Stoll, H.; Preuss, H. *Theor. Chim. Acta* **1990**, *77*, 123−41.
(18) Kallies, B.; Mitzner, R. *J. Phys. Chem. B* **1997**, *101*, 2959−2967.
(19) De Abreu, H. A.; De Almeida, W. B.; Duarte, H. A. *Chem. Phys. Lett.* **2004**, *383*, 47−52.
(20) Gossens, C.; Tavernelli, I.; Rothlisberger, U. *Chimia* **2005**, *59*, 81−84.
(21) Gossens, C.; Tavernelli, I.; Rothlisberger, U. *J. Chem. Theory Comp.* **2007**, *3*, 1212−1222.
(22) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995−2001.
(23) Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. *J. Comput. Chem.* **2003**, *24*, 669−681.
(24) Namazian, M.; Halvani, S.; Noorbala, M. R. *THEOCHEM* **2004**, *711*, 13−18.
(25) Daigle, D. J.; Pepperman, A. B., Jr.; Vail, S. L. *J. Heterocycl. Chem.* **1974**, *11*, 407−8.
(26) Daigle, D. J. *Inorg. Synth.* **1998**, *32*, 40−45.
(27) Bennett, M. A.; Smith, A. K. *J. Chem. Soc., Dalton Trans.* **1974**, 233−41.
(28) Dyson, P. J.; McIndoe, J. S. *Inorg. Chim. Acta* **2003**, *354*, 68−74.

Table 1. p*K*$_a$ Values of Investigated Reference Systems[a]

	charge[d]	p*K*$_a$ (calc)	p*K*$_a$ (expt)
pta/{*N*H}$^+$	0	6.9	5.6−6.1[1]
pta/{*P*H}$^+$	0	−2.6[e]	n.a.
pta(*N*H)$^+$/{*N*H}$^{2+}$	+1	−6.3[e]	n.a.
urotropin/{*N*H}$^+$	0	6.4	4.9[31]
pta═O/{*N*H}$^+$	0	2.7	2.5[31]
pta═O/{*O*H}$^+$	0	−6.1[e]	n.a.
[Ru(η^5-C$_5$H$_5$)(H)(pta)$_2$][b]	0	6.2	5.9 < X < 6.5[37]
[*trans*-Ru(OH$_2$)$_3$(OH)(pta)$_2$]$^+$ [b]	+1	4.3	4 < X < 6[32]
[*trans*-Ru(OH$_2$)$_3$(OH)(pta)(pta-H)]$^{2+}$ [c]	+2	4.7	4 < X < 6[32]
[Ru(η^6-benzene)(en)(OH)]$^+$ [c]	+1	6.2	7.7[38]

[a] The conjugated base of the corresponding base/acid pair is listed. [b] Protonation of pta nitrogen atom. [c] Protonation of hydroxo ligand. [d]Charge of indicated base. [e]Values like these merely indicate that the protonation does not occur or only under special/hypothetic conditions.

Table 2. Gas-Phase Dipole Moment and Calculated and Experimental Solution p*K*$_a$ Values of Neutral pta-Containing Complexes[a]

	dipole	p*K*$_a$ (calc)	p*K*$_a$ (expt)
Ru(η^6-benzene)(pta)(F)$_2$	5.88	3.1	n.a.
Ru(η^6-benzene)(pta)(Cl)$_2$	7.41	2.8	3.2[4]
Ru(η^6-benzene)(pta)(Br)$_2$	7.50	2.7	n.a.
Ru(η^6-benzene)(pta)(I)$_2$	7.86	2.5	n.a.
Ru(η^6-benzene)(pta)(CN)$_2$	9.96	0.8	n.a.
Ru(η^6-benzene)(pta)(OH)$_2$ [b]	3.08	16.1	n.a.
Ru(η^6-benzene)(pta)(oxalo)	12.76	2.7	2.4[c 46]
Ru(η^6-*p*-cymene)(pta)(Cl)$_2$	7.49	2.1	3.1[4]/6.5[2]
Ru(η^6-1,3,5-trifluorobenzene)(pta)(Cl)$_2$	6.65	1.9	n.a.
Ru(η^6-benzene-1,3,5-triamine)(pta)(Cl)$_2$	6.59	5.0	n.a.
Os(η^6-benzene)(pta)(Cl)$_2$	7.19	1.5	n.a.
Os(η^6-*p*-cymene)(pta)(Cl)$_2$	7.25	2.5	n.a.

[a] Dipole in debye. [b] Protonation of hydroxo ligand instead of pta nitrogen atom. [c] Arene = *p*-cymene.

a high pH. At any pH, the observed chemical shift is a weighted average of the two extreme values, $\delta(HA^+)$ and $\delta(A)$:

$$\delta_{av} = \frac{\delta(HA^+)[HA^+] + \delta(A)[A^+]}{[HA^+] + [A^+]}$$

The midpoint of the titration occurs when the concentrations of the acid and its conjugate base are equal: $[HA^+] = [A]$, that is, when the pH equals the p*K*$_a$ of the compound. The pH at the midpoint of the curve is corrected by subtracting 0.44[29] from the pD values or by using the formula proposed by Martin[30] (see below), since the measurements were made in D_2O.

$$pK_a(H_2O) = \frac{pK_a(D_2O) - 0.45}{1.015}$$

The final pH values are given as an average of the two values calculated by these two methods.

Results and Discussion

Validation of the Model. The computational scheme has been validated first on the isolated pta ligand and two of its derivatives. These organic molecules, for which experimental p*K*$_a$ values have been published, still resemble those for which the applied solvation method is known to provide good results.[10,13,24] However, their cage-like structure represents a first computational challenge. Subsequently, other ruthenium−pta compounds whose protonation properties have been discussed to some extent in the literature were also included in the initial study (see Tables 1 and 2 for the compounds included).

The isolated pta ligand offers two different types of donor atoms, namely, three nitrogen and one phosphorus atom (Figure 1). There is some disagreement among the experimentally determined p*K*$_a$ values of pta: 5.63,[4] 5.70,[31] 5.89,[32] 6.0,[33] and 6.07.[32] Crystal structures, in addition to spectroscopic methods, have shown the pta nitrogen atoms to be the protonation site.[1,34,35] We calculated an absolute p*K*$_a$ value of 6.9 for this protonation site, which indicates the quality that can be expected for the computation of absolute p*K*$_a$ values in this study. Our results (Table 1) show why the phosphorus atom of pta (calculated hypothetical p*K*$_a$ of −2.6) is not protonated in aqueous solution. The electrostatic potential (ESP) is much more negative at the nitrogen sites than at those of phosphorus (Figure 2). The latter site is therefore significantly less attractive for a proton, and a subsequent (second) protonation would also rather take place at a nitrogen atom than at a phosphorus atom (Table S1). The ESP at the protonated nitrogen site becomes highly positive, as is apparent from an inspection of Figure 2. As can be seen from a calculated hypothetical p*K*$_a$ of −6.3, the second protonation is unlikely to occur in aqueous solution. Both

Table 3. Gas-Phase Dipole Moment and p*K*$_a$ Values of Monohydroxo Complexes Containing Different Halogen Ligands and Arene Ligands[a]

	dipole	p*K*$_a$ (calc)
Ru(η^6-benzene)F(OH)(pta)	4.72	9.8
Ru(η^6-benzene)Cl(OH)(pta)	5.48	9.2
Ru(η^6-benzene)I(OH)(pta)	5.91	8.9
Ru(η^6-1,3,5-trifluorobenzene)Cl(OH)(pta)	4.81	5.1

[a] Dipole in debye. Protonation of hydroxo ligand instead of pta nitrogen atom.

(29) Mikkelsen, K.; Nielsen, S. O. *J. Phys. Chem.* **1960**, *64*, 632−637.
(30) Troullier, N.; Martins, J. L. *Phys. Rev. B* **1991**, *43*, 1993−2006.
(31) Darensbourg, D. J.; Robertson, J. B.; Larkins, D. L.; Reibenspies, J. H. *Inorg. Chem.* **1999**, *38*, 2473−2481.
(32) Kovacs, J.; Joo, F.; Benyei, A. C.; Laurenczy, G. *Dalton Trans.* **2004**, 2336−2340.
(33) Fisher, K. J.; Alyea, E. C.; Shahnazarian, N. *Phosphorus Sulfur Silicon Relat. Elem.* **1990**, *48*, 37−40.
(34) Saysell, D. M.; Borman, C. D.; Kwak, C.-H.; Sykes, A. G. *Inorg. Chem.* **1996**, *35*, 173−8.
(35) Darensbourg, D. J.; Joo, F.; Kannisto, M.; Katho, A.; Reibenspies, J. H.; Daigle, D. J. *Inorg. Chem.* **1994**, *33*, 200−8.

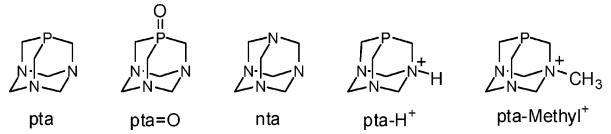


Figure 1. Structures of pta and its investigated derivatives.

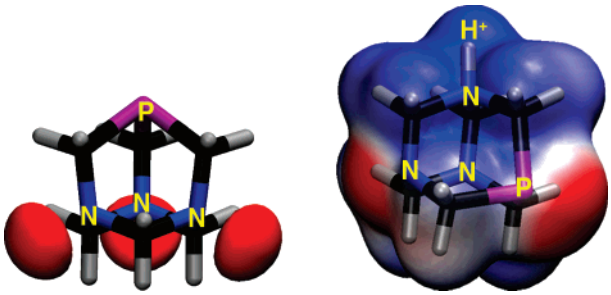


Figure 2. Negative ESP phase (red; isovalue −0.048 au) of pta (left) and ESP of N-protonated pta (right) mapped onto its electron density surface (isovalue 0.004 au). It is colored around the midpoint of 0.1/0.205 au in blue (positive) and red (negative).

observations are in agreement with experimental observations that show such a regioselective protonation behavior of the pta nitrogen atoms.[1,36]

Two pta derivatives have also been studied for comparison purposes. First, the closely related hexamethylenetetramine (urotropin; nta), in which the phosphorus atom is replaced by a nitrogen atom, which is known to be slightly less basic than pta.[31] This relative pK_a change amounts to 0.5 unit in our calculations compared to 0.7−1.2 pK_a units in experiments. Similarly, an oxidation of the phosphorus atom (pta═O) in pta results in even lower basicity of the nitrogen atoms.[31] Our calculated pK_a value of 2.7 captures this effect and is in good agreement with the experimentally measured value of 2.5. For this model system, the calculations are in excellent agreement with reported experimental pK_a values not only in a relative but also in an absolute sense.

Once the pta ligand is bound to a ruthenium(II) center (e.g., as in [Ru(η^6-arene)$(X)_2$(pta)]), a decrease in the nucleophilicity of the complexed pta ligand is expected compared to the isolated ligand.

Frost et al. described the effect of pH on biphasic catalytic hydrogenation using [Ru(η^5-C_5H_5)(H)$(pta)_2$], which contains a η^5-bound cyclopentadienyl ring, two pta ligands that are bound to the ruthenium via their phosphorus atom, and a hydride.[37] Although the authors did not determine the pK_a of this compound explicitly, one can conclude from their NMR study that it should be situated between 5.9 and 6.5. Our calculated value of 6.2 confirms this experimental observation. It shows that even in a neutral, hydride-containing complex, the nucleophilicity of the pta ligand is already reduced compared to the free pta ligand (6.9).

A series of ruthenium−pta compounds, $[Ru(II)(OH_x)_y(pta)_z]^{n+}$ ($x = 1-2$, $y = 1-5$, $z = 1-4$, $n = 0-2$), investigated by Joo et al. exhibited a notable protonation of the coordinated pta ligands below pH 6.5.[32] At pH 4 they obtained crystals of *trans*-$[Ru(OH_2)_4(pta\text{-}H)_2](tos)_4$ suitable for X-ray structural studies, which revealed that the nitrogen of the pta ligand is protonated. We therefore expect a protonation to occur on *trans*-$[Ru(OH_2)_4(pta)_2]^{2+}$ to form *trans*-$[Ru(OH_2)_4(pta)(pta\text{-}H)]^{3+}$. However, our calculations indicate that the species *trans*-$[Ru(OH_2)_3(OH)(pta)(pta\text{-}H)]^{2+}$ is more stable than *trans*-$[Ru(OH_2)_4(pta)_2]^{2+}$. According to our results, protonation occurs in the order $[Ru(OH_2)_3(OH)(pta)_2]^+ \rightarrow [Ru(OH_2)_3(OH)(pta)(pta\text{-}H)]^{2+} \rightarrow [Ru(OH_2)_4(pta)(pta\text{-}H)]^{3+}$. The related calculated pK_a values of 4.3 and 4.7, respectively, are within the experimental range (Table 1). As they are both within our error margin, we expect both protonation reactions to take place around a similar pH.

These results demonstrate that the acidity of the coordinated aqua ligands cannot be neglected and becomes even more relevant since the authors state that the aqua and hydroxo species afford similar ^{31}P NMR spectra, further complicating experimental measurements of the protonation states of hydrolyzed ruthenium−pta complexes.

To investigate the suitability of our pK_a calculations for ruthenium compounds containing an aqua ligand and a η^6-arene moiety, the hydrolyzed species of the monofunctional anticancer compound [Ru(η^6-benzene)(en)(OH_2)]$^{2+}$ was included in our study.[38−41] The deprotonation reaction takes place at the water ligand, and we achieved a reasonable agreement with the experimentally measured pK_a value (see Table 1).

All the above results show that our approach yields surprisingly good absolute pK_a estimations (max. ±1.5 units) combined with a reliable transferability of the method and encouraged us to extend our study to compounds of pharmacological and catalytic relevance.

Ligand Dependence of pK_a Values. Having established that the computational scheme predicts, in general, reliable pK_a values, we explored potential tuning options for the pK_a value of [Ru(η^6-benzene)$(Cl)_2$(pta)]$^{2+}$. To this end, we investigated the effect of varying the chloride ligands with other halides or pseudo-halides on the basicity of the nitrogen sites in the coordinated pta ligand. The results collected in Table 2 show that the aqueous solution pK_a of compounds containing a halide (F, Cl, Br, I) or pseudohalide (CN) decrease in the order F > Cl > Br > I > CN. For X = OH we observed the most basic properties. However, it is not a nitrogen of the pta ligand that is protonated in the dihydroxo complex. Instead the protonation of a hydroxo ligand and the resulting formation of an aqua ligand is more thermodynamically stable (see below). The calculated pK_a of 16.1 suggests that [Ru(η^6-benzene)$(OH)_2$(pta)] is not accessible under physiological conditions.

Replacing benzene with *p*-cymeme or other simple (alkyl-substituted) arenes exerts little influence on the pK_a of the pta ligand in the [Ru(η^6-arene)$(X)_2$(pta)] complexes, in agreement with experimental findings.[4] Addition of aliphatic side chains to the benzene moiety, as is the case in the *p*-cymene ligand of [Ru(η^6-*p*-cymene)$(Cl)_2$(pta)], reduces slightly the gas-phase and solution basicity of [Ru(η^6-arene)$(X)_2$(pta)] compounds. However, our calculations show that significant pK_a changes take place upon addition of electron-withdrawing groups (e.g., F lowers the pK_a) and electron-rich groups (e.g., NH_2 increases the pK_a).

It should be stressed that the calculated pK_a values refer to the nonhydrolyzed RAPTA compounds in aqueous solution, a situation that is only possible in the presence of ca. 100 mM

(36) Darensbourg, D. J.; Yarbrough, J. C.; Lewis, S. J. *Organometallics* **2003**, *22*, 2050−2056.
(37) Mebi, C. A.; Frost, B. J. *Organometallics* **2005**, *24*, 2339−2346.
(38) Wang, F.; Chen, H.; Parsons, S.; Oswald, I. D. H.; Davidson, J. E.; Sadler, P. J. *Chem.−Eur. J.* **2003**, *9*, 5810−5820.
(39) Wang, F.; Habtemariam, A.; van der Geer, E. P. L.; Fernandez, R.; Melchart, M.; Deeth, R. J.; Aird, R.; Guichard, S.; Fabbiani, F. P. A.; Lozano-Casal, P.; Oswald, I. D. H.; Jodrell, D. I.; Parsons, S.; Sadler, P. J. *Proc. Nat. Acad. Sci. U.S.A.* **2005**, *102*, 18269−18274.
(40) Morris, R. E.; Aird, R. E.; Murdoch, P. d. S.; H. Chen, J. C.; Hughes, N. D.; Parsons, S.; Perkin, A.; Boyd, G.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.* **2001**, *44*, 3616−3621.
(41) Yan Yaw, K.; Melchart, M.; Habtemariam, A.; Sadler Peter, J. *Chem. Commun.* **2005**, 4764−76.

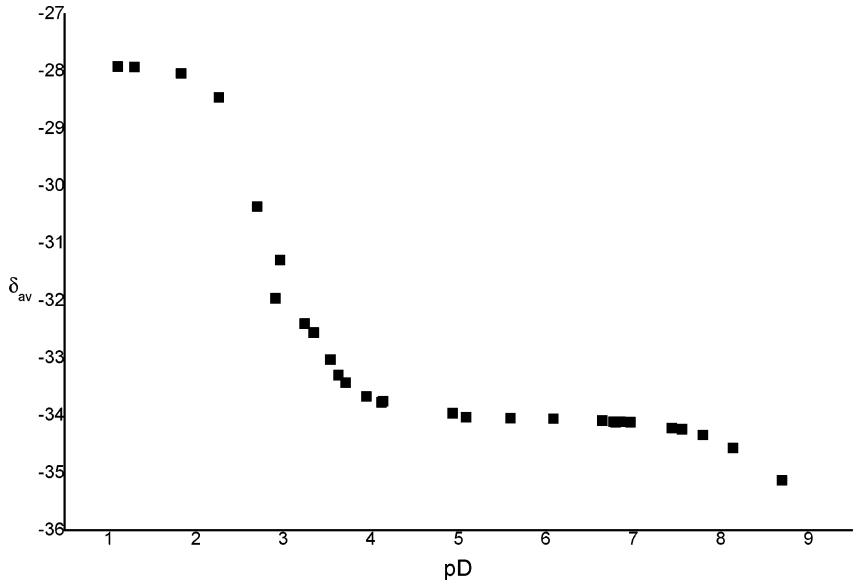


Figure 3. ^{31}P NMR chemical shift versus pD for [Ru(η^6-*p*-cymene)Cl(OH$_x$)(pta)]$^{n+}$.

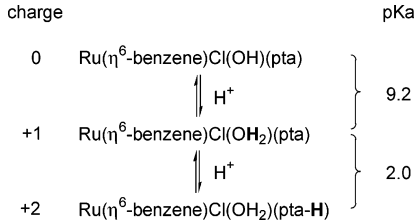


Figure 4. Calculated protonation states and pK_a values of hydrolyzed [Ru(η^6-benzene)(Cl)$_2$(pta)] after the first hydrolysis step.

chloride. Nevertheless, these calculations show that it is possible to make predictions regarding the direction of pK_a changes based on modeling as yet unsynthesized compounds, thereby facilitating the design of compounds with modified pK_a properties (see below).

Hydrolysis of [Ru(η^6-benzene)(Cl)$_2$(pta)]. The calculated pK_a of 2.8 for [Ru(η^6-benzene)(Cl)$_2$(pta)] in aqueous solution matches the experimentally estimated value of 3.2 well. While the [Ru(η^6-arene)(Cl)$_2$(pta)] compounds have the characteristic piano stool geometry, comprising the η^6-arene, pta, and two chloride ligands, once dissolved in water or once inside a cell, the actual species present probably corresponds to the monohydrolyzed species, [Ru(η^6-arene)Cl(OH$_x$)(pta)]$^{n+}$. This hypothesis is tentatively based on ^{31}P NMR spectroscopy, which indicates that [Ru(η^6-arene)Cl(OH$_x$)(pta)]$^{n+}$ is the dominant species in water containing 5 mM chloride,[8] the chloride concentration present in cells. The complete hydrolysis of the second chloride could be achieved only by addition of $AgNO_3$ (see Figure S1 in Supporting Information). However, as stated previously, it is experimentally not possible to distinguish between coordinated water and hydroxo ligands. An electrospray ionization mass spectrometry recorded on a sample of [Ru(η^6-*p*-cymene)(Cl)$_2$(pta)] under the same conditions reveals a low-intensity peak corresponding to [Ru(η^6-*p*-cymene)(OH)(pta)]$^+$, whereas the dominant peak corresponds to [Ru(η^6-*p*-cymene)-Cl(pta)]$^+$, presumably derived from [Ru(η^6-*p*-cymene)Cl(OH$_2$)-(pta)]$^+$.[42] It is probable that the OH ligand is an artifact, derived from water, and therefore these experiments are not conclusive and indicate the difficulties in characterizing such systems. Therefore it remains unclear which species are involved precisely and why only one pK_a value of [Ru(η^6-benzene)(Cl)$_2$-(pta)] was measured under hydrolysis and nonhydrolysis conditions.[4]

The first hydrolysis step involves the displacement of one chloride ligand from [Ru(η^6-benzene)(Cl)$_2$(pta)]. In principle, the resulting product may contain aqua or hydroxo and pta or pta-H$^+$ ligands. The protonation states of these ligands should vary as a function of the pH. Since ^{31}P NMR spectroscopy indicates the monohydrolyzed complex is present almost exclusively, pD measurements and calculations were made to determine the pK_a for the following transformations: [Ru(η^6-*p*-cymene)Cl(OH$_2$)(pta-H)]$^{2+}$ → [Ru(η^6-*p*-cymene)Cl(OH$_2$)-(pta)]$^+$ → [Ru(η^6-*p*-cymene)Cl(OH)(pta)] (Figure 4).

The first pK_a of 2.33 ± 0.07 for pta protonation (Figure 3; experimentally obtained after converting from pD to pH) is lower by ca. 0.8 than the one for [Ru(η^6-*p*-cymene)(Cl)$_2$(pta)], which was previously determined in the presence of 100 mM chloride in order to suppress hydrolysis.[4]

The second pK_a is of particular potential since it could be linked to the anticancer activity of [Ru(η^6-arene)(Cl)$_2$(pta)]. These compounds were shown to exhibit pH-dependent DNA-binding and DNA-damaging properties *in vitro* (below a pH of 7).[2] As for the anticancer drug cisplatin, the hydroxide adducts could be less reactive and therefore less cytotoxic than the aqua adducts.[43] In this respect, Sadler has already shown that the chloride ligand in the monofunctional ruthenium(II)−arene anticancer compounds [Ru(η^6-arene)Cl(en)] (en = ethylenediamine) studied by his group also undergoes hydrolysis. The resulting aqua ligand does not undergo deprotonation at physiological pH's, whereas the osmium analogues are converted to the hydroxo derivatives, which are inactive *in vitro*.[38,39,44]

An analogous process represents a potential path for deactivation of [Ru(η^6-arene)(Cl)$_2$(pta)] drugs by deprotonation of their hydrolyzed derivatives. The above-mentioned second pK_a of the coordinated water, however, was experimentally not observed due to side reactions of the complex at basic pD higher than

(42) It must be noted that coordinated water is usually too weakly bound to be observed by mass spectrometry. For example, the ESI-MS spectrum of [Ru(η^6-$C_{10}H_{14}$)$(OH_2)_3$][CF_3SO_3]$_2$ shows only a peak corresponding to [Ru(η^6-$C_{10}H_{14}$)(CF_3SO_3)]$^+$ in which all three aqua ligands are absent.

(43) Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J. *Chem. Commun.* **1992**, 789−91.

(44) Peacock, A. F. A.; Habtemariam, A.; Fernandez, R.; Walland, V.; Fabbiani, F. P. A.; Parsons, S.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. *J. Am. Chem. Soc.* **2006**, *128*, 1739−1748.

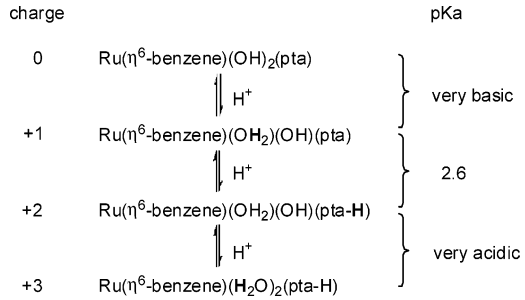


Figure 5. Calculated protonation states and pK_a values of fully hydrolyzed [Ru(η^6-benzene)(Cl)$_2$(pta)] after the second hydrolysis step.

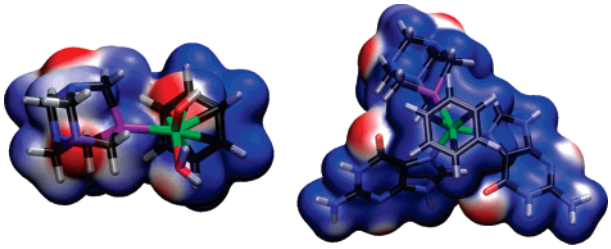


Figure 6. ESP of [Ru(η^6-benzene)(OH$_2$)(OH)(pta)]$^+$ (left) and [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ (right).

ca. 8. At least four ^{31}P NMR peaks were observed at pD 8.7. Additional characterization by ESI-MS could not clearly reveal their nature. Nevertheless, one product should result from the above-mentioned deprotonation of the aqua ligand in [Ru(η^6-*p*-cymene)Cl(OH$_2$)(pta)]$^+$ (for which we computed a pK_a value of 9.2; Figure 4). Another possible reaction is the hydrolysis of the remaining chloride due to the increased concentration of hydroxide ions in the solution.

To investigate the first hydrolysis step further, we computed the thermodynamically most stable protonation states and the corresponding pK_a values (Figure 4) for the monohydrolyzed species. At high pH, the deprotonated complex [Ru(η^6-benzene)(Cl)(OH)(pta)] is the most stable (assuming that it does not undergo any further ligand exchange reaction, e.g., with hydroxide). Lowering the pH results in protonation of the hydroxo ligand, which yields [Ru(η^6-benzene)Cl(OH$_2$)(pta)]$^+$. Finally, at even lower pH the pta ligand is protonated and [Ru(η^6-benzene)Cl(OH$_2$)(pta-H)]$^{2+}$ is formed. A pK_a of 2.0 was calculated for the protonation of the pta ligand. This pK_a value is close to the one for the unhydrolyzed complex [Ru(η^6-benzene)(Cl)$_2$(pta)], for which a pK_a of 2.8 was calculated. On the basis of these calculations, both complexes, [Ru(η^6-benzene)(Cl)$_2$(pta)] and [Ru(η^6-benzene)(Cl)(OH$_2$)(pta)]$^+$, could coexist in solution at the same time and result in the experimentally measured titration curve.[4]

At very low chloride concentration or high concentration of hydroxide ions, a second hydrolysis step may take place in which the remaining chloro ligand is replaced.[4] Again, the resulting product depends on the pH of the solution. We identified four thermodynamic stable protonation states among all possible hydrolysis products (Figure 5). The fully deprotonated complex [Ru(η^6-benzene)(OH)$_2$(pta)] may exist only at very high hydroxide concentrations (pK_a of 16). At lower pH, the pta is not protonated first; instead one of the hydroxo ligands is protonated. The resulting [Ru(η^6-benzene)(OH$_2$)(OH)(pta)]$^+$ complex is stable over a wide pH range. Its ESP shows a significant difference between the hydroxide and aqua ligand sites (Figure 6). Therefore, a nucleophile (e.g., guanine) attacking the ruthenium center should prefer the aqua site over the hydroxide site of the complex, in agreement with Sadlers' observations.[38,44] The calculated pK_a of 2.6 suggests protonation in acidic solution, and at this reduced pH it is the pta that attracts a proton more than the remaining hydroxo ligand; thus [Ru(η^6-benzene)(OH$_2$)(OH)(pta-H)]$^{2+}$ is formed. Finally, at very acidic conditions calculations indicate that the remaining hydroxo ligand is protonated to yield [Ru(η^6-benzene)(OH$_2$)$_2$(pta-H)]$^{3+}$. However, the calculated pK_a value of -1 indicates that this protonation is very unlikely to occur under ambient conditions.

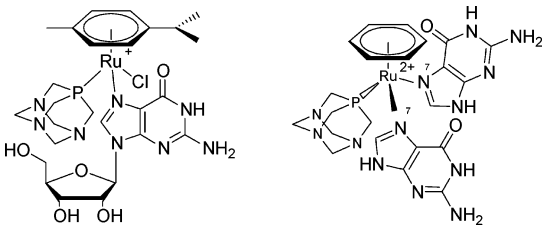


Figure 7. Guanine complexes [Ru(η^6-*p*-cymene)Cl(N7{guanosine})(pta)]$^+$ (left) and [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ (right).

The computed pK_a of 2.6 for the protonation of [Ru(η^6-benzene)(OH$_2$)(OH)(pta)]$^+$ to form [Ru(η^6-benzene)(OH$_2$)(OH)(pta-H)]$^{2+}$ is in the range of the calculated pK_a of 2.8 for the unhydrolyzed complex [Ru(η^6-benzene)(Cl)$_2$(pta)]. This estimate is in excellent agreement with experimental observations, which determined a pK_a of 3.2 for [Ru(η^6-benzene)(Cl)$_2$(pta)] under both hydrolysis and nonhydrolysis conditions,[4] although the experiment could not clarify exactly which hydrolyzed species was measured. Our calculations suggests an interpretation in which both protonation reactions could occur simultaneously, giving rise to only one "averaged" shift of the ^{31}P NMR signal as observed.

The pK_a calculations identified three different protonation reactions that can occur in the same pK_a range between 2.0 and 2.8. In all three reactions it is the pta ligand that is protonated: (i) in the unhydrolyzed [Ru(η^6-benzene)(Cl)$_2$(pta)] at a pK_a of 2.8, (ii) in the partially hydrolyzed [Ru(η^6-benzene)Cl(OH$_2$)(pta)]$^+$ at a pK_a of 2.0, and (iii) in the fully hydrolyzed [Ru(η^6-benzene)(OH$_2$)(OH)(pta)]$^+$ at a pK_a of 2.6. Due to inherent inaccuracies of the computational method applied, these small differences should not be overestimated. Since titration experiments monitored by ^{31}P NMR spectroscopy were not able to discriminate between protonation of the pta ligand under hydrolysis and nonhydrolysis conditions,[4] it is possible that the pK_a's of the three above-mentioned complexes are so similar that they cannot be differentiated. The calculations also suggest that it should be possible to stabilize a desired hydrolysis product at a certain pH by selecting the appropriate co-ligands, which could be important in catalytic or biological applications.

pK_a of [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$. In the context of the pharmacological properties of these types of compounds it was speculated that the low pK_a for pta protonation of the [Ru(η^6-arene)(Cl)$_2$(pta)] compounds might increase once they are bound to DNA.[4] The N7 atom of guanine is a known target for such ruthenium arene compounds,[3,45] and the pK_a of the pta ligand in [Ru(η^6-*p*-cymene)Cl(N7{guanosine})(pta)]$^+$ was experimentally determined by ^{31}P NMR spectroscopy in D$_2$O as 2.5 (see Figure 7 and S2 in the Supporting Information).

The pK_a for the protonation of the pta ligand in compounds in which one or two guanine molecules bind to the ruthenium

(45) Chen, H.; Parkinson, J. A.; Morris, R. E.; Sadler, P. J. *J. Am. Chem. Soc.* **2003**, *125*, 173−186.
(46) Ang, W. H.; Daldini, E.; Scolaro, C.; Scopelliti, R.; Juillerat-Jeannerat, L.; Dyson, P. J. *Inorg. Chem.* **2006**, *45*, 9006−9013.

center via the N7 atom has also been calculated. The ESP of [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ shows potential protonation sites at the pta nitrogen atoms and at the guanine O6 and N3 atoms (Figure 6). The computed pK_a value of −1.2 signifies that [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ is one of the most acidic compounds in the study. Consequently, a protonation of the coordinated pta should not occur under physiological conditions once the compound is bound to two guanine bases. A similar result is expected for DNA in general, as long as the RAPTA scaffold remains unchanged. The environment for the [Ru(η^6-benzene)(pta)]$^{2+}$ moiety bound to helical DNA is different from that of [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ in aqueous solution; however, the polarity is not likely to be very different. In contrast, in a highly apolar environment, the positive charge of the [Ru(η^6-benzene)(pta)]$^{2+}$ moiety is no longer screened. Consequently, the pK_a difference between the dication [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ and a neutral RAPTA complex, e.g., [Ru(η^6-benzene)(Cl)$_2$(pta)], approaches the gas-phase limit. For instance, a hypothetical "pK_a" of −48.5 was computed for [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$ in benzene ($\epsilon = 2.247$). Therefore in a less polar environment protonation is even less likely. For [Ru(η^6-benzene)Cl(N7{guanine})(pta)]$^+$, a higher pK_a value of 1.9 is predicted, which is in good agreement with the above-mentioned experimental value of 2.5 for [Ru(η^6-*p*-cymene)Cl(N7{guanosine})(pta)]$^+$. Nevertheless, the diguanine complex is a better model for a biological system, as it has been shown in previous studies that [Ru(η^6-arene)(Cl)$_2$(pta)] compounds bind preferentially to two nucleobase bases in an oligonucleotide.[3]

The hypothetical complex [Ru(η^6-benzene)(NH$_3$)$_2$(pta)]$^{2+}$ has been studied for comparison purposes. This compound contains two ruthenium−nitrogen bonds and carries a charge of +2 and therefore resembles [Ru(η^6-benzene)(N7{guanine})$_2$(pta)]$^{2+}$. The complex [Ru(η^6-benzene)(NH$_3$)(NH$_2$)(pta-H)]$^{2+}$ is thermodynamically less stable than [Ru(η^6-benzene)(NH$_3$)$_2$(pta)]$^{2+}$, and therefore protonation occurs on the pta ligand. The corresponding pK_a of −0.7 is similar to the one calculated for the diguanine complex and indicates that protonation occurs only under highly acidic conditions. In the gas phase, the diamine complex is even significantly more acidic than the diguanine complex. Again, this shows the importance of the solvation contribution. Nevertheless, these two examples show that aromatic and aliphatic nitrogen donors reduce the pK_a of the pta moiety relative to halide, aqua, or hydroxo ligands.

Basicity of Methylated pta Compounds. The N-methylated pta ligand (Figure 1) was recently used to model the antitumor activity of [Ru(η^6-arene)(Cl)$_2$(pta)] compounds in the sense that it mimics a pH-independent N-protonated pta ligand. Previously we showed that N-protonated and N-methylated η^6-arene−ruthenium−pta derivatives have nearly identical ligand binding energies.[3] Also, our pK_a calculations (Table S1) show very close similarities between both pta derivatives. Both, N-protonated pta and N-methylated pta (predicted pK_a of −6 and −7, respectively) are extremely acidic and, in agreement with experiments, should not be susceptible to further protonation. The same holds for these ligands when bound to the [Ru(η^6-benzene)(Cl)$_2$] moiety, since the predicted pK_a values of the resulting compounds are even lower (−11 and −12, respectively). Although the reported absolute values should not be overestimated due to the employed neutral water model, they indicate that the N-protonated pta and the N-methylated pta are very acidic and should not be susceptible to protonation in aqueous solution. These results support the use of N-methylated pta ligands for the pH-independent study of pta ligands with respect to their basicity. It should be noted that in the gas phase the pta derivatives become more basic once they are coordinated to ruthenium. In contrast, a lower pK_a was calculated for coordinated pta derivatives in solution indicating a higher acidity.

Predictions and Concluding Remarks. The calculations and experimental work described above suggest that the pH-dependent DNA binding of the [Ru(η^6-arene)(X)$_2$(pta)] compounds below a pH of ∼7 is not due to protonation of the pta ligand, at least while the arene remains coordinated to the ruthenium(II) center. Among the hydrolyzed ruthenium compounds the pair [Ru(η^6-benzene)Cl(OH)(pta)] ↔ [Ru(η^6-benzene)Cl(OH$_2$)(pta)]$^+$ with a calculated pK_a around 9 is the best candidate to explain this experimental observation. Since the aqua ligand (dominant at pH <9) is a better leaving group than the hydroxo ligand, the former should show increased reactivity with DNA.

While such a mechanism might explain the observed selectivity, the calculated pK_a of around 9 is not optimal, and a compound with a pK_a value around 7 would be more desirable. Our calculations show that the pK_a value of monohydrolyzed ruthenium complexes can be modified by exchange of the halide ligand (Table 3). However, such a strategy would not be sustainable in a biological environment since halide exchange is most likely to occur due to the high levels of chloride ions in the blood. An effective way to modulate the pK_a of the chloro-aqua complex is via modification of the arene ligand. Indeed, for a complex containing a 1,3,5-trifluorobenzene, a pK_a of 5.1 is predicted compared to the benzene analogue for which a pK_a of 9.2 was calculated (Table 3).

In summary, we have observed good agreement between experimental and calculated absolute pK_a values (±1 unit) over a series of ruthenium compounds. The precision and transferability of the method shown in this work should encourage its application to answer related questions.

Moreover, in this way it becomes possible to fine-tune the pK_a at which the deprotonation of the coordinated water ligand takes place. Our preferred method to control the pK_a of the [Ru(η^6-arene)(X)$_2$(pta)] compounds is by modifying the substituents attached to the arene ring. Calculations have guided us toward using F-substituted rings to lower the pK_a (e.g., for medicinal applications). In contrast NH$_2$-substituted rings should increase the pK_a, which is desirable for, for example, catalysis of CO_2 reduction (due to the maximum of hydrogen bicarbonate concentration at a pH of around 8.3).

Acknowledgment. Support from the Swiss National Science Foundation is gratefully acknowledged.

Supporting Information Available: ^{31}P NMR spectra, additional pK_a data, and energies of all calculated compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

OM700364S