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Complexation of Molybdenum(VI) with Bis(3-hydroxy-4-pyridinone)amino Acid Derivatives

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The molybdenum(VI) binding properties of a series of 3-hydroxy-2-methyl-4-pyridinones (3,4-HP), namely bis(3,4-HP) chelators, were investigated in solution and as solids; in particular, the complexation of Mo^{VI} with iminobis{acetyl[1-(3′-aminopropyl)-3-hydroxy-2-methyl-4-pyridinone]}, IDAPr-(3,4-HP)$_2$, and ethylenediamino-*N*,*N*′-bis(methylcarboxyl)-*N*,*N*′-bis{acetyl[1-(3′-aminopropyl)-3-hydroxy-2-methyl-4-pyridinone]}, EDTAPr(3,4-HP)$_2$, as well as three model compounds: 1,2-dimethyl-3-hydroxy-4-pyridinone (DFP), iminodiacetic acid (IDA), and ethylenediaminodiacetic acid (EDDA). Solution studies show that the complexation occurs at low pH values by the coordination modes of the hydroxypyridinone moiety, and with the formation of bischelated MoO_2^{2+} species. As the pH is increased, hydrolytic processes start with the formation of monochelated MoO_3 complexes and is followed by the total recovery of the free ligand, at pH > 8 for most of the systems, but at pH 10 for EDTAPr-(3,4-HP)$_2$. The obtained solid molybdenum complexes showed evidence for the presence of mononuclear species in solution, whereas the dinuclear species, $(MoO_2)_2$EDTAPr(3,4-HP)$_2$, were only detected for the EDTA derivative. Aqueous solution studies were performed by pH–potentiometry, spectrophotometry, ^{1}H NMR and ^{17}O NMR spectroscopy, and solid complex species were characterized by elemental analysis, IR spectroscopy, and ESI-MS.

Introduction

3-Hydroxy-4-pyridinones (3,4-HP) are compounds of considerable biological interest because of their ability to form very stable complexes with several metal ions, in particular iron(III) and group 13 metal ions. They have been identified as the main candidates for medical application, either for the control of metal toxicity situations in the body (e.g. Fe[1–3] and Al[4–6]), or for clinical diagnosis (e.g. ^{67,68}Ga, ^{111}In).[7,8] In particular, the commercially available deferiprone (2-methyl-3-hydroxy-4-pyridinone) has been used as an orally active chelating agent for the decorporation of iron from iron-overload disease situations.[2,3] As part of an ongoing project aimed at the development of new polydentate 3-hydroxy-4-pyridinone-based derivatives,[9,10] two tetradentate bis(3,4-HP)amino acid derivatives were recently obtained. They present well-balanced lipo–hydrophilic character and improvement on metal-chelating efficacy in solution and in vivo relative to the bidentate monoderivatives; thus, good perspectives of their potential clinical use in much lower doses, to minimize drug-induced toxicity, are envisaged.[11,12]

Molybdenum complexation with 3,4-HP-based ligands has emerged as an important research target. In fact, molybdenum is of great biological relevance because of its role as the cofactor component in various redox enzymes,[13] as a determinant factor for siderophore production (e.g. secretion of azotochelin by *Azotobacter vinelandii*),[14] and also as an insulin mimetic for the prevention or amelioration of cardiac dysfunction in diabetic hearts.[15] However, research on molybdenum(VI) complexation with 3,4-HP-based ligands is quite scarce and is limited to solid-state studies with monochelators,[14–16] which is in contrast to Mo complexation with other O,O-donor ligands (e.g. hydroxamate and cathecholate siderophore analogues, for which solution studies are known[14,17,18]). Furthermore, the bis(3,4-HP) ligands are expected to form stable 1:1 complexes, and so they are better suited for optimal complexation with the MoO_2^{2+} unit (with four available coordination sites) than the corresponding monoderivatives.[15,16,19] Thus, we present herein a molybdenum(VI) complexation study with two bis(3,4-HP)amino acid derivatives recently reported.[11,12] These polydentate compounds have two 3,4-HP binding groups attached to linear hydrophilic amino–carboxylic backbones (see Scheme 1), namely iminodiacetic acid (IDA) and ethylenodiaminotetraacetic acid (EDTA),

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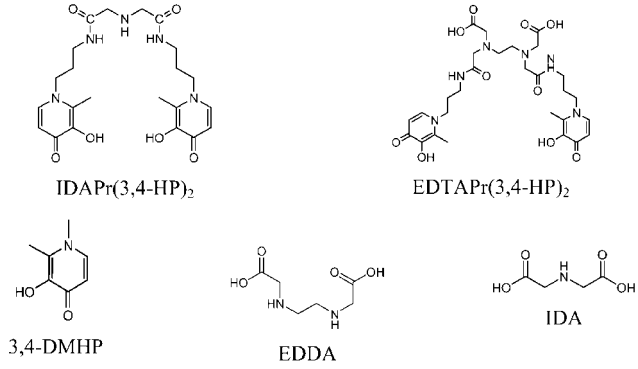
to give iminobis{acetyl[1-(3′-aminopropyl)-3-hydroxy-2-methyl-4-pyridinone]}, IDAPr(3,4-HP)$_2$, and ethylenediamino-*N*,*N*′-bis(methylcarboxyl)-*N*,*N*′-bis{acetyl[1-(3′-aminopropyl)-3-hydroxy-2-methyl-4-pyridinone]}, EDTAPr(3,4-HP)$_2$, respectively. To aid in the rationalization of the results, analogous studies are also carried out for some model compounds, namely 1,2-dimethyl-3-hydroxy-4-pyridinone (3,4-DMHP or DFP), iminodiacetic acid (IDA), and ethylenediaminodiacetic acid (EDDA). The aqueous solution study involves potentiometric and spectroscopic techniques (UV/Vis, ^{1}H and ^{17}O NMR) to evaluate the chelating affinity and the coordination modes. Several solid complexes are also characterized by elemental analysis, IR spectroscopy, and ESI-MS.



Scheme 1.

Results and Discussion

Before starting the analysis and discussion of the results, some peculiar properties of the hydrolytic species associated with the Mo^{VI}-containing systems should be noted. In aqueous solution, numerous molybdenum species are present, whereas the free metal ion does not exist. As usual, MoO_4^{2-} is chosen as the metal component (M) in the calculations. In aqueous solution above pH 7, MoO_4^{2-} predominates, whereas below pH 7 some mononuclear species can exist but polyoxomolybdates are the main species. The equilibrium model determined previously under the conditions used in the present investigation, and the calculated overall stability constants ($\log\beta$) are summarized in Table S1. All these hydrolytic species were included in the equilibrium models to assess the complex species associated to the molybdenum(VI) ligand systems because there is always competition between the hydrolytic processes and the complex formation reactions with molybdenum(VI).[20]

One of the main objectives of this work is to study the molybdenum-binding capabilities of a set of bis(hydroxypyridinone) chelators with the IDA and EDTA backbones, namely in comparison with mono(hydroxypyridinone) derivatives. Therefore, for the rationalization of the complexation results, the interaction of MoO_4^{2-} with mono(3-hydroxy-4-pyridinone), 3,4-DMHP, as well as with some parent compounds (EDDA and IDA) was also studied. The results are also compared with those previously obtained for mono- and bis(hydroxamate) ligands which proved to form very stable complexes with Mo^{VI}.[21]

Mono(3-hydroxy-4-pyridinone): 3,4-DMHP

The protonation constants of 1,2-dimethyl-3-hydroxy-4-pyridinone (3,4-DMHP, Table 1) were calculated by best-fit analysis of the pH–potentiometric curve. As expected, two protons can react with the anionic form of a 3,4-DMHP molecule, and the corresponding $\log K_i$ constants are assigned to the protonation of the enolate group (9.74) and the pyridine N atom (3.66). These are in close agreement with the values previously reported (9.77 and 3.68, respectively).[22]

Table 1. Stepwise protonation constants ($\log K_i$) of the ligands 3,4-DMHP, IDAPr(3,4-HP)$_2$, EDTAPr(3,4-HP)$_2$, EDDA, and IDA, and global stability constants ($\log\beta$) for the corresponding molybdenum(VI) complex species (I = 0.2 M KCl, T = 25 °C).

Ligand	$\log K_i$	Species	$\log\beta$
3,4-DMHP	9.749(4), 9.77[a]	$MoO_2(L)_2$	40.22(4)
(HL)	3.655(7), 3.68[a]	$MoO_3(L)$	20.0(1)
IDAPr(3,4-HP)$_2$	9.91(3), 9.94[b]		
(H_2L)	9.37(3), 9.44[b]	$MoO_2(LH)$	40.33(4)
	5.34(4), 5.42[b]	$MoO_2(L)$	36.41(7)
	3.46(5), 3.54[b]	$[MoO_3(LH_2)]$	
	3.06(6), 3.11[b]		
EDTAPr(3,4-HP)$_2$	10.12(1), 10.12[c]		
(H_4L)	9.54(8), 9.53[c]	$MoO_2(LH)$	45.02(2)
	7.05(1), 7.04[c]	$MoO_3(LH_2)$	38.31(2)
	4.02(2), 4.03[c]	$MoO_3(LH)$	30.53(3)
	3.39(1), 3.38[c]	$MoO_3(L)$	22.12(4)
	2.85(2), 2.84[c]		
EDDA	9.524(4), 9.60[d]	$MoO_2(L)$	26.74(4)
(H_2L)	6.489(6), 6.51[d]	$MoO_3(LH)$	23.38(5)
	2.169(9), 2.12[d]	$MoO_3(L)$	19.18(3)
IDA	9.17(2), 9.34[e]	$MoO_2(L)$	22.73(5)
(H_2L)	2.53(3), 2.62[e]	$MoO_3(L)$	18.15(4), 18.3[f]

[a] Ref.[22] I = 0.10 M KCl, T = 25.0 °C. [b] Ref.[11] I = 0.10 M KNO_3, T = 25.0 °C. [c] Ref.[12] I = 0.20 M KCl, T = 25.0 °C. [d] Ref.[24] I = 0.10 M KNO_3, T = 25.0 °C. [e] Ref.[25] I = 0.20 M KCl, T = 25.0 °C. [f] Ref.[26] I = 0.15 M KNO_3, T = 25.0 °C.

Under the analytical concentration conditions usually used in the pH–potentiometric measurements for the complexation studies, there was some precipitation of the Mo^{VI}–3,4-DMHP complexes and this is indicated by the dashed lines in the pH–metric titration curves (Figure 1). Therefore, pH–metric experimental data was not used to calculate the equilibrium constants for any of the Mo^{VI}–3,4-DMHP complexes formed in solution. However, as complexes containing the dioxomolybdenum core usually have intense UV bands with λ_{max} at ca. 290 nm (molar absorptivity ca. 2500 $M^{-1}cm^{-1}$),[16,20] this system could be studied by spectrophotometry. In this UV region, there are no measurable bands for the polyoxomolybdates and molybdate itself,[20] but for the ligand. Therefore, the spectral parameters (molar absorptivities, λ_{max} values) of the pro-

tonated and nonprotonated forms of 3,4-DMHP need to be independently determined. Figure 2a shows spectra recorded at several pH values for 3,4-DMHP. The individually calculated spectra (molar absorptivity versus λ_{max}) for H_2L^+, HL, and L^- are presented in the inset of this figure. The high molar absorptivities allowed the use of quite low analytical concentrations (ca. 10^{-5} M) during the UV spectroscopic measurements and so, under these conditions, precipitation did not occur in the solutions containing Mo^{VI} and 3,4-DMHP. Representative spectra recorded for this system at various pH values are shown in Figure 2b. Comparison of Figure 2a and Figure 2b shows significant differences between the spectra obtained, namely in the acidic solutions.

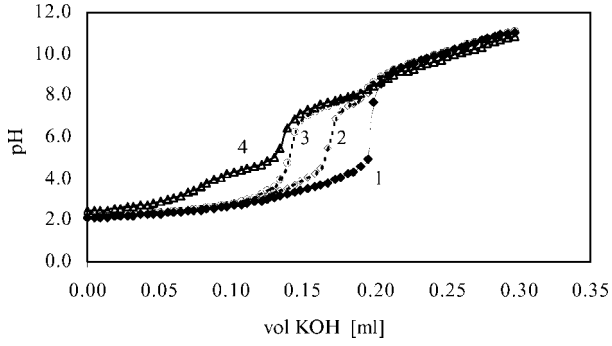


Figure 1. Potentiometric titration curves of aqueous solutions containing Mo^{VI} and 3,4-DMHP. Symbols ♦ and △ represent titration points where no precipitation occurred. 1: $C_L = 4.647 \times 10^{-3}$ M; 2: $C_L = 4.650 \times 10^{-3}$ M, $C_M = 4.633 \times 10^{-3}$ M; 3: $C_L = 4.647 \times 10^{-3}$ M, $C_M = 2.315 \times 10^{-3}$ M; 4: $C_L = 1.164 \times 10^{-3}$ M, $C_M = 4.658 \times 10^{-3}$ M.

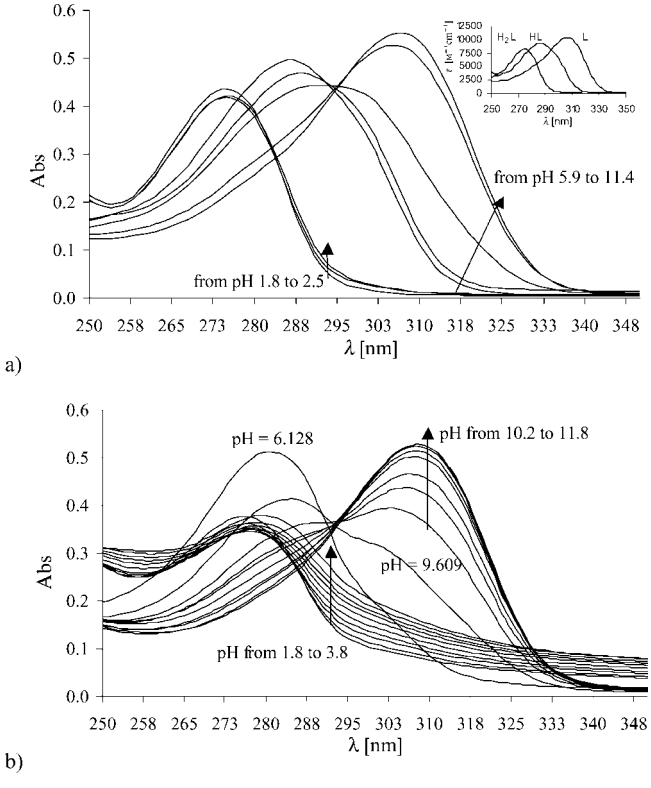


Figure 2. UV spectra for aqueous solutions containing: a) 3,4-DMHP, $C_L = 5.297 \times 10^{-5}$ M; b) Mo^{VI} and 3,4-DMHP, at indicated pH values, $C_L = 4.946 \times 10^{-5}$ M, $C_M = 2.468 \times 10^{-5}$ M. ($I = 0.2$ M (KCl), $T = 25.0$ °C). The inset in a) represents individually calculated spectra for the H_2L^+, HL, and L^- forms of the ligand.

The calculated ligand absorptivity was included in the input files of the PSEQUAD program,[23] together with the set of spectrophotometric data for the ligand in the presence of molybdenum, to fit the spectra obtained for the Mo^{VI}–3,4-DMHP system. The best fit was obtained with the equilibrium model and the stability constants shown in Table 1.

According to the results, within the experimental concentration range, the complexation of Mo^{VI} with 3,4-DMHP involves the formation of the mono- and bischelated complexes. The stability constants obtained are presented in Table 1, and they correspond to the equilibrium processes in Equations (1) and (2).

$$MoO_4^{2-} + 2\,L^- + 4H^+ \rightleftarrows [MoO_2(L)_2] + 2\,H_2O \quad (1)$$

$$MoO_4^{2-} + 2\,L^- + 2H^+ \rightleftarrows [MoO_3(L)]^- + H_2O \quad (2)$$

Analysis of the concentration distribution curves (Figure 3a) show that if the ligand-to-metal ion concentration ratio is at least 2:1, even at very low analytical concentrations ($C_{Mo} = 2.468 \times 10^{-5}$ M), the bischelated species $[MoO_2(L)_2]$ predominates below ca. pH 4. This complex starts do decompose above ca. pH 4 by formation of the monochelated species $[MoO_3(L)]^-$. Above ca. pH 8, there is no Mo^{VI}–3,4-DMHP complex in solution.

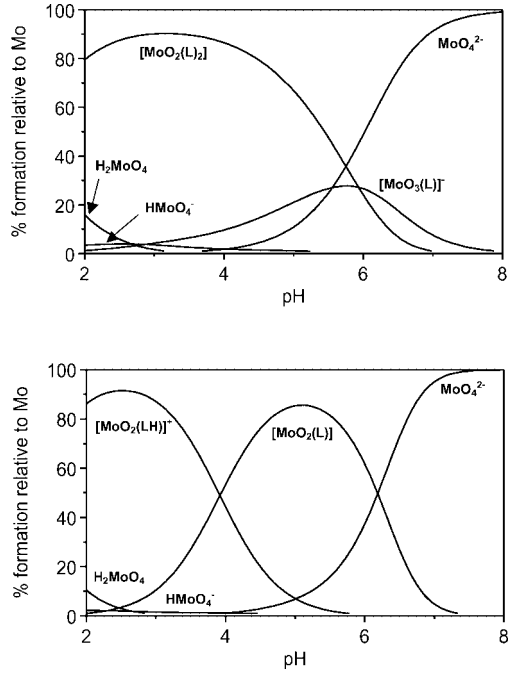


Figure 3. Concentration distribution curves of aqueous solutions containing Mo^{VI} and a) 3,4-DMHP at 2:1 ligand-to-metal molar ratio and $C_M = 2.47 \times 10^{-5}$ M; b) IDAPr(3,4-HP) at 1:1 ligand-to-metal molar ratio and $C_M = 3.52 \times 10^{-3}$ M.

Similar results were previously obtained for the complexation of Mo^{VI} with hydroxamate-based ligands in aqueous solution, where the bischelated complexes, MoO_2–(hydrox-

amate)$_2$, predominate in acidic solution (generally below pH 3), but decompose through the formation of a trioxomolybdenum-containing monohydroxamate species, MoO_3–hydroxamate, at ca. pH 8.[21]

Bis(3-hydroxy-4-pyridinone)s: IDAPr(3,4-HP)$_2$ and EDTAPr(3,4-HP)$_2$

There are three main objectives directed at the design of these bis(hydroxypyridinone)s:[11,12] (1) the improvement of the metal-binding capability, which is higher for the multichelators than the corresponding monochelators, at least in the dilute conditions that prevail under physiological conditions, (2) the reinforcement of the hydrophilic character of the ligand–metal complexes, (3) the capacity for further backbone extra-functionalization for biological interactions or pharmacokinetic modifications. Therefore, two chelating moieties are appended to hydrophilic polyaminocarboxylic acid scaffolds, IDA and EDTA. The chain size of the propylene linker was considered to be long enough to allow the wrapping of the metal moiety by the hydroxypyridinone chelating units without interference of the amino acid backbone, which could be used for further biological interactions.

Mo^{VI}–IDAPr(3,4-HP)$_2$ System

The fully protonated form of this ligand involves five dissociable protons. Although only the protonation macroconstants were determined, chemical evidence and ^{1}H NMR spectra recorded at different pH values indicate that the two pyridine N atoms are the less basic amino groups, whereas the hydroxy groups present the highest basicity. The imino N atom is much less basic in this molecule ($\log K$ = 5.34) than it is in the parent molecule, IDA ($\log K$ = 9.34[25]), which may be attributed to the amidation of the neighbor carboxylic groups. In spite of the quite hydrophilic nature of the IDA-based backbone of the molecule, the Mo^{VI} complexes with IDAPr(3,4-HP)$_2$ are not water-soluble under the usual concentrations used for pH–metric measurements. Therefore, as spectrophotometry allows the use of much lower analytical concentrations, as in the case of the Mo^{VI}–(3,4-DMHP) system, this method was used to determine the stoichiometry and stability constants of the Mo^{VI}–IDAPr(3,4-HP)$_2$ complexes. Comparison between the spectra obtained for the free ligand (Figure S1a) and the molybdenum-containing systems (Figure S1b) in the acidic pH range shows significant differences, which can be used to establish the equilibrium model and to determine the corresponding stability constants, as well as the concentration distribution curves (Figure 3b).

For the solution containing Mo^{VI} and IDAPr(3,4-HP)$_2$, the spectrum obtained at pH 9.10 corresponds to the spectrum of the free ligand (cf. Figure S1a and S1b). In the pH range 6 to 9, the spectra could not be recorded because of the absence of any buffer capacity of the solutions. By fitting all the spectra recorded in the pH range 1.7 to 5.5, the equilibrium model and overall stability constants shown in Table 1 were determined. According to this equilibrium model, two Mo^{VI} complexes are formed in the acidic pH range, which corresponds to the equilibrium processes represented by Equations (3) and (4):

$$MoO_4^{2-} + L^{2-} + 5H^+ \rightleftarrows [MoO_2(LH)]^+ + 2\ H_2O \quad (3)$$

$$MoO_4^{2-} + L^{2-} + 4H^+ \rightleftarrows [MoO_2(L)] + 2H_2O \quad (4)$$

For the species $[MoO_2(LH)]^+$, both hydroxypyridinonate moieties of a single molecule are most probably coordinated to the same dioxomolybdenum core, and the imino N atom is protonated. In $[MoO_2(L)]$, this proton is released. Comparison of the $\log\beta$ values obtained for this ligand with those of the monohydroxypyridinone, and the observed decrease in the imino N basicity upon coordination with the metal ion, agree with the proposed equilibrium model and binding mode. However, it should be emphasized that it is not possible to differentiate between processes depicted in Equations (4) and (5) as both have the same pH effect.

$$MoO_4^{2-} + L^{2-} + 4H^+ \rightleftarrows [MoO_3(LH_2)] + 2H_2O \quad (5)$$

To obtain further support for this equilibrium model, ^{1}H NMR titrations were carried out for ligand solutions in the absence, and in the presence of Mo^{VI}, to support the identification of the different species in solution as well as their pH dependence. Because the metal coordination involves the hydroxypyridinone oxygen atoms, the peaks associated with the pyridinic protons (5-H and 6-H of the hydroxypyridinone ring) are the most informative ones. Therefore, for the ^{1}H NMR titration curves those chemical shifts were registered as a function of pD* (Figure S2). Analysis of these ^{1}H NMR titration curves shows that in the range ca. pH 2 to 4 only one complex species is formed, which corresponds to the bischelated species. Above pH 4 a new species is detected, with a deshielding effect on those protons, whereas the free ligand also starts to be formed. The observation of this second complex species agrees with the hypothetical formation of the monochelated trioxo species. The decomposition of the complex is completed between pH 6 and 8. Thus, the profiles of these ^{1}H NMR titration curves support the model involving the three species suggested by the spectrophotometric results.

Mo^{VI}–EDTAPr(3,4-HP)$_2$ System

In its fully protonated form, EDTAPr(3,4-HP)$_2$ has eight dissociable protons. According to our results, two of them are released below the measurable pH region. Because the protonation microconstants were not evaluated, we cannot discuss much about the acid–base properties of the individual groups. However, on the basis of chemical evidence,[12,14] it is possible to conclude that the two hydroxy groups are the most basic groups, and that the value $\log K$ = 7.04 should be mostly attributed to the deprotonation of one of the ethylenediamine N atoms.

The EDTA derivative has two hydroxypyridinonate arms, as with IDAPr(3,4-HP)$_2$, but it also contains an EDDA moiety that may offer an alternative coordination mode (at least theoretically) for several metal ions. To compare the molybdenum(VI) binding ability of the two "multichelating parts" of the EDTAPr(3,4-HP)$_2$ molecule, studies of the Mo^{VI}–ethylenediaminodiacetic acid (EDDA) system were also carried out, and calculations were made for the hypothetical ternary system Mo^{VI} –EDDA–(3,4-DMHP).

The potentiometric titrations of the Mo^{VI}–EDDA system were carried out without precipitation problems (some titration curves are included in the Supporting Information, Figure S3). These titration curves could be fitted well with an equilibrium model including the species $[MoO_2(L)]$, $[MoO_3(LH)]^-$, and $[MoO_3(L)]^{2-}$, whose stability constants are shown in Table 1. The concentration distribution curves calculated for the Mo^{VI}–EDDA complex species (Figure S4), under the concentration conditions used herein, indicate that an ethylenediaminodiacetate ligand cannot effectively compete with the Mo^{VI} hydrolytic processes. At 1:1 metal-to-ligand molar ratio and at an analytical concentration of ca. 4×10^{-5} M of the components, the maximum relative amount of the metal complex found is 20% at pH ca. 2. By increasing the pH, the Mo^{VI} hydrolytic species becomes more and more dominant.

Therefore, the results obtained for the Mo^{VI}–EDDA system indicate that, for EDTAPr(3,4-HP)$_2$, only the hydroxypyridinonate arms are the effective binding sites for Mo^{VI}. The preference of the hydroxypyridinone over the EDDA coordination mode can also be clearly illustrated by the concentration distribution curves calculated for the hypothetical ternary system Mo^{VI}–EDDA–(3,4-DMHP) at 1:1:2 molar ratio (Figure S5). According to this speciation curves, the percentage of Mo^{VI}–EDDA complexes formed do not exceed 5–6% of total Mo^{VI}. Consequently, if there is no excess of the metal in solution, we expect simple hydroxypyridinonate-type coordination of EDTAPr(3,4-HP)$_2$ towards Mo^{VI}. Because the formation of dinuclear species can be expected under conditions where excess metal is present, some studies were also performed at a ligand-to-metal ion molar ratio of 1:2.

As the solubility of the complexes formed in the Mo^{VI}–EDTAPr(3,4-HP)$_2$ system are slightly higher than those with the former hydroxypyridinonate derivatives, it was possible to titrate some samples without precipitation (see titration curves in Figure 4).

Besides the pH–potentiometric titrations, spectrophotometric measurements were also performed, and the spectra of the free ligand and the Mo^{VI}–ligand system were recorded at several pH values. (Figure S6).

The best-fit for both the pH–potentiometric and spectrophotometric data was obtained with the equilibrium model and the stability constants shown in Table 1. The corresponding concentration distribution curves are shown in Figure 5.

By taking also into account the results obtained for the model ligands, EDDA and 3,4-DMHP, the following assumptions can be made regarding the equilibrium processes

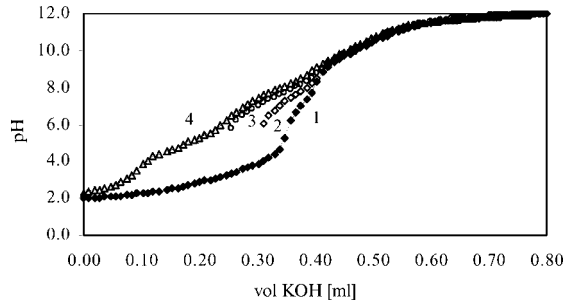


Figure 4. Potentiometric titration curves of aqueous solutions containing Mo^{VI} and EDTAPr(3,4-HP)$_2$: 1: $C_L = 3.523\times10^{-3}$ M; 2: $C_L = 3.451\times10^{-3}$ M, $C_M = 1.654\times10^{-3}$ M; 3: $C_L = 3.523\times10^{-3}$ M, $C_M = 3.355\times10^{-3}$ M; 4: $C_L = 2.864\times10^{-3}$ M, $C_M = 5.732\times10^{-3}$ M.

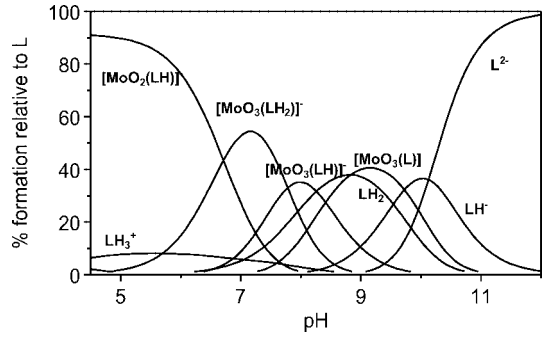


Figure 5. Concentration distribution curves for the complexes formed in Mo^{VI}–EDTAPr(3,4-HP)$_2$ system for solutions containing $C_L = 9.175\times10^{-3}$ M and $C_M = 8.39\times10^{-3}$ M.

and the possible bonding modes of EDTAPr(3,4-HP)$_2$: in the acidic pH range, the complex species $[MoO_2(LH)]^-$ predominates, in which the coordination of the two hydroxypyridinone moieties to the dioxomolybdenum core is the most plausible, while one of the ethylenediamine N atoms remains protonated. The corresponding equilibrium constant in Table 1 corresponds to the process shown in Equation (6):

$$MoO_4^{2-} + L^{2-} + 5H^+ \rightleftarrows [MoO_2(LH)]^- + 2H_2O \quad (6)$$

As the pH is increased, two types of processes can occur: (1) $[MoO_2(L)]^{2-}$ can be formed by the deprotonation of the noncoordinating ethylenediamine N atom,

$$[MoO_2(LH)]^- \rightleftarrows [MoO_2(L)]^{2-} + H^+ \quad (7a)$$

the calculated stability constant corresponding to Equation (7):

$$(MoO_4^{2-} + L^{2-} + 4H^+ \rightleftarrows [MoO_2(L)]^{2-} + 2H_2O) \quad (7)$$

(2) Partial hydrolysis of the complex may occur through formation of a monochelated complex with a trioxomolybdenum core, which has the noncoordinating amino and hydroxy groups in the protonated form.

$$[MoO_2(LH)]^- + H_2O \rightleftarrows [MoO_3(LH_2)]^- + H^+ \quad (8a)$$

In such a hypothesis, the stability constant in Table 1 would correspond to Equation (8):

$$(MoO_4^{2-} + L^{2-} + 4H^+ \rightleftarrows [MoO_3(LH_2)]^- + H_2O) \quad (8)$$

As the formation of $[MoO_2(L)]^{2-}$ and $[MoO_3(LH_2)]^-$ via **8a** and **7a**, respectively, have the same pH effect, they cannot be distinguished by pH-potentiometry. However, analyzing the UV/Vis spectra, we concluded that the best fit of the spectra could be obtained if the latter species was involved in the model. At ca. pH 5.5, there is a change in the UV/Vis spectra, which indicates that free hydroxypyridinone groups start to be present in the system, thus supporting the formation of MoO_3-type complexes in which one of the hydroxypyridinone groups is not coordinated. Further increase in the pH leads to the stepwise deprotonation of the noncoordinated groups in $[MoO_3(LH_2)]^-$ (Equations 9a and 10a), with the final formation of the molybdate anion by the complete decomposition of the metal complexes [Equation (11)].

$$[MoO_3(LH_2)]^- \rightleftarrows [MoO_3(LH)]^{2-} + H^+ \quad (9a)$$

$$(MoO_4^{2-} + L^{2-} + 3H^+ \rightleftarrows [MoO_3(LH)]^- + H_2O) \quad (9)$$

$$[MoO_3(LH)]^{2-} \rightleftarrows [MoO_3(L)]^{3-} + H^+ \quad (10a)$$

$$(MoO_4^{2-} + L^{2-} + 2H^+ \rightleftarrows [MoO_3(L)]^{2-} + H_2O) \quad (10)$$

$$[MoO_3(LH)]^{2-} + H_2O \rightleftarrows MoO_4^{2-} + LH_2 + H^+ \quad (11)$$

Because these solution equilibriums are quite complex and different isomers may correspond to the same stoichiometry, ^1H NMR titrations were also carried out to obtain additional information. These studies were performed for solutions containing free ligand, $EDTAPr(3,4\text{-}HP)_2$, and the Mo^{VI}–$EDTAPr(3,4\text{-}HP)_2$ system, under experimental conditions as close as possible to those used in the potentiometric measurements. The set of results is depicted in Figure 6.

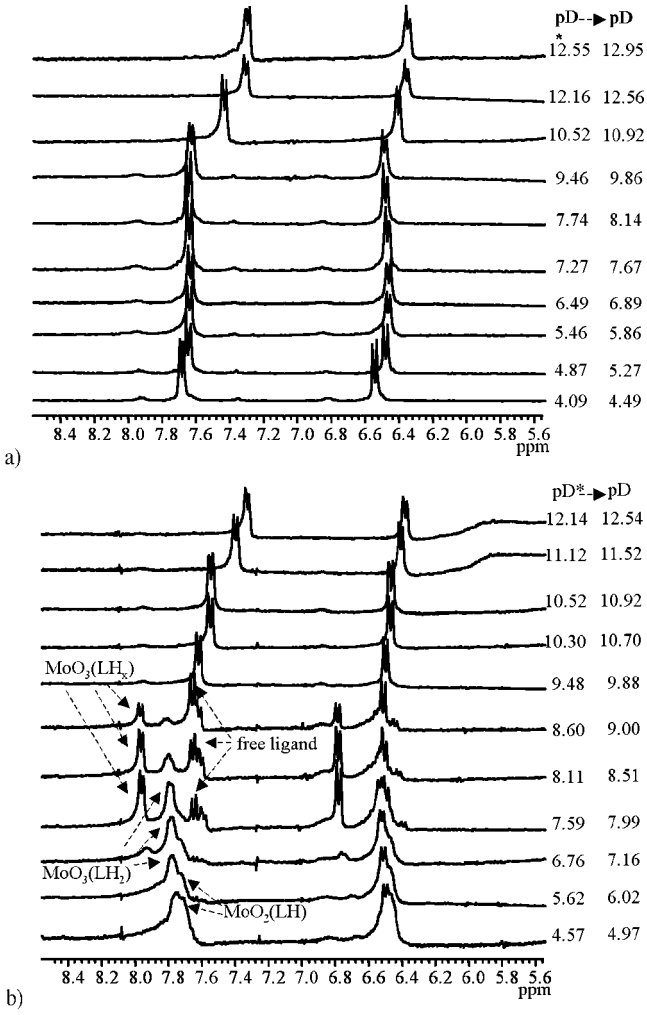


Figure 6. ^1H NMR spectra, at indicated pD* and pD, corresponding to the 5-H and 6-H peaks of the hydroxypyridinone ring of: a) solution containing the free ligand $EDTAPr(3,4\text{-}HP)_2$ $C_L = 8.73 \times 10^{-3}$ M; b) solution containing Mo^{VI} and $EDTAPr(3,4\text{-}HP)_2$ with $C_L = 9.175 \times 10^{-3}$ M and $C_M = 8.39 \times 10^{-3}$ M.

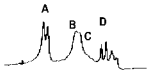
Analysis of the ^1H NMR spectra obtained for the Mo^{VI}–$EDTAPr(3,4\text{-}HP)_2$ system and their comparison with the corresponding free ligand spectra led to the following conclusions: (1) at pH 4, there is only one complex species, (2) at pH ca. 5, another one starts to form, (3) at pH ca. 7, a third distinct complex species appears. These three species are completely hydrolyzed at pH > 9, with the formation of the free ligand. The ligand band is split between pH 6 and 9, thus indicating the existence of two types of "free ligand" hydroxypyridinone moieties in this pH range. One of them should correspond to a noncoordinated hydroxypyridinone and the other one to a species with only one of the noncoordinated arms.

With the use of the Mestre C program,[27] deconvolution of some spectra and a comparative analysis of the relative intensities of the individual peaks and their pH dependence with the species distribution curves enabled the peak attribution to the species present in solution. Table 2 includes the calculated relative percentages for each peak, and the assignment of the corresponding species. We can conclude that there is a good fit between the values obtained for the concentration distribution species on the basis of the deconvolution of the ^1H NMR spectroscopic signals and on the potentiometric results.

To aid in the rationalization of the present results, ^{17}O NMR spectroscopic studies were also performed because the chemical shifts present high-dependence on the number and type of oxo ligands bonded to the molybdenum cores. The chemical shift of MoO_4^{2-} is ca. 532 ppm; *cis* MoO_3 appears at lower field (ca. 700 ppm) followed by the *cis* MoO_2^{2+} core at ca. 900 ppm.[17,19] Therefore, to obtain further evidence for the proposed equilibrium model of the Mo^{VI}–$EDTAPr(3,4\text{-}HP)_2$ system in aqueous solution, two ^{17}O NMR titrations were carried out at 1:1 and 1:2 ligand-to-metal molar ratios. This is shown in Figure S7.

A distinct peak appears at δ = 532 ppm, which corresponds to MoO_4^{2-}; at ca. 680 ppm, the MoO_3^- moiety can be identified between pH 7 and 9. However, it was not possible to observe any peak corresponding to the MoO_2^{2+} core. This is probably due to precipitation and/or some fast chemical exchange between the oxygen atoms involved in the coordination, as a result of the intramolecular exchange between the different bonding isomers formed, which suppresses the ^{17}O NMR spectroscopic signal.

Table 2. Calculated speciation for the hydroxypyridinone-containing species, at indicated pD*, on the basis of the integration of the ^{1}H NMR spectroscopic peaks corresponding to the 5-H and 6-H protons of the hydroxypyridinone ring. The values in parentheses represent the corresponding data obtained from the potentiometric results under the same conditions ($C_L = 9.175 \times 10^{-3}$ M, $C_{Mo}^{VI} = 8.39 \times 10^{-3}$ M).

Peaks 	pD*=8.60	pD*=8.11	pD*=7.59	pD*=6.76	pD*=4.57	Attributions
A	45 (40)	58 (55)	57 (51)	16 (13)	--	MoO_3LH_x, x=0,1
B	5 (10)	10 (4)	18 (21)	54 (55)	0 (2)	MoO_3LH_2
C	--	--	--	30 (20)	100 (90)	MoO_2LH
D	50 (47)	32 (37)	25 (28)	0 (12)	0 (8)	Free ligand

By taking into account the molecular structure of EDTAPr(3,4-HP)$_2$, formation of dinuclear species could be expected for solutions containing this ligand and excess of Mo^{VI}, with the metal centers coordinated either by the hydroxypyridinone groups or the "EDDA skeleton". However, from the experimental data obtained in aqueous solution, calculations with the PSEQUAD program[23] were not successful for all the equilibrium models in which those species were included. This means that, mathematically, it was not possible to obtain any logβ for the dimeric species, thus suggesting a low probability for the existence of dinuclear species in our solution conditions. Then again, if we consider the results obtained in the ^{1}H NMR titration, and if the aliphatic part of the spectra is carefully compared (see Figure S8), there is evidence that, at high pH, the spectrum recorded for the ligand–Mo^{VI} system is the same as that of the free ligand. At pH < 6, only the bands for the protons which feel the coordination of the hydroxypyridinonate chelate (1,6) show significant broadening. However, when the bischelated dioxomolybdenum species starts to decompose, and some monochelated trioxomolybdenum species starts to be formed, the hydroxypyridinonate arms become noncoordinated and different species are suggested by the spectra with the broadening and splitting of the aliphatic peaks. This means that, probably, there is a dynamic intramolecular exchange between the different binding isomers formed, and not a real exchange between the coordination of the hydroxypyridinone and the "EDDA skeleton".

Solid State Studies

As mentioned above, for most of the systems precipitation occurred along the pH–metric titrations. Several of those solids were isolated by performing similar batch experiments, but with the use of higher concentrations of metal ion and ligand. The molybdenum solid complexes obtained were characterized by IR, elemental analysis, and ESI-MS experiments. Some relevant data of the IR results are included in Table 3. The selected IR data are representative of the differences between the free ligand and the complexes. Complex formation is confirmed by the emergence of two strong new bands characteristic of the Mo–O stretching vibrations ($\nu_{Mo=O}$, as strong peaks in the range 800–950 cm^{-1}).[28] Concerning the MoO_2^{2+} core, its characteristic stretching bands, ν_{MoO_2} (symmetric) and ν_{MoO_2} (asymmetric), appear around 920 and 890 cm^{-1}, respectively.[29] For the MoO_3 unit, the characteristic bands normally appear around 890 and 840 cm^{-1}.[30] For the hydroxypyridinone derivatives there is also a characteristic infrared four-band spectral pattern between 1610 and 1400 cm^{-1}.[16,31,32] Those bands can be assigned to $\nu_{C=O}$ and ν_{ring} that are very difficult to differentiate. Upon coordination, this pattern is usually preserved, but a bathochromic shift and a reordering of the peaks can be observed. For the solid complexes obtained, comparison with the free ligand spectra shows that the four-band pattern remains with some change in the intensity of some of the peaks, and some small shifts to lower wavenumbers.

Table 3. Infrared spectroscopic data for the hydroxypyridinone derivatives and the corresponding Mo^{VI} complexes.

Compound	Selected IR data [cm^{-1}]								
	ν_{O-H}	$\nu_{C=C} + \nu_{C=O}$				ν_{C-O}	ν_{Mo-O}		$\nu_{Mo-O-Mo}$
3,4-DMHP	3146(br. s)[a]	1631	1568	1530	1515	1234(s)	–		–
[MoO_2(3,4-DMHP)$_2$·H_2O]	–	1616	1556	1514	1495	–	919(s)	892(s)	–
IDAPr(3,4-HP)$_2$	3227(br. s)[a]	1682	1628	1560	1507	1251(s)	–		–
{Mo_3O_8[IDAPr(3,4-HP)$_2$]·3H_2O}	3412(br. s)[a]	1652	1614	1556	1496	1267(s)	918(m)	891(m)	828, 753(m)
EDTAPr(3,4-HP)$_2$	3328(br. s)[a]	1684	1634	1560	1508	1252(s)	–-		–
{(MoO_2)$_2$[EDTAPr(3,4-HP)$_2$]·7H_2O}	3410(br. s)[a]	1635		1558	1496	–	940(br. m)		899(br. s)

[a] Only one ν_{O-H} vibration band is found in the spectra thus disabling independent attributions to the hydroxypyridinone–OH or other OH groups.

For the Mo^{VI}–(3,4-DMHP)$_2$ complex, elemental analysis fits the formulation as a monohydrated complex in which two 3,4-DMHP molecules are coordinated to one MoO_2^{2+} unit [MoO_2(3,4-DMHP)$_2$·H_2O]. The IR spectrum shows a redshift of the four-band pattern corresponding to the hydroxypyridinone spectra, and two strong peaks are obtained at 919 and 892 cm^{-1}, which can be attributed to the two characteristic IR bands of the MoO_2^{2+} unit.

Concerning the Mo^{VI}–IDAPr(3,4-HP)$_2$ solid complex isolated, an entirely different structure was obtained. From the elemental analysis of the yellow solid we can conclude that a complex with one ligand and three molybdenum atoms was formed: [Mo_3O_8(IDAPr(3,4-HP)$_2$)·3H_2O]. In the IR spectra of the complex, besides the bathochromic shifts of the $\nu_{C=O}$ and ν_{ring} bands, which indicate coordination through the hydroxypyridinone groups, and the two characteristic Mo–O bands at ca. 918 and 891 cm^{-1}, two new bands appear at 828 and 753 cm^{-1} (see Table 3). By taking into account that the IR bands in the 735 to 850 cm^{-1} range have been attributed to Mo–O–Mo stretching modes,[28,33–35] identical assignment can be assumed for [Mo_3O_8(IDAPr(3,4-HP)$_2$)·3H_2O]. Furthermore, on the basis of molecular structures reported in the literature for other molybdenum complexes, such as Na_4[Mo_6O_8(EDTA)$_3$·14H_2O],[36] [$Mo_3Cu_2O_{10}$(pz)]$_n$[28] with pz = pyrazine, and [{MoO_2(MCM(3,4-HP))O}$_2$]$^{2-}$[34] with MCM(3,2-HP) = 1-[(methoxycarbonyl)methyl]-3-hydroxy-2-pyridinone, we suggest that for [Mo_3O_8(IDAPr(3,4-HP)$_2$)·3H_2O], a structure with three Mo^{VI} atoms bonded by oxygen bridges, that is a μ_3-oxo-trismolybdenum(VI) complex exists. However, because no suitable crystals were obtained for X-ray diffraction studies, such an assumption cannot be confirmed.

From solutions containing Mo^{VI} and EDTAPr(3,4-HP)$_2$, a yellow solid complex was obtained at acidic pH. Its elemental analysis indicates a dinuclear structure in which two MoO_2^{2+} units are coordinated by one ligand molecule: [$(MoO_2)_2$(EDTAPr(3,4-HP)$_2$)·7H_2O]. The IR spectra of the solid compound showed bands at 940 and 899 cm^{-1} (Table 3), which are characteristic of the ν_{Mo-O} in the MoO_2^{2+} core.

Some of the solids obtained (dissolved in water) and their mother solutions were also analyzed by ESI-MS. Some ESI mass spectra were obtained from the solutions of "batch" samples of the Mo^{VI}–IDAPr(3,4-HP)$_2$ system, at different pH conditions. One of the spectra (see Figure S9a) contains an intense peak at *m*/*z* = 590, ascribed to the monoprotonated neutral complex {[(MoO_2)(L–2 H)] + H}$^+$, in which L is IDAPr(3,4-HP)$_2$, having the hydroxy groups deprotonated and coordinated to one MoO_2^{2+} unit. The other spectrum (Figure S9b) shows two peaks at *m*/*z* = 484 and 612, which correspond to the ions [L + Na]$^+$ and {[(MoO_2)(L–2 H)] + Na}$^+$, respectively.

The mass spectrum obtained for the solutions containing Mo^{VI} and EDTAPr(3,4-HP)$_2$ (Figure S10a) presents three intense peaks at *m*/*z* = 787, 825, and 863, attributed to the ions {[(MoO_2)(L–2 H)] + K}$^+$, {[(MoO_2)(L–2 H) – H] + 2 K}$^+$, and {[(MoO_2)(L–2 H) – 2H] + 3 K}$^+$, respectively. Also in this case, [(MoO_2)(L–2 H)], in which L is EDTAPr(3,4-HP)$_2$, represents the neutral complex with the two hydroxypyridinone moieties deprotonated and coordinated to the MoO_2^{2+} core, but with the two carboxylic groups protonated. Therefore, the first species is just the neutral complex charged by a potassium cation. As the relative amount of K^+ increases with the addition of KOH to the solutions, the carboxylic groups deprotonate and the proton is substituted by K cations.

Although in the solution-phase molybdenum complexation studies with EDTAPr(3,4-HP)$_2$, dinuclear species were not found, ESI-MS data obtained for the solid material and the corresponding mother solution are in agreement with the elemental analysis results obtained for the solids. The peak at *m*/*z* = 873 presented in the spectrum of this solid (Figure S10b) is assigned to the complex ion {[$(MoO_2)_2$(L–4 H)] + H}$^+$, a dinuclear species $C_{28}H_{37}Mo_2N_6O_{14}^+$. The more intense peak at *m*/*z* = 947 corresponds to the water adduct of the cationic dinuclear complex, {[$(MoO_2)_2$(L–4 H)] + K, 2·H_2O}$^+$. The relative intensities of the isotopic peaks of the molybdenum-containing species agree, within experimental error, with the characteristic patterns resulting from two molybdenum atoms, which provide further support for the above assignments. [$(MoO_2)_2$(L–4 H)] represents the neutral complex with one MoO_2^{2+} core coordinated by the two deprotonated hydroxypyridinone groups, and another one coordinated by the "EDDA part", which involves both the deprotonated carboxylic groups of the ligand. The spectrum also shows a much less intense peak at *m*/*z* = 749 which corresponds to the protonated mononuclear species, {[(MoO_2)(L–2 H)] + H}$^+$, with the relative intensities of the isotopic peaks characteristic for one molybdenum unit. The additional peak at *m*/*z* = 805 is attributed to the water adduct of the mononuclear cationic species {[(MoO_2)(L–2 H)] + K, H_2O}$^+$.

This set of results demonstrates that structure and/or stoichiometry of predominant species in diluted solutions may differ from those of precipitated solids or corresponding mother solutions. The difference observed between solution and solid-state studies can be attributed to the low water solubility of the complexes, thus rendering difficult their detection in solution, at least under dilute experimental conditions.

Conclusions

Two bis(3-hydroxy-4-pyridinone) chelators, having IDA and EDTA backbones with two bearing 3-hydroxy-4-pyridinone chelating moieties, were shown to be able to form quite stable bischelated 1:1 MoO_2 complexes under acidic conditions. An increase in the pH of the solution to basic values leads to the stepwise hydrolysis of the complexes and subsequent formation of monochelate species and the free ligand. Although under dilute conditions only soluble mononuclear species with hydroxypyridinone coordination modes were found, increasing concentration conditions and excess amounts of metal lead to water-insoluble polynuclear

species. Moreover, it was shown that the ligand scaffold determines the tuning of some molybdenum complex properties, namely water solubility and stability to hydrolysis. As compared to the remaining mono- or bischelated systems, the Mo^{VI}–EDTAPr(3,4-HP)$_2$ system presents the most interesting behavior as a result of the EDTA scaffold. In fact, there are fewer water-insolubility problems associated with the complex, and under conditions where there is not an excess of metal, the coordination only involves the hydroxypyridinone moieties, which leaves the EDDA moiety free for further metal-ion coordination or extra-interaction with biological sites.

Experimental Section

General: Unless otherwise indicated, analytical grade reagents were used as supplied. The IDAPr(3,4-HP)$_2$ and the EDTAPr(3,4-HP)$_2$ ligands were synthesized, purified and fully characterized as described previously.[11,12]

The aqueous molybdenum(VI) stock solution was prepared from the required amount of Na_2MoO_4 (Reanal product) dissolved in bidistilled water, and the exact metal-ion concentration was checked gravimetrically by precipitation of its quinolin-8-olate complex. The concentration of each ligand and HCl stock solutions were determined by pH–potentiometry using the Gran's method.[37] For the aqueous solution studies, a MOLSPIN pH-meter, a Methrom 6.0234.100 combined electrode, and a computer-controlled Mol-AcS microburette were used to carry out the potentiometric and also some of the spectrophotometric titrations, at 25.0 ± 0.1 °C and ionic strength of 0.2 M (KCl). The electrode calibrations were made according to the Irving method,[38] and the pH readings could therefore be converted into hydrogen ion concentration. The titrant used was a carbonate-free KOH solution (0.2 M) and its concentration was also determined by potentiometry using the Gran's method.[37] For all samples, the total volume was 3 mL, the ligand concentration was around 4×10^{-3} M and the ligand-to-metal ion molar ratios between 1 and 4. Under the experimental conditions used, the value determined for the ionization constant (pK_w) was 13.76.

Spectral determinations were carried out with a HP 8453 spectrophotometer equipped with 1.0-cm-matched quartz cells at 25.0 ± 0.2 °C and I = 0.2 M KCl. For solutions containing Mo^{VI}, the spectra were recorded in the range 200 to 350 nm by using 3.00 mL samples, ligand concentrations of ca. 5×10^{-5} M and metal concentrations of $2.5–5.0 \times 10^{-5}$ M. The equilibrium models were established and the complex formation constants were calculated with the PSEQUAD computer program[23] by using the previously reported ($\log\beta$) values of the Mo^{VI} hydrolytic species.[20]

A Varian Unity 300 instrument was used to perform the ^{1}H NMR measurements. The titrations were performed at room temperature with ligand concentrations of $5–9 \times 10^{-5}$ M in D_2O with DSS, sodium 3-(trimethylsilyl)-[2,2,3,3–$^2D_4$]propionate, as the internal reference. The direct reading of the H_2O calibrated pH meter, here called pD*, was converted to pD values by using the expression pD = pD* + 0.4.[39]

For the Mo^{VI}–EDTAPr(3,4-HP)$_2$ system, ^{17}O NMR spectroscopic measurements were carried out at 1:1 and 1:2 ligand-to-metal ion ratio with $C_M = 5.0 \times 10^{-2}$ M. The samples were prepared in 82% H_2O, 10% D_2O, and 8% D_2O enriched for ^{17}O (37.8% ^{17}O and 48.8% ^{18}O). The enrichment occurs in the Mo = O oxygen atoms and usually the ligand oxygen atoms are inert towards oxygen exchange. A Bruker AM 360 was used to record the spectra, and the chemical shifts are reported from the signal of the water which is considered as the internal reference, δ = 0 ppm.

The ESI mass spectra were obtained with a LCQ Duo (Finnigan, San Jose, CA, USA) ion trap mass spectrometer equipped with an electrospray ion source, operated in the positive mode. The spray voltage was kept at ±4.5 kV. The temperature of the heated capillary was set at 220 °C. The flow rate of the electrospray solution was 5 μL min^{-1}. Other parameters, including capillary voltage, lens and octapole voltages, and sheath gas flow rate were optimized for maximum abundance of the ions of interest.

The complex [MoO_2(3,4-DMHP)$_2$] was synthesized by a method similar to that described by Griffith,[29] by mixing an ammonium molybdate (5 mL, 230 mg, 1 mmol) aqueous solution with an equivalent amount of 3,4-DMHP (140 mg) previously dissolved in water (10 mL). The yellow solution was stirred for ca. 1 h at room temperature, and the precipitate was obtained, filtered, then washed with water, methanol, and ether, and dried in vacuo. A similar procedure was used for the ligands IDAPr(3,4-HP)$_2$ and EDTAPr(3,4-HP)$_2$, and the corresponding Mo^{VI} complexes were obtained as yellow solids. [MoO_2(3,4-DMHP)$_2$.H_2O]: $C_{14}H_{18}MoN_2O_7$ (422.24): calcd. C 39.82, H 4.30, N 6.63; found C 39.8, H 4.1, N 6.8. [Mo_3O_8(IDAPr(3,4-HP)$_2$)·3H_2O]: $C_{22}H_{35}Mo_3N_5O_{17}$ (929.37): calcd. C 28.43, H 3.80, N 7.56, Mo 30.97; found C 28.2, H 3.9, N 7.7, Mo 31. [(MoO_2)$_2$(EDTAPr(3,4-HP)$_2$)·7H_2O]: $C_{28}H_{50}Mo_2N_6O_{21}$ (998.60): calcd. C 33.68, H 5.05, N 8.42; found C 33.9, H 4.6, N 8.3.

For the Mo^{VI} complexation with ligands IDAPr(3,4-HP)$_2$ and EDTAPr(3,4-HP)$_2$, "batch synthesis" procedures were also carried out. Na_2MoO_4 (0.0414 M; 8.46 mL, 0.350 mmol) was added to an aqueous solution (100 mL) of IDAPr(3,4-HP)$_2$ (0.170 mmol), and the initial pH was 9.98. This solution was divided into 10 mL samples at different pH values, set with 2 N and 0.2 N HCl solutions. When precipitation of the product occurred at pH < 7, the samples were filtered and the solids isolated. For the EDTAPr(3,4-HP)$_2$ system, a similar procedure was used, namely mixing a 0.0014 M ligand stock solution (50 mL, 0.070 mmol) with a 0.016 M (NH_4)$_6$-Mo_7O_{24}·4H_2O solution (1.260 mL, 0.138 mmol of Mo^{VI}) and pH adjustment up to 9.16 with 2 M KOH solution. Several sample solutions were prepared at different pH values by using a 0.1 M HCl solution. Precipitation started at pH < 3, and the yellow solids were filtered and the corresponding mother solutions were preserved. The solutions were analyzed by ESI-MS whereas some of the solids were characterized by IR spectroscopy and elemental analysis.

Supporting Information (see footnote on the first page of this article): Overall stability constants for the species formed in measurable concentrations in the H^+–MoO_4^{2-} equilibrium system; UV spectra for solutions containing free IDAPr(3,4-HP)$_2$ and IDAPr(3,4-HP)$_2$, and Mo^{VI}; graphical representation of the chemical shifts of the 5-H and 6-H of the hydroxypyridinone ring for the free ligand and for the Mo^{VI}–IDAPr(3,4-HP)$_2$ system; potentiometric titration curves of aqueous solutions containing Mo^{VI} and EDDA at different molar ratios; concentration distribution curves for the complexes formed in the Mo^{VI}–EDDA system; concentration distribution curves but for the complexes formed in a hypothetical ternary system; UV spectra for solutions containing EDTAPr(3,4-HP)$_2$ free ligand, and in the presence of Mo^{VI} and EDTAPr(3,4-HP)$_2$. ^{17}O NMR spectra of solutions containing Mo^{VI} and EDTAPr(3,4-HP)$_2$ at the indicated pH values and at ligand-to-metal ratio of 1:1; ^{1}H NMR spectra of the aliphatic region for the same

systems; ESI-MS of Mo^{VI}–IDAPr(3,4-HP)$_2$ and Mo^{VI}–EDTAPr(3,4-HP)$_2$.

Acknowledgments

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