Synthesis and Metal-Complexation Properties of a New Hydroxypyrimidinone-Functionalized Sepharose

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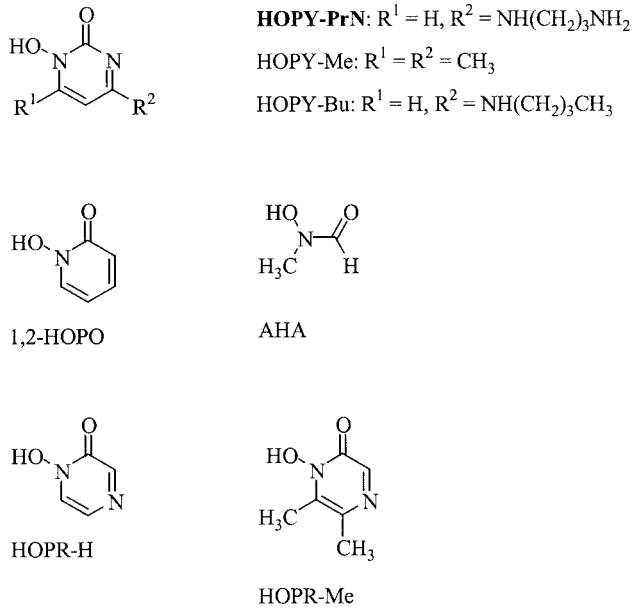
The 1-hydroxy-2-(1*H*)-pyrimidinone derivative 4-(3-aminopropylamino)-1-hydroxy-2-(1*H*)-pyrimidinone (HOPY-PrN) was synthesized and its acid-base and complexation properties towards a set of metal ions (Fe^{III}, Al^{III}, and Th^{IV}) were studied by potentiometry and spectrophotometry. The ligand was further immobilized in an epoxy-activated sepharose by chemical coupling through the aminoalkyl pendent group with the aim of improving its sequestering capacity for residual amounts of metals. The new hydroxypyrimidinone-functionalized sepharose shows a high chelating capacity for metal ions in the pH range 3–8, thus suggesting good perspectives for potential environmental applications.

Introduction

The need to manage, safely store, and limit the potential environmental or injurious effects of toxic metal ions, such as the actinides (e.g.: plutonium) produced from nuclear reactors, is becoming increasingly urgent.[1] Accordingly, the requirement for effective metal-sequestering agents, namely solid supports functionalized with specific chelators, has increased.[2,3] Those materials can find practical applications as extraction agents for the removal of residual amounts of actinides and other hazardous metal ions from waste waters. Immobilized chelators have also been used in biological and pharmacological applications, such as the removal of metal contaminants from biological systems, and as supports for affinity chromatography.[4]

For all these purposes the functionalized solid matrix should have an adequate hydrophilicity and stability, in order to guarantee a good interaction with water, and a low bleeding potential to prevent the introduction of further contaminants into the environment. On the other hand, immobilized metal-chelators should also have important properties such as a high affinity for a specific metal ion (or group of metal ions) and adequate spacer groups to avoid steric hindrance. A variety of supports are known for chelator immobilization, such as silicas, organic polymers, agaroses, and celluloses.[5] The search for specific chelators for hard metal ions has been quite active, and has been especially aimed at finding substitutes for Desferrioxamine B (a hydroxamate-based medicinal chelating drug with limitations due to oral inactivity)[6] and also substitutes of hydroxamate-immobilized matrices for potential environmental applications without their inherent problems of instability and re-usability.[7] *N*-Hydroxyamide-containing heterocycles, such as 1-hydroxy-2-pyridinones (1,2-HOPO),[8] hydroxypyrimidinones (HOPY),[9,10] and hydroxypyrazones (HOPR)[11] (see Scheme 1), have been



Scheme 1. Structural formulae for 4-(3-aminopropylamino)-1-hydroxy-2-(1*H*)-pyrimidinone (HOPY-PrN), 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (HOPY-Me), 1-hydroxy-4-(*N*-butylamino)-2(1*H*)-pyrimidinone (HOPY-Bu), 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO), acetohydroxamic acid (AHA), 1-hydroxy-2(1*H*)-pyrazinone (HOPR-H), and 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (HOPR-Me)

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considered of great interest owing to their effectiveness in the selective sequestration of hard metal ions, particularly Fe^{III}, those of group IIIA, and some actinides (e.g. Pu^{IV} and Th^{IV}).[1] These *N*-hydroxyamide-containing mono- and diazines can be regarded as hydroxamic acids (HAs), but with endocyclic structures, which makes them much more stable in aqueous medium than linear or exocyclic HAs. On the other hand, their π-electron-deficient ring system allows a considerable amount of electron delocalization, which contributes to the high stability of the metal complexes.

Although some 1-hydroxy-2-pyridinone derivatives have already been studied in terms of chelating properties and immobilization onto polystyrene supports for the removal of hard metal-ions (e.g. Pu^{IV}) from water streams,[2,12,13] only a few published data are available on the coordination chemistry of hydroxypirimidinone derivatives.[11,14] Also, to the best of our knowledge, there are no reports of the immobilization of this kind of ligand on solid supports. Hydroxypyrimidinones have an extra nitrogen atom in the heterocyclic ring, which makes them less toxic and more promising compounds for environmental and biomedical applications[9,15−17] by increasing their acidity and water solubility.

As part of an ongoing project on hydroxypyrimidinone-based chelating resins, we present herein the synthesis and study of a water-soluble hydroxypyrimidinone and its sepharose-supported derivative. Thus, 4-(3-aminopropylamino)-1-hydroxy-2-(1*H*)-pyrimidinone (HOPY-PrN) was synthesized and studied in terms of its acid-base and chelating properties towards Fe^{III}, Al^{III}, and Th^{IV}, in aqueous solution, by potentiometric and spectrophotometric techniques. This ligand was further immobilized in an epoxy-activated sepharose and then the new functionalized support was studied with regards to its ligand density, stability, and capacity for the removal of some hard metal ions from aqueous medium. In these studies, Th^{IV} was chosen as a model of Pu^{IV}. In fact, isotopes of thorium are formed as daughter products of the most important isotopes of uranium, and although thorium is also a potential contaminant in the reprocessing of nuclear fuels, it has a very low specific radioactivity and so it is a quite safe actinide ion for model studies.

Results and Discussion

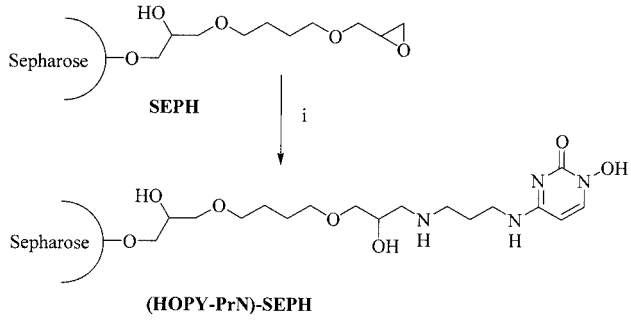
Synthesis of HOPY-PrN

The general procedure for the synthesis of HOPY-PrN is outlined in Scheme 2.

1-(Benzyloxy)uracil (**1**), which was synthesized by a previously reported procedure,[11] was condensed with 1,2,4-triazole in the presence of 4-chlorophenyldichlorophosphate in dry pyridine[18] to give 1-(benzyloxy)-4-(1′,2′,4′-triazol-1′-yl)-2(1*H*)-pyrimidinone (**2**) in 50% yield. To enable the posterior coupling of the ligand to the solid support, an *N*-protected alkylamine segment was introduced as a heterocyclic substituent group by reacting **2** with 3-[(benzyloxycarbonyl)amino]propylamine[19] in refluxing THF to give 4-{3-[(benzyloxycarbonyl)amino]propylamino}-1-(benzyloxy)-2(1*H*)-pyrimidinone (**3**) in 80% yield. Finally, HOPY-PrN was obtained by removing the protecting benzyl groups from both benzyloxycarbonyl/amino and hydroxyl groups of **3** by standard catalytic hydrogenolysis in methanol.[11]

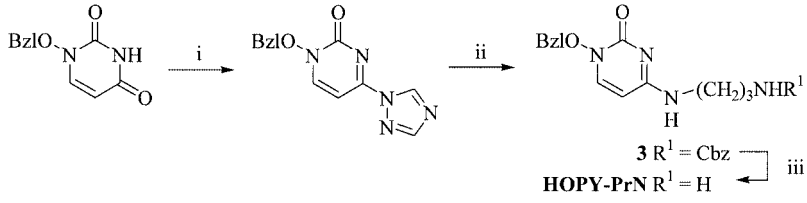
Immobilization of HOPY-PrN

The coupling reaction of the ligand to the commercially available 6B epoxy-sepharose (SEPH, Scheme 3) was carried out under slightly basic conditions according to a published procedure.[20] Care was taken in the selection of the pH for the coupling reaction because it should be just high enough to ensure the deprotonation of the amino group but not so high to promote coupling of the hydroxyl group or even the degradation of the ligand. Therefore, the epoxy sepharose gel was added to a solution of HOPY-PrN in a buffer solution at pH 9 and the mixture was shaken in a water bath at 40 °C for 48 h.



Scheme 3. Immobilization of HOPY-PrN in epoxy-activated 6B sepharose (SEPH): (i) universal buffer, pH 9, $T = 40$ °C (48 h)

A series of preliminary coupling reactions were performed to find the dependence of the ligand concentration on the extent of the HOPY-PrN immobilization. As is shown in Figure 1, a 60-fold ligand excess, relative to the expected amount of active sites in the epoxy-sepharose (ca. 103 μmol active sites per gram dry gel with additives),[20]



Scheme 2. Synthesis of HOPY-PrN: (i) 1,2,4-triazole, 4-chlorophenyldichlorophosphate, pyridine; (ii) $NH_2(CH_2)_3NHCbz$, THF, Δ; (iii) 10% Pd/C, H_2, MeOH

gave the highest (HOPY-PrN)-SEPH density [263 μmol ligand per gram (dry weight)], which corresponds to 54% conversion of the active sites. The ligand density in the functionalized sepharose was easily calculated from the nitrogen amount determined by elemental analysis, since the ligand is the only source of this element in the matrix.

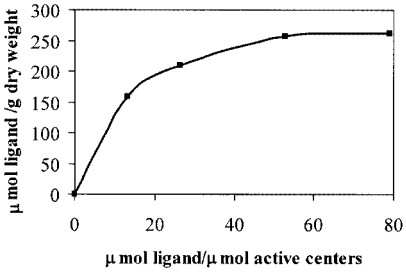


Figure 1. Plot of ligand density in (HOPY-PrN)-SEPH vs. ligand concentration in the coupling reaction

Stability of the Functionalized Support (HOPY-PrN)-SEPH

The stability of the functionalized support in aqueous solution was tested under different pH conditions (pH 3, 7, and 9) at room temperature. The support was added to buffered aqueous solutions at the selected pH and the suspensions were left shaking at room temperature for 24 h. To calculate the amount of ligand released into solution the suspensions were periodically centrifuged during the experiment and the absorbance of the supernatants was measured at the maximum absorption wavelength (λ_{max} = 318 nm) for HOPY-PrN. Plots of percentage of ligand released vs. time (Figure 2) showed that, after 24 h, less than 0.5% of the ligand had been released from the solid matrix for all the experimental pH values, thus indicating that the functionalized sepharose is quite stable.

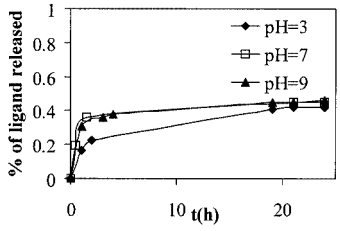


Figure 2. Plot of the percentage of ligand released from the functionalized sepharose vs. time in aqueous solution at pH 3, 7, and 9

Equilibrium Solution Studies

Acid-Base Properties

The ligand was obtained as a neutral species even though, in the fully protonated form, it has three dissociable protons. The corresponding protonation constants were determined from the fitting analysis of the potentiometric titration curve, and its protonation sequence was deduced from the ^{1}H NMR titration curves (Figure 3). The protonation sequence of the ligand is in agreement with the chemical evidence, namely the downfield shifts of the labile protons, which follow the order terminal *N*-amine group (at pD ≈ 10.1−12.5 peaks *c*, *d*, *e*), hydroxy group (at pD ≈ 6−8.8 peaks *a* and *b*), and 4-imine group (at pD ≈ 1.5−4.1 all the peaks, but mostly on *a* and *b*, probably due to the enamine-imine equilibrium). This ^{1}H NMR titration was checked for reversibility and the ligand proved to be stable under both acidic and basic pH conditions.

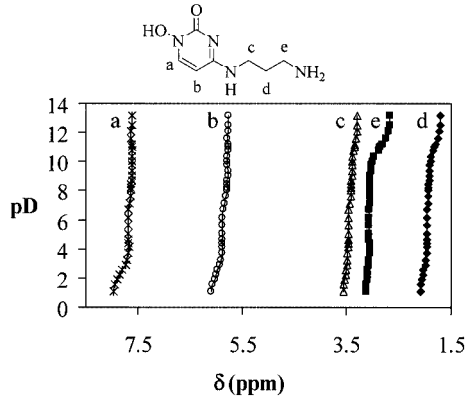


Figure 3. ^{1}H NMR titration curves for HOPY-PrN

The calculated protonation constants (log K_i,) for the ligand are reported in Table 1, which also includes the corresponding values for some analogous compounds, namely 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (HOPY-Me),[14] 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO),[8,21] 1-hydroxy-2(1*H*)-pyrazinone (HOPR-H),[9] 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone (HOPR-Me),[9] and acetohydroxamic acid (AHA).[22]

Table 1. Stepwise protonation constants (log K_i) of HOPY-PrN and other ligands, for comparison, as well as global formation constants (log $\beta_{M_mH_hL_l}$) of the corresponding Fe^{3+}, Al^{3+}, and Th^{4+} complexes (I = 0.1 M KNO_3, T = 25.0 ± 0.1 °C)

Ligand	H^+ log K_i	Fe^{3+}	Al^{3+} (m,h,l) log $\beta_{M_mH_hL_l}$	Th^{4+}
HOPY-PrN	10.11(2)	(111) 19.88(1)	(111) 16.84(2)	(111) 18.74(6)
	6.84(4)	(122) 38.12(6)	(122) 34.04(4)	(122) 37.14(4)
	2.21(5)	(123) 48.04(3)	–	(123) 47.70(6)
		(133) 55.26(5)	(133) 49.67(2)	(133) 53.91(5)
		–	–	(144) 69.54(6)
HOPY-Me[14]	6.1	(103) 22.1	–	–
1,2-HOPO	5.78[8]	(101) 9.0[8]	(101) 8.16[21]	–
	5.86[21]	10.6[21]	(102) 5.54[21]	–
		(102) 16.6[8]	(103) 21.59[21]	
		20.1[21]		
		(103) 26.9[8]		
		27.2[21]		
HOPR-H[9]	4.4	(103) 18.2	–	–
HOPR-Me[9]	4.7	(103) 20.2	–	–
AHA[22][a]	9.36	(101) 11.42	(101) 7.95	
		(102) 21.10	(102) 15.29	–
		(103) 28.33	(103) 21.47	

[a] At 20 °C.

The terminal *N*-amine group of HOPY-PrN is slightly more acidic (log K = 10.11) than that of propylamine (10.57)[23] or 1,3-diaminopropane (10.49)[24] due to the electron-withdrawing effect of the pyrimidinone ring. The log K_2 value (6.84), which corresponds to the protonation of the hydroxy group, is lower than that of an aliphatic hydroxamic acid, such as acetohydroxamic acid (log K = 9.36),[22] due to the stabilization of the negative charge of the conjugated base by the electron-withdrawing and resonance effects of the aromatic ring. It is also lower than that of HOPY-Bu (7.5)[11] due to the electron-donating effect of the butyl group. However, log K_2 is higher than the reported values for the corresponding hydroxy groups of HOPY-Me (6.1), 1,2-HOPO (5.78,[8] 5.86[21]), HOPR-Me (4.7), and HOPR-H (4.4) due to the interplay of different effects such as the inductive/electron-donating effect of the substituting groups at the C-4 position, the electron-withdrawing effect of the additional ring-nitrogen atom and its different positioning relative to N-OH group (e.g. *meta*- or *para*-positioned for HOPY and HOPR derivatives, respectively, with a concomitant difference in the resonance stabilization). From the ^{1}H NMR titration curves there is also evidence of a third labile proton (log K_3 = 2.21), which was determined after addition of excess of mineral acid. It is attributed to the 4-amino group, and its high acidity should be due to the enamine/imine equilibrium and the electron-withdrawing resonance effect of the aromatic ring.

Complexation Studies

The stability constants of the M^{3+} (M = Fe, Al) and Th^{4+} complexes with HOPY-PrN were determined by potentiometric and spectrophotometric techniques; they are collected in Table 1, together with reported values for some structural analogs (HOPY-Me, 1,2-HOPO, HOPR-H, HOPR-Me, AHA), all of which form five-membered chelate rings through the adjacent *N*-hydroxy- and keto-oxygen atoms. For all M/(HOPY-PrN) systems studied herein, the potentiometric titration curves show that the complex formation is already underway at the beginning of the titration. They also indicate the existence of a break at a = 0 (a = mol of base per mol of ligand), which suggests the non-involvement of the appended amine groups in the metal coordination, and also the apparent formation of mixed hydroxo-ligand complexes before the complete deprotonation of the fully coordinated complex species. At the beginning of the titration of the Fe^{3+}/HOPY-PrN or Th^{4+}/HOPY-PrN systems the extent of complexation was too high to allow the use of a direct potentiometric method. Therefore, spectrophotometric titrations at 1:1 metal-to-ligand molar ratios were performed in order to determine the formation constants of the corresponding MHL (L being the fully deprotonated form of a ligand) species. On the other hand, since precipitation started at around pH 4 during the potentiometric titration of the Fe^{3+}/ligand system with a 1:3 metal-to-ligand molar ratio, the formation constants of the bis- and tris-chelated species were obtained from the fitting analysis of the spectrophotometric titration data for a 10-fold ligand excess, but keeping the previously determined log β_{FeHL} value constant. Figure 4 shows the pH dependence of the spectrum during this titration. There is a blue shift of the absorption band with increasing pH, thus indicating a concomitant increase of the coordination number. Along the range of pH 2.5−5, there is one isosbestic point at around $\lambda \approx 510$ nm, which is associated with the interconversion from bis- to tris-chelated complexes. Over the range of pH 5.3−7.7 there is no apparent change in the λ_{max} value, thus indicating the presence of the same chromophoric species. The observed spectral parameters (λ_{max} = 465 nm, ε = 5624 $M^{-1}\cdot cm^{-1}$) are comparable to the reported values for the charge-transfer (CT) bands of ferric tris-chelated complexes with hydroxypyrimidinone analogs (HOPY, HOPR),[9,11] which indicates the existence of a tris-chelated species, although with different protonation extents. Above a pH of about 8 the formation of Fe^{3+} ligand-hydroxide mixed complex species and their precipitation is indicated by the intensity decay and hypsochromic shift of the absorption bands.

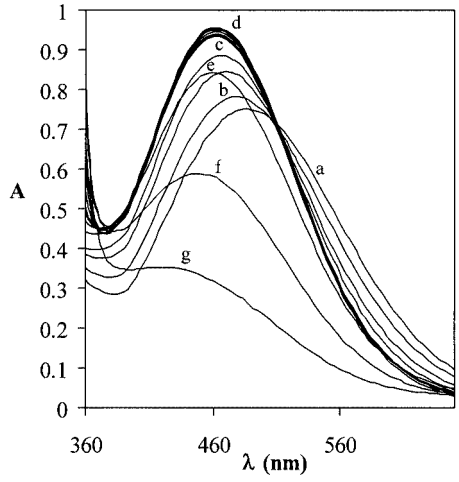


Figure 4. Spectrophotometric absorbance curves at various pH values (a = 2.53, b = 2.78, c = 3.42, d = 5.31−7.72, e = 8.56, f = 9.04, g = 9.31) for the Fe^{3+}/HOPY-PrN system (C_L/C_{Fe} = 10, $C_L = 1.7 \times 10^{-3}$ M)

For the Th^{4+}/HOPY-PrN system the stability constants of the complexes with 1:2, 1:3, and 1:4 metal-to-ligand stoichiometries were evaluated from the potentiometric titration curves for 1:4 and 1:8 metal-to-ligand molar ratios while holding constant the β_{ThHL} value previously determined by spectrophotometry.

The stability constants for the Al^{3+}/HOPY-PrN system were determined by a fitting analysis of the potentiometric data obtained for the 1:1, 1:3, and 1:6 stoichiometries.

A rough comparison between the stability constants of the ligand-metal complexes and the corresponding values for the analogous systems (see Table 1) can be made, assuming that the terminal amine group in the ligand is not

involved in the coordination and so $\log \beta_{MHL} = \log \beta_{ML} + \log K_1$. Analysis of Table 1 indicates that the stability constants for the ligand-metal complexes follow the order Fe > Th > Al, in accordance with the corresponding log K_{MOH} values reported for the same metal ions (11.8, 10.8, and 9.0 respectively).[25] This feature reflects the ionic nature of this type of complex, with no preponderance of the crystal-field stabilization effect. The speciation at different pH values for the ligand (HOPY-PrN) and the corresponding ligand/metal-ion systems are illustrated in Figure 5. For all the metal/ligand systems studied herein, Figure 5 clearly evidences the strong chelating capacity of HOPY-PrN, with the complexation starting in quite acidic conditions (even at pH ≈ 2, for M = Fe^{3+} and Th^{4+}). The speciation curves for the iron and thorium systems confirm that, at pH 2, MHL is already formed, thus rendering impossible the determination of log β_{MHL} by potentiometry, as stated above.

Analysis of the speciation curves further shows the existence of a stepwise formation of $M(HL)_i$ species whose metal-ligand binding mode can be derived from chemical evidence, namely a protonated ligand molecule with the hydroxypyrimidinone moiety coordinated to the metal ion but the terminal amino groups protonated. For the thorium system there is a minor species present above pH 5 which can be ascribed either to a partially amino-deprotonated tris-chelated species or to a hydroxo-ligand mixed complex such as $[Th(HL)_3(OH)]$. However, those species are indistinguishable by potentiometry and they are just assigned as ThH_2L_3.

Since comparison between the metal-binding affinity of different ligands must take into account their distinct proton concentration dependency, pM values (pM = −log [M] for $C_L/C_M = 10$ and $C_L = 10^{-5}$ M) have been calculated at different pH conditions for HOPY-PrN and its analogs. Plots of pM (M = Fe, Al) versus pH have been calculated and are shown in Figure 6 and 7. It must be mentioned that these pM values were calculated based on the complex stability and ligand-protonation constants calculated herein or reported in the literature (Table 1), as well as the corresponding hydrolytic species used above for the equilibrium models, but neglecting effects of different ionic strengths and temperatures (as in the case of AHA) on the complexation models and assuming that no precipitation takes place.

Analysis of Figure 6 shows that all these *N*-hydroxamide compounds present a steady variation of pFe with pH. Furthermore, among these compounds (excluding 1,2-HOPO which clearly presents the highest pFe value), HOPY-PrN seems to be the most promising chelating agent in the pH range 2−3.9 and above 5. For pH values between 3.9 and 5 HOPR-Me has slightly higher pFe values, probably due to the electron-donation effect of the ring methyl groups. Therefore the inclusion of methyl groups as substituents in the HOPY-PrN ring should also be expected to improve the chelating capacity of the ligand; this will be a challenge for future work.

Figure 7 indicates that although 1,2-HOPO presents higher pAl values than HOPY-PrN in the acid region, under neutral and basic pH conditions both these ligands and AHA possess the same aluminum-complexation strength.

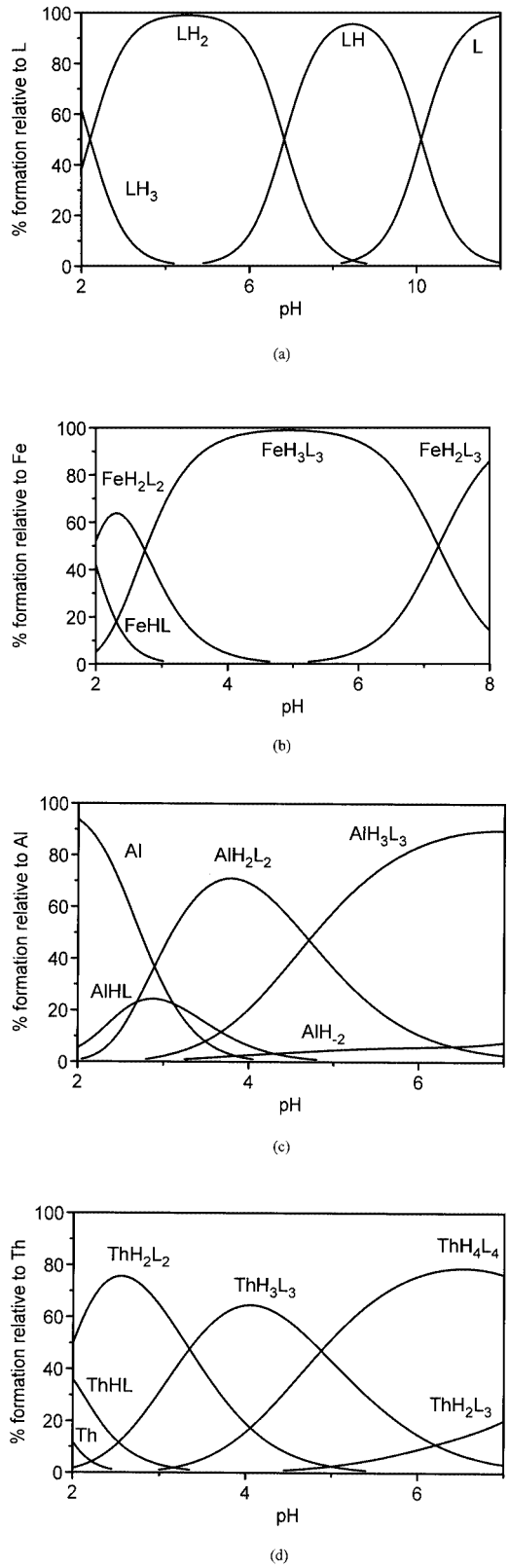


Figure 5. Species-distribution diagrams for HOPY-PrN (a) and M/HOPY-PrN systems with M = Fe^{3+} ($C_L/C_{Fe} = 10$; b); M = Al^{3+} ($C_L/C_{Al} = 3$; c); M = Th^{4+} ($C_L/C_{Th} = 4$; d); $C_L = 2 \times 10^{-3}$ M

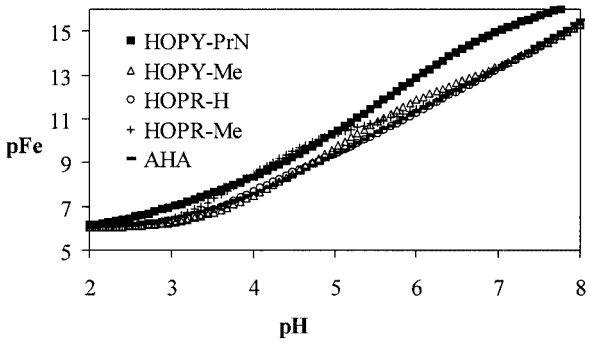


Figure 6. Metal-complexation strength, reported as pFe, versus pH, for HOPY-PrN and some of the hydroxypyrimidinone analogs presented in Table 1; $C_L/C_M = 10$ and $C_L = 10^{-5}$ M

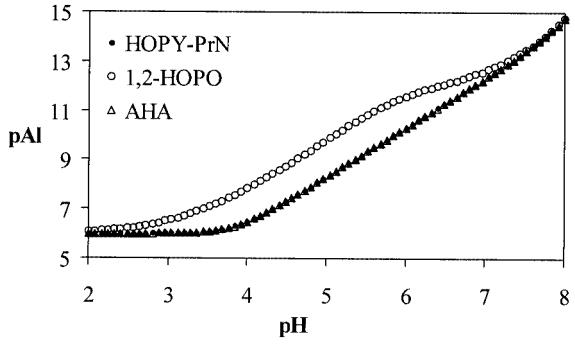


Figure 7. Metal-complexation strength, reported as pAl, versus pH, for HOPY-PrN, 1,2-HOPO, and AHA; $C_L/C_M = 10$ and $C_L = 10^{-5}$ M

Chelating Capacity of (HOPY-PrN)-SEPH

To evaluate the chelating capabilities of (HOPY-PrN)-SEPH towards Fe^{III}, Al^{III}, and Th^{IV}, batch experiments were used. Typically, for Fe^{III}, the functionalized support was added to a universal buffer solution at pH 7 containing Fe^{III}-citrate in fourfold excess relative to the amount of ligand immobilized in the solid matrix, and the mixture was shaken at room temperature for 4 h. During the experiment it could be observed that the solid support gained the characteristic red color of the iron complex almost immediately upon addition of the iron solution. After filtration and careful washing of the functionalized support to remove the excess of metal ions, the Fe^{III} retained on (HOPY-PrN)-SEPH was released by treatment with an acidic solution. The Fe-chelating capacity of the support was calculated from the iron content of the collected filtrates, which was determined by atomic absorption spectrophotometry (AAS). The obtained values (Table 2) indicate that the metal complexes possess an approximate 1:1 ligand-Fe^{III} stoichiometry. Comparison of the ligand density in the modified sepharose with the corresponding chelating capacity indicates that, at neutral pH, (HOPY-PrN)-SEPH is 100% efficient in iron removal, but at pH 3 its chelating efficiency is slightly lower, as expected from the pH dependence of the pM (Figure 6). However, it should be emphasized that the presence of the citrate buffer at pH 3 could be responsible for some underevaluation of the chelating efficacy of these functionalized supports because citrate can also compete with the ligand for the metal ions.

Table 2. Chelating capacity of (HOPY-PrN)-SEPH towards Fe^{III}, Al^{III}, and Th^{IV} at pH 3 and 7

Ligand density (μmol/g dry weight)	pH	Chelating capacity (μmol/g dry weight)		
		Fe^{III}	Al^{III}	Th^{IV}
263 ± 8	3	(16 ± 1) × 10	<70	90 ± 8
263 ± 8	7	(26 ± 1) × 10	(24 ± 2) × 10	(25 ± 2) × 10

The chelating capacities of the functionalized sepharose for Al^{III} and Th^{IV} were also calculated by following the same procedure; the results show that the complexation behavior of the supported hydroxypyrimidinone towards these metal ions is similar to that found for Fe^{III}. Interestingly, the metal chelating capacities at pH 7 follow the same order ($Fe^{III} > Th^{IV} > Al^{III}$) found for the stability constants of the corresponding metal-ligand complexes in aqueous solution (Table 1). So, although comparisons between the solution and the two-phase conditions have to be made with care, the eventual formation of ligand-hydroxo mixed species in the solid phase is only expected at pH values above 8, but without the precipitation problems found in the solution studies, which means that this functionalized resin can be used in the pH range 3−8. The fact that both the ligand and the functionalized resin proved to be very stable under acid conditions is noteworthy. Furthermore, an extra experiment showed that, upon removal of the iron under very acidic conditions, the iron-chelating capacity was kept constant (within the experimental error range) and so there are good perspectives for its reutilization. This feature is very important and it represents an advantage of this chelating support over other ones, such as the linear hydroxamate-based supports, because these groups are easily hydrolysable under such acidic conditions.

Conclusion

Solution studies of a 1-hydroxy-2-(1*H*)-pyrimidinone substituted with an alkylamino group at the C-4 position, HOPY-PrN, by potentiometry and spectrophotometry have shown a strong metal-binding affinity for hard metal-ions, particularly Fe^{III}, Al^{III}, and Th^{IV}, and its ability to compete with other analogous *N*-hydroxamide ligands. This compound was then immobilized in an epoxy-activated sepharose by chemical coupling through the aminoalkyl pendent group. This new hydroxypyrimidinone-functionalized sepharose shows high stability in water over a wide pH range (3−9), a high sequestering capacity for this type of hard metal-ions at mildly basic or even acidic pH conditions, and an ability for reutilization. Therefore, there are good perspectives for its potential use for the removal of traces of toxic hard metal-ions from aqueous media, and thus for environmental purposes.

Experimental Section

Materials: 6B Epoxy-sepharose was purchased from Amersham Pharmacia Biotech. All reagents were analytically pure. Thin Layer Chromatography (TLC) was performed on silica gel 60 F254 plates with 0.2 mm layer thickness from Macherey−Nagel and the compounds visualized by illumination under UV light at 254 nm. Column chromatography (CC) was carried out with Macherey−Nagel Si gel 60 (230−400 mesh). Whenever necessary, solvents were dried according to standard methods.[26] 1-(Benzyloxy)uracil (**1**) was prepared according to the literature.[11]

The 0.050 M Al^{3+} and Th^{4+} solutions were prepared from the corresponding nitrate salts and were standardized by atomic absorption and inductively coupled plasma emission, respectively. The 1000 ppm $Fe(NO_3)_3$ standard solution was purchased from Merck. The solutions of trivalent metal ions were prepared with an excess of nitric acid to prevent hydrolysis. The exact concentration of HNO_3 in each solution was determined by titration with 0.1 M HNO_3 (Titrisol, 0.1 M HNO_3 ampoules) for values of pH ≥ 2. The titrant was prepared from carbonate-free commercial concentrate (Titrisol, KOH 0.1 M ampoules) and was standardized by titration with a solution of potassium hydrogenphthalate. KOH solutions were discarded whenever the percentage of carbonate, determined by Gran's method,[27] was superior to 0.5% of the total amount of base.

Instrumentation and General Information: FTIR spectra were recorded on a Perkin−Elmer 1725 spectrometer and UV/Vis spectra on a Hitachi 150−20 spectrophotometer. Melting points were determined on a Reichert Thermovar melting-point apparatus and are uncorrected. Fourier transform (FT) NMR spectra were run at ambient temperature on a General Electric QE-300 spectrometer with a resonance frequency of 300.65 for ^1H and 75.6 MHz for ^{13}C, using an appropriate solvent. The chemical shifts are reported in δ (ppm) relative to internal references (TMS for organic solvents or sodium 3-trimethylsilyl-D_4-propionate for aqueous solutions). Coupling constants (J) are expressed in hertz. Electrospray ionization mass spectra (ESI-MS) were determined on a Bruker Esquire 3000 mass spectrometer.

1-(Benzyloxy)-4-(1′,2′,4′-triazol-1′-yl)-2(1*H*)-pyrimidinone (2): 1,3,4 Triazole (0.509 g, 7.28 mmol) was added to a solution of 1-(benzyloxy)uracil (**1**; 0.500 g, 2.46 mmol) in dry pyridine (32 mL) at room temperature. Then, 4-chlorophenyldichlorophosphate (0.50 mL, 3.69 mmol) was added and the reaction mixture stirred for 3 d at room temperature. Water was added to the mixture and the solvents evaporated. A saturated $NaHCO_3$ solution was added to the residue and this aqueous suspension extracted with $CHCl_3$ (5 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4. After evaporation of the solvent, the resulting residue was purified by CC on silica gel using a mixture of ethyl acetate/*n*-hexane (2:1) as eluent to give pure **2** as a yellowish solid (0.443 g; yield 67%), m.p. 202−205 °C (206−209 °C).[11] ESI-MS: m/z = 270 $[M^+ + 1]^+$. FT-IR (KBr): $\tilde{\nu}$ = 1453, 1541, 1616, 1692, 2930, 3099 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 5.38 (s, 2 H, $PhCH_2O$), 6.73 (d, J = 8.5 Hz, 1 H, 5-H), 7.39 (s, 5 H, ArH), 7.50 (d, J = 8.5 Hz, 1 H, 6-H), 8.09 (s, 1 H, 3′-H or 5′-H), 9.22 (s, 1 H, 5′-H or 3′-H) ppm.

4-{3-[(Benzyloxycarbonyl)amino]propylamino}-1-(benzyloxy)-2(1*H*)-pyrimidinone (3): A solution of **2** (0.50 g, 1.86 mmol) and 3-[(benzyloxycarbonyl)amino]propylamine[19] (0.50 g, 2.4 mmol) in dry THF (15 mL) was stirred overnight at reflux temperature, under N_2. The solvent was then evaporated and H_2O added to the residue. The aqueous layer was extracted with $CHCl_3$ (5 × 20 mL) and the combined organic extracts were washed successively with 5% citric acid solution, H_2O, and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product recrystallized from MeOH to afford **3** as a pale-yellow solid (0.60 g; yield 80%), m.p. 154−156 °C. ESI-MS: m/z = 408 $[M + H]^+$, 301 $[M - 107]^+$. ^1H NMR ($CDCl_3$): δ = 1.74 (quin, J = 6.15 Hz, 2 H, $CH_2CH_2CH_2$), 3.28 (q, J = 6.30 Hz, 2 H, $NHCH_2$), 3.53 (q, J = 6.30 Hz, 2 H, CH_2NH), 5.11 (s, 2 H, $PhCH_2O$), 5.22 [s, 2 H, $NHC(O)OCH_2$], 5.91 (m, 1 H, 5-H), 6.89−6.92 (m, 1 H, 6-H), 7.36−7.39 (m, 10 H, 2 ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 29.2 ($CH_2CH_2CH_2$), 38.0 ($NHCH_2$), 38.1 (CH_2NH), 66.8 [$C(O)OCH_2$], 78.5 (OCH_2), 94.4 (C-5), 128.0, 128.2, 128.6, 128.9, 129.5, 130.3 (ArCH),142.6 (C-6), 154.6 (C-2), 157.7 [C(O)O], 162.8 (C-4) ppm. $C_{22}H_{24}N_4O_4$ (408.45): calcd. C 64.69, H 5.92, N 13.72; found C 64.66, H 5.89, N 13.52.

4-(3-Aminopropylamino)-1-hydroxy-2-(1*H*)-pyrimidinone (HOPY-PrN): A suspension of 10% Pd/C (0.23 g) in dry MeOH (50 mL) was prehydrogenated with H_2 (1 atm) for 30 min. A solution of **3** (0.40 g; 1.06 mmol) in dry MeOH (200 mL) was added to this suspension and the mixture stirred under H_2 (1 atm) for 3 h at room temperature. The catalyst was removed by filtration and the solvent evaporated under reduced pressure. The residue obtained was recrystallized from H_2O/MeOH to afford pure HOPY-PrN (0.185 g; yield 95%), m.p. 216−218 °C. ESI-MS: m/z = 185 $[M + H]^+$, 168 $[M - 16]^+$. FT-IR (KBr): $\tilde{\nu}$ = 2926−3401, 1626, 1524 cm^{-1}. ^1H NMR (D_2O): δ = 1.82 (quin, J = 5.30 Hz, 2 H, $CH_2CH_2CH_2$), 2.93 (t, J = 5.30 Hz, 2 H, CH_2NH_2), 3.31 (t, J = 4.80 Hz, 2 H, $NHCH_2$), 5.64 (dd, J = 7.2, 1.9 Hz, 1 H, 5-H), 7.48 (dd, J = 7.2, 1.9 Hz, 1 H, 6-H) ppm. ^{13}C NMR (D_2O): δ = 29.6 ($CH_2CH_2CH_2$), 39.2 (CH_2NH_2), 39.4 ($NHCH_2$), 95.1 (C-5), 147.2 (C-6), 160.1 (C-2), 163.4 (C-4) ppm. $C_7H_{12}N_4O_2$ (184.20): calcd. C 45.64, H 6.57, N 30.42; found C 45.52, H 6.66, N 30.20.

General Procedure for the Preparation of (HOPY-PrN)-SEPH: Epoxy-activated sepharose 6B freeze-dried powder (500 mg) was washed with distilled H_2O (100 mL) in a sintered glass filter and then suspended in coupling buffer (10 mL of universal buffer, pH 9).[28] HOPY-PrN (500 mg) was dissolved in coupling buffer and added to the matrix suspension. The coupling mixture was adjusted to pH 9 and then shaken in a shaker water bath at 40 °C for 48 h. After that time, the mixture was filtered and the excess ligand was washed away with coupling buffer, distilled H_2O, or, alternatively, with 0.1 M $NaHCO_3$ containing 0.5 M NaCl (pH 8) and 0.1 M acetate buffer containing 0.1 M NaCl, (pH 4) at least three times. The functionalized gel obtained was freeze-dried and stored at 0−4 °C.

Stability Studies of (HOPY-PrN)-SEPH: Freeze-dried (HOPY-PrN)-SEPH (15 mg) was placed in distilled water at pH 3, 7, and 9 and the suspensions shaken at room temperature for 24 h. Aliquots (3 mL) were taken several times, diluted with water, and the absorbance of the resulting solutions measured at λ = 318 nm. Plots of percentage of ligand released from the solid support vs. time were obtained from the values of ligand concentration in solution for each aliquot calculated from a calibration curve.

^1H NMR Titration of HOPY-PrN: ^1H NMR spectra were recorded with a Varian Unity 300 spectrometer at probe temperature. Solutions of HOPY-PrN (approx. 0.02 M) were prepared in D_2O using DSS as internal reference. The pD was adjusted by addition of DCl or CO_2-free KOD, using a Crison 2001 instrument fitted with a combined Mettler Toledo U402-M3 S7/200 microelectrode. This microelectrode was previously calibrated with standard buffered aqueous solutions; $-\log[D^+]$ was measured in the NMR tubes. The final values of pD were determined from the equation pD = pD*

+ 0.40,[29] in which pD* corresponds to the reading of the pH meter previously calibrated with aqueous buffers at pH 4 and 7.

Potentiometric Measurements: The equipment used has been described before.[30] All the potentiometric measurements were carried out under thermostatted conditions ($T = 25.0 \pm 0.1$ °C) at an ionic strength, I, of 0.10 M (KNO_3); the experiments were monitored by computer. Atmospheric CO_2 was excluded from the cell during the titration by passing purified N_2 across the top of the experimental solution in the reaction cell. The potentiometric measurements were performed at ligand concentrations of 2.0×10^{-3} M, first in the absence of metal ions and then in the presence of each metal ion with corresponding C_M/C_L ratios (1:3 for Fe^{3+}; 1:3 and 1:6 for Al^{3+}; 1:4 and 1:8 for Th^{4+}). The $[H^+]$ of the solutions was determined by measuring the electromotive force of the cell, as described previously;[31] the determined value of K_w used in the computations was $10^{-13.80}$.
At the beginning of the titrations of the ligand with Fe^{3+} and Th^{4+}, the extent of formation of the metal complexes was too high to allow the use of the direct potentiometric method, therefore spectrophotometric titrations were performed.

UV Spectrophotometric Measurements: Electronic spectra were recorded with a Perkin−Elmer model Lambda 9 spectrophotometer, using aqueous solutions of the complexes, in 1 cm path-length cells. The temperature of the solutions was kept at 25.0 ± 0.1 °C, using a Grant W6 thermostat, and $I = 0.10$ M (KNO_3). The spectra were recorded in the range 250−350 or 350−650 nm for the Th^{4+} and the Fe^{3+} systems, respectively. The solution of the Fe^{3+} complex (1:10 stoichiometry) was prepared as indicated for potentiometric measurements. For the 1:1 Fe^{3+}/ligand and Th^{4+}/ligand systems, the measurements were made for $0.8 \leq pH \leq 2$ and the amount of acid to be added (from standard solutions of 0.097 or 1 M HNO_3) was calculated for the total volume solution under study.

Calculation of Equilibrium Constants: Ligand protonation constants, $K_i = [H_iL]/[H_{i-1}L][H]$, were calculated by fitting the potentiometric data obtained for the free ligand using the program HYPERQUAD version 2.1.[32] Stability constants of the metal-ion complexes formed in solution were determined from the experimental data corresponding to the titration of solutions with different ligand and metal-ion ratios, also with the aid of the same program. Whenever a high extent of formation of the metal complexes at the beginning of the titration was present, the PSEQUAD program[33] was used to treat the spectrophotometric data. The results were obtained in the form of overall stability constants or $\beta_{M_mH_hL_l} = [M_mH_hL_l]/[M]^m[H]^h[L]^l$. In the analysis of the potentiometric as well as the spectrophotometric data, the hydrolytic species[31,34−36] of the respective metal ions were considered. Species-distribution curves were plotted with the HYSS program.[32] The errors quoted are the standard deviations of the overall stability constants given directly by the program. In the case of K_i, the standard deviations were determined by the normal propagation rules and do not represent the total experimental errors.

Determination of the Chelating Capacity of (HOPY-PrN)-SEPH: To evaluate the Fe^{III} chelation, (HOPY-PrN)-SEPH freeze-dried gel (50 mg) was placed in a buffer solution at pH 7 containing 90 ppm Fe^{III} citrate, and the suspension was shaken at room temperature for 4 h. The gel was filtered, washed with deionized water, and HNO_3 was added to the filtrate solution. Then, the gel was washed with an acidic solution of HNO_3 to release the complexed Fe^{III}. The Fe^{III} chelating capacity of the gel was calculated from the difference in the iron content of the filtrate solutions determined by AAS. To determine the Al^{III} chelation, a procedure identical to that described above was followed but with Al^{III} citrate[37] instead of Fe^{III} citrate. As for the evaluation of the Th^{IV} chelation, the same technique used for the Fe^{III} system was applied, but the Th^{IV} citrate was generated by preparing an aqueous solution containing equimolar amounts of Th^{IV} nitrate and citric acid (1.72×10^{-3} M).

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