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Expanding Monomeric Pyrophosphate Complexes beyond Platinum

Nadia Marino, Anthony R. Vortherms, Amanda E. Hoffman, and Robert P. Doyle*

Department of Chemistry, Syracuse University, Syracuse, New York 13244-4100

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Four new monomeric pyrophosphate complexes, namely [Co(phen)$_2$($H_2P_2O_7$)]·4H_2O (**1**·4H_2O), [Ni(phen)$_2$($H_2P_2O_7$)]·8H_2O (**2**·8H_2O), [Cu(phen)(H_2O)($H_2P_2O_7$)] (**3**) and {[Cu(phen)(H_2O)(P_2O_7)][Na_2(H_2O)$_8$]}·6H_2O (**4**·14H_2O) have been isolated and structurally characterized. The impact of pH and stoichiometry in obtaining **1**-**4** is described. These complexes have been tested against the adriamycin-resistant ovarian cancer cell line A2780/AD, revealing highly significant (nM) IC_{50} values, compared to μM IC_{50} values for cisplatin controls.

Pyrophosphate (PPi) compounds have been investigated extensively over the past decade, with a focus on gaining insight into the virtually unexplored coordination chemistry of PPi and the magnetic properties of PPi-bridged paramagnetic metal complexes.[1] More recently, these species have garnered attention as anticancer agents, driven by major contributions by Bose et al.[2] and Doyle et al.[3] Studies by these two groups have revealed significant toxicity in drug-resistant cancer cell lines of monomeric Pt(II)/Pt(IV) or dimeric Co(II)/Ni(II)/Cu(II) pyrophosphate systems, respectively. Solubility and stability (pH-dependent) in aqueous solution has been reported for both series of compounds and preliminary mechanistic investigations have been undertaken. The platinum-pyrophosphate complexes of Bose showed no evidence of covalent binding to DNA. Although the actual cytotoxicity of the most effective species, the pyrophosphate-oxaliplatin analogous [Pt(dach)($H_2P_2O_7$)] (where dach is trans-1,2-cyclohexanediamine), is only half that of cisplatin in the cisplatin/carboplatin-resistant A2780/C30 cell line (IC_{50} value of 48 vs. 100 μM, respectively), the lack of DNA-binding suggests these pyrophosphate compounds exemplify a new generation of alternative anticancer platinum agents.

The results of the parallel research conducted by Doyle et al. on the cytotoxicity of the 1,10′-phenanthroline- (phen) containing dimeric complexes {Co(phen)$_2$]$_2$(μ-P_2O_7)}·6CH_3OH, {Ni(phen)$_2$]$_2$(μ-P_2O_7)}·27H_2O and {Cu(phen)(H_2O)]$_2$-(μ-P_2O_7)}·8H_2O appear even more extraordinary, with time dependent nano- and pico-molar toxicity recorded against the adriamycin-resistant A2780/AD cell line for the copper(II) and cobalt(II) species, respectively (compared to an inhibitory concentration of 11 μM for the cisplatin control). DNA interactions (*via* binding as well as intercalation routes), topoisomerase I enzyme inhibition and oxidative stress have all been shown to contribute in determining the overall toxicity of these compounds. Hydrolysis has been proposed as the most likely mechanism of cellular internalization and activation of these complexes.[3] Different possible species may be postulated as the result of the hydrolysis/aquation of the pyrophosphate dimers (see Scheme SI) and from here, a cascade of other derivatives incorporating available *in situ* nucleophiles may be easily formed and participate in the observed cell death through diverse mechanisms. So far, only one possible Co(II) mononuclear hydrolysis-derivative, the fluorescent literature compound {[Co(phen)$_2$(H_2O)Cl]Cl}[4] has been resynthesized and tested in our laboratory, revealing significant activity at 72 h.[3] More recently, as part of our ongoing research focused on the exploration of pyrophosphate-, non Pt-based drugs, we set out to investigate the monomeric analogues of our previously reported Co(II), Ni(II) and Co(II) dimeric species. This work is motivated by the desire to investigate a possible (-pyrophosphate) hydrolysis product of the dimer which is more likely to passively diffuse into the cell. Note that a monomeric pyrophosphate species may be derived from the dimer hydrolysis at one metal center instead of at the P−O−P pyrophosphate level (Scheme SI).

The synthesis, single-crystal X-ray structural analysis and cytotoxicity investigation of three new pyrophosphate monomers, namely [Co(phen)$_2$($H_2P_2O_7$)] (**1**), [Ni(phen)$_2$($H_2P_2O_7$)] (**2**) and [Cu(phen)(H_2O)($H_2P_2O_7$)] (**3**), is presented herein. A copper(II) disodium adduct, Na_2[Cu(phen)(H_2O)(P_2O_7)] (**4**), has been also synthesized and tested for activity for comparison to **3**.

Compounds **1**-**3** are all neutral species, with the metal ion coordinated to a dianionic dihydrogen pyrophosphate group, phen and, in the case of **3**, a water molecule. Compound **4** is a

*To whom correspondence should be addressed. Prof. Robert P. Doyle, E-mail: rpdoyle@syr.edu ; Phone: (1)-315-443-4070; Fax: (1)-315-443-4070.

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molecular salt being the dianionic copper(II) complex charge compensated by the presence of two equivalents of sodium cation.

1-3 were synthesized from acidic aqueous solutions of $CoSO_4 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$ or $CuNO_3 \cdot 2.5H_2O$, phen and sodium pyrophosphate typically added in a nonstoichiometric ratio, the pH being lowered with concentrated H_2SO_4 (**1**), HCl (**1** and **2**) or $HClO_4$ (**3**) to fall in the range 2−4.5. A basic environment (pH∼9) was required instead for the synthesis of **4**. An excess of the pyrophosphate salt was used to help prevent the formation of undesired kinetic products, identified as the dimeric species $\{[(phen)_2Co]_2(\mu\text{-}P_2O_7)\}$ in the case of **1**, or the very stable tris-phen species $\{[Ni(phen)_3]Cl_2\}$ in the case of **2** [compounds $\{[(phen)_2Co]_2(\mu\text{-}P_2O_7)\} \cdot 16H_2O$ (**5**) and $[Ni(phen)_3]Cl_2 \cdot 6.5H_2O$ (**6**), see Supporting Information]. In each case, the hydrolysis of the pyrophosphate anion has been avoided by reacting the metal salt first with the phen ligand and then with the pyrophosphate salt, following our established strategy in the field.[3,9−14]

The structural analysis of **1-4** revealed the complexes typically crystallized as hydrates, with respective formula $[Co(phen)_2(H_2P_2O_7)] \cdot 4H_2O$ (**1**·4H$_2$O),[5] $[Ni(phen)_2(H_2P_2O_7)]$ $8H_2O$ (**2**·8H$_2$O),[6] $[Cu(phen)(H_2O)(H_2P_2O_7)]$ (**3**)[7] and $\{[Cu(phen)(H_2O)(P_2O_7)][Na_2(H_2O)_8]\} \cdot 6H_2O$ (**4**·14H$_2$O).[8]

1 and **2** crystallize in the triclinic $P\bar{1}$ and monoclinic $P2_1/c$, respectively. The crystal structures are made up of neutral monomeric units of general composition $[M(phen)_2(H_2P_2O_7)]$ (with M = Co(II) in **1** and Ni(II) in **2**) held together by $\pi-\pi$ stacking between adjacent phen molecules and hydrogen bonds involving the water molecules of crystallization and the dihydrogen-pyrophosphate ligand. The metal ion shows a distorted octahedral geometry, being coordinated to two independent *cis* phen ligands and one dihydrogen-pyrophosphate group (see Figure 1(a-b)). The Co−N and Co−O bond distances (Tables S1) are sufficiently close to those found in the corresponding dimeric species **5** (Table S3) and in the literature Co(II) complexes $\{[(phen)_2Co]_2(\mu\text{-}P_2O_7)\} \cdot 6MeOH$ (**5a**)[9] and $\{[Co_2(\mu\text{-}P_2O_7)(bpym)_2] \cdot 12H_2O\}_n$ (bpym = 2,2' bipyrimidine),[14] featuring the bridging pyrophosphate tetraanion, while the Ni−N and Ni−O bond lengths (Tables S1) fit well with the previously reported $\{[(phen)_2Ni]_2(\mu\text{-}P_2O_7)\} \cdot 27H_2O$ species.[12] A combination of intermolecular phen−phen $\pi-\pi$ stacking interaction [interplanar distance in the range 3.3−3.4 Å] and hydrogen bonds between adjacent dihydrogen-pyrophosphate groups (Table S4) in both structures contribute to define supramolecular channels running along *a* (**1**) or *c* (**2**) and hosting the molecules of solvent (Figure S1).

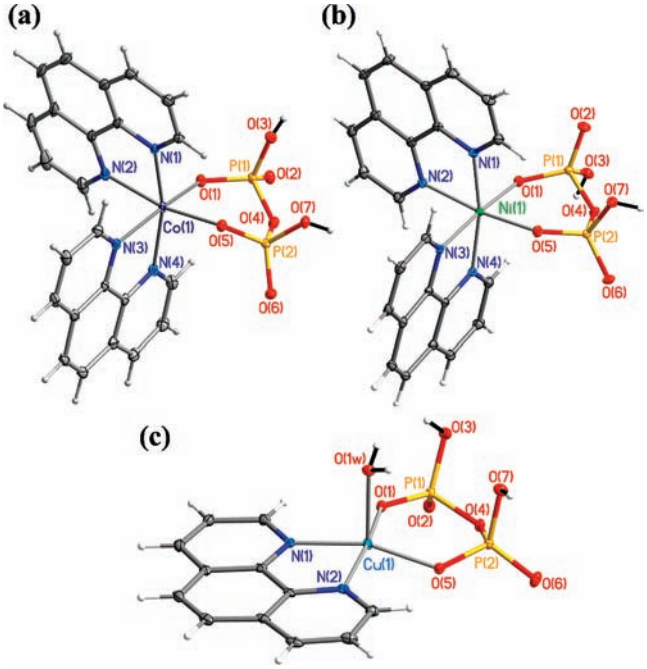

Figure 1. ORTEP plot (30% probability level) of (a) **1**, (b) **2** and (c) **3**, showing the atom labeling scheme. Refer to the Supporting Information file for compound **4**.

Compound **3** and **4** crystallize in the monoclinic $P2_1/n$ and triclinic $P\bar{1}$ space groups, respectively. The crystal structures are made up of neutral (**3**)/dianionic (**4**) monomeric units of formula $[Cu(phen)(H_2O)(H_2P_2O_7)]$ and $[Cu(phen)(H_2O)(P_2O_7)]^{-2}$ (Figures 1c and S2a, respectively), in which the five-coordinated copper(II) ion adopts a classical distorted square-pyramidal geometry (trigonal parameter[15] $\tau = 0.15$ for **3** and 0.11 for **4**) with two nitrogen atoms of a single phen ligand and two oxygen atoms of the dihydrogen (or tetranionic) pyrophosphate group occupying the equatorial positions and a water molecule occupying the apical one. Sodium cations (Table S2) and water molecules of crystallization are

(5) Crystal data ($\lambda = 0.71073$ Å and $T = 98(2)$ K) for **1**·4H$_2$O: $C_{24}H_{26}CoN_4O_{11}P_2$, $M_r = 667.36$, triclinic $P\bar{1}$, $a = 10.323(1)$, $b = 10.433(1)$ and $c = 12.746(1)$ Å, $\alpha = 80.459(2)$, $\beta = 85.572(2)$ and $\gamma = 82.841(2)°$, $V = 1340.9(2)$ Å^3, $Z = 2$, $D_c = 1.653$ g cm^{-3}, $F(000) = 686$, $\mu(Mo\text{-}K\alpha) = 0.829$ mm^{-1}, Refl. collected = 11809, Refl. indep. (R_{int}) = 5459 (0.0222), Refl. obs. [$I > 2\sigma(I)$] = 4744, refinement method = full-matrix least-squares on F^2, R_1 [$I > 2\sigma(I)$](all) = 0.0464 (0.0540), wR_2[$I > 2\sigma(I)$](all) = 0.1184 (0.1236), GoF = 1.059. CCDC 770952.

(6) Crystal data ($\lambda = 0.71073$ Å and $T = 98(2)$ K) for **2**·8H$_2$O: $C_{24}H_{34}N_4NiO_{15}P_2$, $M_r = 739.20$, monoclinic $P2_1/c$, $a = 12.365(1)$, $b = 24.118(2)$, $c = 10.944(7)$ Å, $\beta = 113.916(1)°$, $V = 2983.3(4)$ Å^3, $Z = 4$, $D_c = 1.646$ g cm^{-3}, $F(000) = 1536$, $\mu(Mo\text{-}K_\alpha) = 0.838$ mm^{-1}, Refl. collected = 28948, Refl. indep. (R_{int}) = 7364 (0.0610), Refl. obs. [$I > 2\sigma(I)$] = 5919, refinement method = full-matrix least-squares on F^2, R_1 [$I > 2\sigma(I)$](all) = 0.0400 (0.0536), wR_2[$I > 2\sigma(I)$](all) = 0.0968 (0.1037), $GoF = 1.025$. CCDC 770953.

(7) Crystal data ($\lambda = 0.71073$ Å and $T = 98(2)$ K) for **3**: $C_{12}H_{12}CuN_2O_8P_2$, $M_r = 437.72$, monoclinic $P2_1/n$, $a = 8.1826(9)$, $b = 20.643(2)$ and $c = 8.930(1)$ Å, $\beta = 91.123(2)°$, $V = 1508.1(3)$ Å^3, $Z = 4$, $D_c = 1.928$ g cm^{-3}, $F(000) = 884$, $\mu(Mo\text{-}K_\alpha) = 1.709$ mm^{-1}, Refl. collected = 13793, Refl. indep. (R_{int}) = 3596 (0.0557), Refl. obs. [$I > 2\sigma(I)$] = 2813, refinement method = full-matrix least-squares on F^2, R_1 [$I > 2\sigma(I)$](all) = 0.0308 (0.0442), wR_2[$I > 2\sigma(I)$](all) = 0.0655 (0.0698), $GoF = 0.928$. CCDC 770954.

(8) Crystal data ($\lambda = 0.71073$ Å and $T = 98(2)$ K) for **4**·14H$_2$O: $C_{12}H_{38}CuN_2Na_2O_{22}P_2$, $M_r = 733.90$, triclinic $P\bar{1}$, $a = 6.9426(5)$, $b = 10.9941(7)$ and $c = 19.421(1)$ Å, $\alpha = 100.248(1)$, $\beta = 90.574(1)$ and $\gamma = 97.102(1)°$, $V = 1340.9(2)$ Å^3, $V = 1446.7(2)$ Å^3, $Z = 2$, $D_c = 1.685$ g cm^{-3}, $F(000) = 762$, $\mu(Mo\text{-}K_\alpha) = 0.988$ mm^{-1}, Refl. collected = 12512, Refl. indep. (R_{int}) = 5908 (0.0171), Refl. obs. [$I > 2\sigma(I)$] = 5427, refinement method = full-matrix least-squares on F^2, R_1 [$I > 2\sigma(I)$](all) = 0.0380 (0.0413), wR_2[$I > 2\sigma(I)$](all) = 0.0989 (0.1036), $GoF = 1.036$. CCDC 770955.

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Table 1. IC_{50} values (μM) for **1-4** in A2780/AD cell line. Cisplatin controls and dimeric complexes previously reported by this author are also shown for comparison

	6 h	24 h	72 h	ref
$[Co(phen)_2(H_2P_2O_7)]$ (**1**)	> 1000	5 ± 1	0.03 ± 0.02	This work
$[Ni(phen)_2(H_2P_2O_7)]$ (**2**)	> 1000	> 1000	630 ± 390	This work
$[Cu(phen)(H_2O)(H_2P_2O_7)]$ (**3**)	14 ± 6	0.7 ± 0.3	0.14 ± 0.05	This work
$Na_2[Cu(phen)(H_2O)(P_2O_7)]$ (**4**)	15 ± 6	0.6 ± 0.6	0.08 ± 0.03	This work
Cisplatin	> 160	200 ± 16	13 ± 5	This work
$\{Co(phen)_2]_2(\mu\text{-}P_2O_7)\}$	-	2	1.7×10^{-4}	3
$\{Ni(phen)_2]_2(\mu\text{-}P_2O_7)\}$	-	590	304	3
$\{Cu(phen)(H_2O)]_2(\mu\text{-}P_2O_7)\}$	-	0.6	1.8×10^{-3}	3

also present in the crystal lattice of **4**. The Cu−N and Cu−O bond distances (Table S1) are in agreement with literature values,[10,11,16] including the parent dimer $\{[(phen)Cu(H_2O)]_2(\mu\text{-}P_2O_7)\}\cdot 8H_2O\}$.[3] As in **1** and **2**, the phen ligands in **3-4** are involved in significant $\pi-\pi$ interactions [interplanar distance of 3.4−3.6 Å] which delineate supramolecular 1D motifs running along the crystallographic *a* axis (Figure S3a-b). An extensive network of hydrogen bonds finally connects these chains in the *bc* plane, ensuring the three-dimensional cohesion (Tables S4−S5), with the solvent-free **3** network showing a much higher density compared to **1**, **2** and **4** (1.93 g cm^{-3} in **3** vs 1.65 g cm^{-3} in both **1** and **2** and 1.69 g cm^{-3} in **4**). No substantial differences were noted between **4** and the monomeric unit of a previously reported bipy analogue $\{[(bipy)Cu(H_2O)(P_2O_7)Na_2(H_2O)_6]\cdot 4H_2O\}$[11] other than the pyrophosphate anion in **4** does not coordinate the sodium cations as observed in the bipy structure. In fact, in **4** the sodium cations are connected only by bridging water molecules in order to form zigzag chains, which grow in the *b* directions (Figure S2b) and contribute to strongly separate the supramolecular monomeric chains along both the *b* and *c* axes (Figure S3b).

Detailed synthetic information for **1-6** and full crystallographic information are available in the Supporting Information file.

Table 1 shows the results obtained from the testing of **1-4** against the drug resistant ovarian cell line A2780/AD. Cisplatin was used as a control and for comparative purposes.

While **2** revealed little activity, the profiles of **1** and **3**/**4** show IC_{50} values reaching the low nanomolar range. However, at earlier time points they exhibit marked differences in cytotoxicities. At 6 h only the copper(II) species show activity with IC_{50} values of 14−15 μM, compared to over 1 mM for **1** and **2**. At 24 h both **1** and **3** demonstrate low μM activity, 5 and 0.7 μM, respectively. At 72 h the cytotoxicity observed for **1** and **4** becomes statistically similar, between 30 and 80 nM. Note that the two copper(II) species **3** and **4** have a similar cytotoxicity profile, suggesting that the anionic or neutral nature of the starting material is not critical for activity. The overall differences between the monomeric systems shown here may be attributed to the activation of different cytotoxicity pathways depending on the metal center, as already suggested for the dimeric analogues,[3] with redox chemistry/oxidative stress playing an important role. When comparing the monomers and dimers activities the cytotoxicity of both systems is similar at 24 h, however the dimers show increased cytotoxicity at 72 h, suggesting that kinetic control is involved. This also supports the idea that a hydrolysis mechanism is important for the activation of the dimers.[3]

In conclusion, this work further demonstrates the potential of pyrophosphate complexes as antitumor agents and also represents another step toward the understanding of the extremely high toxicity exhibited by the dimer complexes of Cu(II) and Co(II), listed and referenced in Table 1. The synthesis, characterization and cytotoxicity study of a series of other possible hydrolysis/aquated species such as the mono- or diphosphate mononuclear complexes (see Scheme SI) is ongoing in our laboratory. The investigation of the impact of the capping ligand on the activity of pyrophosphate-based drugs is also currently being explored, as is the reason(s) for the diverse activity noted for certain metal ions.

Finally, of interest to us is the fact that the synthesis of the monomers as described herein suggests a potentially facile route (utilizing pH) to mixed metal pyrophosphate dimers, a feat not yet reported in the literature for such coordination complexes. Given the extraordinary toxicity of the pyrophosphate based compounds reported to date and described herein, such complexes hold great potential.

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Supporting Information Available: Crystallographic data in CIF format; Scheme SI (postulated monomeric species formed upon the hydrolysis/aquation of pyrophosphate dimers); experimental details (synthesis of **1-6**, structural refinement of **1-6**, cell testing of **1-4**); selected bond distances and angles, and hydrogen bonds for **1-5** (Tables S1−S6); Figure S1−S6 (packing diagrams of **1-6**, ORTEP plot of **4-6**); TGA data. This material is available free of charge via the Internet at http://pubs.acs.org.

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