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Tetra to Dodecanuclear Oxomolybdate Complexes with Functionalized Bisphosphonate Ligands: Activity in Tumor Cell Killing

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Abstract

We report the synthesis and characterization of five novel Mo-containing polyoxometalate (POM) – bisphosphonate complexes with nuclearities ranging from 4 to 12 and with either fully reduced, fully oxidized or mixed valence (Mo^V, Mo^{VI}) molybdenum in which the bisphosphonates bind to the POM cluster via their two phosphonate groups as well as a deprotonated 1-OH group. Compounds were synthesized in water from the reaction of [Mo^V₂O₄(H₂O)]²⁺ or [Mo^{VI}O₄]²⁻ with H₂O₃PC(C₃H₆NH₂)OPO₃H₂ (alendronic acid) or its aminophenol derivative, and were characterized by single crystal X-ray diffraction and ³¹P NMR spectroscopy. (NH₄)₆[(Mo^V₂O₄)(Mo^{VI}₂O₆)₂(O₃PC(C₃H₆NH₃)OPO₃)₂].12H₂O (**1**) is an insoluble mixed valence species. [(C₂H₅)₂NH₂]₄[Mo^V₄O₈(O₃PC(C₃H₆NH₃)OPO₃)₂].6H₂O (**2**) and [(C₂H₅)₂NH₂]₆[Mo^V₄O₈(O₃PC(C₁₀H₁₄NO)OPO₃)₂].18H₂O (**4**) contain a similar tetranuclear reduced framework. Li₈[(Mo^V₂O₄(H₂O))₄(O₃PC(C₃H₆NH₃)OPO₃)₄].45H₂O (**3**) and Na₂Rb₆[(Mo^{VI}₃O₈)₄(O₃PC(C₃H₆NH₃)OPO₃)₄].26H₂O (**5**) are alkali salts of, respectively, fully reduced octanuclear and fully oxidized dodecanuclear POMs. The activities of **2–5** (which are water soluble) against three human tumor cell lines were investigated *in vitro*. **2–4** had weak but measurable activity but **5** had IC₅₀ values of ~10 μM, about four times the activity of the parent alendronate molecule, on a per- alendronate basis, opening up the possibility of the development of novel drug leads based on Mo- bisphosphonate clusters.

Keywords

anticancer agents; bisphosphonates; molybdenum; ³¹P NMR spectroscopy; polyoxometalates

Introduction

Bisphosphonates such as alendronate (Fosamax[®]), risedronate (Actonel[®]), ibandronate (Boniva[®]) and zoledronate (Zometa[®]) are important drugs used to treat osteoporosis,

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Paget's disease and hypercalcemia due to malignancy.[1] In addition, they have been found to kill tumor cells,[2] they activate $\gamma\delta$ T cells (containing the $V\gamma 2V\delta 2$ T cell receptor) to kill tumor cells and zoledronate has been found to switch tumor associated macrophages from an M2, tumor promoting to an M1, tumor killing phenotype.[3] Furthermore, they are active in killing a broad range of protozoan parasites both *in vitro*[4] and *in vivo*,[5] and are thus of interest as both anti-cancer as well as anti-infective agents. All commercially available bisphosphonate drugs are geminal bisphosphonates and have a common P-C-P backbone (Figure 1a) together with an R_1 hydroxyl group which increases the affinity of bisphosphonates for bone mineral and is called the "bone hook". R_2 determines the potency of the bisphosphonates, all of which function by inhibiting the enzyme farnesyl diphosphate synthase, in osteoclasts.[1] Alendronate ($R_1 = OH$, $R_2 = CH_2CH_2CH_2NH_2$) is one of the most popular bisphosphonates and has been sold for many years as a treatment for osteoporosis. Bisphosphonates are also known to be good metal complexing agents, and many bisphosphonate compounds have been structurally characterized, varying from molecular complexes to three-dimensional coordination frameworks.[6] Among these complexes, polyoxometalates (POMs) are of interest since POMs themselves have interesting medicinal properties, including anti-tumoral and anti-retroviral activity[7], and POM - hybrid clusters lead to selective cell adhesion to surfaces.[8]

Polyoxometalates are often considered as soluble molecular oxides. They are built by connecting $\{MO_x\}$ polyhedra, where M is a d-block element in a high oxidation state, usually $V^{IV,V}$, Mo^{VI} or W^{VI} . [9] In earlier work, Sergienko et al. reported the crystallographic structures of the reaction products of Mo^{VI} and W^{VI} ions with bisphosphonates, mainly etidronate.[10] In parallel, Kortz and Pope isolated the polymeric W^{VI} derivative $\{[(O_3PCHN(CH_3)_2PO_3)W_2O_6]^{4-}\}_\infty$, [11] as well as the hexanuclear Mo^{VI} POM, $[(O_3PCH_2PO_3)Mo_6O_{18}(H_2O)_4]^{4-}$. [12] More recently, Wang et al. described the synthesis of two mixed V^{IV}/Mo^{VI} species, one with etidronate, the other with alendronate. [13] In our group, we initiated a study of the reactivity of the reduced dinuclear $\{Mo^V_2O_4\}^{2+}$ group with bisphosphonates, starting with the simplest species, methylenediphosphonate ($R_1 = H$, $R_2 = H$), where we reported a large family of ring-shaped reduced POMs built up by the alternation of organic ligands and dimers of edge-sharing Mo^V octahedra, having the general formula $\{(Mo^V_2O_4)(O_3PCH_2PO_3)\}_n$ ($n = 3, 4, 6, 10$). [14] Some of these compounds encapsulated extra ligands such as carbonate, [14a] sulfite [14b] or methylarsenate. [14c] These anions have been used successfully as both reductants and as stabilizing agents for the synthesis of metallic nanoparticles (Pd^0 , Pt^0 , Ag^0), [14c, 15] and we recently extended this family of compounds by using the commercial drug, etidronate ($R_1 = OH$, $R_2 = CH_3$). [16]

Among the POMs isolated so far with etidronate, only that with a cyclohexane-like shape is analogous to those obtained with methylenediphosphonate. The other species that have been isolated are the tetranuclear anion $[(Mo^V_2O_4)(O_3PC(CH_3)O)PO_3]^{6-}$, and the tetradecanuclear ring $[(Mo^V_2O_4)_7(O_3PC(CH_3)(OH)PO_3)_2(CH_3COO)_7]^{21-}$. To date, none of the POMs with bisphosphonate ligands have been studied for activity in cells. We thus decided to explore the reactivity of Mo^V and Mo^{VI} ions with more complex bisphosphonates which, on their own, possess tumor cell killing activity, starting with alendronate ($R_1 = OH$, $R_2 = (CH_2)_3NH_2$), a drug used commercially, [17] the aim being to see if more potent activity could be obtained. Here, we describe the synthesis, crystallographic and ^{31}P NMR characterizations of four novel POMs containing alendronate (noted here as **Ale**): two with Mo^V , in addition to one with Mo^{VI} and an insoluble mixed-valence POM. We also report the synthesis of an aminophenol derivative (noted here **MP-Ale**) of the alendronate ligand, with enhanced binding capacity to transition metal ions, as well as a tetranuclear Mo^V compound incorporating this new ligand. Compounds were then screened for tumor cell killing ability, *in vitro*.

Results and Discussion

Crystal structure

In the five crystal structures determined, the 1-hydroxyl group of the bisphosphonate ligand is found to be deprotonated and coordinates to either one or two metal ions, as illustrated in Scheme 1. Valence bond calculations[18] (Figures SI1-SI5) support the proposed oxidation states of Mo in **1–5**, as well as the deprotonation of the hydroxyl groups, and also indicate the presence of terminal water molecules, in **3**. The anion in **1** (Figure 2a) is built up by the connection of three dimeric units: one contains two edge-sharing Mo^V octahedra, the others are two face-sharing Mo^{VI} octahedra. Each Mo^V octahedron shares one vertex with one Mo^{VI} octahedron of each Mo^{VI} dimer. The {PO₃} groups of the bisphosphonate ligands are equivalent and connect two Mo^{VI} ions and a Mo^V ion, as illustrated in Scheme 1a. The deprotonated hydroxyl group of **Ale** binds to one Mo^{VI} ion. The six Mo ions are coplanar, but the organic ligands are not located in this plane and the anion in **1** contains only one symmetry element, namely a C₂ axis passing through the center of the Mo^V-Mo^V bond (Figure 2a). The NH₃⁺ groups of the organic ligand are involved in hydrogen bonds with a water molecule and with oxygen atoms of neighbouring POMs, as illustrated in Figure 2b.

The anions in **2** and **4** possess the same compact Mo^V framework (Figures 3a, 4a), similar to the structure of the tetranuclear anion [(Mo^V₂O₄)(O₃PC(CH₃)O)PO₃)₂]⁶⁻, except that the etidronate ligands are replaced by **Ale** or **MP-Ale** ligands, respectively. They contain a centrosymmetric rhomboid {Mo^V₄O₁₆} core, commonly encountered in Mo^V chemistry.[19]

The crystallographically equivalent bisphosphonate ligands act as tetradentate ligands (Scheme 1b) and connect dissymmetrically to the Mo ions. Indeed, one {PO₃} group is monocoordinated to a Mo^V center, while the other phosphonato group is coordinated via two oxygen atoms to an adjacent metallic center. Additionally, the deprotonated hydroxyl group acts as a μ₂-O ligand. In both structures, the counter-ions are dimethylammonium cations which establish hydrogen bonds with the POMs (Figures 3b, 4b). However, these two compounds are not isostructural, and their three dimensional structures are also different. Indeed, in **2** the Mo frameworks of the POMs are parallel while in **4** they are perpendicular, probably because the ligand in **4** is far bulkier than that in **2**.

The structure of the octanuclear anion in **3** (Figure 5a) has not been observed previously in this family of Mo^V/bisphosphonate complexes. It is built up from four Mo^V dimers which do not share any oxygen atoms, and are only linked by **Ale** molecules, acting as pentadentate ligands (Scheme 1c). One of the Mo^V ion is bound to three oxygen atoms of the organic ligand, while the other is only bound to two oxygen atoms of **Ale**, and is also connected to a terminal water molecule. The positions of the –CH₂-CH₂-CH₂-NH₃⁺ arm of the organic ligand around the circular Mo framework evoke a paddle wheel shape. The Mo ions are not coplanar, however, and the wheel is twisted. There are no crystallographic symmetry elements in the structure of **3**. However, the anion possesses a pseudo-S₄ axis passing through the center of the POM framework (Figure 5b), and in the three-dimensional structure of **3**, the anions assemble in centrosymmetric structures and interact via N-H...O hydrogen bonds (Figure 5c).

The structure of the anion in **5** (Figure 6a) can be best described as the assembly of four trimeric units. A trimeric unit is built of a dimer of face-sharing Mo^{VI} octahedra which has a common vertex with a monomeric Mo^{VI} octahedron (Figure 6b). The **Ale** ligand acts as a hexadentate ligand with a coordination mode similar to that encountered in **1** (Scheme 1a), together with an additional connection to the monomeric Mo^{VI} ion of a neighboring trimeric group, via a terminal oxygen atom of one of the P=O groups (indicated with an arrow in Figure 6b). Among the six rubidium and two sodium counter-ions, an octa-coordinated

sodium ion occupies a special position at the center of the POM, acting as a template ion (shown in purple in Figure 6a). Intramolecular N-H...O hydrogen bonds between the amino group of the **Ale** ligand and terminal or bridging atoms of the POM core stabilize this dodecanuclear architecture (Figures 6c and SI6), and we have recently found evidence for their key role in the intrinsic photochromic properties of these Mo^{VI} species.[20] Although the anion does not lay on any crystallographic symmetric element, a pseudo-S₄ axis can be identified, which passes through the central sodium ion (Figure 6a), and the three dimensional structure (Figure 6d) shows a complex network of polyoxometalate ribbons.

³¹P NMR characterization

The synthetic pathways to **1–5** are shown in Figure 1. We characterized these species, as described above, in the solid state by using single crystal X-ray diffraction and in solution, by ³¹P NMR. As described previously,[14b,21] ³¹P NMR is a powerful tool to determine whether structures characterized in the solid state are maintained in solution, for investigation of the stability of isolated compounds in solution, and for characterizing the nature of the species present in these solutions. In the case of **1**, we find that this compound is rather insoluble in all common solvents. Nevertheless, two features can be seen: i) the ³¹P NMR spectrum of a freshly prepared solution of **1** indicates the presence of the octanuclear anion [(Mo^V₂O₄(H₂O))₄(O₃PC(C₃H₆NH₃)OPO₃)₄]⁸⁻, which crystallizes as **3** (Figure SI7) and ii) the synthetic process leading to **1** requires slow air oxidation of the reacting solution. Formation of mixed-valence species from solutions of [Mo₂O₄(H₂O)₆]²⁺ and methylenediphosphonate ligands has been observed previously and permitted isolation of the triangular anion [(Mo^V₂O₄)₃(Mo^{VI}O₄)(O₃PCH₂PO₃)₃]⁸⁻, which encapsulates a {Mo^{VI}O₄} tetrahedron, as well as the rectangular [(Mo^V₂O₄)₂(Mo^{VI}O₃)₂(O₃PCH₂PO₃)₂(HO₃PCH₂PO₃)₂]¹⁰⁻ species, containing two Mo^{VI} centers.[14b] The ³¹P NMR spectra of freshly prepared synthetic solutions of **2** and **4** (Figure SI8) are similar to those of crystals of **2** and **4** dissolved in water (Figure 7). These spectra exhibit a doublet of doublets, in agreement with the presence of two non-equivalent and coupled phosphorus atoms within the bisphosphonate ligand. No change is observed over a period of one week, showing that **2** and **4** are stable in pure aqueous solutions. The chemical shifts and coupling constants of **2** and **4** are very similar, and are also similar to those of the etidronate derivative [(Mo^V₂O₄)₂(O₃P(C(CH₃)O)PO₃)₂][6–16] (Table 1).

In contrast, the ³¹P NMR spectrum of **2** dissolved in the presence of Li⁺ changes rapidly with time: a solution of **2** in 1M LiCl (Figures 8 and SI9) indicates a degradation of **2** into three species. Slow evaporation of a solution of **2** in 1M LiCl afforded crystals of **3**, and a comparison of the ³¹P NMR spectrum of **3** redissolved in water with that of a solution of **2** in 1M LiCl permits an identification of **3** as one of the three species. The other two are unknown. The ³¹P NMR spectrum of **3** (Figure 7) also exhibits a doublet of doublets (as with **2** and **4**), but the splitting between the two doublets is smaller, and the coupling constant is larger. As expected, like **2**, **4** degrades slowly in 1M LiCl. With **5**, this salt is sparingly soluble in water, but dissolves readily in a 1M NaOAc/AcOH buffer and 1M NaCl. As for **2–4**, the ³¹P NMR spectrum of **5** (Figure 7) exhibits a doublet of doublets, in accord with the presence of two non equivalent phosphorus atoms. Moreover, solutions of **5** are stable over a period of at least one week.

Cell Growth Inhibition

In order to determine whether **1–5** had any activity in tumor cell growth inhibition, we screened all compounds against three tumor cell lines: NCI-H460 (human non-small lung cancer); MCF-7 (human breast cancer) and SF-268 (human CNS cancer). **1** was poorly soluble and no inhibition activity was found, however, **2–5** all had measurable activity (Table 2). For **2–4** the activity was worse than that of alendronate, especially when

converted to a per-alendronate basis. However, **5** was clearly more active than was alendronate. Averaging over the three cell lines, alendronate had an IC_{50} of $\sim 160 \mu M$ while that for **5** was $\sim 10 \mu M$, or $\sim 40 \mu M$ on a per-alendronate basis, a factor of four increase in potency. Whether this enhanced activity is due to dissociation of the complex outside the cell (and uptake of alendronate and a molybdate), to dissociation inside the cell, or to POM-complex activity *per se* (as with e.g. $Mo_7O_{24}^{2-}$ [7c]) is unknown at this time. However, the comparison of the activity of **2–5** with that of the commercial $(NH_4)_6Mo^{VI}_7O_{24}$ and of $LiNa[Mo^V_4O_8(OH)_2(H_2O)_2(C_4O_4)_2]$ [22] which do not contain any bisphosphonate ligands suggest that the POM and the alendronate ligand have a synergic effect. In conclusion, the observation that bisphosphonate activity is enhanced is of interest, and suggests that more lipophilic bisphosphonate[2] POM complexes may have much better activity.

Conclusion

Hybrid bisphosphonate-POM complexes built by condensing Mo octahedra and bisphosphonate ligands having nuclearities from 4 to 12, either fully reduced, mixed-valence or fully oxidized, have been isolated, and show the strong chelating ability of this class of ligand. As observed with previously reported Mo/bisphosphonate complexes, the geometry of the POM is strongly dependent on the nature of the ligand and counter-ion. While most bisphosphonate-POM compounds reported in the literature are insoluble, with the exception of **1**, the Mo^{VI} and Mo^V complexes reported here are soluble and stable in aqueous solution, as evidenced by ^{31}P NMR. Our results thus open the way to the synthesis of a large family of functionalized Mo/bisphosphonates in which the nature of the organic ligand is varied. It may thus be possible to deliver hybrid POM-bisphosphonates containing redox-active POMs, which have potent anti-tumor activity as well as potent bisphosphonates, all packed in a lipophilic complex, increasing cell uptake, as well as bisphosphonate potency, and further work on such systems is underway.

Experimental Section

Chemicals and reagents

Alendronic acid, $H_2O_3PC(C_3H_6NH_2)(OH)PO_3H_2$ (**H₄Ale**), was synthesized as described in the literature.[23] All other chemicals were used as purchased without further purification. The synthetic pathways to the aminophenol derivative of **Ale** (**MP-Ale**) and to **1–5** are given in Figure 1.

Preparation of a 0.10M solution of $[Mo_2O_4(H_2O)_6]^{2+}$ in 4 M HCl

Hydrazine hydrate $N_2H_4 \cdot H_2O$ (210 μL , 4.29 mmol) was added to a suspension of MoO_3 (2.30 g, 16.0 mmol) in 4M HCl (80 mL). The mixture was then stirred at $60^\circ C$ for 3 h and the resulting deep red solution allowed to cool to room temperature, before further use.

Synthesis of $Na_2HO_3PC(C_{10}H_{14}NO)(OH)PO_3H \cdot 2H_2O$ (Na_2H_2 (MP-Ale))

H₄Ale (5g, 20 mmol) and salicylaldehyde (2.5 mL, 24 mmol) were dissolved in a mixture of methanol (100 mL) and triethylamine (17 mL, 120 mmol). The deep orange solution was refluxed for two hours, then cooled to room temperature before addition of sodium borohydride (1.14 g, 30 mmol), in small portions. The resulting yellow suspension was refluxed overnight, then the pale yellow solid (5.9 g, yield 73%) was filtered under vacuum, thoroughly washed with methanol, then dried with diethyl ether. Anal. calc. for $C_{11}H_{17}NNa_2O_8P_2$ (found): C 30.36 (29.71), H 4.86 (4.73), N 3.22 (3.12), Na 10.56 (9.75), P 14.23 (14.29). 1H NMR (D_2O): δ (ppm) = 1.96 (m, 4H); 3.08 (t, 2H); 4.21 (s, 2H); 6.96 (m, 2H); 7.34 (m, 2H). ^{31}P NMR (D_2O): δ (ppm) = 21.1 (s).

Synthesis of $(\text{NH}_4)_6[(\text{Mo}^{\text{V}}_2\text{O}_4)(\text{Mo}^{\text{VI}}_2\text{O}_6)_2(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_2]\cdot 12\text{H}_2\text{O}$ (1)

H₄Ale (0.156 g, 0.625 mmol) was dissolved in a 0.1M solution of $[\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6]^{2+}$ (6.25 mL, 0.625 mmol) synthesized as described above. A 33% ammonium hydroxide solution (1.8 mL, 15 mmol) was then added dropwise to pH = 7.3. The resulting deep red solution was filtered, then left to evaporate at room temperature. After 2 days, large red parallelepipedic crystals (0.122 g, 37% based on Mo) were collected, by filtration. Anal. calc. for $\text{C}_8\text{H}_{66}\text{O}_{44}\text{N}_8\text{P}_4\text{Mo}_6$ (found): C 5.72 (6.10), H 3.96 (4.06), N 6.68 (6.95), P 7.38 (7.69), Mo 34.30 (35.71). IR (KBr pellets): ν (cm^{-1}) = 1458 (m), 1398 (s), 1268 (w), 1136 (s), 1076 (s), 1053 (vs), 1021 (s), 988 (m), 923 (s), 912 (s), 874 (vs), 816 (s), 787 (sh), 752 (w), 734 (sh), 706 (m), 668 (m).

Synthesis of $[(\text{C}_2\text{H}_5)_2\text{NH}_2]_4[\text{Mo}^{\text{V}}_4\text{O}_8(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_2]\cdot 6\text{H}_2\text{O}$ (2)

H₄Ale (0.156 g, 0.625 mmol) was dissolved in a 0.1 M solution of $[\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6]^{2+}$ (6.25 mL, 0.625 mmol) prepared as described above. Diethylamine (2.8 mL, 27 mmol) was added dropwise to pH = 6.8. The resulting deep red solution was filtered and left to evaporate at room temperature. Small, red, parallelepipedic crystals (0.211 g, 48% based on Mo) were collected by filtration the following day. Anal. calc. for $\text{C}_{24}\text{H}_{78}\text{Mo}_4\text{N}_6\text{O}_{28}\text{P}_4$ (found): C 20.59 (20.76), H 5.59 (5.52), Mo 27.28 (27.64), N 5.97 (6.05), P 8.81 (8.92). IR (KBr pellets): ν (cm^{-1}) = 1558 (w), 1453 (m), 1391 (w), 1165 (s), 1139 (vs), 1113 (s), 1058 (sh), 1046 (s), 1029 (s), 965 (vs), 936 (s), 891 (m), 845 (w), 811 (m), 737 (s). ^1H NMR (D_2O): δ (ppm) = 1.14 (t, 24H); 1.97 (m, 4H); 2.34 (m, 2H); 2.96 (q, 16H).

Synthesis of $\text{Li}_8[(\text{Mo}^{\text{V}}_2\text{O}_4(\text{H}_2\text{O}))_4(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_4]\cdot 45\text{H}_2\text{O}$ (3)

2 (0.2 g, 0.14 mmol) was dissolved in 1M LiCl (14 mL). 0.1 M hydrochloric acid was added dropwise to pH = 4.0. The red solution was left to evaporate at room temperature. Large, red, parallelepipedic crystals (0.142 g, 68% based on Mo) were collected by filtration after 2 days. Anal. calc. for $\text{C}_{16}\text{H}_{134}\text{Li}_8\text{Mo}_8\text{N}_4\text{O}_{93}\text{P}_8$ (found): C 6.53 (6.67), H 4.59 (4.09), Li 1.89 (1.88), Mo 26.09 (26.11), N 1.90 (1.82), P 8.42 (8.68). IR (KBr pellets): ν (cm^{-1}) = 1518 (m), 1470 (w), 1451 (w), 1396 (w), 1153 (s), 1127 (s), 1065 (vs), 1029 (s), 948 (vs), 917 (sh), 745 (m). ^1H NMR (D_2O): δ (ppm) = 2.26 (m, 4H); 3.07 (t, 2H).

Synthesis of $[(\text{C}_2\text{H}_5)_2\text{NH}_2]_6[\text{Mo}^{\text{V}}_4\text{O}_8(\text{O}_3\text{PC}(\text{C}_{10}\text{H}_{14}\text{NO})\text{OPO}_3)_2]\cdot 18\text{H}_2\text{O}$ (4)

Na₂H₂(MP-Ale) (0.544 g, 1.25 mmol) synthesized as described above was dissolved in a 0.1M solution of $[\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6]^{2+}$ (12.5 mL, 1.25 mmol). Diethylamine (5.4 mL, 52 mmol) was added dropwise to pH = 7.7. The resulting deep red solution was filtered and left to evaporate at room temperature. Red hexagonal crystals (0.178 g, 14% based on Mo) were collected by filtration after two days. Anal. calc. for $\text{C}_{46}\text{H}_{136}\text{Mo}_4\text{N}_8\text{O}_{42}\text{P}_4$ (found): C 27.88 (27.93), H 6.92 (6.57), Mo 19.37 (17.05), N 5.66 (6.22), P 6.25 (6.16). IR (KBr pellets): ν (cm^{-1}) = 1506 (w), 1461 (m), 1389 (w), 1256 (m), 1160 (sh), 1139 (s), 1120 (s), 1045 (vs), 1026 (s), 967 (vs), 945 (sh), 926 (s), 876 (m), 804 (m), 766 (m), 733 (s). ^1H NMR (D_2O): δ (ppm) = 1.25 (t, 36H); 2.05 (m, 4H); 2.30 (m, 2H); 3.03 (q, 24H); 4.14 (s, 4H); 6.92 (m, 4H); 7.29 (m, 4H).

Synthesis of $\text{Na}_2\text{Rb}_6[(\text{Mo}^{\text{VI}}_3\text{O}_8)_4(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_4]\cdot 26\text{H}_2\text{O}$ (5)

To a solution of $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ (2.72 g, 11.2 mmol) in 30 mL of water was added **H₄Ale** (0.929 g, 3.73 mmol). Concentrated hydrochloric acid was added dropwise to pH = 3.0. The solution was then stirred for 30 min before addition of solid RbCl (0.9 g, 7.44 mmol). The solution was allowed to stir for another 2 hours, during which time a fine white powder slowly precipitated. This solid was filtered off and the filtrate left to evaporate at room temperature. Colourless crystals were collected the following day. Yield 0.429 g (12% based on Mo). Anal. calc. for $\text{C}_{16}\text{H}_{88}\text{Mo}_{12}\text{N}_4\text{Na}_2\text{O}_{86}\text{P}_8\text{Rb}_6$ (found): C 5.23 (5.05), H 2.42 (1.94),

Mo 31.36 (32.16), N 1.53 (1.43), Na 1.25 (1.75), P 6.75 (6.56), Rb 13.97 (13.59). IR (KBr pellets): ν (cm⁻¹) = 1505 (m), 1469 (m), 1452 (m), 1397 (w), 1333 (w), 1169 (s), 1128 (s), 1051 (s), 1026 (vs), 964 (sh), 927 (vs), 884 (vs), 762 (s), 743 (w), 697 (s), 652 (s), 598 (w), 555 (s).

X-ray crystallography

Data collection was carried out by using a Siemens SMART three-circle diffractometer for all the structures except for **4** and **5** for which data were collected using a Bruker Nonius X8 APEX 2 diffractometer. Both were equipped with a CCD bidimensional detector using the monochromatized wavelength λ (Mo K α) = 0.71073 Å. Data were collected at room temperature for **1–3** and **5**, and at 100 K for **4**, since these crystals were unstable due to loss of water of hydration. Absorption corrections were based on multiple and symmetry-equivalent reflections in the data set using the SADABS program,[24] based on the method of Blessing.[25] Structures were solved by direct methods, and refined by full-matrix least-squares using the SHELX-TL package.[26] In all the structures, there is a discrepancy between the formulae determined by elemental analysis and that deduced from the crystallographic atom list because of the difficulty in locating all the disordered water molecules and alkali counter-ions. Disordered water molecules and alkali counter-ions were thus refined with partial occupancy factors, and with isotropic thermal parameters. In the structure of **1**, NH₄⁺ and H₂O could not be distinguished based on the observed electron densities; therefore, all positions were labeled as O and assigned the oxygen atomic diffusion factor. Crystallographic data are given in Table 3.

CCDC-779541 – 779545 contain the supplementary crystallographic data of **1–5**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

NMR measurements

³¹P NMR spectra were recorded with ¹H decoupling on a Bruker AC-300 spectrometer operating at 121.5 MHz, using 5 mm tubes. ³¹P chemical shifts were referenced to the external standard of 85 % H₃PO₄. For all compounds, ~20 mg of sample were dissolved in 700 µL of D₂O, except for **5**, which was dissolved in 700 µL of a 10% D₂O/90% 1M NaOAc/AcOH buffer mixture. The concentrations thus varied between 2–8 mM. For the kinetic study, 20 mg (1.34 10⁻⁵ mol) of **2** was dissolved in 600 µL of 1M LiCl solution (pH = 3.7) to which 100 µL of D₂O was added.

Infrared spectra

IR spectra were recorded on an IRFT Magna 550 Nicolet spectrophotometer, using KBr pellets.

Cell Growth Inhibition Assays

Human tumor cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (lung large cell), and SF-268 (central nervous system glioblastoma) were obtained from the National Cancer Institute and maintained at 100% humidity and 5% CO₂ at 37 °C. Cell lines were cultured in RPMI-1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum (Gibco, Grand Island, NY) at 37°C in a 5% CO₂ atmosphere with 100% humidity. Compound stock solutions were typically prepared in water at a concentration of 0.04 M. A broth microdilution method was used to determine the bisphosphonate growth inhibition IC₅₀ values. Compounds were half log serial diluted using cell culture media into 96-well TC-treated round bottom plates (Corning Inc., Corning, NY) typically from 1264 µM to 40 nM, but in some cases compounds were run over a larger concentration range to enable

accurate IC₅₀ determinations. Cells were plated at a density of 5000 cells/well. Cells were then incubated under the same culture conditions for 4 days at which time an MTT ((3-(4,5-dimethylthi-azole-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay (ATCC, Manassas, VA) was performed to obtain dose response curves. Briefly, cells were incubated for 2 h under culture conditions with the tetrazolium salt, lysed with detergent, incubated overnight, protected from light at room temperature. The absorbance at 600 nm was read the following day using a SpectraMax Plus 384 spectrophotometer (Molecular Devices, Sunnyvale, CA). The compound concentration for 50% growth inhibition values (IC₅₀) were obtained by fitting absorbance data to a rectangular hyperbolic function: $I = (I_{\max})(C)/IC_{50} + C$ where I is the percent inhibition, $I_{\max} = 100\%$ inhibition and C is the concentration of the inhibitor, using GraphPad PRISM 3.0 software for windows (Graphpad Software Inc., San Diego, CA).

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Figure 1.

a) General formula of the geminal bisphosphonates used for medical applications. b) Reaction scheme for the synthesis of the bisphosphonate ligand $\text{H}_4\text{O}_3\text{PC}(\text{C}_{10}\text{H}_{14}\text{NO})(\text{OH})\text{PO}_3$ (**MP-Ale**). c), d) Synthetic pathways for polyoxometalates **1-5**.

Figure 2.

a) Mixed ball-and-stick and polyhedral representation of the mixed-valence anion $[(\text{Mo}^{\text{V}}_2\text{O}_4)(\text{Mo}^{\text{VI}}_2\text{O}_8)_2(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_2]^{6-}$ in **1**. The arrow indicates the position of the symmetry axis. b) 3D structure of **1** with intermolecular hydrogen bonds. Some H atoms have been omitted for clarity.

Figure 3.

a) Mixed ball-and-stick and polyhedral representation of the fully reduced tetranuclear anion $[\text{Mo}_4^{\text{V}}\text{O}_8(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_2]^{4-}$ in **2**. b) 3D structure of **2** with intermolecular hydrogen bond interactions. Some H atoms have been omitted for clarity.

Figure 4. a) Mixed ball-and-stick and polyhedral representation of the fully reduced tetranuclear anion $[\text{Mo}^{\text{V}}_4\text{O}_8(\text{O}_3\text{PC}(\text{C}_{10}\text{H}_{14}\text{NO})\text{OPO}_3)_2]^{6-}$ in **4**. b) 3D structure of **4** with intermolecular hydrogen bond interactions. Some H atoms have been omitted for clarity.

Figure 5.
 a) and b) Mixed ball-and-stick and polyhedral representations of the paddle wheel shaped $[(\text{Mo}^{\text{V}}_2\text{O}_4(\text{H}_2\text{O}))_4(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_4]^{8-}$ anion in **3** with two different orientations. The arrow indicates the position of the pseudo symmetry axis. c) 3D structure of **3** with intermolecular hydrogen bond interactions. Some H atoms have been omitted for clarity.

Figure 6. Mixed ball-and-stick and polyhedral representation of a) the Mo^{VI} anion $[(\text{Mo}^{\text{VI}}\text{O}_8)_4(\text{O}_3\text{PC}(\text{C}_3\text{H}_6\text{NH}_3)\text{OPO}_3)_4]^{8-}$ in **5**; the arrow indicates the position of the symmetry axis and b) the trimeric building unit in **5**; the arrow indicates the position of the oxygen atom of the $\text{P}=\text{O}$ group involved in a $\text{P}-\text{O}-\text{Mo}$ bond with an adjacent trimeric unit. c) Representation of the anion in **5** showing the intramolecular hydrogen bonds. d) 3D structure of **5**. Some H atoms have been omitted for clarity.

Figure 7.
 $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of **2–4** dissolved in water and **5** dissolved in 1M HOAc/NaOAc buffer solution.

Figure 8.
Evolution with time of a solution of **2** dissolved in 1M LiCl (pH = 3.7) at room temperature; X1 and X2 are unidentified intermediate species.

Scheme 1.

Representation of the coordination modes of the bisphosphonate ligands in a) complexes **1** and **5**, b) complexes **2** and **4**, c) complex **3**.

4	5	$[(\text{Mo}^{\text{V}}_2\text{O}_4)_2(\text{O}_3\text{PC}(\text{CH}_3)\text{O})\text{PO}_3]^{6-}$	$[(\text{Mo}^{\text{V}}_2\text{O}_4)_6(\text{OH})_6(\text{O}_3\text{PCH}_2\text{PO}_3)_6]^{18-}$	$[(\text{Mo}_2\text{O}_4)_3(\text{O}_3\text{PCH}_2\text{PO}_3)_3(\text{CH}_3\text{AsO}_3)]^{8-}$
this work	this work	16	14c	14c

4	5	[(Mo ^V ₂ O ₄) ₂ (O ₃ PC(CH ₃)O)PO ₃] ⁶⁻		[(Mo ^V ₂ O ₄) ₆ (OH) ₆ (O ₃ PCH ₂ PO ₃) ₆] ¹⁸⁻	[(Mo ₂ O ₄) ₃ (O ₃ PCH ₂ PO ₃) ₃ (CH ₃ AsO ₃)] ¹⁸⁻
H ₂ O	1M	H ₂ O	1M NaCl	H ₂ O	H ₂ O
		NaOAc/HOAc			
30.91	25.02	32.28	24.52	25.55	
30.62	24.52	32.10	24.40	25.42	
20.20	19.93	22.12	19.82	23.52	
19.92	19.43	21.93	19.70	23.39	
23.4	40.6	22.1	13.7	15.6	

Table 2

Cell growth inhibition assay results for **2–5** compared to alendronate and Mo^V or Mo^{VI} bisphosphate-free POMs.

Compound	NCI-H460	MCF-7	SF-268
	IC ₅₀ (μM)	IC ₅₀ (μM)	IC ₅₀ (μM)
2	201 ±16	171±8	105±32
3	109±75	107±21	365±43
4	443±67	126±10	39±3
5	12±4	11±1	7±1
Alendronate	202±43	126±2	136±13
LiNa[Mo ^V ₄ O ₈ (OH) ₂ (H ₂ O) ₂ (C ₄ O ₄) ₂]	476±60	605±1	457±14
(NH ₄) ₆ [Mo ^{VI} ₇ O ₂₄]	199±5	283±29	183±4

Table 3

Crystallographic data for **1** – **5**.

	1	2	3	4	5
Empirical formula	C ₈ H ₆₆ Mo ₆ N ₈ O ₄₄ P ₄	C ₂₄ H ₇₈ Mo ₄ N ₆ O ₂₈ P ₄	C ₁₆ H ₁₃₄ Li ₈ Mo ₈ N ₄ O	C ₄₆ H ₁₃₆ Mo ₄ N ₈ O	4 ₂ P ₄ C ₁₆ H ₈₈ Mo ₁₂ N ₄ Na ₂ O
Formula weight, g	1678.18	1406.56	2942.07	1981.26	3670.72
Crystal system	orthorhombic	Triclinic	triclinic	triclinic	monoclinic
Space group	P2 ₁ 2 ₁ 2	P-1	P-1	P-1	P2 ₁ /c
<i>a</i> /Å	14.274(2)	9.4664(8)	17.0185(1)	13.970(1)	20.7814(6)
<i>b</i> /Å	17.412(2)	11.0739(9)	17.248(1)	14.063(1)	21.2334(6)
<i>c</i> /Å	9.583(1)	14.062(1)	17.610(2)	20.784(2)	29.3749(7)
<i>α</i> /deg	90	78.103(2)	96.874(2)	72.948(4)	90
<i>β</i> /deg	90	72.168(2)	104.411(2)	74.989(4)	134.944(1°)
<i>γ</i> /deg	90	85.285(2)	94.772(1)	61.029(4)	90
<i>V</i> /Å ³	2381.8(6)	1372.9(2)	4936.1(8)	3381.7(5)	9174.4(2)
<i>Z</i>	2	1	2	2	4
<i>ρ</i> _{calc} /g cm ⁻³	2.322	1.687	1.979	1.544	2.417
<i>μ</i> /mm ⁻¹	1.793	1.091	1.243	0.901	4.997
Data/Parameters	7022/289	7750/291	27807/1123	12086/761	16137/1098
<i>R</i> _{int}	0.0624	0.0374	0.0298	0.0520	0.0488
GOF	1.081	0.995	1.032	1.095	1.185
<i>R</i> (>2σ(<i>I</i>))	<i>R</i> _I = 0.0599	<i>R</i> _I = 0.0585	<i>R</i> _I = 0.0519	<i>R</i> _I = 0.0533	<i>R</i> _I = 0.0664
	<i>wR</i> ₂ = 0.1340	<i>wR</i> ₂ = 0.1381	<i>wR</i> ₂ = 0.1441	<i>wR</i> ₂ = 0.1561	<i>wR</i> ₂ = 0.1682