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Ca\textsuperscript{2+} metal ion adducts with cytosine, cytidine and cytidine 5’-monophosphate. A comprehensive study of calcium reactivity towards building units of nucleic acids.

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This manuscript is dedicated to Prof. Miguel Julve to celebrate his 60th birthday.

Six new Ca(II) adducts of formulae [Ca\textsubscript{2}(cyt\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})\textsubscript{2}][ClO\textsubscript{4}\textsubscript{2}]·2cyt·2H\textsubscript{2}O (1), [Ca\textsubscript{2}(cyt\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})(ClO\textsubscript{4})\textsubscript{2}] (2), [Ca\textsubscript{2}(cyt\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})Cl\textsubscript{2}] (3), [Ca(H\textsubscript{2}cyd\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})][ClO\textsubscript{4}\textsubscript{2}]·3H\textsubscript{2}O (4), [Ca(H\textsubscript{2}cyd\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})Cl\textsubscript{2}·3H\textsubscript{2}O (5) and [Ca\textsubscript{2}(CMP\textsubscript{2})(H\textsubscript{2}O\textsubscript{11})]·5H\textsubscript{2}O (6) [cyt = cytosine, H\textsubscript{2}cyd = cytidine, CMP = cytidine 5’-monophosphate] have been synthesized and structurally characterized. They reveal classical as well as uncommon structures, with H\textsubscript{2}cyd and CMP showing unprecedented binding sites for the calcium ion. The structure of compound 1 consists of monomeric [Ca\textsubscript{2}(cyt\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})\textsuperscript{2+}] cations, uncoordinated ClO\textsubscript{4}\textsuperscript{-} anions as well as lattice nucleobases molecules. The structures of compounds 2 and 3 contain either neutral (2) or cationic (3) dinuclear entities. They have in common a bis-\textmu-carboxilate bridged [Ca\textsubscript{2}(cyt\textsubscript{2})\textsuperscript{2+}] dinuclear core, where each cytosine molecule shows coordination simultaneously through O\textsubscript{2}N\textsubscript{3}. The coordination sphere of each calcium ion in 2 is completed by two \textit{cis} water molecules and two ClO\textsubscript{4}\textsuperscript{-} groups, the latter either in a mono- or bis-monodentate fashion. In the structure of 3, the dinuclear entities are cationic due to the direct metal-coordination of only two Cl\textsuperscript{-} anions over four, the remaining two being engaged as counterions in hydrogen bonds with the metal complex. The coordination sphere of each calcium ion in 3 is completed by two \textit{trans} water molecules and an additional cytosine molecule, coordinated this time \textit{via} O\textsubscript{2} only. Compounds 4 and 5 are, like 1 and 3, ionic salts. They share the same [Ca(H\textsubscript{2}cyd\textsubscript{2})(H\textsubscript{2}O\textsubscript{4})\textsuperscript{2+}] cationic unit, and differ by the supramolecular packing motif generated with the aid of water molecules of crystallization and the specific counterions in each case [ClO\textsubscript{4}\textsuperscript{-} in 4 and Cl\textsuperscript{-} in 5]. The structure of compound 6 consists of neutral [Ca\textsubscript{2}(CMP\textsubscript{2})(H\textsubscript{2}O\textsubscript{11})] asymmetric moieties and crystallization water molecules. Each dimer contains two Ca(II) ions in a slightly different coordination environment and two CMP di-anions exhibiting the chelating coordination mode through the ribose O\textsubscript{2} and O\textsubscript{3}’ hydroxyl groups and interacting with a calcium ion each. One of them is further coordinated \textit{via} the nucleobase O\textsubscript{2} oxygen atom toward the exogenous calcium ion, thus building up the dinuclear unit. The lack of coordination of Ca\textsuperscript{2+} ions towards phosphate groups observed in 6 is unusual.

Introduction

Studies on metal ion-nucleic acid interactions have been of great interest since metal ions play a crucial role in the structure and function of nucleic acid and genetic information transfer.\textsuperscript{1-4} Both experimental and theoretical studies have been performed on these systems.\textsuperscript{1-10} The coordinative site depend on the nature of the metal ion, and is non-innocent, generating a stabilizing or destabilizing effect on DNA, correlated to its ability to supports or prevents the formation of base pairing. Generally, metal ions bounded to
phosphate groups stabilize the double helical structure of the DNA while base-binding destabilize this structure.1−3 Furthermore, at high metal concentrations, an excessive charge neutralization in the helix promotes the formation of further hydrogen-bonding base pairs not so stable as the Watson-Crick ones, and mispairing is often chosen. On the other hand, metal ions compete with the hydrogens involved in H-bonds for the electron donor sites on nucleobases and, as a consequence of hydrogen displacements, metals can build crosslinks between strands.3,4 The simultaneous picture of structure, stability and electronic properties of the studied systems obtained through detailed computational analysis is extremely useful in order to have a better insight in their biochemical functions. However it is still important to increase records of experimental data, when possible, in physiological DNA and RNA. For this purpose, many research efforts, based on crystallographic studies, have been dedicated to the rational design, preparation and characterization of bio-mimetic systems based on the interaction of fragments or constituents of DNA, as nucleobases and their derivatives, with a wide range of metal ions.1−5,11−14 On the other hand, new generations of metal complexes containing biomolecules, such as amino acids, peptides, proteins, etc. have emerged in supramolecular coordination chemistry as nonthreatening building blocks, useful to develop new materials with tailored architectures and properties. The intrinsic self-assembling features of these molecules offer the possibility to achieve a fine control over the structure of the material at the nanoscale level. The incorporation of transition metal ions into pre-organized structures allows the introduction of addressable functionality and properties.15−16 Among molecules from the biological world, key constituents of nucleic acids, such as nucleobases, and related nucleosides and nucleotides, have accessible nitrogen and oxygen electron lone pairs, which allow these molecules to be suitable candidates to act as multidentate organic ligands. Their rich metal binding and H-bonding capabilities, together with the rigidity of their molecular structures, make them ideal bio-linkers for constructing topologically diverse families of coordination compounds potentially useful in the field of nanotechnology.17−26

Then, motivated by the importance of the topic, with the aim to contribute rationalizing the reactivity of the Ca2+ ion towards a series of related nucleobase - nucleoside - nucleotide ligands, we set out to explore its behaviour towards cytosine (cyt), cytidine (H2cyd) and cytidine 5'-monophosphate (CMP) in the presence of either chloride or perchlorate anions. In reference to the alkaline-earth metal ions, many X-ray structures of compounds containing Mg2+, Ca2+ and Ba2+ with nucleic acids constituents have been reported.27−34 Focusing on Mg(II) and Ba(II), three structures are known. One of Mg2+ with the nucleobase cytosine,27 and two of Ba2+ with cytidine-5'-phosphate and uridine-5'-phosphate28. The binding mode of the Mg2+ metal ion with base atoms in cytosine is unidentate via O(2). Ba2+ exhibits the expected coordination to phosphate group together with bind to O2' and O3' atoms of the sugar moiety. From these results it is evident that the Mg2+ ion shows, with cytosine, a behavior similar to that of Mn2+ and Co2+ and Ni2+, giving rise to compounds in which the nucleobase is coordinated via O(2).29 Few examples of Ca2+ adducts with nucleotides, have been structurally studied.30−33 In these compounds Ca2+ cations are bound to phosphate anions. Remarkable exceptions are given by two compounds where Ca2+, in addition to the coordination through the phosphate group, is bound to O3’ of the sugar moiety and to the exocyclic O(2) atom of the nucleobase of deoxythymidine-5'-monophosphate,33 and to O2' and O3' atoms of the sugar in the adduct with guanosine-5'-monophosphate.32 As far as we know, no example of adducts of Ca2+ with nucleosides have been reported in literature. Limiting our attention to nucleobases, only one Ca2+ compound and cytosine, is known,4 containing N(3)-O(2) and O(2) bridge co-ordination modes simultaneously.

In the present work we report our first results concerning synthesis and X-ray structure analyses of six new adducts of Ca2+. Among them there are the first isolated cytidine-containing calcium(II) complexes. They exhibit a rare and unpredictable binding to the sugar moieties both with the cytidine and CMP. This occurs in water, without sugar deprotonation and, with CMP, no-binding of the phosphate group is observed.
Results and discussion

Structure of [Ca(cyt)2(H2O)4][ClO4]2 ·2cyt ·2H2O (1).

Compound 1 consists of monomeric [Ca(cyt)2(H2O)4]2+ moieties, uncoordinated ClO4- anions and nucleobases and crystallization water molecules (Fig. 1). It is isostructural with the related Mg(II), Mn(II), Co(II) and Ni(II) compounds. The Ca2+ ion lies on an inversion centre and is hexacoordinated in an octahedral environment, being linked to four water and two cytosine molecules trans-coordinated via oxygen atoms. The octahedral geometry around Ca(1) may be described as slightly elongated, the best equatorial plane being defined by O(1W1), O(1W2), O(2), O(2a) set of atoms. The Ca-O(eq) [2.303(4) Å, 2.380(4) Å] and Ca-O(2W) [2.343(4) Å] distances (Table S1) are longer than those found in the magnesium compound as well as those found in manganese, cobalt and nickel compounds, but they are in the same range found in the reported example of Ca-cyt compound.

Four hydrogen bonds, in which coordinated water molecules and N(1) and N(1a) nitrogen atoms are involved, contribute to the stabilization of the structure. Two uncoordinated cytosine molecules are joined to the coordinated ones by hydrogen bonds in such a way as to form two base pairs. Two crystallization water molecules and two perchlorate anions are linked to each other and are also linked to the [Ca(cyt)2(H2O)4]2+ ion and to the base pairs so as to form two

![ORTEP drawing of the structure of 1 with atoms numbering scheme. (Thermal ellipsoids are plotted at 30% probability). [Symmetry code: (a) - x, - y, - z].](image-url)
rings (Fig. 1) (See Table S7). The formation of these rings, most likely, stabilizes this structure giving rise to an unusual hexacoordination for Ca$^{2+}$. The coordination exhibited in the other compounds hereunder reported, in which no base-pairs are formed, is thoroughly altered. The bond lengths and angles in the cytosine ligand are in agreement with those reported in the literature.\textsuperscript{27,29,34}

In the crystal packing further hydrogen bonds involving O21 oxygen atom and N3 nitrogen atoms of the non-coordinated cyt and water molecules together with parallel-displaced π-stacked arrangement of the uncoordinated cytosine nucleobase rings and coordinated and non-coordinated cyt rings occur (Figure 2). The average inter-ring C–C distance is 3.46 Å, and the angles between the centroid-centroid vectors from the facing nucleobase rings and their normal ($\theta$) are 36.5(1) and 41.6(1)°.

In the structure of 3, the dinuclear entities are cationic due to the direct coordination of two chloride anions (Figure 4a). Furthermore the two chloride counterions interacts, indirectly, with dinucleo moieties by means of H-bonds involving coordinated water molecules (Figure 5).

![Fig. 3 ORTEP drawing of the structure of 2 with atoms numbering scheme. (Thermal ellipsoids are plotted at 30% probability). [Symmetry code: (a) - x, - y, - z].](image)

The bidentate chelation mode of cyt ligand has been found in {[Ca(cyt)Cl$_2$·H$_2$O]}$_n$ (3a)\textsuperscript{34}, the unique structurally characterized compound of Ca$^{2+}$ with this nucleobase, but, despite the similarity with 2 and 3, the previously reported compound 3a show a chain motif. Compound 3 is different with respect to the previous known compound because of the further coordination of a monodentate nucleobase and a water molecule replacing a coordinated chloride in 3a, eluding the polymerization.

The Ca–N3 [2.555(3) (2), 2.573(4) Å (3)] and the Ca–O$_2$ bridging distances for 2 and 3 [Ca–O(2) 2.540(3) and Ca–O(2a) 2.323(3)
Å, (a): -x, -y, -z; (2), Ca–O(21) 2.524(3) and Ca–O(21a) 2.404(3) Å, (a): -x, -y, -z + 1; (3)] (see Figs 3 and 4 and Tables S2 and S3) are in the same range found in 3a.

Fig. 4 ORTEP drawing of the structure of 3 with atoms numbering scheme. (Thermal ellipsoids are plotted at 30% probability). [Symmetry code: (a) -x, -y, -z + 1].

In 3 the Ca-O(2) distance related to the monodentate cyt is 2.312(3) Å shorter than that found for the bridging cyt, as expected. The Ca–O_wa_ le bond lengths are slightly longer in 2 [2.396(3) and 2.421(3) Å] in respect to those found in 3 [2.327(3) and 2.356(3) Å]. The monodentate perchlorate anion in 2 exhibits a shorter bond distance [2.423(3) Å] in respect to those observed for the bidentate one [2.521(3) and 2.722(4) Å]. The Ca-Cl bond length in 3 [2.817(2) Å] is in agreement with those found in previous reported compounds34. The terminal cyt rings in 3 are tilted, bent around the trans conformation with a dihedral angle with chelating cyt of 24.32°(1) (Fig. 5b).

The intramolecular Ca(1)–Ca(1a) distances (r) are 3.977(1) and 4.158(1) Å in 2 and 3 respectively. The shortest intermolecular Ca(1)–Ca(1b) separations are 7.042(1) and 6.542(1) Å (b = -1 + x, y, z (2); -x, 1-y, 1-z (3)).

Fig. 5 a) Front view b) side view of the indirect interactions to the dinuclear core of chloride atoms in 3.

In the crystal packing of 2 the monodentate perchlorate anions interact with the ~NH₂ groups of adjacent dimers, building double H-bonds bridges (Fig. 6). Further H-bonds interactions involving perchlorate anions and water molecules stabilize the whole lattice (see Table S8).

In the crystal packing of 3 two hydrogen bonds contribute to the stabilization of the dimers (Fig. 5) involving both chloride anions (see Table 7) and coordinated water molecules, leading to layers lying in the bc crystallographic plane (Figure 7). In addition, parallel-displaced π-stacked arrangement of the chelating cytosine nucleobase rings exists. The average interring C–C distance is 3.40 Å, and the angle between the centroid-centroid vector from the facing nucleobase rings and their normal (θ) is 32.3(1)°.

Fig. 6 Perspective view of the crystal packing of 2.

The influence of the anions is pretty evident comparing 2 and 3. It is worth noting that they contend with monodentate cyt for the coordination toward Ca²⁺. Keeping in mind the 1:1 M/L molar ratio used in the preparation of both compounds, and the common dimeric core [Ca₃(cyt)₂(H₂O)ₓX₂]²⁻ (where X = Cl⁻ (2) and Cl⁻ (3)), it is evident that 3 is formed because of the ligand cyt succeeds in the competition with chloride. It can be realized that monodentate cyt ligand appears to be less coordinative than ClO₄⁻ anion but definitively more efficient of Cl⁻ in binding Ca²⁺ ion.

Fig. 7 Perspective view of the crystal packing in 3.
Structures of [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$](ClO$_4$)$_2$·3H$_2$O (4) and [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]Cl$_2$·3H$_2$O (5).

Compounds 4 and 5 are constructed by [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]$^{2+}$ cationic units, perchlorate (4)/chloride (5) as counterions and crystallization water molecules. In the asymmetric unit two crystallographically non-equivalent cationic units are present. The asymmetric cationic entity with atom numbering scheme are depicted in Fig. 8 and Fig. 9, for 4 and 5, respectively.

**Fig. 8** ORTEP drawing of the cationic [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]$^{2+}$ units in 4 with atoms numbering scheme. (Thermal ellipsoids are plotted at 30% probability).

The mononuclear [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]$^{2+}$ unit contains two H$_2$cyd neutral ligands chelating the metal ion through the O(2') and O(3') hydroxyl groups of ribonucleosides. Consequently, the Ca(1) and Ca(2) atoms result involved in the formation of five-membered chelate rings. The coordination around Ca$^{2+}$ ions is completed by four oxygen atoms of water molecules. Both in 4 and 5 structures, each calcium(II) results octacoordinated, with highly distorted geometry. In 4 Ca(1) lie in a plane defined by O(1w), O(3w), O(2') and O(3'), atoms, quite perpendicular (91.3°) to the plane defined by O(2w), O(4w), O(2'1), O(3'1) set of atoms. Concerning the environment of Ca(2), O(2'), O(2'1) and O(3'1), O(2'3), O(2'2), O(3'2) set of atoms define the equatorial plane containing the metal ion. The other four atoms, O(6w), O(8w), O(2'3) and O(3'3) hardly define a good plane with significant deviations up to 0.197(1) Å. The Ca-O(2') bond lengths in 4 [mean value of 2.469(3) Å] (see Table S4) are quite similar to those observed for Ca-O(3') [mean value of 2.460(3) Å] except the Ca(1)-O(2'1) [2.531(3)] that is somewhat longer. The Ca-O$_{\text{water}}$ distances are very similar to each other varying in the range 4 Å 2.413(3)-2.483(3).

**Fig. 9** ORTEP drawing of the cationic [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]$^{2+}$ units in 5 with atoms numbering scheme. (Thermal ellipsoids are plotted at 30% probability).

In 5 the coordination geometry around each Ca(II) may be considered as derived by a pentagonal bipyramid with an axial position split. The equatorial positions are defined by O(2'), O(2'1) and O(3'1), and O(2'3), O(2'2), O(3'2) set of atoms from two H$_2$cyd molecules, for Ca(1) and Ca(2), respectively and two oxygen atoms of water molecules [O(3w) and O(4w), for Ca(1)], [O(5w) and O(6w), for Ca(2)]. An axial position is filled by O(3') [Ca(1)] and O(3'3) [Ca(2)] oxygen atoms. The split axial positions are occupied by O(1w) and O(2w), for Ca(1), O(7w) and O(8w), for Ca(2). Unlike in 4, in compound 5 structure the Ca-O(3') bond lengths [mean value of 2.525(2) Å]
are longer than those for Ca-O(2') [mean value of 2.435(2) Å] and Ca-Owater [range 2.386(2)-2.462(2) Å] (see Table S5).

In both structures the pyrimidine rings from the nucleobase are planar and exhibit an anti-disposition. The ribose moiety is puckered with a C2'-endo conformation and its absolute configuration is C(1')R, C(2')R, C(3')R, C(4')R.

Although the chelation through the O(2') and O(3') hydroxyl groups of ribonucleosides towards Ca2+ ions is known, it is still rare. On the other hand, even that, to the best of our knowledge, no examples of nucleoside and Ca2+ adducts exist in literature, the chelating coordination mode in 4 and 5 is most likely the favourite binding way due to the chelate effect.

In the crystal packing of 4 an extended network of hydrogen bonds together with very efficient stacking interactions contribute to stabilize the whole lattice. Because of thermal and static disorder, it was not possible to clearly define the positions of some of non-coordinated perchlorate and crystallization water molecules. Our best model contains only a disordered perchlorate anion, lying on a twofold axis, interacting with cationic entities by means of host-guest interactions.

In addition, these wheel supramolecular systems, are stabilized by intra- and intermolecular hydrogen bonds involving coordinated water molecules and the O(2) oxygen atoms of the pyrimidine rings pointing toward the center of the cavities (Owater···Oanions distances varying in the range of 2.84(2)-3.14(1) Å). Each wheel includes perchlorate anions, that act as templating ions, showing receptor properties through multiple H-bonding interactions toward anions, which occupy the centers of the tetranuclear motifs.

These tetranuclear wheels contain eight H2cyd molecule as in the octanuclear Cu(II) cytidine complex, where, other than the different nature of metal ions, the dissimilar nuclearity is likely due to the deprotonation of cytidine that support the unusual O(2') bridging coordination mode observed in the Cu(II) polynuclear compound.

Neighboring tetranuclear wheels lead to layers in the ab plane with a AB stacking sequence running along the crystallographic b-axis, likely interacting by further H-bonds and built up to a porous 3D motif (total potential solvent and anion accessible voids of 29% of the volume of the unit cell) (Fig. 11).

Fig. 10 View along c crystallographic axes of the supramolecular wheel motif in 4. [Symmetry code: (a) -x + 1, -y + 1, z].

As shown in Fig. 10, four cationic Ca(H2cyd)2(H2O)2 Ca2+ units are arranged in a wheel supramolecular shape, built up through π interactions between cytosine rings lying in a parallel face-to-face fashion at interplanar distances of 3.36(1) and 3.50(1) Å. The average inter-ring C-C distance is 3.45 Å, and the angle between the centroid-centroid vector from the facing nucleobase rings and their normal (θ) of 7.46(1) and 12.38(1)°.

Fig. 11 View along c crystallographic axes of the layers of tetranuclear pseudo-wheels in 4.

The resulting overall packing suggests the occurrence of a number of crystallization water molecules, placed inside and outside of the wheels affected by thermal and static disorder.

The different nature, size and shape of the counterions in 5 are the main reasons to a pretty dissimilar crystal packing in respect to 4.

In 5 concerted stacking interactions between pyrimidine rings (Fig. 12) and H-bonds lead to an overall 1D motif.
As shown in Fig. 13, adjacent cationic \([\text{Ca}(\text{H}_2\text{cyd})_2(\text{H}_2\text{O})_4]^{2+}\) units are arranged in stranded base-stacked ribbons, in which pyrimidine bases are aligned parallel to one another with interplanar distances of 3.44(1) and 3.55(1) Å and an average inter-ring C–C distance of 3.45 Å.

Structure of \([\text{Ca}_2(\text{CMP})_2(\text{H}_2\text{O})_{11}] \cdot 5\text{H}_2\text{O}\) (6).

The structure of compound 6 consists of neutral \([\text{Ca}_2(\text{CMP})_2(\text{H}_2\text{O})_{11}]\) moieties and crystallization water molecules. Each molecule contains two Ca\(^{2+}\) ions, and two CMP anions exhibiting different coordination mode, together with eleven coordinated water molecules (Figure 14). Remarkably no coordination of Ca\(^{2+}\) ions towards phosphate groups occurs.

Fig.14 ORTEP drawing of the \([\text{Ca}_2(\text{CMP})_2(\text{H}_2\text{O})_{11}] \cdot 5\text{H}_2\text{O}\) molecules in 6 with atom numbering scheme. (Thermal ellipsoids are plotted at 30% probability).

One of the CMP ligand acts as bridge, chelating through the O(2') and O(3') hydroxyl groups of the ribose at Ca(2) and, simultaneously, coordinating via O(2) at Ca(1) of the nucleobase. The other CMP ligand chelates Ca(1) through the O(2') and O(3') atoms. Consequently the two bridged Ca\(^{2+}\) ions are crystallographically not equivalent. Each Ca\(^{2+}\) ion is octacoordinated Ca(1) is linked to oxygen atom O(2) of the pyrimidine base of the bridging CMP anion and O(2') and O(3') of the ribose of the auxiliary CMP, having completed the coordination environment by five solvent
molecules. Ca(2) is linked only to a chelating CMP ligand and to six water molecules (Fig. 13).

The coordination geometry around Ca(1) may be described as derived by a highly distorted pentagonal bipyramid with equatorial positions defined by O(1W), O(2W), O(3W), O(4W) and O(5W) set of atoms, an axial position defined by O(21) oxygen atom of the coordinated nucleobase and the second one split in two positions, defined by O(2') and O(3') hydroxyl groups. In regards to Ca(2) surrounding, no ideal geometry can be found: the oxygen atoms O(2'1) and O(3'1) of CMP are on the opposite side of the O(11w) oxygen atom, but the other five oxygen atoms of water molecules hardly define a plane. The Ca-Owater bond lengths vary in the range 2.370(5) - 2.608(5) Å. The Ca-O(2') bond lengths [mean value of 2.540(2) Å] are longer than those for Ca-O(3') [mean value of 2.404(4) Å].

Meaningfully the Ca(1) - O(21) bond length is the shortest one [2.361(2) Å] (see Table S6).

In the whole molecule the pyrimidine rings from the nucleobase are planar and exhibit a conformation anti with respect to the sugar ring. The ribose moiety is puckered with a C2'-endo and gauche-gauche conformation about the C4'-C5' bond. Its absolute configuration is C(1')R, C(2')R, C(3')R, C(4')R. Intramolecular H-bonds among O(1W) and O(9W) water molecules from the Ca(1) and Ca(2) coordination spheres, respectively (Fig. 14), contribute to enhance the stability of dimers. In the crystal packing of 6, the [Ca2(CMP)2(H2O)11] molecules are joined by stacking interactions between pyrimidine rings, arranged in a offset parallel face-to-face mode giving rise to a 1D ribbon-like motif (Fig. 15). The observed interplanar distances among aromatic rings vary in the range 3.48(1) – 3.70(1) Å. The angle between the centroid-centroid vector from the closest facing nucleobase rings and their normal (θ) is of 19.9° (Fig. 15b). H-bond interactions, involving crystallization water molecules and oxygen atoms of the phosphate groups, ensure the cohesion between chains (see Table S11).

The lack of Ca2+ ions coordination towards phosphate groups observed in 6 demonstrates that the binding mode through chelation via O(2') and O(3') hydroxyl groups, observed also in the structures of compounds 4 and 5, is a “credible” coordination mode towards Ca2+ ions even in presence of the phosphate groups. In other words, the O(2') site competes with the phosphate group for the coordination and, sometimes, can succeed, which is, most likely, due to the chelate effect. Furthermore, compound 6 represents the first evidence of Ca2+ ion coordinated to nucleobase and ribose moiety, while the phosphate group, surprisingly, is only “endorsed” to engage H-bonds interactions.

Conclusions

In the present work we have reported the synthesis and X-ray structure analysis of six new adducts of Ca2+ with cytosine (compounds 1-3), cytidine (compounds 4-5) and cytidine 5'-monophosphate (compound 6). These nucleic acid constituents show similarities, offering more and more possibilities for coordination from cyt to H2cyd and CMP. There are many structural studies on cyt, H2cyd and CMP compounds with transition metal ions, but very few of those with alkaline earth metal ions remain. In particular, restricting ourselves to the Ca2+ ion, only a structure of a cytosine compound, as chelating ligand, via N3-O2, and, simultaneously, via O2, giving rise to a chain motif, is known. Compounds 2 and 3 confirm this coordination mode, even if they are dinuclear species in which the different nature, size and shape of anions play an active role in the overall structures. Interestingly, compound 2 was obtained in mixture with the mononuclear compound 1. In the structure of 1, Ca2+ exhibits an atypical hexa-coordination, that is most likely driven by the formation of supramolecular assemblies containing crystallization water molecules and non-innocent perchlorate anions. Fascinatingly, the motif is analogues to those previously
reported for Mg(II), Mn(II), Co(II) and Ni(II), despite the different nature of the metal ions involved.\(^{27,29}\)

Cytidine Ca(II) compounds (4-5) are substantially different if compared to those of cytosine. In this case, both using perchlorate or chloride as counterions, analogous structural units are obtained. These units contain \([\text{Ca(H2cyd)}_2(\text{H}_2\text{O})]^{2+}\) moieties, where neutral cytidine molecules coordinate via O(2') and O(3') atoms of the ribose. As far as we are aware, they are the first isolated cytidine-containing calcium(II) complexes. It is noteworthy as, although containing similar structural units, they show a different crystal packing. Both are packed by means of stacking interactions between the pyrimidine rings and hydrogen bonds in which perchlorate (4) or chloride (5) anions are involved, but the resulting motif is totally different.

In 4, four cationic \([\text{Ca(H2cyd)}_2(\text{H}_2\text{O})]^{2+}\) units are arranged, by means of stacking interactions between the pyrimidine rings, in a wheel-like supramolecular system. Each pseudo-wheel represents the first evidence of Ca\(^{2+}\) coordination to nucleobase and ribose moieties without involvement of phosphate groups. Then, the donor sites competition of the nucleoside cytidine towards metal ions is a never-ending story, being still active and fruitful even in presence of the favourite winner as the phosphate group.

The outcomes reported are certainly not predictive for nucleic acids behaviour in their biologically environment, due to the presence in them of more stable hydrogen bonds, electrostatic interactions and steric hindrances, that may prevent the metal ions, in the hydrated form, to reach the likely binding sites. However, the results reported in this work, can contribute to a better knowledge of the ground rules relating donor sites of DNA and RNA bricks with metal ions. Indeed, it is really surprising to see that the sugar moieties in the cytidine and, even more so, in CMP, is always linked. Noteworthy, this binding mode occurs: i) in water ii) with no sugar deprotonation and, what is astonishing is that, the phosphate group, “chooses” to be engaged, exclusively, in hydrogen bonds.

**Experimental Section**

**Materials and equipment.**

The cyt, H2cyd and cytidine 5'-monophosphate disodium salt (CMP) ligands were purchased of reagent grade and used without further purification. The elemental analysis (C, H, N) were performed on a Perkin-Elmer 2400 elemental analyzer. The IR spectra of compounds 1-6 were recorded with a Nicolet 5700 FTIR spectrometer with pressed KBr pellets in the 4000–500 cm\(^{-1}\) region.

**Synthesis of \([\text{Ca}(\text{cyt})_2(\text{H}_2\text{O})]^{2+}\) (4)** and \([\text{Ca}(\text{cyt})_2(\text{H}_2\text{O})_4]^{4+}\) \((\text{ClO}_4^-)_2\) (5). An aqueous solution (5 mL) of cyt (0.2 mmol, 0.023 g) obtained after gently warming to facilitate the base dissolution, was added to an aqueous solution (5 mL) of Ca(ClO\(_4\))\(_2\)·4H\(_2\)O (0.2 mmol, 0.062 g) dropwise under stirring at room temperature. The resulting colourless solution gave X-ray quality colourless tiny needles of 4 in mixture with irregular parallelepipeds of 2 upon slow evaporation after two weeks. The solids were recovered by filtration, and air dried. Crystals of 1 and 2 were separated by hand (compound 1 was the minor product of the reaction that gave crystals of 2 as main product).

All attempts to obtain compound 1 in a higher yield, starting from the M:L ratio 1:4 or 1:2, were unsuccessful and constantly the unreacted cytosine nucleobase precipitate as colourless plate crystals. Analytical data for 1: Anal. Calcd for Ca\(_2\)(H\(_2\)cyd)\(_2\)·H\(_2\)O: C, 24.28; H, 4.08; N, 22.76%; IR (KBr): \(\nu = 3474 (\text{O–H}), 1648, 1607 \text{ cm}^{-1}\). Anal. Calcd for Ca\(_2\)(H\(_2\)cyd)\(_2\)(H\(_2\)O)\(_4\): C, 26.02; H, 3.82; N, 22.67%; IR (KBr): \(\nu = 3432 (\text{O–H}), 1718, 1678 \text{ cm}^{-1}\). Anal. Calcd for Ca\(_2\)(H\(_2\)cyd)\(_2\)(H\(_2\)O)\(_4\)Cl\(_2\): C, 26.09; H, 3.75; N, 22.56%; IR (KBr): \(\nu = 3432 (\text{O–H}), 1718, 1678, 1623 \text{ cm}^{-1}\).

**Synthesis of \([\text{Ca}(\text{cyt})_2(\text{H}_2\text{O})_4]\) \((\text{ClO}_4^-)_2\) \((\text{Cl})_2\) \((\text{Cl})_2\) \((\text{Cl})_2\) (6).** An aqueous solution (5 mL) of cyt (0.2 mmol, 0.022 g) obtained after gently warming (T = 40 °C), was added to an aqueous solution (5 mL) of CaCl\(_2\)·2H\(_2\)O (0.2 mmol, 0.030 g), dropwise under stirring at room temperature. The resulting colourless solution gave X-ray quality colourless irregular parallelepipeds of 3 upon slow evaporation after two weeks together with traces of plate-like crystals of compound of formula \([\text{Ca}(\text{cyt})_2\text{Cl}_2]\)·H\(_2\)O\(_4\) (3a) previously reported.\(^{14}\) The solid was recovered by filtration, air dried and crystals of 3 were separated by hand (70% yield). It is worth to point out that, when the reaction solution was heated at 80 °C, crystals of 3a were precipitated with a good yield. Their structure was confirmed by determination of cell parameters of a selected plate-like crystal. Anal. Calcd for Ca\(_4\)(H\(_2\)cyd)\(_2\)N\(_2\)O\(_4\)Cl\(_4\): C, 26.02; H, 3.82; N, 22.76%; Found: C, 26.09; H, 3.75; N, 22.67%. IR (KBr): \(\nu = 3432 (\text{O–H}), 1718, 1678, 1623 \text{ cm}^{-1}\).
C_{13}H_{46}Ca_{6}N_{12}O_{12}Cl_{2} (5): C, 29.88; H, 5.57; N, 11.62%. Found: C, 30.00; H, 5.26; N, 11.66%. IR (KBr): 3520 (O–H), 1752, 1603 cm–1 (C=O).

Synthesis of [Ca_{2}(CMP)_{2}(H_{2}O)_{11}]·5H_{2}O (6). An aqueous solution (10 mL) of Na_{2}Ca_{6}H_{2}O (0.2 mmol, 0.074 g) was added to an aqueous solution (10 mL) of CaCl_{2}·2H_{2}O (0.2 mmol, 0.030 g) dropwise under stirring at room temperature. The colourless reaction mixture was further stirred for 20 min under gentle warming and then, after two weeks, upon slow evaporation at room temperature X-ray quality colourless prisms of 6 appeared. The same product has been obtained starting from Ca(ClO_{4})_{2}·4H_{2}O as source of Ca^{2+} ions and running with the same reaction conditions (70% yield). (6) Anal. Calcd for C_{13}H_{46}Ca_{6}N_{12}O_{12}Cl_{2}: C, 21.39; H, 5.58; N, 8.31%. Found: C, 21.08; H, 5.69; N, 8.21%. IR (KBr): 3410 (O–H), 1638, 1603 cm–1 (C=O), 1070, 1113 cm–1 (P=O).

X-ray crystallography. Single-crystal X-ray diffraction data of 1-6 were generally collected at room temperature on either a Bruker Kappa automatic four-circle (1-3) or a Bruker-Nonius X8APEXII CCD area detector diffractometer (4-6) using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å); data for compound 4 were collected at 100 K. Lorentz-polarization and empirical absorption corrections through the SHELXTL software package. All non-hydrogen atoms were refined anisotropically. In compounds 4 and 6 two sets for oxygen atoms of the perchlorate anion have been modelled with refined occupancy factors. A double position has been refined also for the O(5′) oxygen atom of a ligand in 4 and for the lattice molecule O(16)w in 5.

The hydrogen atoms were set in calculated positions and refined as riding atoms. The hydrogen atoms on water molecules, when assigned, were refined with restraints on O-H distances and H-O-H angles. In compound 4 the contribution to the diffraction pattern from the perchlorate anion and the water molecules of crystallization (96 molecules of H_{2}O and 56 perchlorate anions located in the voids of the lattice that amount to 29 % percentage void volume of the unit cell), were subtracted from the observed data by using the SQUEEZE method, as implemented in PLATON. The residual agreement factors for reflections with I > 2σ(I) for 4 were R_{I} = 0.0771 and wR_{2} = 0.2157 before SQUEEZE whereas they were and R_{I} = 0.0597 and wR_{2} = 0.1704 after SQUEEZE. The final formulation of the compound is in agreement with the residual electron density and volume.

The final geometrical calculations and the graphical manipulations were carried out with PARST97 and DIAMOND programs, respectively. The crystal data are presented in Table 1.

Acknowledgements

This work was supported by the MiUR (Italy) and by the European Community’s Seventh Framework Program (FP7 2007-2013) through MATERIA Project (PONa3_00370). Thanks are due to the European Commission, FSE (Fondo Sociale Europeo) and Calabria Region for a fellowship grant to N.M.


Ca$^{2+}$ metal ion adducts with cytosine, cytidine and cytidine 5'-monophosphate. A comprehensive study of calcium reactivity towards building units of nucleic acids.

Nadia Marino, Donatella Armentano, Claudia Zanchini, and Giovanni De Munno

This manuscript is dedicated to Prof. Miguel Julve to celebrate his 60th birthday.

Six new Ca(II) adducts of formulae [Ca(cyt)$_2$(H$_2$O)$_4$][ClO$_4$]$_2$·2cyt·2H$_2$O (1), [Ca$_2$(cyt)$_2$(H$_2$O)$_6$(ClO$_4$)$_4$] (2), [Ca$_2$(cyt)$_4$(H$_2$O)$_4$Cl]Cl$_2$ (3), [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$][ClO$_4$]$_2$·3H$_2$O (4), [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]Cl$_2$·3H$_2$O (5) and [Ca$_2$(CMP)$_2$(H$_2$O)$_{11}$]·5H$_2$O (6) [cyt = cytosine, H$_2$cyd = cytidine, CMP = cytidine 5'-monophosphate] have been synthesized and structurally characterized. They reveal classical as well as uncommon structures, with H$_2$cyd and CMP showing unprecedented binding sites for the calcium ion. The structure of compound 1 consists of monomeric [Ca(cyt)$_2$(H$_2$O)$_4$]$_{2+}$ cations, uncoordinated ClO$_4^-$ anions as well as lattice nucleobases molecules. The structures of compounds 2 and 3 contain either neutral (2) or cationic (3) dinuclear entities with a bis-µ-carboxilate bridged [Ca$_2$(cyt)$_2$]$_{4+}$ dinuclear core, where each cytosine molecule shows coordination simultaneously through O$_2$.N$_3$. Compounds 4 and 5 are ionic salts. They share the same [Ca(H$_2$cyd)$_2$(H$_2$O)$_4$]$_{2+}$ cationic unit, and differ by the supramolecular packing motif generated with the aid of water molecules of crystallization and the specific counterions in each case [ClO$_4^-$ in 4 and Cl$^-$ in 5] (see picture below). The structure of compound 6 consists of neutral [Ca$_2$(CMP)$_2$(H$_2$O)$_{11}$] asymmetric dimers and crystallization water molecules. The lack of coordination of Ca$^{2+}$ ions towards phosphate groups observed in 6 is unusual.