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N,N,O and N,O,N meridional *cis* coordination of two guanines to copper(II) by d(CGCGCG)₂

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Abstract

Many research groups study the generation of supramolecular *n*-dimensional arrays by combining metals with DNA building blocks. Most of the time, the natural nucleobases are modified in order to obtain higher affinity metal binding sites. Using unmodified nucleobases avoids a potentially difficult synthesis, however they have the possible disadvantage of a less defined and/or weaker coordination mode of the metal. Structural studies on the behaviour of copper(II) as a linking metal and guanine as the natural ligand for metals in unmodified DNA are reported. Previously, the ability of mono- and dinuclear metal complexes to induce Z-DNA has been explored [Medina-Molner, A.; Spingler, B. *Chem. Commun.* **2012**, 48, 1961; Medina-Molner, A.; Rohner, M.; Pandiarajan, D.; Spingler, B. *Dalton Trans.* **2015**, 44, 3664]. Herein, X-ray crystallographic studies of the structures resulting from the combination of copper(II) ions with DNA hexamers of the general sequence d(CG)₃ are presented. Three different packing motifs were observed in three crystal structures with resolutions ranging from 2.15 Å to 1.45 Å. The motifs are dependent upon other cations being present and/or the crystallization conditions. The

first examples of intramolecular O6,N7-chelates of a neutral purine nucleobase to copper(II) were obtained as well as the first meridional N,N,O and N,O,N coordination modes of two guanines to copper(II). The fascinating coordination chemistry of copper(II) complexes generated by the Z-DNA oligonucleotides and the differences to simple nucleobases complexes with copper(II) are discussed in detail.

Introduction

Linking modified or unmodified DNA building blocks with metals in order to generate supramolecular *n*-dimensional arrays is a very active field of research (see Figure 3).¹ The use of unmodified nucleobases avoids a potentially difficult synthesis, however these simple nucleobases have the disadvantage of a less defined coordination mode of the metal.² In the following, we will concentrate on copper(II) as the linking metal and guanine in unmodified DNA as the natural ligand for metals. The binding of two guanines that are part of two different oligonucleotides to one copper(II) center presents a simple way of building up supramolecular structures. Additionally, the sterically more demanding oligonucleotides might induce more unusual coordination geometries at the copper center compared with the bis-coordination of copper(II) by two simple building blocks, such as 9-methylguanines^{3,4}, other guanine analogues⁵ or 5'-monophosphate-2'-deoxy-guanosine^{6,7}, all of which have been studied in the crystalline state.

Previously, we have explored the ability of mono-⁸ and dinuclear^{9,10} metal complexes to induce the B- to Z-DNA transition. The mononuclear building block (**A**), presented in Figure 1, consists of a [12]aneN₃ azamonocycle with either zinc(II), nickel(II) or copper(II) as the metal centre. The dinuclear version (**B**) of the mononuclear complexes **A** were synthesized using bicyclic ligands

that displayed a varying linker length between the two [12]aneN₃ units. The transition from the B- to the Z-DNA conformation induced by these metal complexes **A** and **B** was followed using circular dichroism.¹⁰ The dinuclear complexes were chosen with the following principles in mind: a) a metal-metal distance of about 5-7 Å, which corresponds to the N7-N7 interstrand distance in a GC base pair step in Z-DNA, and b) a flexible linker. It was found that the dinuclear complexes **B**, irrespective of the metal center, were able to induce Z-DNA, whereas the mononuclear complexes **A** were not. Varying the linker length from ethylene to propylene and butylene did not show a dramatic difference in the Z-DNA induction. Among the metals studied, copper(II) was found to be the most efficient followed by nickel(II) and zinc(II) (more or less following the trend of the Irving-Williams series).¹⁰

Figure 1. Mono- (**A**) and dinuclear (**B**, n : 0-2) zinc(II), nickel(II) and copper(II) complexes tested for their ability to induce the conformational change from B- to Z-DNA.¹⁰ Complex **C** was used for co-crystallization with DNA.

An extensive study conducted by Gao *et al.* investigated the binding modes of several metal ions to short DNA strands.¹¹ They found that copper(II) and cobalt(II) exclusively bound to N7 of guanine. Pavelka *et al.* studied the coordination modes of Cu(II) to guanine and water by DFT calculations.¹² They found several chelating Cu-(N7-guanine) complexes with Jahn-Teller distortion at the sites of the coordinated water ligands. Roelfes and co-workers have discovered that copper(II) complexes together with DNA or oligonucleotides catalyze in water the Michael

or Diels-Alder reaction with high enantioselectivity. In the presence of only DNA and copper(II) ions, the reactions proceed but with very low enantioselectivity.¹³

This publication reports X-ray crystallographic studies of the structures resulting from the combination of copper(II) ions with DNA hexamers^{14,15} of the general sequence d(CG)₃. Three different packing modes were observed in three crystal structures with resolutions ranging from 2.15 Å to 1.45 Å. The motifs are dependent upon other cations being present and/or the crystallization conditions; one, two or four double stranded DNA oligomers were found in the asymmetric units. These are referred to as **am_one**, **am_two** and **am_four** (pdb codes: 4XSN, 5IHD and 4XQZ respectively). The first examples of intramolecular O6,N7-chelates of a neutral purine nucleobase to copper(II) were obtained as well as the first meridional N,N,O and N,O,N coordination modes of two guanines to copper. The fascinating coordination chemistry of copper(II) generated by the Z-DNA oligonucleotides is discussed in detail.

Experimental Section

Unless otherwise stated, all chemicals were of reagent grade and purchased from Sigma–Aldrich. The DNA oligomers were obtained from Eurogentec, Belgium, in HPLC purified form. The solutions were prepared with double deionised H₂O and sterile filtered before use. The solutions were stored at room temperature in the dark.

Co-crystallisation of DNA with the dinuclear copper complex C. For the co-crystallisation of the dinuclear copper complex **C** (Figure 1) with DNA, about 1500 starting conditions were screened at the Hauptman-Woodward Institute (HWI) in Buffalo, NY, USA. A high-throughput screen (HTS) method was used as described in the literature¹⁶, and the conditions yielding

crystals were further refined in-house (Table 1). The DNA sample sent to the HWI was prepared as follows: 1.51 mg of copper complex **C** were dissolved in 293 μl H_2O leading to a 5.2 mM solution. 4000 μg of $\text{d}(\text{CG})_3$ were dissolved in 242 μl H_2O and 208 μl of the aqueous copper complex solution were added (2.4 mM dsDNA, 2.4 mM Cu_2L).

In-house crystallisation conditions for am_one. The crystal of **am_one** was obtained by the hanging-drop vapour diffusion method using 24-well VDX plates (Hampton Research). The DNA solution contained 0.625 mM $\text{d}(\text{C-8BrG-CGCG})$ (2.22 $\mu\text{g}/\mu\text{l}$) and 0.34 mM **C**. The precipitant solution consisted of 40 mM Na-cacodylate pH 7, 10 % MPD, 80 mM KCl and was mixed 1:1 with the DNA solution. A 3.6 μl drop of this mixture was equilibrated against 800 μl of a reservoir solution consisting of 30 % MPD.

In-house crystallisation conditions for am_two and am_four. In order to obtain crystals of **am_two**, 1 μl of a DNA solution of 2.4 mM $\text{d}(\text{CG})_3$ (8.88 $\mu\text{g}/\mu\text{l}$) and 2.4 mM **C** and 1 μl of the precipitant solution consisting of 500 mM 2-(N-morpholino)ethanesulfonic acid (MES) pH 6, 50 mM $\text{Ca}(\text{S-lactate})_2$ and 5 % (v/v) EtOAc were mixed. This solution was placed in an Imp@act 96 well plate without a reservoir (Greiner) and 3 μl paraffin oil were added to cover the drop (sitting drop method). Crystals of **am_four** were obtained in the same way as for **am_two** but using dioxane instead of ethyl acetate.

Data Collection in-house. Crystallographic data for **am_one**, **am_two** and **am_four** were collected at 183(2) K with graphite-monochromated Cu $\text{K}\alpha$ radiation ($\lambda = 1.54184 \text{ \AA}$) on a Stoe IPDS2 diffractometer. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), placed on a nylon loop that was mounted on a CrystalCap MagneticTM (Hampton Research) and immediately transferred to the diffractometer. Data were processed with the X-Area software suite¹⁷ and were corrected for Lorentz and polarisation effects (Table 1).

	am_one	am_two	am_four
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁	P2 ₁
a [Å]	18.79	27.45	35.34
b [Å]	29.08	35.62	35.25
c [Å]	43.28	31.08	47.64
β [°]	90	107.31	98.28
V [Å ³]	23649	29013	53743
resolution [Å]	1.45	1.57	2.15
double strands of d(CG) ₃ per asym. unit	1	2	4
V [Å ³] per ds d(CG) ₃	5912	7253	6718
solvent [%]	47.7	57.2	53.9
copper(II) per asym. unit	1	4	6
calcium(II) per asym. unit	0	2	2
Chloride per asym. unit	0	0	5
water molecules per asym. unit	98	136	57
pdb code	4XSN	5IHD	4XQZ
crystallisation conditions:			
DNA solution	0.625 mM d(C-8BrG-CGCG) 0.34 mM C	2.4 mM d(CG) ₃ 2.4 mM C	2.4 mM d(CG) ₃ 2.4 mM C
Precipitant	40 mM Na- cacodylate pH 7 80 mM KCl 10 % MPD	0.5 M MES pH 6 50 mM Ca(lactate) ₂ 5% EtOAc	0.5 M MES pH 6 50 mM Ca(lactate) ₂ 5% dioxane
Reservoir	30 % MPD	as precipitant	as precipitant

Table 1. Crystallographic data for **am_one**, **am_two** and **am_four**.

Structure Solution and Refinement of am_one, am_two and am_four

All three structure were solved by molecular replacement and refined with the SHELXL program¹⁸, in which the commands ANIS and RIGU^{19,20} were applied for the anisotropic refinement of all DNA strand atoms and the copper(II) ion. Detailed descriptions of the solution and refinement procedure can be found in the supporting information. All figures of structure models, if not otherwise stated, were created using CCP4mg²¹.

Results and Discussion

Structure of **am_one**

The structure of **am_one** was solved and refined at a resolution of 1.45 Å. In the asymmetric unit, there is one double strand of 5'-d(C(BGM)CGCG)-3' in the Z-DNA conformation and one copper(II) ion (Figure 2). The 8-bromo guanosine (BGM) was initially chosen to simplify the structure solution with the help of the MAD procedure.²² However, the structure could be solved by molecular replacement. In 2012, the group of Prof. Sheldrick included improved rigid-bond restraints in a new version of SHELXL¹⁸ (the RIGU¹⁹ instruction) and showed these restraints to be applicable for protein structures that have a resolution of 1.7 Å or better. In the context of this work, the refinement model of the copper-DNA structure **am_one** was improved in SHELXL by applying these RIGU restraints as well as anisotropic displacement parameters for all non-hydrogen atoms of the DNA, the copper(II) ion and the bromine atoms (Table S1). The positions of the metal ion and the bromine atoms were confirmed by an anomalous density map²³, which was calculated with help of the program ANODE²⁴ (Figure 2). The data set had been collected using a normal copper sealed X-ray tube at $\lambda = 1.54$ Å (Cu K α), a wavelength at which, apart from copper, phosphorus and bromine also contribute to the anomalous signal, due to their anomalous scattering factor f' . At a wavelength of 1.54 Å, the anomalous scattering coefficient f' of bromine is higher (1.08e⁻) than those of copper (0.51e⁻) or phosphorus (0.44e⁻). In the PDB, only 4 Z-DNA structures with a brominated nucleobase (1VTW²⁵, 1DA1²⁶, 242D²², 2OBZ no

publication) can be found. Furthermore, this is the first time that the presence of 8-bromo-2'-deoxy-guanosine (BGM) in a DNA-only X-ray structure has been reported. The supramolecular arrangement of DNA and copper ions is shown in Figure 3.

Figure 2. Final crystal structure model for **am_one** (copper (II) ion shown in green) together with an anomalous density map of **am_one** at 3σ in dark red. The map was clipped so as to show just the metal ion and the DNA model. Water molecules were omitted for clarity.

Figure 3. The supramolecular arrangement (simplified depiction) consisting of DNA oligomers (cylinders), copper ions (blue spheres) and calcium (gold spheres) in **am_one** (left) and **am_two** (right).

Figure 4. Metal interactions within **am_one**. Copper(II) ions are shown in green and water molecules are omitted for clarity. Left: The interaction of copper(II) ions along the x-axis. Right: The interaction of copper(II) ions along the z-axis viewed along the x-direction.

The sequence 5'-d(CGCGCG)-3', when in the Z-DNA conformation, preferentially crystallizes in the space group $P2_12_12_1$. This is the case for 61 out of 96 Z-DNA-only structures found in the PDB (Figure S4). The copper(II) ion binds to two symmetry-related DNA double strands hence acting as a linking moiety in the x and y direction (Figure 4). The stacking of the double strands can be seen clearly in Figure S4 left. The strands perfectly stack end to end in the z direction forming pseudo-continuous helices. As can be seen in the NUCPLOT²⁷ scheme (Figure S5), the metal ion is coordinated to the sixth guanine of chain B and three water molecules. The missing two coordination sites of the copper(II) ion are occupied by the symmetry-generated fourth

guanine of chain B. In Figure 5, the coordination sphere of the copper (II) ion is presented in detail and Table 2 depicts the binding distances. All binding ligands are fully occupied and have comparable B-factors. As a control, the valency of the metal center was calculated with the parameters given in the literature²⁸ and the calculation confirmed the assignment as a copper(II) ion. The coordination of the metal by the nucleobases is rather unusual, as the two N7 atoms occupy *cis* positions in the coordination octahedron of the copper(II) ion. This *cis* coordination geometry of purine bases to copper(II) has been found only thrice²⁹⁻³¹ in the CSD³² (February 2016) compared with 11 *trans* structures. The binding distances of the water molecules of 1.95(4) Å and 2.07(4) Å fall in the shorter of the two subpopulations for copper(II) water distances found for the analogous distances in the CSD (Figure S6). Such guanine(N7) – metal ion – guanine(N7) bridges are possible in B-DNA only for terminal guanines.¹¹ The overall geometry of the copper(II) coordination is a Jahn-Teller distorted octahedron with O102 and N7/dG/B/4 forming the elongated axis and O100, O101, N7/dG/B/6 and O6/dG/B/4 as the equatorial ligands (Figure 5). Since the two purine bases coordinate in a N,N,O mode all within one plane, the three water molecules also occupy the remaining three positions in a meridional fashion. This represents a new coordination geometry mode for copper(II) with two neutral purine nucleobases involving N7,O6 chelation. The structures most similar to this motive are the two small-molecule structures found in the CSD (Figure S7, ESIWOT³⁰ and DABXOT²⁹). As can be seen in Figure S7, the purine bases in these two complexes are not coplanar but rotate out of a common plane to prevent steric clashes between the bases. In both cases, the O6 atoms of the purines do not coordinate to the metal center and only water molecules or an ethylenediamine complete the coordination octahedron. The octahedral ESIWOT structure is also Jahn-Teller distorted, but along the O1-Cu-O3 axis (Figure S7 left), in contrast to the distortion at Cu1 along N7/dG/B/4-Cu-O102 in **am_one** (Figure 5). The coordination found in the structure of **am_one** is probably

due to the geometry of the Z-DNA forcing the two purine bases to stay in one plane and the O6 atom to coordinate to the copper(II) cation.

Figure 5. Copper(II) coordination sphere in **am_one**.

Metal ID	Inner sphere ligands	Distance [Å]	Occupancy	B-factor	Valency ^a
Cu/1			1	13.9	1.85
	N7/dG/B/6	2.01(4)	1	14.8	
	O6/dG/B/4_\$5 ^b	2.01(3)	1	12.7	
	N7/dG/B/4_\$5 ^b	2.57(4)	1	12,9	
	O/100	1.95 (4)	1	14.5	
	O/101	2.07(4)	1	14.2	
	O/102	2.37(4)	1	13.6	

Table 2. Coordination parameters of the inner coordination sphere of the copper(II) ion in **am_one**. a) the valency was calculated as the sum of all bond valencies: $S = \exp((R_0 - R)/b)$.²⁸ b) symmetry operation \$5: x-0.5, -y+1.5, -z+1

Searching the PDB for structures of Z-DNA with the sequence 5'-d(CGCGCG)-3' and copper(II) coordination, the entry 1D39¹⁴ was found. The unit cell of 1D39 is slightly bigger ($a = 18.01$ Å, b

= 31.03 Å, c = 44.80 Å, $\alpha = \beta = \gamma = 90^\circ$, V = 25036 Å³) than the unit cell of **am_one** (a = 18.79 Å, b = 29.08 Å, c = 43.28 Å, $\alpha = \beta = \gamma = 90^\circ$, V = 23648 Å³). As no cryo-protection procedure is mentioned in the publication of 1D39, we assume that the measurement was performed at room temperature. This may lead to an increase in the unit cell dimensions and the thermal expansion ellipsoids of the atoms. The metal incorporation in the case of 1D39 was achieved by soaking Z-DNA crystals with a 1 mM CuCl₂ solution for one week. The structure of 1D39 was then solved by molecular replacement with the native 5'-d(CGCGCG)-3' double strand as a model. Here, six copper(II) ions were found coordinating to the N7 atoms of six bases. These copper(II) ions are partially occupied with an occupancy between 6 and 40 %. Looking also at the symmetry-related atoms of the structure, it is observed that the copper(II) ions 14 and 15 (naming from the pdb) are only 1.55 Å apart (Figure 6 top left), which is only possible with occupancies lower or equal than 50% (both 39%). On the bottom left of Figure 6, the same close-up is presented for **am_one**. The main differences of the two close-ups are visible in the symmetry-related strand on the right, which is also part of strand B. The overlay of the structure model **am_one** and 1D39 (Figure 6 right) shows very similar positions for the copper(II) ion in **am_one** and Cu/15 in 1D39. This overlay also shows that the two models vary very little in their geometry, except that Cu(14) in 1D39 does not bind to O6 of guanine (G10/B) (distance of 3.54 Å). The water molecules in the two structures have been ignored in this analysis. Mainly the backbone of strand B shows some variations, which were already found in the comparison of the two coordination spheres of Cu/15 in 1D39 with that of the copper(II) ion in **am_one**. The six copper(II) ions of 1D39 follow the rotation of the helix, coordinating to every guanine base. It is possible that, due to the slight differences in the backbone geometry, there is no space for six copper(II) ions to coordinate in **am_one** (Figure 6 right). The coordination sites are either occupied with water or not occupied at all, as they are very close to symmetry-related strands. The rather low occupancy of the metals in 1D39 could indicate that the soaking was not yet finished at the time of the X-ray measurement

or that these positions, or at least the access to them, are not favored. Still, it is remarkable that two methods of metal ion incorporation, namely soaking (in 1D39) and co-crystallization (in **am_one**) lead to similar but in terms of coordination extensively different structures. In another study by the same group¹⁵, a copper(II) ion (Cu12) was found to coordinate in a meridional mode to two N7 of guanine and a water molecule with a Jahn-Teller distortion along the axial chloride ions. The coordination geometry was found to be a distorted trigonal bipyramid, but no coordination of O6, as in the case of **am_one**, was found.

Figure 6. Overlay of the model 1D39¹⁴ in blue and **am_one** in purple. Copper(II) ions of 1D39 are shown in dark green, those of **am_one** in bright green, sodium ions in orange and water molecules are omitted for clarity. Top left: Close up of the metal coordination site in 1D39. The two partially occupied copper(II) ions of 1D39 are separated by only 1.55 Å. Bottom left: Close-up of the fully occupied metal coordination site in **am_one**. Right: Whole model overlay of 1D39 and **am_one** generated by least square superposition using COOT³³

Structure of **am_two**

The structure **am_two** was solved at a resolution of 1.57 Å. In the asymmetric unit, there are, apart from the found water molecules, two double strands of 5'-d(CGCGCG)-3' in the Z-DNA conformation, four copper(II) ions, two calcium(II) ions, two *S*-lactate ligands and two carboxylate ligands (see discussion below). The refinement was done with the help of SHELXL¹⁸ and PHENIX.REFINE³⁴ (Figure 7). The positions of the copper(II) and calcium(II) ions were confirmed by the calculation of an anomalous density map with the help of the program ANODE²² (Figure 8). The data set had been collected with a copper X-ray source (Cu K α), $\lambda = 1.54$ Å), where, apart from copper (0.51e⁻), phosphorus (0.44e⁻) also contributes to the anomalous signal, as described in the discussion of structure **am_one**. The anomalous density map for **am_two** was very noisy compared to the map obtained for **am_one**. This is probably due to the lower resolution of the data. On the other hand, one would expect a strong anomalous signal from calcium (1.28e⁻), however the observed signal was weaker than for copper(II). The positions of the calcium(II) ions were supported by the composition of the coordination spheres and the difference electron density map.³⁵ As already mentioned in 1994³⁶, following the HSAB principle the hard calcium(II) shows a higher affinity for the hard oxygen atoms of the phosphate groups than for the softer N7 atoms of guanine. The coordination to several phosphate groups was thus suggested as the preferred coordination site for calcium(II) (see further below).

During the refinement, the two anions coordinating to two copper(II) ions between the two double strands, initially assigned as formate ions, were replaced by acetate anions, but ongoing refinement of the model indicated further electron density between the acetate ligands, as well as the problem that the two methyl groups of the acetate moieties were too close to each other. This lead to the final conclusion that there was one succinate ligand interconnecting the two copper(II) ions (Figures 7 and 8). As described in the experimental part, no succinate was used in the

crystallization conditions of **am_two**. The following calculations show that it is reasonable to suggest impurities in $\text{Ca}(S\text{-lactate})_2$ are a source of the succinate: A succinate impurity of 1% (declared maximal impurity from MERCK) within $\text{Ca}(S\text{-lactate})_2$ would lead to 0.5 mM succinate in the crystallization precipitant solution at a 50 mM concentration of $\text{Ca}(S\text{-lactate})_2$. The DNA solution contained double stranded DNA at a concentration of 2.4 mM. This amount of succinate is certainly not enough to crystallize one succinate molecule per two double strands (asymmetric unit). However, for this explanation it is assumed that all the declared impurity is succinate and every DNA molecule is involved in crystallization. As both assumptions are not true, it is still possible that the crystallization conditions at some point fulfill the criteria for the incorporation of succinate in the crystal structure. In the PDB, currently (February 2016) no DNA-only structure with succinate can be found. The only relevant references are the following RNA structures³⁷ (PDB codes 3SKI, 3SKZ, 3SLM, 3SLQ). All four structures present a RNA riboswitch bound to 2'-deoxyguanosine-5'-monophosphate at resolutions of between 2.17 and 2.5 Å. However, also in these cases the succinate was not added to the crystallization solution and its presence could not entirely be explained. Additionally, none of the four crystal structures contained a complete succinate molecule. In summary, we present here the first nucleic acid structure with a complete succinate molecule.

Figure 7. Final crystal structure model for **am_two**. Copper(II) ions are indicated in green, calcium(II) ions in magenta. Water molecules are omitted for clarity.

Figure 8. Close up of the succinate ligand in **am_two** interconnecting the two copper(II) ions (see text for detailed discussion). The anomalous density map in purple is drawn at 3σ and the electron density (feature-enhanced map³⁸, cyan) at 1σ . Both maps were clipped so as to show just the ligands and the metals respectively. For an orthogonal view see Figure S9.

Further discussion about the differences of the structures **am_one**, **am_two** and **am_four** will be presented below. The NUCPLOT²⁷ scheme (Figure S10) shows that the two calcium(II) ions coordinate to phosphate groups, as described above. The copper(II) ions on the other side coordinate to the bases and the oxygen atoms of the ligands (2op for *S*-lactate and Sin for succinate).

Table S2 presents the inner sphere binding geometry and the valency of the metal ions in the crystal structure of **am_two**. The valency of the metal ions was calculated using the same procedure²⁸ as described for **am_one**. As Cu/3 is only 50 % occupied, here the valency

calculation formula is not valid. However, for all the other metal ions the calculated valency is in an acceptable range and thus confirms the assigned copper(II) and calcium(II) valency. As can be seen in Table S2, the B-factors of the ligands are comparable to the B-factors of the coordinated metal ion. The occupancy for two phosphate ligands is lower than 1. This is due to a disorder in the phosphate groups of the backbone (not further discussed here). The coordination sphere for all copper(II) ions, except Cu/3 is complete, i.e. octahedral coordination.

The Cu/1 (Figure 9 left) is coordinated to two purine bases of two neighboring strands. The coordination is similar to the copper(II)-coordination in **am_one**, however Cu/1 in **am_two** binds to the two purine bases via the N7 atoms in a *trans* fashion. As the O6 atom of one of the purine bases is also coordinating to the copper(II) to give a N7,O6 chelate ring, there is a meridional N,O,N coordination mode by the two purine bases. The alcohol oxygen and one of the carboxylate oxygens of a *S*-lactate ligand (2OP/F/2) and O2 of the succinate ligand occupy the other three meridional coordination sites, hence completing the octahedron. As can be seen from Table S2, the octahedron is Jahn-Teller distorted along the axis of O6-Cu-OHN. This is probably due to steric hindrance preventing O6 from coordinating closer to the copper(II) ion as this would cause a clash of the two strands. Also the linearity of this coordination axis is slightly distorted as the OHN of the *S*-lactate is moved out of plane of the N,O,N coordination of the nucleobases. The reason for this steric distortion is the coordination of the carboxylate oxygen of the *S*-lactate ligand. As the efficiency of the coordination of this oxygen would suffer from the tilting, the distortion along the O6-Cu-OHN axis is preferred in order to reach maximal coordination symmetry.

The coordination sphere of Cu/2 of **am_two** (Figure 9 right) is very similar to that of Cu/1 in the same structure. The purine bases bind to the copper(II) in a meridional way forming a N,O,N plane and the succinate and another *S*-lactate complete the octahedron. The coordination

distances are comparable and the coordination mode is the same as found for Cu/1. Again, the O6 atom of one of the purine bases and the OHN of the first *S*-lactate (2OP/F/1) ligand form the elongated axis of the octahedron (Table S2). In our search in the CSD for similar small-molecule structures, we could not find any example of a copper(II) complex with 2-hydroxy-acetate as a substructure of any ligand. The PDB also does not contain a nucleic acid structure with a free lactate ligand (February 2016). The supramolecular arrangement based upon these two copper ions and the DNA oligos is shown schematically in Figure 4.

Figure 9. Labeling of the atoms is according to Table S2. Left: Coordination sphere of Cu/1 in **am_two**. Right: Coordination sphere of Cu/2 in **am_two**.

Cu/3 (Figure 10 left) has a low occupancy of only 50 %. Probably as a consequence, a complete coordination sphere could not be found for Cu/3, since the half occupied water molecule was diffracting too weakly. Another specialty of Cu/3 is its coordination to a phosphate group. The copper(II)-OH₂ distances are all in the range of the found values in the CSD (Figure S6). Cu/3 is directly coordinating to atom N7 of the sixth base of strand B, the phosphate group of the fifth

base of stand B and three water molecules. The purine base visible at the top of Figure 10 left is too far away to form an effective coordination to the copper(II) ion. The overall geometry of Cu/3 is a highly distorted square pyramid. This coordination of copper(II) is only found in one structural arrangement in the CSD (February 2016, CUGMPP⁶ Figure S11 A, redeterminations: CUGMPP02³⁹ and CUGMPP11³⁹), but in our case the geometry is a perfect square pyramid. The fourth copper(II) ion (Cu/4, Figure 10 right) is coordinated to two purine bases via their N7 atoms in a *trans* arrangement. Four water molecules complete the octahedron of Cu/4. As can clearly be seen in Figure 10 B, the water molecules O/140 \$2 and O/202 form an elongated axis of the octahedron presumably as a consequence of a Jahn-Teller distortion. The other four ligands have almost the same binding distance to Cu/4 (Table S2). The positions of the two water molecules O/204 and O/203 are stabilized by weak H-bonding interactions between the water molecules and the O6 atoms of the two bases, thus preventing a distortion along this axis. In the case of Cu/4, both bases are tilted in such a way that atom O6 is not coordinating and thus the base suffers from less bond strain (compared to Cu/1 and Cu/2). In the cases of Cu/1 and Cu/2 of **am_two**, the *S*-lactate and the succinate need more space and hence force the bases to coordinate atom O6. For the coordination geometry of Cu/4, three comparable structures were found in the CSD (February 2016, BAHMAY⁷, NAMGCU⁴, TAHYPC⁴⁰). In all three structures (Figure S11 B, C and D), the guanine bases were tilted in such a way that atom O6 could not coordinate to the copper(II) center and the water molecules in the O4 and O2 positions were stabilized by H-bonding interactions. This supports the suggestion that this is the most stable binding mode with the least strain on the base. The overall coordination geometry of the three structures is again a Jahn-Teller distorted octahedron along the O1-Cu-O3 axis, as for Cu/4.

Figure 10. Labeling of the atoms is according to Table S2. Left: Coordination sphere of Cu/3 in the crystal structure of **am_two**. Right: Coordination sphere of Cu/4 in the crystal structure of **am_two**.

The bigger Van-der-Waals radius of calcium(II) (1.74 Å) allows for a higher coordination number than that of copper(II) (1.40 Å). However, the calcium(II) ions found in the crystal structure of **am_two** do not show this feature, rather they also possess six-fold coordination. Ca/5 coordinates to two phosphate groups from symmetry-related strands and four water molecules. The binding distances (Table S2) of the two phosphate groups are similar and are also in line with the statistics found in the CSD (Figure S12). The overall coordination geometry of Ca/5 is trigonal prismatic (Figure 11 left). In contrast to Ca/5, Ca/6 (Figure 11 right) shows an octahedral coordination sphere with three phosphate groups in meridional coordination and three water molecules completing the octahedron. As can be seen in Table S2, almost no distortion of the octahedron is observed. Though in Figure 11 it looks as if the two fully occupied calcium(II) ions are rather close together, they are actually 5.20 Å apart. They are coordinating to the same phosphate groups of dG/A/2 and dG/C/2.

Figure 11. Labeling of the atoms is according to Table S2. Left: Coordination sphere of Ca/5 in the crystal structure of **am_two**. Right: coordination sphere of Ca/6 in the crystal structure of **am_two**.

Overall, it can be said that the calcium ions interconnect the DNA double strands in the z-direction and the copper(II) ions in the x- and y-directions. It has to be mentioned, that this crystal was colorless initially and only after some time did it change its color to green. Therefore, we assume that copper(II) ions, provided by decomplexation from complex **C**, diffused into the crystalline lattice originally set up only by the DNA and the calcium ions. The copper ions occupied the softer binding nitrogen sites, unlike the calcium ions, which were coordinating exclusively to oxygen atoms and thereby linking together the DNA oligomers. The binding constant of Cu(II) to [12]aneN₃ to form complex **A** was determined to be $13.2 \pm 0.2 \text{ M}^{-1}$.⁴¹ We conclude that the binding sites of copper within the DNA crystal lattice have higher binding constants to the metal than the ligand [12]aneN₃.

Structure of **am_four**

The crystal structure of **am_four** could be solved with molecular replacement in PHENIX.MRAGE⁴² using the pdb file of **am_two** without any solvent molecules and cations as the input model. Further comparison of the three structures will be presented below. The structure was then refined with PHENIX.REFINE³⁴ and validated with the help of the online validation service of the PDB.⁴³ The asymmetric unit of **am_four** contains four double strands of 5'-d(CGCGCG)-3', six copper(II) ions, two calcium(II) ions, five chloride ions (two of them are disordered), one MES ligand and 57 solvent molecules, one of which is methanol (Figure S13). The chloride ions originate from complex **C**.

The positions of the metal ions were confirmed by the calculation of an anomalous density map with the help of the program ANODE²⁴ (Figure S14). The data set had also been collected with a copper X-ray source (Cu K α , $\lambda = 1.54 \text{ \AA}$). As in the crystal structure of **am_two**, the positions of the copper(II) ions ($0.51e^-$) were easily depicted from the anomalous density map, but the calcium(II) ions were not scattering as expected from their scattering factor ($1.28e^-$). Therefore, the positions of the calcium(II) ions had to be assigned according to the difference electron density map and their coordination sphere.

Figure 13. Left: Crystal packing of **am_four** along the z-axis. The double strands stack end to end, forming continuous helices. Right: Stacking in xy direction of **am_four** viewed along the z-axis.

The crystal structure of **am_four** was solved in the space group $P2_1$ as was also the case for **am_two**. Figure 13 left depicts the stacking of the DNA strands along the z-axis of the unit cell in the crystal structure of **am_four**. The strands perfectly stack along the z-axis end-to-end forming pseudo-continuous helices. In Figure 13 right, the stacking in the xy direction is viewed along the z-axis. The NUCPLOT schemes in Figure S18 show that the copper ions are mainly coordinating to guanine bases. The chloride ions are coordinating to DNA in the proximity of the copper(II) ions and the MES ligand is coordinated to Cu/4. The calcium(II) ions Ca/9 and Ca/10 coordinate to phosphate groups of the backbone. Compared to the NUCPLOT schemes of **am_one** and **am_two**, significantly less water molecules coordinating to the DNA strands could be found. This is mainly due to the low resolution (2.15 Å) of the crystal structure of **am_four**. In Table S3 we present the distances of the inner sphere ligands to the metal ions in **am_four**. The valency of the metal ions was calculated as described for **am_one** and **am_two**. The coordination sphere of Cu/1 in **am_four** consists of two water molecules, one chloride ion and two purine

bases (Figure 14 left). The chloride ion was formerly assigned as a water molecule but the B-factor indicated higher electron density. The distance between the metal and the chloride is 3.34 Å (Table S3), which is rather long compared with the analogous distances in the CSD (Figure S19). The two purine bases are coordinated in a meridional fashion, similar to the coordination geometry of Cu/1 and Cu/2 in **am_two**. The base of strand B is turned slightly out of the plane compared to dG/C/2. Here again, the coordination distance of atom O6 of the purine base is longer than the coordination distance of the two N7 atoms. The copper(II) to water distance of water molecule O/179 is in the longer of the two subpopulations found in the CSD (Figure S6) indicating a distortion of the octahedron. The second water molecule O/133 shows a very long coordination distance and therefore indicates a very weak interaction with the copper(II) ion. The overall coordination geometry is a strongly distorted octahedron with the two water molecules shifted away from the octahedral coordination axis. Due to the low resolution of **am_four**, the exact positions of the water molecules may be inaccurate and hence lead to an imprecise depiction of the coordination geometry.

Figure 14. Left: Inner coordination sphere of Cu/1 in **am_four**. Right: Inner coordination sphere of Cu/2 in **am_four**. The labeling of the atoms is according to Table S3.

The copper(II) ion Cu/2 in **am_four** is coordinated by two purine bases and two water molecules (Figure 14 right). Here none of the O6 atoms of the purine bases coordinates to the metal ion. The two bases show the same coordination geometry as described for Cu/4 in **am_two** (Figure 10 right). The coordination distance of the water molecule O/122 (Table S3) is comparable to the shorter subpopulation of the coordination distances found in the CSD (Figure S6), whereas the water molecule O/153 shows a significantly larger distance from the metal ion. As two coordination sites are not occupied, the geometry can be described as an incomplete octahedron. The ligand at the coordination site along the Cu/2-O/153 axis is probably missing due to steric hindrance of the base dG/D/6. No ligand could be found occupying the sixth coordination site, most likely because of the resolution of **am_four**. The coordination geometry of Cu/3, Cu/4, Cu/5, Cu/6 and the calcium ions is discussed in the supporting information.

Structural Comparison between am_one, am_two and am_four

The asymmetric units of these structures differ significantly in their content. It can be seen from Table 1, that the number of metal ions increases as the number of DNA double strands in the asymmetric unit increases. The volume of the three unit cells is obviously almost a linear function of the number of double-stranded DNA molecules per asymmetric unit multiplied with Z of the three structures. The volume per double strand strictly correlates with the solvent percentage as the DNA itself uses a constant volume. Interestingly enough, **am_four** has a lower solvent content compared with **am_two** despite its relatively poor diffraction ability. This clearly shows that exceptions to the normally inverse relationship between diffracting power and solvent content do exist.⁴⁴

The crystallization condition of **am_one** corresponds with condition 15 of a well known crystallization screen (commercialized under the name of Nucleic Acid Mini Screen) but without spermine⁴⁵. Spermine was excluded as initial experiments showed that the polyamine induces Z-DNA crystals even without copper(II). The crystallization conditions for **am_two** and **am_four** only differ in the additive added. For **am_two** 5 % EtOAc were added and for **am_four** 5 % dioxane.⁴⁶ Crystals grown with dioxane as the added solvent diffracted to a lower resolution. It can be seen that only small variations in the solvent additive can change the crystallization of a structure enormously. The structure of **am_four** contains chloride anions though no chloride salt was added to the mixture for the crystallization. The anions probably were contained in the MES buffer solution or the water. As the space group P2₁2₁2₁ is found for most Z-DNA structures of the sequence 5'-d(CGCGCG)-3', it can be asked why the **am_two** and **am_four** structures crystallized in P2₁. Searching the PDB for Z-DNA structures in the space group P2₁ found six other structures (February 2016, 1JES⁴⁷, 3E9W⁴⁸, 3FQ5 no publication, 4DKZ⁴⁹, 4DWY⁵⁰, 4E4O no publication). Unfortunately, the crystallization procedures were not published for all of these structures. The crystallization conditions found for the published structures are listed in Table S4. It can be seen that the reservoir solution contained only MPD in all cases, which was also the situation for **am_one**. The ingredients of the precipitant solution can be summarized as DNA (ssDNA), buffer, salts and precipitants. Compared to the conditions used for **am_two** and **am_four**, the DNA is much more concentrated for **am_one**. As no explanation for the space group could be found in the comparison with these structures, other reasons must be discussed.

Malinina and Tereshko⁵¹ investigated in 1990 the structural variability in the crystallization of oligonucleotides with the same sequence. They observed different packing patterns for the crystallization of the oligonucleotide with the sequence 5'-d(pCGCGCG)-3' and they investigated the dependence of the DNA packing pattern on the ratio of the concentrations of

spermine and the oligonucleotide. They found a hexagonal double strand packing (P6₅) along the helical axis for a high cation concentration (spermine/DNA ratio = 9.0 and magnesium chloride/spermine ratio = 1.5) and monoclinic packing (P2₁) for a ratio of spermine to DNA of 1.6 and no magnesium chloride. Another packing pattern was obtained at a higher magnesium chloride/spermine ratio of 1.8 and a lower spermine/DNA ratio of 1.6 (P2₁2₁2₁, overall less cations per DNA strand).⁵² The same authors found that several double strands in the asymmetric unit and a face-to-face interaction of the double strands mediated by metal ions leads to denser packing.⁵³ It was stated that the convex surface structure of Z-DNA leads to such strand-to-strand interactions, because the active groups (N7, O6 and N4) are exposed to the surface. Hence, it can be concluded that the ratio of the concentration of the cations and the DNA are critical for the packing of the DNA double strands and therefore the space group. It seems that a lower cation content compared to the DNA, as found in **am_one** and **am_two**, promotes the space group P2₁, as supported by the findings of Malinina et al.⁵¹⁻⁵³ For the crystallization conditions of **am_two** and **am_four** no precipitant, such as spermine or MPD, was used. However, this contradicts the observations in the structures 1JES and 4DKZ, because their conditions are similar those of **am_one**. Comparing the direct coordination sites, it is obvious that all Cu(II) ions coordinate to the N7 atoms of guanine bases. This observation was already reported by Gao *et al.* in 1993.¹¹ A detailed structural comparison between our three structures and five related structures, taken from the PDB, having the same 5'-d(CGCGCG)-3' sequence, but different metals or ligands was conducted with help of the program X3DNA⁵⁴. These results can be found in the SI.

There remains the question, whether partially occupied copper ions are present at occupancies too low to detect them as separate peaks in the residual electron density maps. As already discussed, the structure of **am_one** is similar to pdb entry 1D39, in which partially occupied copper ions were assigned next to every N(7) atom of guanine.¹⁴ The group of Williams proved

the existence of partially occupied thallium ions next to N(7) atom of guanines.⁵⁵ In this context, we have analyzed all N(7) – water contacts of the two higher resolution structures of **am_one** and **am_two**. (Table S5). The water molecules, which could be found next to N(7) of guanine, are all fully occupied. They have all higher B values than their N(7) counterparts, which is not expected if heavier elements would be present instead of the water molecule. The N(7) – water distances are between 2.52-3.19 Å. The shortest N(7) – water distance found in the Cambridge Structure Database³⁸ was 2.61(2) Å.³⁹ In summary, while the electron density gives no indication that partially occupied copper ions are present instead of water molecules, there is a slight indication from the N(7) – water distances that these are shorter than expected in 2 out of 6 cases.

Conclusion

The co-crystallisation studies of copper(II) complexes together with DNA hexamers of the general sequence d(CG)₃ have been described. Three different packing modes, which are dependent upon the crystallisation conditions, were observed in three crystal structures with resolutions ranging from 2.15 Å to 1.45 Å. Two of these structures contained the first examples of an intramolecular O6,N7-chelate of a neutral purine nucleobase to copper. Additionally, the first cases of a meridional N,N,O and N,O,N coordination of 2 guanines to copper were observed. A fascinating and novel coordination chemistry of copper(II) could be achieved by using unmodified Z-DNA oligomers as ligands instead of simple nucleobases, -sides, -tides or B-DNA.

ASSOCIATED CONTENT

Supporting Information. Hardware and software specifications, detailed experimental procedure for solving and refining the X-ray structures, crystal packing figures, CSD and PDB

reference structures, coordination tables, CSD binding distances histograms, NUCPLOT figures, crystallization conditions of reference structures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ABBREVIATIONS

2OP: *S*-lactate three-letter code in PDB; BGM: 8-bromo-2'-deoxyguanosine-5'-monophosphate three-letter code in PDB; CCP4: Collaborative Computational Project 4; COOT: Crystallographic Object-Oriented Toolkit; CSD: Cambridge Structural Database; DNA: deoxyribonucleic acid; EtOAc: ethyl acetate; LLG: log-likelihood-gain; MeOH: methanol; MES: 2-(*N*-morpholino)ethanesulfonic acid; MPD: 2-Methyl-2,4-pentanediol; PDB: Protein Data Bank; SIN: succinate three-letter code in PDB; TFZ: translation function Z-score; XDS: X-ray Detector Software.

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Table of Contents Graphic and Synopsis

Unmodified DNA oligomers as ligands for copper(II) gave rise to unusual coordination chemistry of the metal. We have determined three X-ray crystal structures of DNA hexamers with Cu(II), all having a different packing mode. We report the first crystallographic structures of an intramolecular O6,N7-chelate of a neutral guanine nucleobase to copper (green), as well as the first structures involving meridional N,N,O and N,O,N coordination of two guanines to copper.