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Synthesis and properties of a Cu(II) complexing pyrazole ligandoside in DNA†

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The development of metal base pairs is of immense importance for the construction of DNA nanostructures. Here we report the synthesis of a biaryl pyrazole–phenol nucleoside that forms in DNA a stable self-pair upon complexation of a Cu(II) ion. A sequence with five consecutive pyrazole nucleotides allows the complexation of five Cu(II) ions in a row.

We are currently experiencing the increased use of DNA strands for the defined construction of two and three dimensional nanoobjects.[1–3] Nowadays DNA nanoconstructions allow not only the assembly of nanoobjects with complex shapes, but also of nanosystems that feature new unusual functions.[4–6] Examples include the decoration of DNA structures with catalytically competent or sensoring proteins,[7–9] and the construction of cage-like DNA nanoobjects for the encapsulation of cargo that is released by a defined outside stimulus.[10] Currently it is of interest to create DNA nanostructures that exhibit novel electronic and magnetic properties. This would allow the controlled assembly of nanomagnets or electronically switchable devices. Taking advantage of the interesting electronic and magnetic properties of the metal ions with unpaired electrons, research has focused on the design of metal complexing ligandosides capable of complexing such metal ions in the DNA duplex.[11] Alternatively, the incorporation of metal ions into DNA might allow the design of new catalytically competent nanosystems at the boundary between homogenous and heterogenous catalysis.[12] Fig. 1 shows the ligandosides **1–3**, which were so far designed for the stable incorporation of Cu(II) ions into the duplex.[13–15]

Here we report the preparation of a new Cu(II) complexing ligandoside **4**, and its easy incorporation into the DNA duplex by using standard phosphoramidite chemistry. With this new biaryl-type ligandoside the system is able to accomodate five Cu(II) ions on top of each other within the duplex.

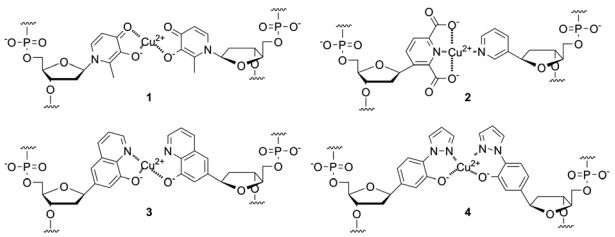

Fig. 1 Currently available Cu(II) complexing ligandosides **1–3** and depiction of the Cu(II) complexing new biaryl-ligandoside **4** described here.

The straightforward synthesis is depicted in Scheme 1A. The starting point is 2-amino-5-bromo-anisole (**5**) which was, after nitrosation, converted to the pyrazole **6** by generation of the diazonium salt. Deprotection of the methoxy group to the phenol derivative **7** followed by silyl protection provided the key intermediate **8** in 58% overall yield. Cuprate based coupling of **8** with the toluoyl-protected α-2′-deoxyribosylchloride **9**[16] led to the β-configured nucleoside **10** in 53% yield.[17] Saponification of the sugar ester groups in methanol to give **11** was followed by 5′-DMT protection (compound **12**) and generation of the desired phosphoramidite **13** using standard procedures. To unambiguously determine the geometry of the ligandoside, compound **11** was deprotected, crystallized and the crystal structure of the resulting compound **14** was solved.

In order to learn more about the complexing behaviour we next synthesized the ligandoside **17** in which the phenolic hydroxyl group is permanently blocked (Scheme 1B). To this end the TIPS and toluoyl-protected intermediate **10** was selectively TIPS-deprotected (compound **15**) and transformed into the anisole derivative **16**. Finally, toluoyl deprotection furnished compound **17**, which provided, after 5′-DMT protection (**18**), the corresponding phosphoramidite **19** under standard conditions. Small crystals of ligandoside **14** were obtained by slow evaporation of the ethyl acetate solution. The structure depicted in Scheme 1A shows that the phenyl ring and the pyrazole ring are just slightly tilted

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† Electronic supplementary information (ESI) available: Experimental procedures for **6–19** and substituted oligonucleotides, characterization data of compounds, crystallographic data, T_M and CD experiments data and NMR spectra. CCDC 965412. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc47561a

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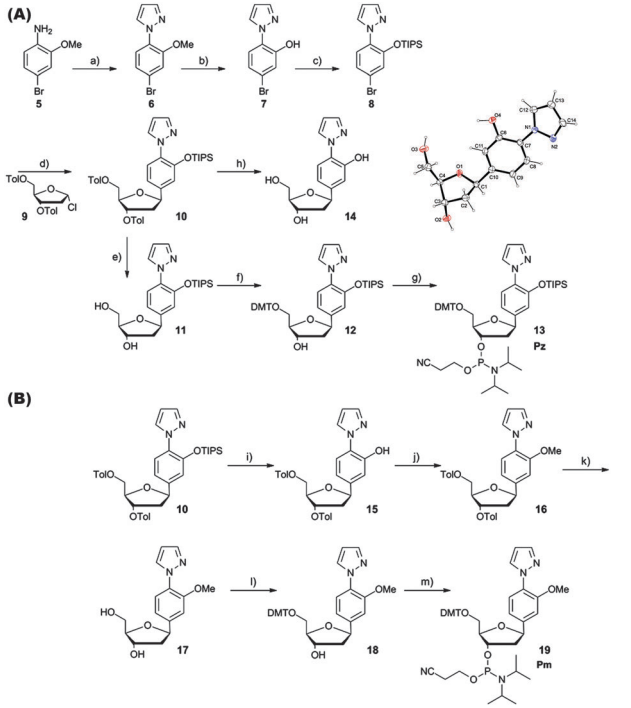

Scheme 1 (A) (a) i. $NaNO_2$, $SnCl_2$, HCl, 0 °C; ii. 1,1,3,3-tetramethoxypropane, EtOH, HCl, reflux, 86%; (b) BH_3, DCM, −78 °C, 68%; (c) TIPSOTf, $NEt(iPr)_2$, DCM, 0 °C, 99%; (d) i. *t*BuLi, Et_2O, −78 °C; ii. CuBr·Me_2S, Et_2O, −30 °C; iii. **9**, DCM, rt, 53%; (e) K_2CO_3, MeOH, rt, 65%; (f) DMT-Cl, $NEt(iPr)_2$, DCM, rt, 74%; (g) $P(OCH_2CH_2CN)(NiPr_2)_2$, diisopropylammoniumtetrazolide, DCM, rt, quant.; (h) TBAF, THF, rt, 87%. DMT = 4,4′-dimethoxytrityl, TIPS = tri-iso-propylsilyl, Tol = Toluoyl. (B) (i) TBAF, THF, rt, 83%; (j) NaH, CH_3I, DMF, 0 °C, 76%; (k) K_2CO_3, MeOH, rt, 90%; (l) DMT-Cl, $NEt(iPr)_2$, DCM, rt, 75%; (m) $P(OCH_2CH_2CN)(NiPr_2)_2$, diisopropylammonium tetrazolide, DCM, rt, quant. DMT = 4,4′-dimethoxytrityl, TIPS = tri-iso-propylsilyl, Tol = Toluoyl.

against each other in the biaryl structure by 24°. Since complexation of metal ions within the duplex requires formation of an almost flat complex that fits into the duplex and that can stack with the canonical bases, this small tilt angle is an important feature of the new pyrazole base Pz. We next used the phosphoramidites **13** (Pz) and **19** (Pm) for the synthesis of the 15-mer oligonucleotides **X1–3** and the complementary counterstrands **Y1–3** (Fig. 2A, Fig. S1, ESI†). The undisturbed duplex, containing a G : C base pair instead of Pz and Pm, showed a melting point of 49 °C (1 μM oligonucleotide in 150 mM NaCl and 10 mM CHES buffer, pH 9). Incorporation of a Pz and Pm self-pair reduced this melting point to 40 °C and 42 °C, respectively. Upon metal complexation we observed an increase of the melting temperature (T_M = 50 °C) for the Pz self-pair, which is slightly above the original T_M of the control strand. This result shows that the complexation of Cu(II) provides a stabilizing interaction. Interestingly, no increased melting temperature was detected for the Pm self-pair (T_M = 42 °C), which shows that the deprotonation of the phenolic hydroxyl groups, which goes hand in hand with charge neutralization, is critical for metal ion binding.

We next scanned different metal ions to compare their complexing properties (Fig. S2, ESI†). From these results we can conclude that the Pz-base binds preferentially to Cu(II)

A

Strands	Sequence of oligonucleotide	T_M /°C	T_M /°C (+Cu^{2+})
X0	5'-CAC ATT A**G**T GTT GTA-3'	48.7	-
Y0	3'-GTG TAA T**C**A CAA CAT-5'		
X1	5'-CAC ATT A***Pz***T GTT GTA-3'	40.5	49.5
Y1	3'-GTG TAA T***Pz***A CAA CAT-5'		
X2	5'-CAC ATT A***Pm***T GTT GTA-3'	41.8	42.2
Y2	3'-GTGTAA T***Pm***A CAA CAT-5'		
X3	5'-GCGCG ***PzPzPzPzPz*** GGCCG-3'	60.8	-
Y3	3'-CGCGC ***PzPzPzPzPz*** CCGGC-5'		

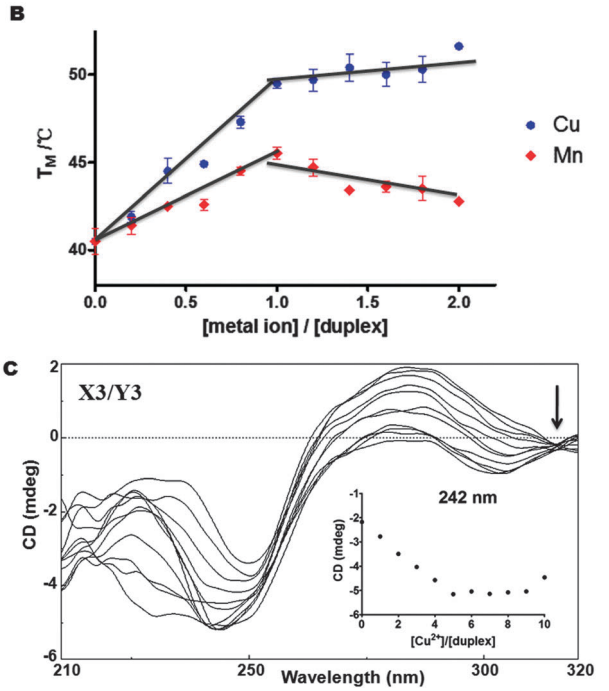

Fig. 2 (A) Sequences of the examined oligonucleotides; (B) Melting temperature titrations for duplex **X2/Y2** as a function of the ratio [metal ion] to [duplex]; (C) CD spectral changes of the duplex **X3/Y3** at various concentrations of Cu^{2+} (steps of 1 eq.). Inset: plot of dichroic changes at 242 nm against the ratio of [Cu^{2+}] to [**X3/Y3**]. Conditions: 150 mM NaCl, 10 mM CHES buffer pH 9, 1 μM oligonucleotide, final volume of 200 μL.

followed by Mn(III), in a similar way to what is observed for the salen ligandoside.[18–20]

The 1 : 1 ratio of duplex **X1**/**Y1**-Cu(II) of the structure was confirmed by UV titration (Fig. 2B). The data clearly confirm the 1 : 1 stoichiometry as the complexation process comes to end after addition of one equivalent of Cu(II). While the titration with $CuSO_4$ gave a clear 1 : 1 result, the titration data obtained with $MnSO_4$ are less clear (Fig. 2B, Fig. S3, ESI†). Although a 1 : 1 stoichiometry can again be deduced from the data, the observed spectral changes indicate complexation independent of further structural changes. We explain the results with the well known oxidation of Mn(II) to Mn(III) and the need for an apical ligand, which will certainly disturb the duplex structure.[21,22]

In order to examine the capability of multiple Pz ligandosides to complex more than one Cu(II), thus creating Cu(II) stacking structures with potentially interesting magnetic properties, we next prepared the oligonucleotide **X3** and its counterstrand **Y3** (Fig. 2A). Upon hybridization, these strands form a structure with five

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consecutive Pz–Pz complexing units. The duplex had a T_M of 60.8 °C, due to the stabilization achieved by the presence of the CG wings. After incorporation of 5 equivalents of Cu^{2+}, the duplex was so stable that the melting temperature could not be measured (Fig. S4, ESI†).

The characteristic changes in the UV-Vis spectrum of duplex **X3/Y3** that occur upon titration with Cu^{2+} ions are shown in Fig. S5 (ESI†). Analysis of the resulting duplex by CD-titration showed that five metal ions are indeed complexed in the structure (see Fig. 2C). The overlaid CD curves exhibit an isosbestic point at λ = 316 nm, in agreement with data obtained for the salen complex[20] and with data of Shionoyas' hydroxypyridine ligandoside.[23,24] However, while for the salen complex, where the Cu(II) ions are likely more rigidly stacked, diamagnetic coupling was observed, the open Shionoya 5 × Cu(II) system displayed paramagnetic coupling. This was recently the subject of excellent theoretical calculations.[25,26] The Pz-system is the third system, which enables complexation of 5 metal ions in a row. EPR studies to investigate the electron couplings are now underway.

In summary we describe the synthesis of a new Pz-self-base pair able to complex up to five metal ions in a row in a DNA-like structure. Complexation requires deprotonation of the phenol hydroxyl groups in order to achieve charge neutralization. Most importantly, the Pz-base is readily available and can be incorporated into oligonucleotides using standard solid phase synthesis. The neutral Pz–Cu–Pz base pair is slightly more stable than a canonical G : C base pair.

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