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Metal Ion Coordination to Azole Nucleosides

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Abstract: To evaluate the possibility of introducing azole nucleosides as building blocks for metal-mediated base pairs in artificial oligonucleotides, imidazole nucleoside, 1,2,4-triazole nucleoside and tetrazole nucleoside have been synthesized and characterized. The X-ray crystal structures of *p*-toluoyl-protected 1,2,4-triazole and tetrazole nucleosides are reported. Contrary to the situation primarily found for deoxyribonucleosides, the sugar moieties adopt C3′-*endo* conformations. The acidity of the β nucleosides increases with increasing number of nitrogen ring atoms, giving pK_a values of 6.01 ± 0.05, 1.32 ± 0.05 and <-3, respectively. This decrease in basicity results in a decreasing ability to form 2:1 complexes with linearly coordinating metal ions such as Ag^+ and Hg^{2+}. In all cases, the Ag^+ complexes are of higher stability than the corresponding Hg^{2+} complexes. Whereas imidazole nucleoside forms highly stable 2:1 complexes with both metal ions (estimated $\log\beta_2$ values of >10), only Ag^+ is able to reach this coordination pattern in the case of triazole nucleoside ($\log\beta_2 = 4.3\pm0.1$). Tetrazole nucleoside does not form 2:1 complexes at all under the experimental conditions used. These data suggest that imidazole nucleoside, and to a lesser extent 1,2,4-triazole nucleoside, are likely candidates for successful incorporation as ligands in oligonucleotides based on metal-mediated base pairs. DFT calculations further corroborate this idea, providing model complexes for such base pairs with glycosidic bond distances (10.8–11.0 Å) resembling those in idealized B-DNA (10.85 Å).

Keywords: azole · bioinorganic chemistry · coordination chemistry · ligand design · nucleosides

Introduction

The development of artificial nucleobases and their insertion into nucleic acids has received much attention over the past few years. Unnatural base pair surrogates developed so far rely variously on hydrogen bonding,[1] hydrophobic interactions[2] or metal ion coordination.[3] In particular, oligonucleotides including metal-mediated base pairs are of great interest with respect to the development of novel self-assembling molecular devices, in that they might extend the repertoire of DNA assembly in structural DNA nanotechnology[4] or nanomechanical devices.[5] Metallation of oligonucleotides could be expected to result in products with interesting electrical or photoelectrical properties, or potentially in the generation of molecular magnets.[6] Such adducts might also turn out to be useful for information storage[7] or as metal ion sensors,[8] or they serve as sources of stabilization of unusual secondary structures.[9]

Despite a rapidly increasing list of ligands used in metal-mediated base pairs that have been incorporated into oligonucleotides by automated solid-phase synthesis—including pyridine, 2-methyl-3-hydroxy-4-pyridone, 6-(2′-pyridyl)purine and 4-(2′-pyridyl)pyrimidinone, bound through N-glycosidic bonds,[3g,10] as well as 2,2′-bipyridine, 5-methyl-2,2′-bipyridine, pyridine-2,6-dicarboxylate, 2,6-bis(ethylthiomethyl)pyridine, pyridine-2,6-dicarboxamide and 8-hydroxyquinoline, bound through C-glycosidic bonds[3b,c,f,11]—no systematic study on the applicability of different groups of ligands has yet been reported. Here we present an exhaustive study of the possibility of using azoles as nucleobase surrogates in metal-mediated base pairs. These monodentate ligands should in principle be able to form both linear complexes, as required for double helix formation, and trigonal adducts, as required to establish a triple helix.[10a]

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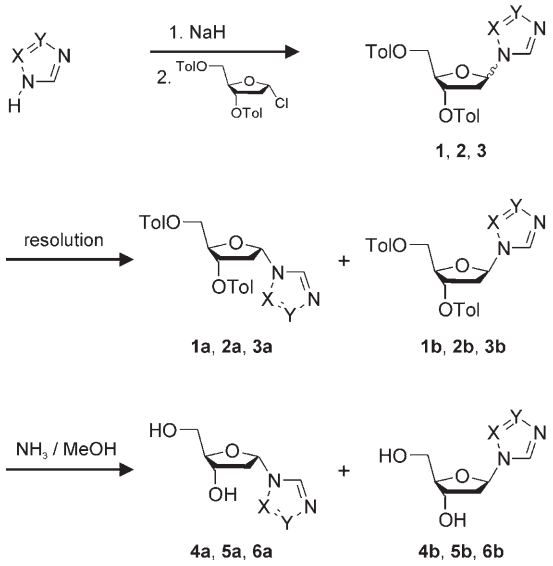
Supporting information (plots of pD-dependent chemical shifts of **4a**, **4b**, **5a**, **5b**, and **6b**, typical ^{1}H,^{1}H NOESY data explaining the ^{1}H NMR spectroscopic differentiation of α and β nucleosides, the output files of EQNMR, and Cartesian coordinates of all calculated structures) for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

Results and Discussion

Synthesis and characterization of azole nucleosides: The synthesis of the azole nucleosides **4b**, **5b**, and **6b** (**4**: imidazole nucleoside, **5**: 1,2,4-triazole nucleoside, **6**: tetrazole nucleoside) from Hoffer's chloro sugar[12] (Scheme 1) was first



Scheme 1. Synthesis of azole nucleosides (modified from reference [13]; Tol = *p*-toluoyl). Compounds **1**, **4**: X = Y = CH (imidazole). Compounds **2**, **5**: X = N, Y = CH (1,2,4-triazole). Compounds **3**, **6**: X = Y = N (tetrazole). In the case of tetrazole as a ligand, additional isomeric products with N2-glycosidic bonds were also obtained.

described in the context of a search for artificial nucleobases that might promote antiparallel triple helix formation.[13] Unlike in that work, we were unable to obtain the desired β nucleoside as the sole product, but only as the main product accompanied by minor amounts of the corresponding α nucleoside, which had to be separated by flash chromatography or crystallization. The epimers were easily distinguished by 2D ^{1}H,^{1}H NOESY spectroscopy (see Supporting Information). In the case of the β isomer, the signals of the sugar protons H1′ and H3′, situated on opposite sides of the mean sugar plane, give rise to intense cross-peaks to the signals of H2″ and H2′, respectively. In the case of the α isomer, in which H1′ and H3′ point in the same direction, intense cross-peaks to the signal of H2′ are observed for both H1′ and H3′. A first hint of the configuration can be inferred from the splitting pattern of the H1′ signal in the ^{1}H NMR spectrum: for the α nucleoside a clear doublet of doublets can be observed, whereas a pseudo-triplet appears for the β nucleoside.

In addition to the α and β diastereoisomers, 1,2,4-triazole and tetrazole can in principle give rise to two positional isomers upon formation of the glycosidic bond. In the case of triazole, only the N1-glycosylated product **2** is observed. This was shown unequivocally by the appearance of two distinct singlets of aromatic protons in the ^{1}H NMR spectrum of **2**. A ^{1}H NMR spectrum of the isomeric N4-glycosylated product would have contained only one aromatic signal of two symmetry-equivalent aromatic protons. The use of tetrazole gives a mixture of N1- and N2-glycosylated products at an approximate ratio of 65:35. To ensure better comparability with the imidazole- and triazole-based nucleosides, we focussed on isolating and characterizing the N1-glycoslated isomer. Differentiation of the isomers was possible by 2D ^{1}H,^{1}H-NOESY spectroscopy, in which the signal of the aromatic proton displays cross-peaks to signals of the sugar protons such as H1′ in the case of the N1-glycosylated product **3**, whereas no such cross-peaks are detected for the N2 isomer.

Crystal structures of protected nucleosides: In the cases of the nucleosides **5b** and **6b**, purification of the β nucleosides was facilitated by the low solubilities of the toluoyl-protected intermediates **2b** and **3b** in chloroform. Crystals of **2b** and **3b** suitable for structure analysis by X-ray diffraction were obtained, and Figure 1 provides a view of the two

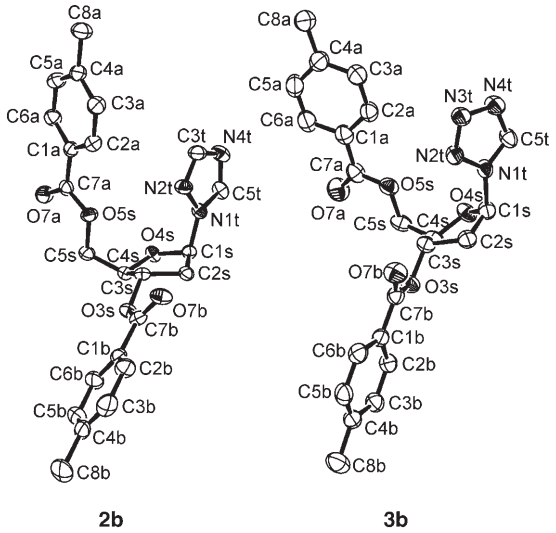


Figure 1. Views of **2b** and **3b** with atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level. The high *anti* orientation of the nucleobase surrogates can clearly be discerned.

structures. Relevant crystallographic data can be found in Table 1, whilst selected interatomic distances and dihedral angles are listed in Table 2. Both compounds display almost identical conformational features, so they will be discussed together. The nucleobase surrogates are found in a high *anti* orientation with respect to the deoxyribose moiety (with glycosidic angles χ (O4s-C1s-N1t-C5t) of −95.5(4)° (**2b**) and −85.3(4)° (**3b**)). The sugars each adopt a C3′-*endo* conformation (N conformation, phase angles of pseudorotation P of 13.0° (**2b**) and 11.7° (**3b**)). A search in the Cambridge Structural Database (CSD version 5.26, November 2004)[14] revealed that other nucleosides bearing a five-membered ring as a nucleobase surrogate prefer *syn* orientations. None of these crystal structures displays a C3′-*endo* conformation,

Table 1. Crystallographic data for **2b** and **3b**.

	2b	**3b**
formula	$C_{23}H_{23}N_3O_5$	$C_{22}H_{22}N_4O_5$
formula weight	421.44	422.44
crystal system	monoclinic	triclinic
space group	$P2_1$ (no. 4)	$P\bar{1}$ (no. 1)
a, b, c [Å]	14.536(3), 5.3625(11), 15.326(3)	5.4380(11), 8.3500(17), 12.549(3)
α, β, γ [°]	90, 117.43(3), 90	105.26(3), 97.29(3), 103.97(3)
V [Å^3]	1060.3(5)	522.3(2)
Z	2	1
ρ_{calcd} [g cm^{-3}]	1.32	1.343
$\mu(Mo_{K\alpha})$ [mm^{-1}]	0.094	0.097
crystal size [mm]	$0.07\times0.09\times0.12$	$0.05\times0.10\times0.50$
temperature [K]	150(2)	150(2)
radiation [Å]	$Mo_{K\alpha}$ (0.71073)	$Mo_{K\alpha}$ (0.71073)
$\theta_{min}, \theta_{max}$ [°]	3.2, 27.5	2.6, 27.0
tot., uniq. data	2644, 2644	2216, 2216
observed data [$I > 4\sigma(I)$]	1881	1397
N_{ref}, N_{par}	2644, 372	2216, 368
R, w$R2$, S	0.0480, 0.0898, 1.09	0.0331, 0.0628, 0.84
min. and max. resd. dens. [e Å^{-3}]	−0.19, 0.19	−0.18, 0.14

Table 2. Selected interatomic distances [Å] and dihedral angles [°] of **2b** (Z = C) and **3b** (Z = N).

	2b	**3b**		**2b**	**3b**
N1t–N2t	1.365(4)	1.358(3)	C4s-O4s-C1s-C2s	2.8(3)	3.7(3)
N2t–Z3t	1.318(4)	1.300(4)	O4s-C1s-C2s-C3s	−20.0(3)	−22.1(3)
Z3t–N4t	1.361(5)	1.363(4)	C1s-C2s-C3s-C4s	28.4(3)	30.8(3)
N4t–C5t	1.312(5)	1.309(4)	C2s-C3s-C4s-O4s	−27.4(3)	−29.2(3)
C5t–N1t	1.340(4)	1.335(4)	C3s-C4s-O4s-C1s	15.5(3)	16.3(3)
C1s–N1t	1.471(4)	1.473(4)	O4s-C1s-N1t-C5t	−95.5(4)	−85.3(4)

however. Prevention of a steric clash between the aromatic base proton at C5t and H3′ in the C3′-*endo* conformation is likely to be the reason for the preference for high *anti* over *syn* in the cases reported here. A comparison of the C1s–N1t bond lengths (1.471(4) and 1.473(4) Å, respectively) with those within the azole rings (1.300(4)–1.365(4) Å) clearly shows a delocalization of the π electrons, although slight differences in bond lengths, fitting a Lewis formula with fixed single and double bonds, can also be discerned (Table 2).

Acidity constants of azole nucleosides: To obtain reliable data on the metal ion coordination behavior of a prospective ligand, its acidity constant(s) need to be known, so that competition between protonation and metallation can be excluded. We therefore decided to determine the pK_a values of **4b**, **5b**, and **6b** by pD-dependent ^{1}H NMR spectroscopy (see Supporting Information). In the cases of imidazole and triazole nucleosides **4b** and **5b**, this gave values of 6.01 ± 0.05 and 1.32 ± 0.05, respectively. As expected from a comparison with the common nucleobases and nucleosides,[15] the deoxyribose-substituted ligands are more acidic than the corresponding methyl-substituted ligands by about one log unit. Tetrazole nucleoside **6b** hydrolyses under both acidic (pD < 5) and alkaline (pD > 10) conditions, so the full pD-dependence of its chemical shifts cannot be compiled. It can, however, be determined that the resonance of its aromatic proton does not display any pD dependence within its detectable range of 1 < pD < 12. Since a pK_a value of −3.00 has been reported for 1-methyltetrazole,[16] this finding is not unexpected. If the greater acidity of nucleosides relative to methylated nucleobases is taken into account,[15] pK_a values of < −3 can be estimated for **6a** and **6b**. With regard to the protonation site, previous experimental and theoretical studies on N1-substituted azoles have both shown that the nitrogen next to the methylene bridge (i.e., N4 in 1,2,4-triazole and tetrazole, N3 in imidazole) is the most or more basic one and hence is protonated first.[17] We assume that the same is true for the nucleosides discussed here. A summary of all pK_a values determined in this work is given in Table 3. The acidity constants of **4b**, **5b**, and **6b** suggest that no competition with ligand protonation should be expected for metallation reactions carried out at neutral pH. On the other hand, the low basicities of **5b** and, especially, **6b** would be likely to go along with low tendencies to coordinate metal ions and hence low association constants.

Table 3. pK_a values of azole nucleosides and 1-methylazoles.[a]

	α Nucleoside (**a**)	β Nucleoside (**b**)	1-Methyl derivative
imidazole (**4**)	6.42 ± 0.05	6.01 ± 0.05	7.20 ± 0.02[b]
1,2,4-triazole (**5**)	1.53 ± 0.05	1.32 ± 0.05	2.31 ± 0.03[c]
tetrazole (**6**)	< −3[d]	< −3[d]	−3.00[e]

[a] The errors given correspond to three times the standard deviations of the mean values. [b] Taken from reference [31]. [c] Taken from reference [32]. [d] Estimate based on the pK_a value of the corresponding 1-methyl derivative. [e] Taken from reference [16].

Metal ion coordination: With the applicability of monodentate azole moieties as nucleobase surrogates in metal-mediated base pairs in mind, Ag^+ and Hg^{2+} appear to be promising candidates for complexation studies. They typically coordinate in a linear fashion, yet are flexible enough to accommodate additional ligands, as is necessary for potential triple helix formation. Furthermore, these metal ions are known to form kinetically labile complexes, making NMR spectroscopy a convenient method for the determination of adduct stoichiometries and stability constants. To this end we carried out several titrations of solutions of **4b**, **5b**, and **6b** in D_2O with $AgNO_3$ and $Hg(CF_3COO)_2$ and monitored the

chemical shift changes of the aromatic protons of the ligands.

Job plots for all possible combinations of azole nucleosides and metal ions were prepared by application of the method of continuous variations.[18] Experiments with Hg^{2+} were performed in triethylammonium acetate buffer (pD 7.0), whereas unbuffered solvent was used for Ag^{+}, due to the formation of precipitates upon addition of $AgNO_3$ to various buffers as observed in blind studies in the absence of any nucleoside. In the latter case, the pD was monitored to verify that potential acidification caused by addition of the metal ion would not give rise to a change in chemical shift that might be misinterpreted as evidence for metal coordination. Figure 2 gives an overview of the results. It can be discerned that imidazole nucleoside **4b** forms 2:1 complexes with both Ag^{+} and Hg^{2+}. The shapes of the continuous variations plots of **4b**, with their sharp maxima, imply high stability constants for the corresponding complexes.[18d] The more pronounced curvature of the plots observed for triazole nucleoside **5b** suggests a decrease in stability upon formal substitution of a CH group by a nitrogen atom in the aromatic ring. With mercuric ions this decrease is so substantial that only a 1:1 complex forms. In conjunction with the decreasing basicity, the tendency of tetrazole nucleoside **6b** to build stable complexes declines even further: only Ag^{+} is able to establish a 1:1 complex; no coordination of Hg^{2+} to **6b** can be detected under the experimental conditions used.

To determine the stability constants of the complexes, solutions of **4b**, **5b**, and **6b** in D_2O were titrated with increasing amounts of metal ion solutions. Again, triethylammonium acetate buffer (pD 7.0) was used for Hg^{2+}, and no buffer for Ag^{+}. Figure 3 shows the titration curves obtained in these experiments. As was already expected from the ap-

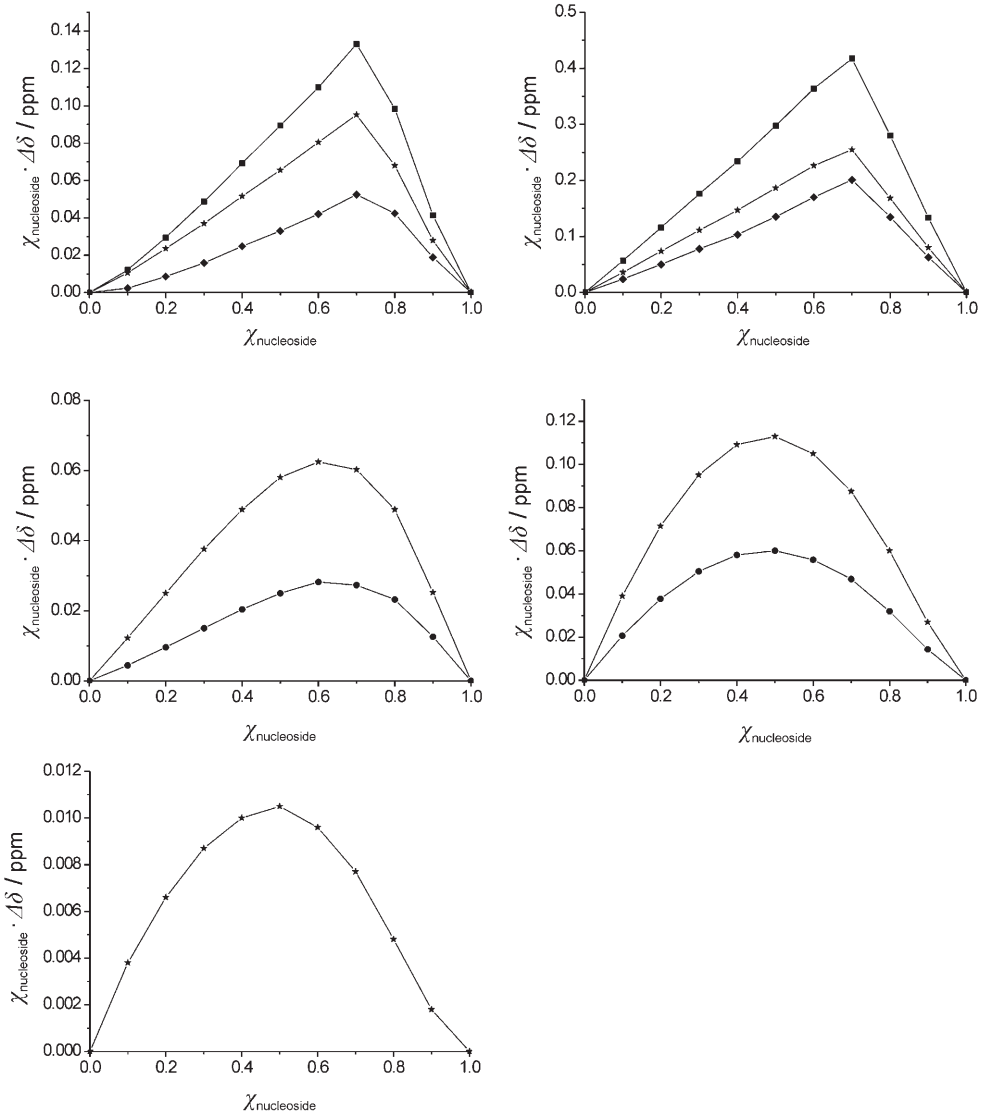


Figure 2. Job plots for treatment of **4b** (top), **5b** (middle), and **6b** (bottom) with Ag^{+} (left) and Hg^{2+} (right). H2: ■, H3: ●, H4: ◆, H5: *. No chemical shift changes were observed upon titration of **6b** with Hg^{2+}. The concentrations at $\chi = 1$ range from 18 to 25 mM.

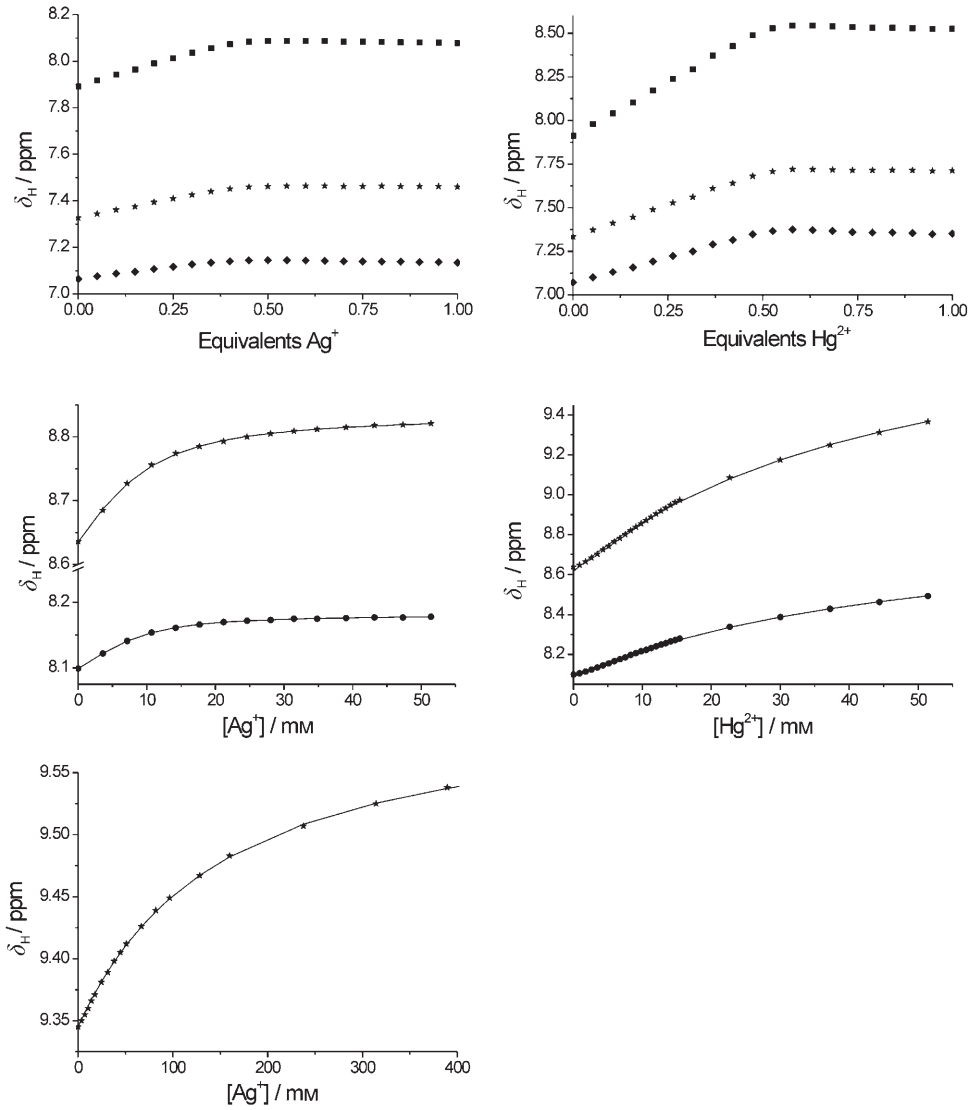


Figure 3. Titration of **4b** (top), **5b** (middle), and **6b** (bottom) with Ag^+ (left) and Hg^{2+} (right). H2: ■, H3: ●, H4: ◆, H5: *. No chemical shift changes were observed upon titration of **6b** with Hg^{2+}. Nucleoside concentrations are 18.0 mM (**5b**) and 16.5 mM (**6b**).

pearance of the associated Job plots, imidazole nucleoside **4b** forms highly stable adducts with the two metal ions. The binding curves display abrupt changes of slope at a ratio of 0.5 metal ions per nucleoside, thereby confirming the results obtained by the method of continuous variations. Because of the absence of any curvature in the two linear fragments of the plot, no stability constants for the metal complexes of **4b** can be determined by NMR spectroscopy. From known limits of this titration method, individual formation constants of $\log K_i > 5$ can be estimated.[18b] In contrast to **4b**, the binding isotherms of **5b** and **6b** do not show any discontinuities. In these cases, overall stability constants could be extracted from the curves by nonlinear least-squares regression implemented in the program EQNMR.[19] The 2:1 complex of triazole nucleoside **5b** with Ag^+ exhibits a formation constant of $\log \beta_2 = 4.3 \pm 0.1$. The value for $\log \beta_1$ as determined by EQNMR is 1.5 ± 0.7. The complex apparently does not obey the generic rule that the second individual stability constant is smaller than the first one. Although similar findings have already been reported for metal complexes of other triazole derivatives,[20] or even for the well known $[Ag(NH_3)_2]^+$ complex ($\log \beta_1 = 3.4$, $\log \beta_2 = 7.2$[21]), this discrepancy might also be due to poor data convergence in the case of the $\log \beta_1$ value caused by almost identical chemical shifts for the complexes $[Ag(\mathbf{5b})]^+$ and $[Ag(\mathbf{5b})_2]^+$ (see Supporting Information). The 1:1 complexes of **5b** with Hg^{2+} and **6b** with Ag^+ display formation constants ($\log \beta_1$) of 1.56 ± 0.04 and 0.86 ± 0.02, respectively. For these last two complexes, identical values are obtained when the experimental data are fitted to mathematical equations derived specifically for a ligand to metal stoichiometry of 1:1.[22]

A summary of all stability constants determined in this work is given in Table 4. Obviously, Ag^+ complexes of the azole nucleosides **5b** and **6b** are higher in stability than the

Table 4. Stability constants for metal ion complexation of **4b**, **5b** and **6b**.[a]

	Ag^+		Hg^{2+}	
	$\log\beta_1$	$\log\beta_2$	$\log\beta_1$	$\log\beta_2$
4b	>5[b]	>10[b]	>5[b]	>10[b]
5b	1.5 ± 0.7	4.3 ± 0.1	1.56 ± 0.04	—
6b	0.86 ± 0.02	—	—	—

[a] The errors given correspond to three times the standard deviations of the mean values or the possible systematic error, whichever is larger. [b] Estimate based on the occurrence of an abrupt change of slope in the binding isotherms of **4b** with Ag^+ or Hg^{2+}.[18b]

corresponding mercuric adducts, a finding that is probably also true for the imidazole nucleoside **4b**. Moreover, the stabilities of the complexes decrease with increasing number of endocyclic nitrogen atoms and hence with decreasing basicity.

DFT calculations: DFT calculations were performed to determine the geometrical parameters of the putative metal-mediated base pairs including two azole nucleosides and Ag^+ or Hg^{2+}. To reduce computing time, 1-methylazoles were used as surrogates for the azole nucleosides. After a full geometry optimization, the energy difference with respect to a planar geometry with cisoid methyl groups as necessary for a Watson–Crick base pair surrogate was determined. Table 5 gives an overview of the calculated energy differences. They are all small enough to allow for reasonably high population of the planar conformation required for implementation into artificial oligonucleotides. In addition, it can be anticipated that the gain in total energy provided through favorable stacking interactions with neighboring base pairs should more than compensate for the loss in energy upon formation of a coplanar complex. The relatively large energy differences involving 1-methyltetrazole can be attributed to the fact that in this case the fully optimized geometry does not show the azole derivatives in a more or less perpendicular orientation with respect to each other. Instead, they are oriented in a fully planar fashion with transoid methyl groups, thereby providing the largest possible distance between the sole remaining aromatic protons.

Table 5. Results of the DFT calculations: energy differences between planar and fully optimized geometries of 2:1 complexes of 1-methylazoles (representing azole nucleosides) [kJ mol^{-1}] with Ag^+ and Hg^{2+} as well as C(methyl)–C(methyl) distances (representing the average distance between glycosidic bonds) [Å].

	Ag^+		Hg^{2+}	
	ΔE	d	ΔE	d
imidazole	1.693	10.8	2.103	11.0
1,2,4-triazole	0.832	11.0	2.082	11.2
tetrazole	4.047	11.4	3.078	11.6

The C(methyl)–C(methyl) distances of 10.8–11.6 Å as found in the calculations (Table 5) are in the range of the average distance between glycosidic bonds of idealized B-DNA (10.85 Å). This is especially true for those azole/metal combinations that were experimentally shown to favor the formation of 2:1 complexes: $[Ag(\mathbf{4b})_2]^+$, $[Hg(\mathbf{4b})_2]^{2+}$, and $[Ag(\mathbf{5b})_2]^+$. It therefore seems likely that imidazole nucleosides or 1,2,4-triazole nucleosides in combination with Ag^+ or Hg^{2+} could be incorporated into artificial oligonucleotides as metal-mediated base pairs.

Conclusion

An investigation of the acid–base properties and metal ion complexation behavior of nucleosides with imidazole, 1,2,4-triazole and tetrazole as nucleobase surrogates suggests that, with Ag^+ as the bridging metal ion in a self-complementary metal-mediated base pair, imidazole and triazole nucleosides should form stable base pairs when incorporated into oligonucleotides. Mercuric ions give less stable adducts, such that only imidazole nucleoside could be shown to form a stable 2:1 complex with Hg^{2+}. The basicity of tetrazole nucleoside is far too low to allow for the formation of 2:1 complexes under the experimental conditions used. The geometries of the metal-mediated base pairs obtained from DFT calculations support their applicability in oligonucleotides. Further investigations into the incorporation of these azole nucleosides into oligonucleotides and the possibility of creating an oligonucleotide-based silver ion sensor making use of the differential stabilization of triazole–M^{n+}–triazole base pairs are currently being pursued.

Experimental Section

Preparations: The azole nucleosides were synthesized by a modified literature procedure (Scheme 1),[13] with the modification referring to the separation of undesired α nucleosides (see below for more details). Because of dissatisfying ^{1}H NMR spectroscopic characterization in the original work, the ^{1}H NMR chemical shifts of compounds **1–6** are communicated below. All nucleosides except for **6a** have been characterized.

Compound 1: Separation of α and β nucleosides **1a** and **1b** was achieved by flash chromatography over silica gel with elution with a gradient from 100% dichloromethane to 100% ethyl acetate.

Compound 1: Elemental analysis calcd (%) for $C_{24}H_{24}N_2O_5$ (420.5): C 68.6, H 5.8, N 6.7; found: C 68.4, H 5.9, N 6.6.

Compound 1a: ^{1}H NMR ($CDCl_3$): δ = 7.93 (d; H_{Tol}), 7.79 (d; H_{Tol}), 7.77 (s; H2), 7.29 (d; H_{Tol}), 7.25 (d; H_{Tol}), 7.16 (s; H5), 7.10 (s; H4), 6.18 (dd; H1′), 5.65 (m; H3′), 4.73 (m; H4′), 4.60 (m; H5′, H5″), 3.00 (m; H2′), 2.63 (m; H2″), 2.43 (s; CH_3), 2.42 (s; CH_3) ppm.

Compound 1b: ^{1}H NMR ($CDCl_3$): δ = 7.95 (d; H_{Tol}), 7.90 (d; H_{Tol}), 7.74 (s; H2), 7.29 (d; H_{Tol}), 7.25 (d; H_{Tol}), 7.09 (s; H4, H5), 6.15 (dd; H1′), 5.66 (m; H3′), 4.62 (m; H4′), 4.60 (m; H5′, H5″), 2.70 (m; H2′, H2″), 2.44 (s; CH_3), 2.42 (s; CH_3) ppm.

Compound 2: Pure β nucleoside **2b** was obtained by crystallization from a solution of a mixture of **2a** and **2b** in chloroform.

Compound 2: Elemental analysis calcd (%) for $C_{23}H_{23}N_3O_5$ (421.5): C 65.6, H 5.5, N 10.0; found: C 65.8, H 5.8, N 9.7.

Compound 2a: ^{1}H NMR ($CDCl_3$): δ = 8.40 (s; H5), 7.94 (s; H3), 7.94 (d; H_{Tol}), 7.63 (d; H_{Tol}), 7.25 (d; H_{Tol}), 7.18 (d; H_{Tol}), 6.32 (dd; H1′), 5.62 (m; H3′), 4.81 (m; H4′), 4.60 (m; H5′, H5″), 3.01 (m; H2″), 2.95 (m; H2′), 2.43 (s; CH_3), 2.39 (s; CH_3) ppm.

Compound 2b: ^{1}H NMR ($CDCl_3$): δ = 8.29 (s; H5), 7.94 (d; H_{Tol}), 7.92 (d; H_{Tol}), 7.91 (s; H3), 7.25 (d; H_{Tol}), 7.20 (d; H_{Tol}), 6.32 (dd; H1′), 5.80 (m; H3′), 4.63 (m; H4′), 4.60 (m; H5′, H5″), 3.16 (m; H2′), 2.74 (m; H2″), 2.43 (s; CH_3), 2.40 (s; CH_3) ppm.

Compound 3: Pure β nucleoside **3b** was obtained by crystallization from a solution of a mixture of **3a** and **3b** in chloroform/hexane (1:1).

Compound 3: Elemental analysis calcd (%) for $C_{22}H_{22}N_4O_5$ (422.4): C 62.6, H 5.2, N 13.3; found: C 62.5, H 5.0, N 13.2.

Compound 3a: ^{1}H NMR ($CDCl_3$): δ = 8.86 (s; H5), 7.94 (d; H_{Tol}), 7.56 (d; H_{Tol}), 7.28 (d; H_{Tol}), 7.20 (d; H_{Tol}), 6.56 (dd; H1′), 5.67 (m; H3′), 4.82 (m; H4′), 4.64 (m; H5′, H5″), 3.18 (m; H2″), 3.04 (m; H2′), 2.44 (s; CH_3), 2.39 (s; CH_3) ppm.

Compound 3b: ^{1}H NMR ($CDCl_3$): δ = 8.81 (s; H5), 7.94 (d; H_{Tol}), 7.81 (d; H_{Tol}), 7.28 (d; H_{Tol}), 7.24 (d; H_{Tol}), 6.55 (dd; H1′), 5.77 (m; H3′), 4.71 (m; H4′), 4.58 (m; H5′, H5″), 3.16 (m; H2′), 2.96 (m; H2″), 2.44 (s; CH_3), 2.41 (s; CH_3) ppm.

Compound 4: Elemental analysis calcd (%) for $C_8H_{12}N_2O_3$ (184.2): C 52.2, H 6.6, N 15.2; found: C 52.2, H 6.4, N 15.4.

Compound 4a: ^{1}H NMR (D_2O, pD 9.1): δ = 7.92 (s; H2), 7.42 (s; H5), 7.06 (s; H4), 6.16 (dd; H1′), 4.47 (m; H3′), 4.24 (m; H4′), 3.69 (m; H5′, H5″), 2.85 (m; H2′), 2.36 (m; H2″) ppm.

Compound 4b: ^{1}H NMR (D_2O, pD 8.1): δ = 7.79 (s; H2), 7.22 (s; H5), 6.96 (s; H4), 6.19 (dd; H1′), 4.52 (m; H3′), 4.06 (m; H4′), 3.72 (m; H5′, H5″), 2.67 (m; H2′), 2.41 (m; H2″) ppm; HRMS (FAB): found 185.0904 $[M+H]^+$; $C_8H_{13}N_2O_3$ calcd 185.0926.

Compound 5: Elemental analysis calcd (%) for $C_7H_{11}N_3O_3$ (185.2): C 45.4, H 6.0, N 22.7; found: C 45.0, H 5.8, N 22.7.

Compound 5a: ^{1}H NMR (D_2O, pD 7.1): δ = 8.67 (s; H5), 8.11 (s; H3), 6.34 (dd; H1′), 4.46 (m; H3′), 4.32 (m; H4′), 3.71 (m; H5′, H5″), 2.82 (m; H2′), 2.52 (m; H2″) ppm.

Compound 5b: ^{1}H NMR (D_2O, pD 7.1): δ = 8.64 (s; H5), 8.10 (s; H3), 6.37 (dd; H1′), 4.60 (m; H3′), 4.11 (m; H4′), 3.72 (m; H5′, H5″), 2.79 (m; H2′), 2.53 (m; H2″) ppm; HRMS (FAB): found: 186.0868 $[M+H]^+$; $C_7H_{12}N_3O_3$ calcd 186.0879.

Compound 6: Elemental analysis calcd (%) for $C_6H_{12}N_4O_4$ (**6**·H_2O, 204.2): C 35.3, H 5.9, N 27.4; found: C 35.2, H 5.5, N 27.3.

Compound 6b: ^{1}H NMR (D_2O, pD 6.9): δ = 9.35 (s; H5), 6.62 (dd; H1′), 4.66 (m; H3′), 4.17 (m; H4′), 3.72 (m; H5′, H5″), 2.95 (m; H2′), 2.69 (m; H2″) ppm; HRMS (FAB): found: 187.0816 $[M+H^+]$; $C_6H_{11}N_4O_3$ calcd 187.0831.

Instrumentation: ^{1}H NMR spectra were recorded on Varian Mercury 200 and Bruker DRX 400 spectrometers. Chemical shifts were referenced to residual $CHCl_3$ ($CDCl_3$, δ = 7.26 ppm) or sodium 3-(trimethylsilyl)propanesulfonate (D_2O, δ = 0 ppm). pD values were obtained by adding 0.4 to the pH meter reading.[23] The pK_a values in H_2O were calculated from the pK_a* values in D_2O according to pK_a* = 1.015 pK_a + 0.45.[24] Microanalyses were measured on a Leco CHNS 932 instrument.

X-ray crystallography: Crystal data were collected at 150 K on an Enraf–Nonius–KappaCCD diffractometer with use of graphite-monochromated $Mo_{K\alpha}$ radiation (λ = 0.71073 Å). For data reduction and cell refinement, the Bruker–Nonius HKL 2000 suite was used. The structures were solved by direct methods and subsequent Fourier syntheses and were refined by full-matrix, least squares on F^2 by use of the SHELXTL PLUS and SHELXL-97 programs.[25] All non-hydrogen atoms were refined anisotropically, whilst hydrogen atoms were refined isotropically. Relevant crystallographic data are listed in Table 1. CCDC-270927 and CCDC-270928 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Calculations: Starting geometries for the DFT calculations were created by use of Molden's[26] built-in geometrical parameters. These geometries were fully optimized at the DFT level by use of Becke's three-parameter hybrid exchange functional (B3LYP)[27] as implemented in the Gaussian suite of programs,[28] and frequency calculations were run to confirm these structures as energy minima. For the metals, a LANL2DZ basis set was used,[29] other atoms were described by a 6–31G* basis set.[30] For the geometry optimization of the structures with cisoid methyl groups, the previously obtained fully optimized structures were used as starting geometries. The cisoid arrangement was achieved by constraining one dihedral angle (imidazole: C2a-N3a-N3b-C2b, triazole and tetrazole: C5a-N4a-N4b-C5b, set to 0°) during the optimization. Thus, no frequency calculations were performed for these geometries. The relative energies given refer to the electronic energies of the obtained geometries.

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