ChemPubSoc Europe

DOI: 10.1002/chem.201402221

CHEMISTRY
A European Journal
Full Paper

Bioinorganic Chemistry

A Family of "Click" Nucleosides for Metal-Mediated Base Pairing: Unravelling the Principles of Highly Stabilizing Metal-Mediated Base Pairs

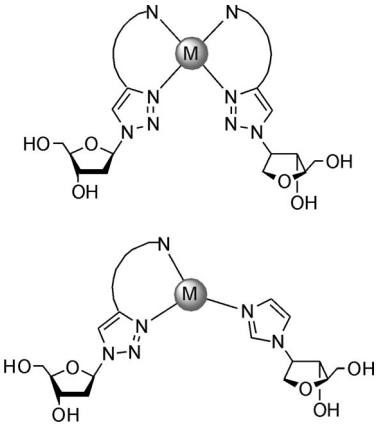
Tim Richters,[a] Olga Krug,[b] Jutta Kösters,[a] Alexander Hepp,[a] and Jens Müller*[a]

Abstract: A family of artificial nucleosides has been developed by applying the Cu^{I}-catalyzed Huisgen 1,3-dipolar cycloaddition. Starting from 2-deoxy-β-D-glycosyl azide as a common precursor, three bidentate nucleosides have been synthesized. The 1,2,3-triazole involved in all three nucleobases is complemented by 1,2,4-triazole (**TriTri**), pyrazole (**TriPyr**), or pyridine (**TriPy**). Molecular structures of two metal complexes indicate that metal-mediated base pairs of **TriPyr** may not be fully planar. An investigation of DNA oligonucleotide duplexes comprising the new "click" nucleosides showed that they can bind Ag^{I} to form metal-mediated base pairs. In particular the mispair formed from **TriPy** and the previously established imidazole nucleoside is significantly stabilized in the presence of Ag^{I}. A comparison of different oligonucleotide sequences allowed the determination of general factors involved in the stabilization of nucleic acids duplexes with metal-mediated base pairs.

Introduction

Nucleic acids are important building blocks in nanotechnology.[1] They are regularly used for the directed assembly of functional moieties.[2] One prominent possibility to introduce metal-based functionality is the incorporation of a metal-mediated base pair into a nucleic acid.[3] Metal-mediated base pairs are formed when transition-metal ions coordinate to donor atoms of complementary nucleobases. The corresponding nucleosides can be either natural,[4] or they can comprise specifically designed ligands.[5] Depending on the type of ligand, a metal-mediated base pair may contain up to two transition-metal ions.[6] Moreover, different metal-mediated base pairs can be incorporated into one duplex.[7] The structures of DNA duplexes with metal-mediated base pairs show that the regular B-type geometry is not necessarily disrupted by the introduction of the transition-metal ions.[8] This may be a reason why metal-mediated base pairs can even be recognized and processed by polymerases.[9] Several interesting applications have been proposed for metal-mediated base pairs,[10] including the introduction of improved charge-transfer properties into DNA[11] as well as various sensor applications.[10] DNA with metal-mediated base pairs is also relevant from a fundamental point of view: Several experiments and calculations have shown that metal ions in neighboring metal-mediated base pairs can interact with one another, for example, by ferromagnetic or antiferromagnetic coupling.[12]

To increase the scope of metal-mediated base pairing, we devised a new family of bidentate triazole-based nucleosides. They are capable of engaging in either a [2+2] or a [2+1] coordination mode, depending on the complementary nucleoside (Scheme 1).



Scheme 1. Metal-mediated base pairs with bidentate triazole-based nucleosides: [2+2] and [2+1] coordination mode.

Results and Discussion

Synthesis of the nucleosides

Three 1,2,3-triazole-based "click" nucleosides were synthesized by using Cu^{I}-catalyzed Huisgen 1,3-dipolar cycloaddition

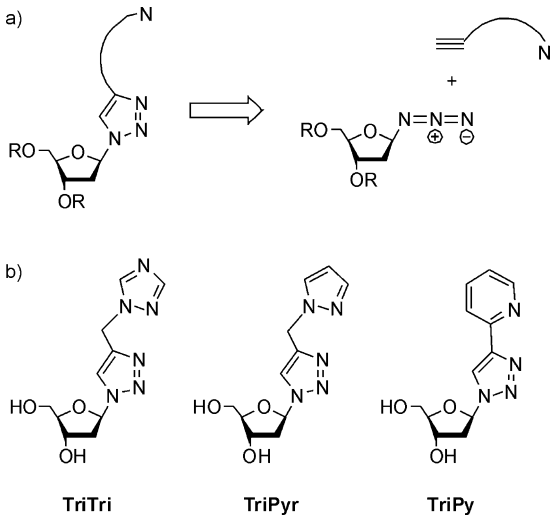
[a] T. Richters, J. Kösters, Dr. A. Hepp, Prof. Dr. J. Müller
Institut für Anorganische und Analytische Chemie
Westfälische Wilhelms-Universität Münster
Corrensstrasse 28/30, 48149 Münster (Germany)
Fax: (+49) 251-8336007
E-mail: mueller.j@uni-muenster.de

[b] Dr. O. Krug
Fakultät Chemie und Chemische Biologie
Technische Universität Dortmund
Otto-Hahn-Strasse 6, 44227 Dortmund (Germany)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201402221.

(Scheme 2a). Depending on the alkyne, the 1,2,3-triazole moiety is complemented by 1,2,4-triazole (**TriTri**), pyrazole (**TriPyr**), or pyridine (**TriPy**) (Scheme 2b). The use of 2-deoxy-β-D-glycosyl azide as a common precursor allows the modular synthesis of a variety of 1,2,3-triazole nucleosides.

The synthesis of **TriTri** (and its phosphoramidite required for automated DNA synthesis) has been reported recently.[13] The other two nucleosides **TriPyr** and **TriPy** have been synthesized accordingly (Scheme 3). The use of the **TriPy** nucleoside has recently also been reported as a ligand for the generation of a luminescent probe for the study of nucleoside transporters.[14] Its incorporation into nucleic acids and its capability to form metal-mediated base pairs has not yet been investigated though.



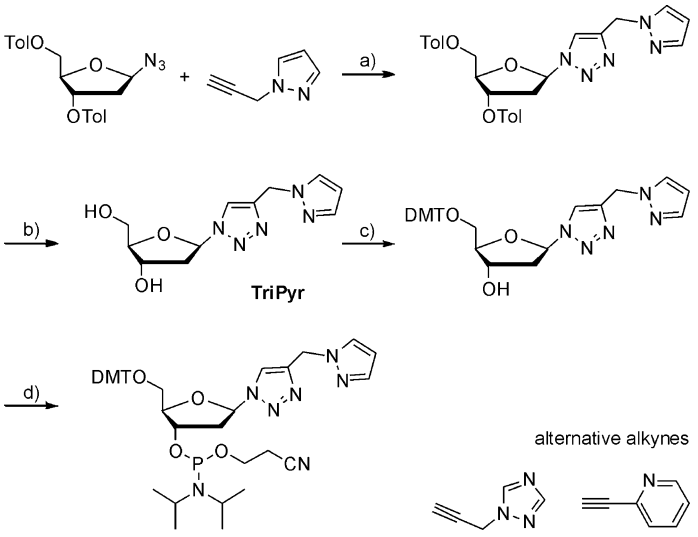
Scheme 2. a) Retrosynthesis of the bidentate triazole-based nucleosides; b) Nucleosides reported in this work.

Molecular structures of the model complexes

It is important to note that the methylene spacer in **TriTri** and **TriPyr** might lead to the formation of a non-planar metal complex. Such behavior has been reported for related ligands bearing an alkyl or aryl substituent instead of the deoxyribose.[15] These systems can be considered model nucleobases, which are regularly applied to study the molecular structures of metal complexes of nucleosides.[16] Such a model nucleobase representing **TriPy** is known to support both fully planar and tetrahedral complexes.[15,17] Accordingly, we synthesized a model nucleobase for **TriPyr** (by formally replacing the sugar moiety with a benzyl substituent) and investigated its metal-binding properties. Two metal complexes of the resulting ligand **BnTriPyr** are displayed in Figure 1 and S1 (the Supporting Information). In both molecular structures, the M–N distances are in the normal range (Table 1). The Cu^{II} ion is weakly coordinated by two additional solvent molecules in the axial positions, thereby completing a distorted octahedral coordination environment. These methanol ligands are easily cleaved off, as confirmed by mass spectrometry and elemental analysis of the complex (see the Supporting Information). Hence, they would not prohibit the formal incorporation of such a metal complex as a metal-mediated base pair into a nucleic acid duplex. However, the *transoid* orientation of the benzyl substituents observed in the molecular structure is not compatible with the regular B-DNA conformation, which requires a *cisoid* orientation of the glycosidic bonds. The *transoid* orientation is probably being adopted to avoid a steric clash of two closely located benzyl groups. As such a steric clash does not exist in B-DNA, the complex still appears to be a valid structural model for the corresponding potential metal-mediated base pair. Neither in the cation of $[Pd(\mathbf{BnTriPyr})_2]Cl_2{\cdot}2H_2O$ (Figure S1, the Supporting Information) nor in that of $[Cu(\mathbf{BnTriPyr})_2(CH_3OH)_2](NO_3)_2$ (Figure 1) is the **BnTriPyr** ligand found to be planar. In the Pd^{II} complex, the triazole and pyrazole moieties of this ligand are tilted towards one another with an angle of 54.25(6)°. The corresponding angle in the Cu^{II} complex is a bit smaller, amounting to 47.41(9)°. As we will see, the non-planarity does not prohibit the use of **TriPyr** in metal-mediated base pairs within an oligonucleotide duplex, even though fully planar metal complexes can be additionally stabilized by π-

Table 1. Selected bond lengths [Å] in the molecular structures of $[Pd(\mathbf{BnTriPyr})_2]Cl_2{\cdot}2H_2O$ and $[Cu(\mathbf{BnTriPyr})_2(CH_3OH)_2](NO_3)_2$.

	$[Pd(\mathbf{BnTriPyr})_2]Cl_2{\cdot}2H_2O$	$[Cu(\mathbf{BnTriPyr})_2(CH_3OH)_2](NO_3)_2$
M1–N3t	1.9988(10)	2.0109(15)
M1–N2p	2.0102(10)	2.0232(16)
M1–O2m	–	2.4346(15)



Scheme 3. Synthesis of the artificial nucleosides and their phosphoramidites. a) THF/2-propanol/H_2O, $CuSO_4$, sodium ascorbate, 12 h, RT; b) MeOH, NH_3, 18 h, RT; c) Dimethoxytrityl chloride (DMT-Cl), 4-dimethylaminopyridine (DMAP), pyridine, 3 h, RT; d) 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (CEDIP-Cl), diisopropylethylamine (DIPEA), CH_2Cl_2, 30 min, RT.

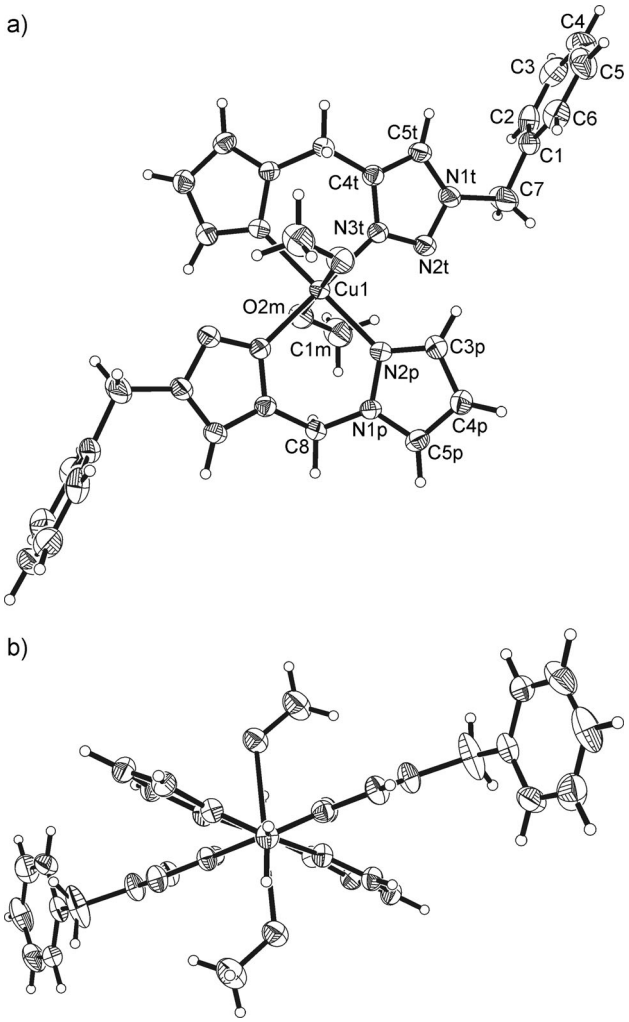


Figure 1. Molecular structure of the cation of $[Cu(\mathbf{BnTriPyr})_2(CH_3OH)_2](NO_3)_2$ viewed from two different orientations.

stacking interactions with neighboring base pairs. In contrast, the introduction of such non-planar nucleoside into an oligonucleotide helps to unravel the principles of the stabilization of metal-mediated base pairs.

Investigation of the oligonucleotide duplex I

Two different oligonucleotide duplexes were prepared by using automated solid-phase synthesis according to the phosphoramidite method. Both duplexes comprise one artificial metal-mediated base pair, six G:C base pairs, and six A:T base pairs (G = guanine, C = cytosine, A = adenine, T = thymine). The natural nucleosides are arranged in a way that the duplex consists of one purine strand and one pyrimidine strand. Hence, the two duplexes have an identical overall composition and differ (amongst others) by the identity of the two canonical base pairs located adjacent to the artificial base pair (Scheme 4). One of these duplexes has previously been used in other studies,[13,16b] thereby enabling a direct comparison of the melting temperatures with those of nucleic acids comprising other metal-mediated base pairs. The other duplex has

Duplex **I**
5'-d(AGA AAG **X**GA GGG A)
d(TCT TTC **Y**CT CCC T)-5'

Duplex **II**
5'-d(GAG GGA **X**AG AAA G)
d(CTC CCT **Y**TC TTT C)-5'

Scheme 4. Oligonucleotide duplexes under investigation (**X**, **Y** = **TriTri**, **TriPyr**, **TriPy**, or **Imi**).

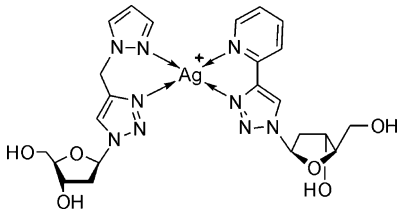
been introduced in this study to enable an investigation of nearest-neighbor effects. In addition to the bidentate triazole-based nucleosides, the well-established monodentate imidazole nucleoside **Imi**[8a,b,18] has also been included in this study.

To probe the overall metal-binding preferences of the artificial nucleosides, the thermal stability of duplex **I** with **X** = **TriPyr** and **Y** = **TriPy** was determined by temperature-dependent UV spectroscopy in the presence of various transition-metal ions.[25] Table 2 shows the resulting melting temperatures

Table 2. Melting temperature T_m [°C] of duplex **I** with **X** = **TriPyr** and **Y** = **TriPy** (Scheme 5) in the absence and presence of various transition-metal ions.[a]

Metal ion	T_m (0 equiv)	T_m (1 equiv)	ΔT_m
Ag^I	32.6	40.0	7.4
Hg^{II}	32.2	32.2	0.0
Cu^{II}	32.1	32.2	0.1
Zn^{II}	32.2	32.2	0.0
Ni^{II}	32.1	32.1	0.0

[a] Conditions: oligonucleotide duplex (1 μM), $NaClO_4$ (150 mM), MOPS (5 mM; pH 6.8). The estimated standard deviation of T_m amounts to 1 °C.



Scheme 5. Proposed structure of the Ag^I-mediated base pair from **TriPyr** and **TriPy**.

T_m. As can be seen, only Ag^I has a stabilizing effect on the oligonucleotide duplex (Scheme 5). The same observation was made for other combinations of the triazole-based bidentate nucleosides. Hence, only the Ag^I-mediated base pairs were studied subsequently in more detail. It is interesting to note that Cu^{II} does not stabilize any of the base pairs, despite the fact that a corresponding model structure could be structurally characterized (Figure 1) and that several examples exist in the literature of Cu^{II}-mediated base pairs.[7,9c,11b,12,21] Various explanations are possible: 1) A Cu^{II}-mediated base pair is formed but does not bring about a net stabilization of the duplex because its formation is accompanied by other destabilizing conformational changes; 2) Most previously reported Cu^{II}-mediated base pairs involve negatively charged ligands, that is, lead to the

formation of a neutral complex. Such neutral entities might be more easily accommodated within the base pair stack compared with the potential Cu^{II}-mediated base pairs investigated here carrying two positive charges.

Table 3 lists the increase in melting temperature (ΔT_m) upon the addition of one equivalent of Ag^I to duplex **I** with all possible combinations of **TriTri**, **TriPyr**, **TriPy**, and **Imi** in the positions **X** and **Y**.

Table 3. Increase of T_m [°C] of duplex **I** upon the addition of one equivalent of Ag^I.[a]

	X = Imi	X = TriTri	X = TriPyr	X = TriPy
Y = Imi	9.5	2.2	1.1	12.6
Y = TriTri	6.4	2.1	2.9	1.5
Y = TriPyr	1.5	1.9	2.8	4.1
Y = TriPy	12.6	1.3	7.4	1.4

[a] Conditions: oligonucleotide duplex (1 μM), $NaClO_4$ (150 mM), MOPS (5 mM; pH 6.8), $AgNO_3$ (1 μM). The estimated standard deviation of T_m amounts to 1 °C. The absolute melting temperatures in the absence and presence of one equivalent of Ag^I are listed in Tables S1 and S2 (the Supporting Information).

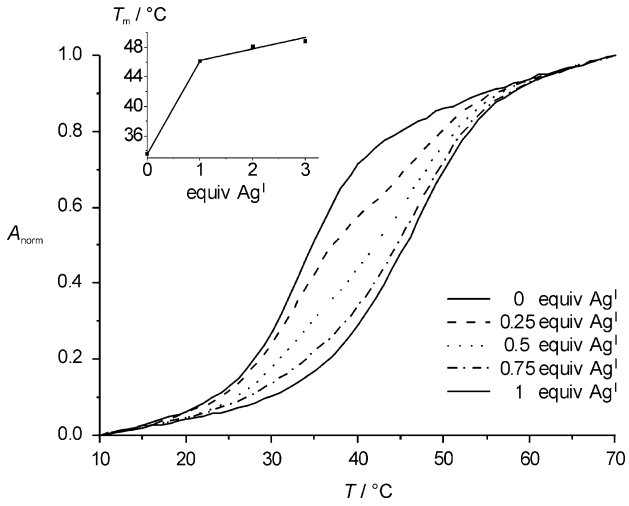
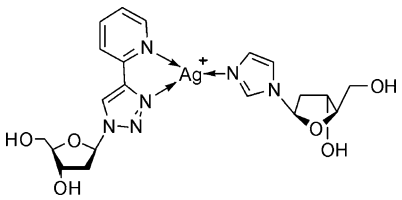


Figure 2. Melting curves of duplex **I** with **X** = **Imi** and **Y** = **TriPy** in the presence of various amounts of Ag^I. Inset: Dependence of melting temperature T_m on number of added equivalents of Ag^I. Conditions: oligonucleotide duplex (1 μM), $NaClO_4$ (150 mM), 4-morpholinepropanesulfonate (MOPS; 5 mM; pH 6.8).

Several interesting conclusions can be drawn from the data shown in Table 3. Firstly, the most stabilizing combination is that of **Imi** and **TriPy**. Secondly, the well-known **Imi**-Ag-**Imi** base pair is also significantly stabilized. Thirdly, not all combinations of nucleosides lead to stabilizing metal-mediated base pairs. Finally, not all ΔT_m values are arranged symmetrically in the Table. Estimating a standard deviation of T_m of 1 °C, the stabilization of two base pairs depends on the positioning of the nucleosides in the purine or pyrimidine strand: the duplex comprising **TriPyr**-Ag-**TriPy** ($\Delta T_m = 7.4$ °C) is stabilized to a larger extent than that with **TriPy**-Ag-**TriPyr** ($\Delta T_m = 4.1$ °C). Similarly, the stabilization observed for **Imi**-Ag-**TriTri** ($\Delta T_m = 6.4$ °C) is much larger than that for **TriTri**-Ag-**Imi** ($\Delta T_m = 2.2$ °C). These findings will be discussed individually in the following.

Observation 1

A more detailed investigation of the Ag^I-mediated base pair formed from **Imi** and **TriPy** (Scheme 6) indicates that it comprises a kinetically inert metal complex. This kinetic effect may be a significant contributor to the observed large thermal stabilization. As can be seen from the melting curves of duplex **I** with an **Imi**-Ag-**TriPy** base pair, biphasic melting occurs when substoichiometric amounts of Ag^I are present (Figure 2). The biphasic melting is a result of the kinetically inert **Imi**-Ag-**TriPy** complex. As long as less than one equivalent of Ag^I is present per base pair, two types of double helices exist, namely metal-free ones (with an **Imi**:**TriPy** mispair) and metal-containing ones (with an **Imi**-Ag-**TriPy** base pair). As the exchange of Ag^I is slow, these duplexes do not interconvert during the recording of the melting curves. A similar behavior had previously been reported for metal-mediated salen base pairs.[19] As can be seen from the inset of Figure 2, a significant increase in T_m is observed up to one equivalent of Ag^I only. Excess Ag^I leads to very little additional stabilization. This nicely confirms the structure of the base pair proposed in Scheme 6. The additional stabilization conferred by excess Ag^I may be explained by non-specific binding events between the positively charged metal ion and the polyanion DNA.[16b]



Scheme 6. Proposed structure of the Ag^I-mediated base pair from **Imi** and **TriPy**.

Observation 2

As **Imi**-Ag-**Imi** base pairs have been found to be stabilizing in a series of different oligonucleotide sequences,[8a,18b] it is not surprising to see the formation of stable base pairs of this type also for the sequence investigated here. Nonetheless, the stabilization observed here ($\Delta T_m = 9.5$ °C) is the largest one found as yet for **Imi**-Ag-**Imi** base pairs.

Observation 3

Imidazole and pyridine are more basic ligands than 1,2,4-triazole and pyrazole.[20] Accordingly, it is not surprising that **Imi** and **TriPy** are involved more often in the formation of strongly stabilizing metal-mediated base pairs than **TriTri** and **TriPyr**. Moreover, it appears that non-planar metal-mediated base pairs obtained by combining two "click" nucleosides compris-

ing a methylene linker do not have a significant stabilizing effect, as they cannot interact efficiently with neighboring base pairs through π-stacking. Similarly, the combination of two **TriPy** nucleosides is expected to lead to a tetrahedral Ag^I complex, in analogy to a previously reported structure of a related model nucleobase.[17] Hence, no significant increase in T_m is observed upon the addition of Ag^I to duplex **I** with **X**=**Y**=**TriPy**.

Observation 4

Duplex **I** with **Imi**-Ag-**TriTri** is stabilized to a larger extent than duplex **I** with **TriTri**-Ag-**Imi** (Table 3). Figure 3 confirms this by showing the dependence of T_m on the number of added

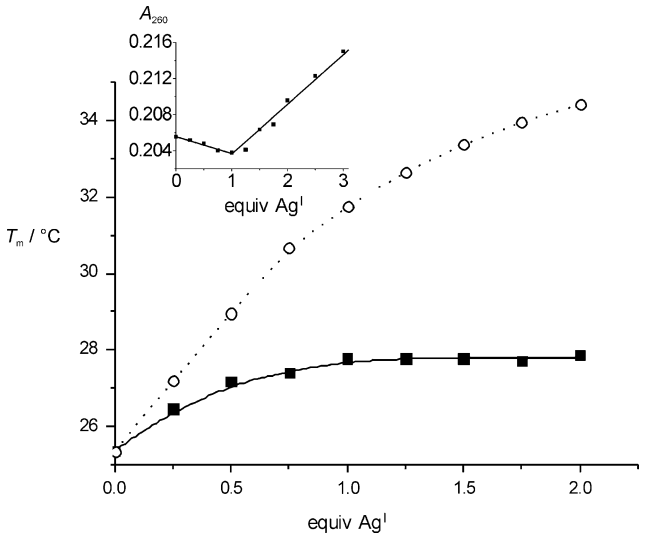


Figure 3. Melting temperature T_m of duplex **I** with **Imi**-Ag-**TriTri** (○) or **TriTri**-Ag-**Imi** (■), depending on the number of added equivalents of Ag^I. Inset: Change of the UV absorbance at 260 nm upon the addition of Ag^I to duplex **I** with **X**=**TriTri** and **Y**=**Imi**.

equivalents of Ag^I for these two duplexes. The plot suggests that in these base pairs the Ag^I ion is bonded in a labile fashion, as T_m increases rather smoothly. Nonetheless, the 1:1 binding stoichiometry can clearly be discerned from the inset of Figure 3, showing the change of the UV absorbance upon the addition of Ag^I. A possible explanation for the differential stabilization could be an additional coordination of the metal ion by a donor atom above or below the base pair. In asymmetrical [2+1] base pairs, the metal ion is not necessarily located directly in the center of the duplex, hence different additional donor atoms might be present depending on the orientation of the base pair. A similar behavior had been reported for other metal-mediated base pairs before.[12c,21] Unfortunately, no structural information exists on the systems studied here, so no final conclusion can be drawn at this stage.

Comparison of duplexes I and II

As mentioned before, duplexes **I** and **II** have the same overall composition and differ by the identity of the canonical base pairs flanking the metal-mediated base pair. More precisely, two guanine residues are located next to nucleoside **X** in duplex **I** whereas **X** is surrounded by adenine residues in duplex **II**. This difference has important effects on the thermal stability of the duplexes. Table 4 lists the melting temperatures

Table 4. Melting temperature T_m [°C] and increase of T_m (ΔT_m, °C) of duplexes **I** and **II** (**Y**=**Imi**) upon the addition of one equivalent of Ag^I.[a]

X	T_m (0 equiv Ag^I)		T_m (1 equiv Ag^I)		ΔT_m	
	duplex **I**	duplex **II**	duplex **I**	duplex **II**	duplex **I**	duplex **II**
Imi	29.8	25.4	39.3	39.1	9.5	13.7
TriTri	25.4	21.7	27.6	27.7	2.2	6.0
TriPyr	25.6	23.4	26.7	27.4	1.1	4.0
TriPy	33.6	30.4	46.2	43.7	12.6	13.4

[a] Conditions: oligonucleotide duplex (1 μM), $NaClO_4$ (150 mM), MOPS (5 mM; pH 6.8). The estimated standard deviation of T_m amounts to 1 °C.

T_m as well as ΔT_m for both duplexes. In all cases, **Imi** acts as artificial nucleoside **Y** in the pyrimidine strand.

Judging by the increase in T_m upon the addition of Ag^I (shown in the two columns on the right), it appears that the degree of thermal stabilization depends on the oligonucleotide sequence. In all cases, duplex **II** is stabilized to a larger extent than duplex **I**. However, a close inspection of the underlying melting temperatures shows that both duplexes have identical melting temperatures in the presence of Ag^I for **X**=**Imi**, **TriTri**, and **TriPyr** (within standard deviation). Hence, the differences in ΔT_m must arise from different stabilities of the metal-free **X**:**Y** mispairs. In the absence of transition-metal ions, duplex **I** is consistently more stable than duplex **II**. This is likely to be the result of better π-stacking capabilities of the flanking guanine residues in duplex **I** compared with the flanking adenine residues in duplex **II**.[22] As long as no metal ion is coordinated, the nucleobases **TriTri** and **TriPyr** can adopt a planar conformation and can therefore participate in π-stacking. Comprising five-membered azole moieties only, their general π-stacking capability is low, as is that of **Imi**. The fact that the T_m value is identical for both duplexes if **X**=**Imi**, **TriTri**, or **TriPyr** in the presence of Ag^I indicates that non-planar metal-mediated base pairs are formed, so that no additional π-stacking interactions are possible. Hence, for duplex **I** the formation of a metal-mediated base pair is accompanied by a decrease in stability due to the loss of π-stacking interactions with guanine and a concomitant increase in stability due to the formation of coordinative bonds. In contrast, only the stabilization resulting from the coordinative bonds is relevant for duplex **II**, because comparatively weak π-stacking interactions with adenine residues are lost.

In this context it is interesting to discuss also the base pairs involving **TriPy**. This artificial nucleobase is fully planar, as are its [2+1] metal complexes. Moreover, being a six-membered

aromatic heterocycle, the pyridine moiety is a good π-stacking entity. Hence, no differential stabilization as discussed above for **Imi**, **TriTri**, and **TriPyr** should be expected as both the metal-free and the metal-containing base pairs are planar. Indeed, the duplexes comprising **TriPy** have the largest melting temperature of all duplexes listed in Table 4. Furthermore, both duplexes are stabilized by $\approx 13\,^{\circ}C$ upon the addition of Ag^{I}. Hence, for a planar base pair such as **TriPy**-Ag-**Imi** the stabilizing effect of the formation of coordinative bonds is relevant only.

Conclusion

To increase the scope of metal-mediated base pairing, a new family of 1,2,3-triazole-based "click" nucleosides was developed. The use of 2-deoxy-β-D-glycosyl azide as a common precursor allows the modular synthesis of a family of 1,2,3-triazole nucleosides through Cu^{I}-catalyzed Huisgen 1,3-dipolar cycloaddition. Three of these nucleosides (**TriTri**, **TriPyr**, **TriPy**) were tested with respect to their applicability in metal-mediated base pairs. Several conclusions can be drawn that are important for the future development of new metal-mediated base pairs and for the characterization of metal-mediated base pairs in general.

1) The introduction of "click" chemistry into the generation of metal-mediated base pairs enables a fast and easy synthesis of a variety of closely related artificial nucleosides. Hence, a series of ligands can be created with a gradually changing metal-binding behavior. Such a series of nucleosides may be useful for the development of tailored metal ion sensors based on nucleic acids with metal-mediated base pairs. So far, nucleobases containing nitrogen donor atoms only have been synthesized with the "click" approach. The introduction of another donor atom such as oxygen is a goal to be established next.
2) The formation of asymmetric metal-mediated base pairs may result in a significantly different stabilization of the double helix, depending on whether **X**-M-**Y** or **Y**-M-**X** is formed. Structural information will be necessary to elucidate whether this effect can be explained by the presence of additional axial ligands.
3) Metal-mediated base pairs do not necessarily have to be fully planar to be incorporated into a double helix. If the energy gained from forming the coordinative bonds is sufficiently large to compensate the loss of π-stacking interactions, slightly tilted metal-mediated base pairs are acceptable, too.
4) It is important to choose properly the oligonucleotide sequence when investigating metal-mediated base pairs. Particularly in those cases in which a metal-mediated base pair is not necessarily planar, the stabilization of the DNA duplex may depend significantly on the stability of the metal-free mispair. Taking this idea to the extreme, a relatively stable mispair in combination with an only slightly stabilizing metal-mediated base pair may lead to the incorrect conclusion that no metal-mediated base pair is formed at all.

Experimental Section

DNA syntheses were performed in the DMT-off mode on a K&A Laborgeräte H8 DNA/RNA synthesizer by following standard protocols.[16b] The oligonucleotides were identified by MALDI-TOF mass spectrometry (see the Supporting Information). MALDI-TOF mass spectra were recorded on a Bruker Reflex IV instrument using a 3-hydroxypicolinic acid/ammonium citrate matrix and applying a commercially available oligonucleotide with a molecular mass of 4577 Da as internal reference. NMR spectra were recorded using Bruker Avance(I) 400 and Bruker Avance(III) 400 spectrometers at 300 K. Chemical shifts were referenced to residual CD_3OH (CD_3OD, $\delta = 4.78$ ppm), TSP (D_2O, $\delta = 0$ ppm), TMS ($CDCl_3$, $\delta = 0$ ppm), or H_3PO_4 (^{31}P NMR, $\delta = 0$ ppm). UV/Vis spectra were recorded on a Varian CARY BIO 100 spectrophotometer. Temperature-dependent UV spectra were recorded between 10 and 70 °C with a heating/cooling rate of $1\,^{\circ}C\,min^{-1}$ and a data interval of 0.5 °C. Absorbance was normalized according to $A_{norm} = (A - A_{min})/(A_{max} - A_{min})$ at 260 nm. Melting temperatures have been determined as the maximum of the derivative of the annealing curves. 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl azide, orthogonally protected imidazole nucleoside, 1-propargyl-1*H*-pyrazol, 1-propargyl-1*H*-1,2,4-triazole, and **BnTriPyr** were prepared according to literature procedures, respectively.[18a, 23]

In the following, only the general syntheses are reported. The spectral data of the resulting compounds are listed in the Supporting Information.

Syntheses

Toluoyl-protected nucleosides: *(Scheme 3, reaction a)*: To a mixture of alkyne (1.2 equiv) and 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentafuranosyl azide (1 equiv) in THF/isopropanol (40 mL, 4/1), a solution of $CuSO_4 \cdot 5H_2O$ (0.2 equiv) in H_2O (8 mL) and sodium ascorbate (0.4 equiv) were added. After stirring for 12 h at room temperature the reaction was quenched by addition of EtOAc (150 mL). The organic layer was washed with aqueous ethylenediaminetetraacetate (EDTA) solution (0.5 %) until the aqueous layer was colorless. The organic phase was dried ($MgSO_4$). After filtration the solvent was removed. The crude product was purified by column chromatography.

Free nucleosides: *(Scheme 3, reaction b)*: The toluoyl-protected nucleoside was dissolved in MeOH (100 mL), and aqueous NH_3 (25 %, 50 mL) was added. After stirring overnight, the solvent was removed and the crude product was purified by column chromatography.

DMT-protected nucleosides: *(Scheme 3, reaction c)*: Free nucleoside (1 equiv) was co-evaporated three times with dry pyridine (20 mL) and then dissolved in dry pyridine (50 mL). To this reaction mixture, catalytic amounts of 4-dimethylaminopyridine (DMAP) were added under an argon atmosphere, followed by the addition of 4,4′-dimethoxytrityl chloride (DMT-Cl; 1.2 equiv). The mixture was stirred for 3 h at room temperature. The solution was diluted with CH_2Cl_2 (100 mL) and washed with water (3 × 20 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness. The crude product was purified with column chromatography.

Orthogonally protected nucleosides: (*Scheme 3, reaction d*): The DMT-protected nucleoside (1 equiv) was dissolved in freshly dis-

tilled CH_2Cl_2 (35 mL). *N*,*N*-Diisopropylethylamine (DIPEA; 3 equiv) and *N*,*N*-diisopropylchlorophosphoramidite (CEDIP-Cl; 2 equiv) were then added to the nucleoside/CH_2Cl_2 solution under an argon atmosphere. After 30 min of stirring at room temperature the mixture was diluted with EtOAc (75 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ solution (30 mL) and dried ($MgSO_4$). The solvent was evaporated to dryness and the crude product was purified by column chromatography.

Metal complexes: A metal salt/metal precursor complex (1 equiv) in MeOH (5 mL) was added to a solution of **BnTriPyr** (2 equiv) in MeOH (5 mL). After stirring for 3 h with heating at reflux the solution was kept at 4 °C for several days, until crystals suitable for single-crystal X-ray diffraction are obtained.

Crystal structure determination

Crystal data were collected at 153(2) K with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) with a STOE StadiVari (Cu complex) and a Bruker D8 Venture (Pd complex) diffractometer. The structures were solved by direct methods and were refined by full-matrix, least-squares on F^2 by using the SHELXTL and SHELXL-97 programs.[24] All non-hydrogen atoms were refined anisotropically, whereas hydrogen atoms were calculated on ideal positions. Relevant crystallographic data are listed in Table 5. CCDC-986937 ([Pd(**BnTriPyr**)$_2$]Cl_2·2 H_2O) and CCDC-986936 ([Cu(**BnTriPyr**)$_2$-(CH_3OH)$_2$](NO_3)$_2$) 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.

Table 5. Crystallographic data for the metal complexes.

	[Pd(**BnTriPyr**)$_2$]Cl_2·2 H_2O	[Cu(**BnTriPyr**)$_2$(CH_3OH)$_2$](NO_3)$_2$
empirical formula	$C_{26}H_{30}Cl_2N_{10}O_2Pd$	$C_{28}H_{32}CuN_{12}O_8$
formula weight	691.90	728.19
crystal system	triclinic	monoclinic
space group	$P\bar{1}$	$P2_1/c$
a, b, c [Å]	7.2416(5), 9.5423(7), 11.1250(8)	10.510(2), 9.572(2), 16.176(3)
α, β, γ [°]	91.448(2), 105.758(2), 101.912(2)	90, 100.17(3), 90
V [Å^3]	721.23(9)	1601.9(6)
Z	1	2
ρ_{calcd} [g cm^{-3}]	1.593	1.510
$\mu(Mo_{K\alpha})$ [mm^{-1}]	0.873	1.510
crystal size [mm]	0.48×0.23×0.16	0.22×0.20×0.07
θ_{min}, θ_{max} [°]	2.78, 30.07	2.5, 39.9
dataset	−10:10, −13:13, −15:15	−13:13, −11:12, −21:16
tot., uniq. data	11582, 4186	14411, 3790
observed data [$I>2\sigma(I)$]	4162	2491
N_{ref}, N_{par}	4186, 195	3790, 224
R, wR_2, S [$I>2\sigma(I)$][a]	0.0212, 0.0566, 1.139	0.0320, 0.0672, 0.805
min. and max. resd. dens. [e Å^{-3}]	−0.77, 0.46	−0.27, 0.54

[a] $R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (MU 1750/2–1).

Keywords: bioinorganic chemistry · click chemistry · cycloaddition · DNA · silver

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Received: February 17, 2014
Published online on May 18, 2014