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Synthesis of nucleosides and dNTPs bearing oligopyridine ligands linked through an octadiyne tether, their incorporation into DNA and complexation with transition metal cations†

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Modified nucleosides (**dA^R**s and **dC^R**s) bearing bipyridine or terpyridine ligands attached through an octadiyne linker were prepared by single-step aqueous-phase Sonogashira cross-coupling of 7-iodo-7-deaza-2′-deoxyadenosine and 5-iodo-2′-deoxycytidine with the corresponding bipyridine- or terpyridine-octadiynes and were triphosphorylated to the corresponding nucleoside triphosphates (**dA^{R}TP**s and **dC^{R}TP**s). The modified **dN^{R}TP**s were successfully incorporated into the oligonucleotides by primer extension experiment (PEX) using different DNA polymerases and the PEX products were used for post-synthetic complexation with divalent metal cations. The complexation of these DNAs containing flexibly-tethered ligands was compared with the previously reported ones bearing rigid acetylene-linked ligands suggesting the possible formation of both inter- and intra-strand complexes with Ni^{2+} or Fe^{2+}.

Introduction

DNA is a suitable template for construction of nanomolecular components, which assemble in a deliberately designed manner,[1] due to its nanometric dimension, high stability, selective recognition of the complementary strand through the Watson–Crick base-pairing and facile synthesis. Incorporation of transition metals into these biomacromolecules allows DNA to form many other spatial arrangements[2,3] due to the geometrical preference of metal ions in coordinating with different ligands. Moreover, incorporation of transition metals into DNA can lead to photoactive,[4] electroactive[5] or even catalytically active[6] species and can significantly influence the stability.[7] Transition metals can be placed within (metal–base-pairs[8] or intercalators[9]) or outside (metal complex covalently attached to a nucleobase, sugar or phosphate)[10] the DNA duplex. Considering metal-induced assembly of conjugates of nucleic acids and metal-chelating moieties, it is important to point out that most of the studies have been carried out with terminally modified oligonucleotides *via* long and flexible linkers. Recently, we,[11] Wagenknecht[12] and Stulz[13] independently published enzymatic or chemical synthesis of oligonucleotides bearing terpyridine (tpy) ligands linked through a short and rigid acetylene tether and their post-synthetic side by side complexation with M^{2+} ions in order to prepare intermolecular DNA-complexes. However, presumably due to the rigidity of the linker and rather short distance from the DNA duplex, the complexation properties of these DNA constructs were rather limited. Using a longer and more flexible linker might enhance the complexation ability and even allow formation of cyclic metal–DNA chelates, which were reported very rarely.[14] Therefore, we report here on the enzymatic synthesis of oligonucleotides bearing oligopyridine ligands attached through a long and flexible 1,7-octadiyne linker, which is often used[15] for click-conjugations, and their post-synthetic complexation with diverse transition metals.

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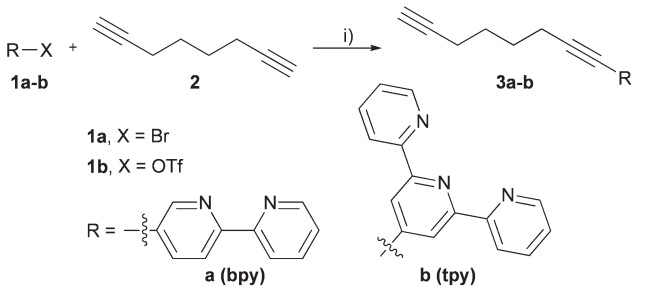
Results and discussion

Synthesis of ligand building blocks

In order to prepare functionalized nucleosides bearing oligopyridine ligands attached *via* a long and flexible linker, suitable ligand–octadiyne building blocks had to be synthesized first. The Sonogashira cross-coupling reactions of an activated oligopyridine **1a–b**[16] with 3 equiv. of 1,7-octadiyne (**2**) in the



Scheme 1 Synthesis of ligand building blocks. *Reagents and conditions*: (i) $Pd(PPh_3)_2Cl_2$ (5 mol%), CuI (5 mol%), Et_3N (10 equiv.), THF, 3 h, yields: 66% (**3a**); 75% (**3b**).

presence of $Pd(PPh_3)_2Cl_2$, CuI and Et_3N (Scheme 1) were performed at 70 °C for 3 h. Desired products were obtained in good yields of 66% for **3a** or 75% for **3b**.

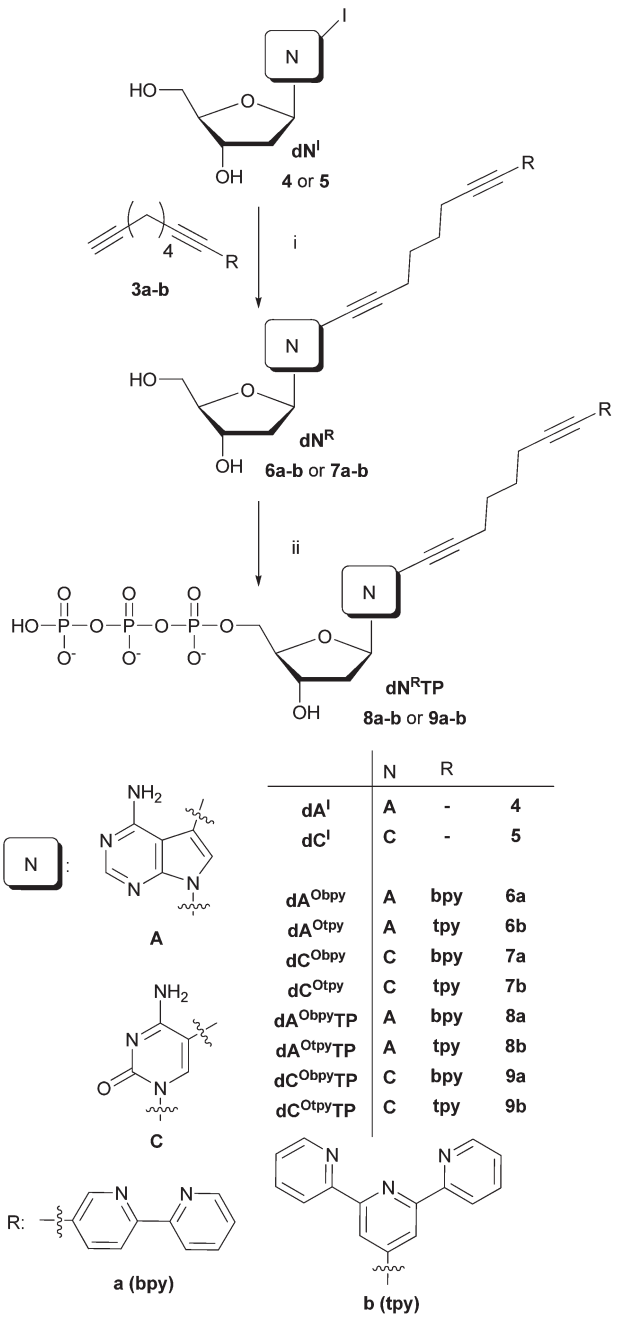
Synthesis of modified nucleosides and dNTPs

The Sonogashira cross-coupling reaction was also successfully used for the attachment of oligopyridine–octadiyne building blocks to position 5 of 2′-deoxycytidine and to position 7 of 7-deaza-2′-deoxyadenosine. The Pd-catalyzed Cu-mediated cross-coupling reactions of 5-iodo-2′-deoxycytidine (**dC^{I}**, **5**) or 7-iodo-7-deaza-2′-deoxyadenosine (**dA^{I}**, **4**) with **3a** or **3b** were performed in the presence of 5 mol% $Pd(OAc)_2$, water soluble tris(3-sulfonatophenyl)phosphine (TPPTS), CuI and Hünnig's base in DMF at 75 °C for 2 h to reach full conversion (Scheme 2, Table 1) in analogy to previously developed procedures.[17] Products were purified by flash chromatography on reverse phase and after the crystallization were isolated as brownish solids in acceptable yields (taking into account that non-polar building blocks were attached to polar nucleoside in a single-step reaction without any use of protecting groups) (Table 1).

A previously developed procedure of direct functionalization of halogenated dNTPs by aqueous-phase Sonogashira cross-coupling[11,18] could not be used due to low solubility of ligand building blocks in the mixture water–acetonitrile (2 : 1) which resulted in very poor yields even after extensive optimizations. Therefore, the desired modified triphosphates **dN^{R}TPs** (**8a–b** and **9a–b**) were prepared by classical triphosphorylation[19] of modified nucleosides (Scheme 2). The solution of **dN^{R}** (**6a–b** and **7a–b**) in $PO(OMe)_3$ was treated with $POCl_3$ at 0 °C for 1 h, followed by an addition of a pre-formed mixture of tributylammonium pyrophosphate in DMF with an addition of Bu_3N. The reaction mixture was stirred at 0 °C for another 1.5 h and quenched by an addition of 2 M TEAB. Desired products were isolated in the yields shown in Table 2. The rather moderate yields are the consequence of difficult isolation of amphiphilic compounds **8a–b** and **9a–b**, involving 2 step purification using DEAE Sephadex and semi-preparative RP HPLC.

Incorporation of dN^{R}TPs by DNA polymerase

The enzymatic incorporation of all four functionalized **dA^{R}TPs** (**8a–b**) and **dC^{R}TPs** (**9a–b**) was tested by using thermostable



	N	R	
dA^{I}	A	-	4
dC^{I}	C	-	5
dA^{Obpy}	A	bpy	6a
dA^{Otpy}	A	tpy	6b
dC^{Obpy}	C	bpy	7a
dC^{Otpy}	C	tpy	7b
dA^{Obpy}TP	A	bpy	8a
dA^{Otpy}TP	A	tpy	8b
dC^{Obpy}TP	C	bpy	9a
dC^{Otpy}TP	C	tpy	9b

Scheme 2 Reagents and conditions: (i) $Pd(OAc)_2$ (5 mol%), TPPTS (2.5 equiv. to Pd), CuI (10 mol%), $Et(i\text{-}Pr)_2N$ (10 equiv.), DMF, 75 °C, 2 h; (ii) 1. $PO(OMe)_3$, $POCl_3$ (1.3 equiv.), 0 °C, 1 h; 2. $(NHBu_3)_2H_2P_2O_7$ (5 equiv.), Bu_3N (4.5 equiv), DMF, 0 °C, 1.5 h; 3. 2 M TEAB.

Table 1 The Sonogashira cross-coupling reaction of nucleosides **dC^{I}** (**5**) and **dA^{I}** (**4**)

Entry	Nucleoside	Oligopyridine	Product	Yield
1	**dA^{I}** (**4**)	**3a**	**dA^{Obpy}** (**6a**)	46%
2	**dC^{I}** (**5**)	**3a**	**dC^{Obpy}** (**7a**)	38%
3	**dA^{I}** (**4**)	**3b**	**dA^{Otpy}** (**6b**)	45%
4	**dC^{I}** (**5**)	**3b**	**dC^{Otpy}** (**7b**)	72%

Table 2 Synthesis of modified **dN^{R}TP**s by triphosphorylation of **dN^{R}** (**6a–b** and **7a–b**)

Entry	Nucleoside	dNTPs	Yield
1	**dA^{Obpy}** (**6a**)	**dA^{Obpy}TP** (**8a**)	35%
2	**dA^{Otpy}** (**6b**)	**dA^{Otpy}TP** (**8b**)	14%
3	**dC^{Obpy}** (**7a**)	**dC^{Obpy}TP** (**9a**)	39%
4	**dC^{Otpy}** (**7b**)	**dC^{Otpy}TP** (**9b**)	31%

Table 3 Oligo-2′-deoxyribonucleotides used or synthesized in this study[a]

prim^{rnd}	5′-CATGGGCGGCATGGG-3′
prim^{comp}	5′-CATGGGCGGCATCTC-3′
temp^{rnd16}	5′-**CTAGCATGAGCTCAGT***CCCATGCCGCCCATG*-3′
temp^{comp3gA}	5′-**CAGACCAGCCCTCCC***GAGATGCCGCCCATG*-3′
temp^{A}	5′-**CCCT***CCCATGCCGCCCATG*-3′
temp^{C}	5′-**CCCG***CCCATGCCGCCCATG*-3′
temp^{A1}	5′-**T***CCCATGCCGCCCATG*-3′
temp^{C1}	5′-**G***CCCATGCCGCCCATG*-3′
temp^{compA1}	5′-**T***GAGATGCCGCCCATG*-3′
DNA1	5′-CCCTCCCATGCCGCCCATG-3′
	3′-GGGAGGGTACGGCGGGTAC-5′
DNA2	5′-CCCGCCCATGCCGCCCATG-3′
	3′-GGGCGGGTACGGCGGGTAC-5′
DNA3	5′-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′
	3′-GATCGTACTCGAGTCAGGGTACGGCGGGTAC-5′
DNA4	5′-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′
	3′-GATCGTACTCGAGTCAGGGTACGGCGGGTAC-5′

[a] In the template (temp) ON segments that form a duplex with the primer are printed in italics, the replicated segments are printed in bold. In synthesized DNAs (**DNA1-4**), the underlined letters indicate modifications. For magnetic separation of the extended primer strands, the templates were 5′-end biotinylated. The acronyms used in the text for primer products are analogues to those introduced for templates (*e.g.* the PEX product pex^{rnd16} was synthesized on the temp^{rnd16} template).

DNA polymerases in primer extension experiment (PEX). Similarly to our previous work,[11,18] each PEX experiment, analyzed by denaturing polyacrylamide gel electrophoresis (PAGE), was compared with positive (all four natural dNTPs) and negative control experiments (absence of one natural dNTP) in order to exclude any miss-incorporation. Single and multiple incorporations of oligopyridine-functionalized triphosphates were tested (for sequences of primer and templates see Table 3).

Single nucleotide extension experiments were tested separately with all modified **dN^{R}TP**s (**8a–b** and **9a–b**) by using 19-mer templates temp^{A} and temp^{C} and four different DNA polymerases: Pwo, DyNAzyme II, KOD XL and Deep Vent. While experiments using Pwo polymerase (Fig. 1) were successful to give fully extended products for all of the dNTPs and the lack of extension in negative control (A- or C-) proving that no miss-incorporation occurred, experiments using other DNA polymerases (see ESI†) lead to the mixture of products with different lengths or to some miss-incorporations and therefore could not be used for direct functionalization of DNA.

To compare the efficiency of incorporation in the natural and oligopyridine-modified dNTPs, we performed a simple kinetic study in single-nucleotide PEX-experiment. The experiments were performed using Pwo polymerase and a temp^{A1} template for experiments with natural and modified dATPs,

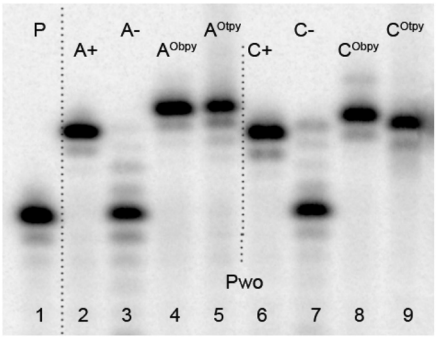


Fig. 1 Denaturing PAGE analysis of PEX experiment synthesized on temp^{A} (lanes 2–5) and temp^{C} (lanes 6–9) with Pwo polymerases. 5′-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: primer; A+: natural dATP, dGTP; A–: dGTP; A^{Obpy}: **dA^{Obpy}TP** (**8a**), dGTP; A^{Otpy}: **dA^{Otpy}TP** (**8b**), dGTP; C+: natural dCTP, dGTP; C–: dGTP; C^{Obpy}: **dC^{Obpy}TP** (**9a**), dGTP; C^{Otpy}: **dC^{Otpy}TP** (**9b**), dGTP.

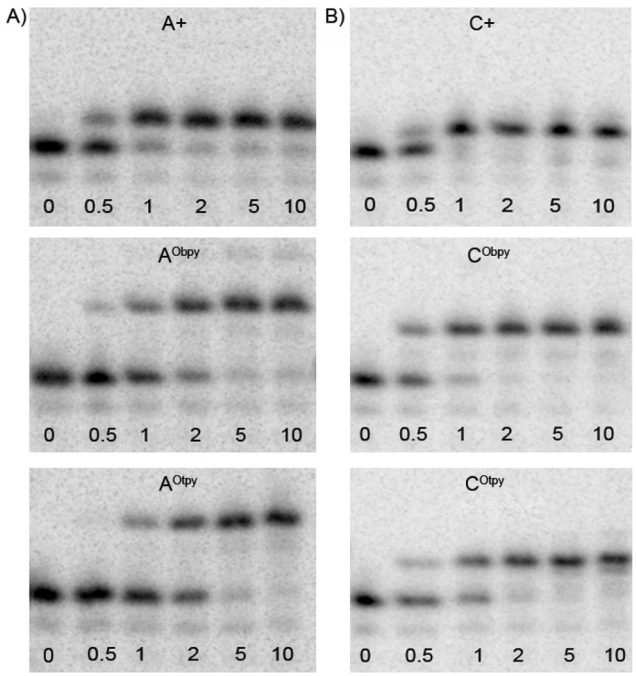


Fig. 2 Comparison of the rate of the single-nucleotide PEX using Pwo polymerase: (A) with natural A+ (dATP) and modified **dA^{R}TP**s (**dA^{Obpy}TP**, **8a** and **dA^{Otpy}TP**, **8b**) nucleotides using temp^{A1}; (B) with natural C+ (dCTP) and modified **dC^{R}TP**s (**dC^{Obpy}TP**, **9a** and **dC^{Otpy}TP**, **9b**) nucleotides using temp^{C1}. The reaction mixtures were incubated for time intervals indicated (in min), followed by stopping the reaction by addition of PAGE loading buffer and immediate heating.

whereas the temp^{C1} template was used for experiments with natural and modified dCTPs (Fig. 2). The PEX with natural dNTPs was finished within 1 min, whereas the PEX with **dC^{R}TP** (**9a–b**) or **dA^{R}TP** (**8a–b**) took 2 or 5 min, respectively. Due to the slower incorporations of modified **dN^{R}TP**s (**8a–b** and **9a–b**) in comparison to the natural dNTPs, the reaction time for multiple incorporations must be prolonged to 30 min to ensure full length product formation.

Multiple incorporations were tested on 31-mer template temp^{rnd16} requiring incorporation of four modified dNTPs in

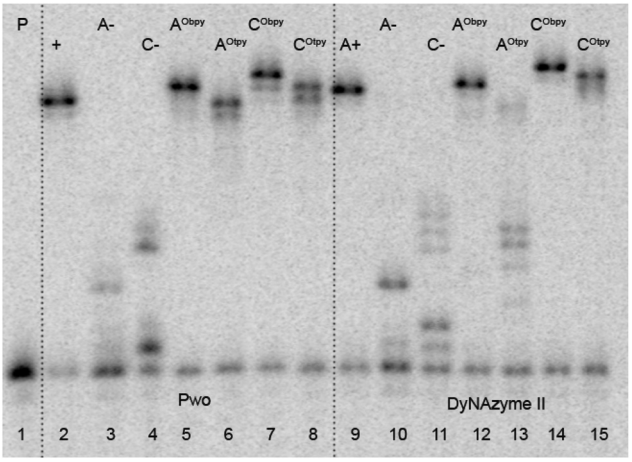


Fig. 3 Denaturing PAGE analysis of PEX experiment synthesized on temp^{rnd16} with Pwo (lanes 2–8) and DyNAzyme II (lanes 9–15) polymerases. 5′-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: P: primer; A+: unmodified DNA (dATP, dTTP, dCTP, dGTP); A–: unmodified DNA (dTTP, dCTP, dGTP); C–: unmodified DNA (dATP, dTTP, dGTP); A^{Obpy}: **dA^{Obpy}TP** (**8a**), dTTP, dCTP, dGTP; A^{Otpy}: **dA^{Otpy}TP** (**8b**), dTTP, dCTP, dGTP; C^{Obpy}: dATP, dTTP, **dC^{Obpy}TP** (**9a**), dGTP; C^{Otpy}: dATP, dTTP, **dC^{Otpy}TP** (**9b**), dGTP.

separate positions. Modified **dA^{R}TPs** (**8a–b**) were successfully incorporated into the ONs by Pwo, KOD XL as well as Deep Vent polymerases. Using these polymerases gave fully extended ONs (for using Pwo polymerase see Fig. 3, lanes 5–6), while incorporation of **dA^{tpy}TP** (**8b**) using DyNAzyme II polymerase was less feasible and resulted in early termination of PEX (lane 13). For incorporation of modified **dC^{R}TP** (**9a–b**) only DyNAzyme II polymerase was suitable (lanes 14 and 15). Experiments using other polymerases lead to the mixture of ONs with different lengths (for incorporation using KOD XL or Deep Vent see ESI†).

The products of PEX slightly differ in electrophoretic mobilities visible on gel, due to the higher molecular weight of modified ONs in combination with possible formation of different secondary structures. Therefore, the successful incorporations and full-length product formations were verified by measurement of MALDI mass spectra of PEX products. Single stranded DNA was prepared by PEX with biotinylated templates and then isolated by magnetoseparation.[18b] The correct masses were confirmed for all products (see ESI†).

Complexation studies

All oligopyridine-modified nucleosides **dN^{R}** (**6a–b** and **7a–b**) were tested as model compounds for further complexation studies proceeded on modified oligonucleotides. Methanolic solutions of **dN^{R}** (**6a–b** and **7a–b**) were mixed with 0.5 equiv. of divalent metal ions, *i.e.* Cu^{2+}, Ni^{2+}, Zn^{2+} and Fe^{2+}. After incubation for 10 min at room temperature, the complex-formation was detected by UV/Vis spectroscopy. The spectra were recorded for non-metalated as well as for metalated nucleosides. Due to the fact, that an oligopyridine unit is not conjugated with the nucleobase (in contrast to the previous study

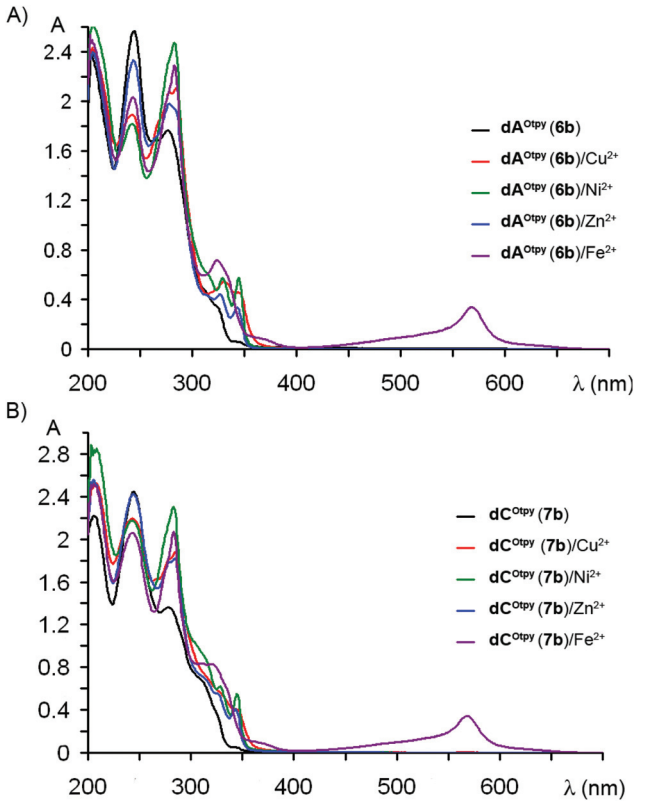


Fig. 4 UV/Vis spectra of oligopyridine-modified nucleosides: (A) **dA^{Otpy}** (**6b**), (B) **dC^{Otpy}** (**7b**) with metal cations.

when the oligopyridine unit was attached to the nucleobase *via* an acetylene linker[11]), the dominating absorbance is shifted to lower wavelengths and MLCT bands of all metal complexes of **dN^{Otpy}** (**6b** and **7b**) can be easily detected (Fig. 4). For UV/Vis spectra of **dN^{Obpy}** (**6a** and **7a**) with metal cations see ESI.†

After successful complexation of nucleosides, we proceeded to complexation of Otpy-modified oligonucleotides. For complexation studies with UV/Vis detection, pex^{rnd16} products were prepared in bigger scale by using Deep Vent (for incorporation of **dA^{Otpy}TP 8b**, synthesis of **DNA3**) or DyNAzyme II polymerases (for incorporation of **dC^{Otpy}TP 9b**, synthesis of **DNA4**). Oligonucleotides were well purified from unreacted **dN^{Otpy}TPs** (**8b** or **9b**) before complexation. After addition of 0.5 equiv. of $M(BF_4)_2 \cdot nH_2O$ per each modification to the aqueous solution of modified oligonucleotide, the mixture was incubated overnight at room temperature. The UV/Vis spectra were recorded for non-metalated and metalated DNA duplexes, either natural or modified ones (Fig. 5). The dominant absorbance at *ca.* 260 nm originated from the absorbance of natural nucleotides (grey line) while the small absorbance band at *ca.* 350 nm is due to the presence of Otpy-modification (black line). While MLCT bands of complexes formed by Otpy-modified ON with Cu^{2+} (red line), Ni^{2+} (green line), Zn^{2+} (blue line) are partially overlapped with the absorbance band of Otpy-modification, the complex formed by mixing Otpy-modified ON with Fe^{2+} can be easily detected due to the characteristic absorbance at

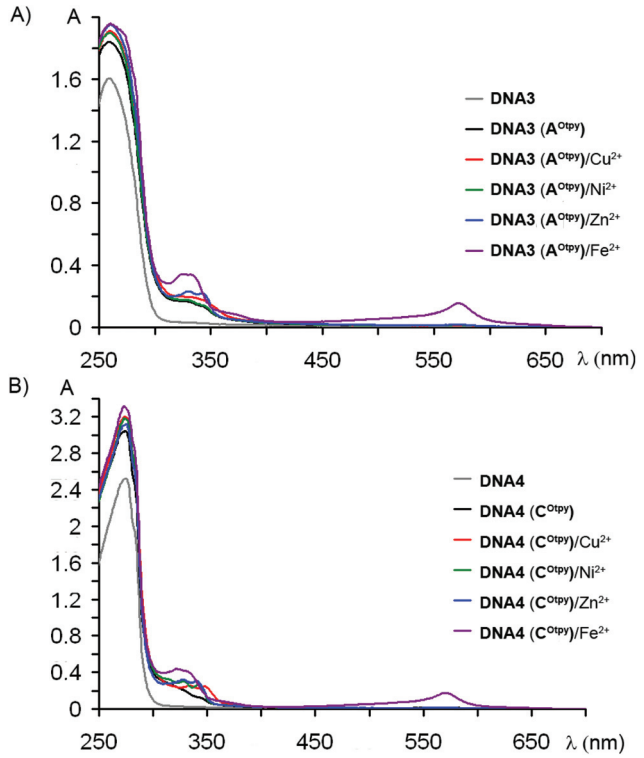


Fig. 5 UV/Vis spectra of oligopyridine-modified DNA: (A) **DNA3** (**A^{Otpy}**), (B) **DNA4** (**C^{Otpy}**) with divalent metals.

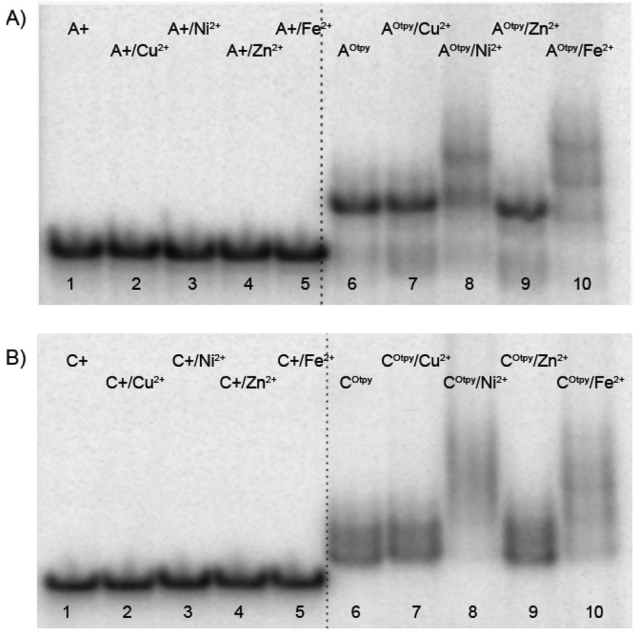


Fig. 6 Non-denaturing gel electrophoresis (8% SB_PAGE) of DNA duplexes in the absence and in the presence of M^{2+} for pex^{rnd16}. 5′-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA (dATP, dTTP, dCTP, dGTP); A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Otpy}: Otpy-modified DNA (**dA^{Otpy}TP 8b**, dTTP, dCTP, dGTP); A^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals; C+: unmodified DNA (dATP, dTTP, dCTP, dGTP); C+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; C^{Otpy}: Otpy-modified DNA (dATP, dTTP, **dC^{Otpy}TP 9b**, dGTP); C^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals.

580 nm[20] (magenta line). Similar MLCT bands were not observed for a natural DNA duplex mixed with the corresponding metals (see ESI†).

Complex formation was also detected by native polyacrylamide gel electrophoresis. The products of monoincorporations were prepared by PEX experiment using temp^{A} and dATP (natural DNA) or **dA^{Otpy}TP**, **8b** (modified DNA, **DNA1**), or temp^{C} and dCTP (natural DNA) or **dC^{Otpy}TP**, **9b** (modified DNA, **DNA2**). PEX products were directly, without previous purification, mixed with 1 equiv. of $M(BF_4)_2{\cdot}nH_2O$ (calculated to the amount of modified **dN^{Otpy}TP** in PEX experiment) and incubated at room temperature overnight. Despite longer time of incubation, compared to the time required for successful complexation of acetylene linked derivatives,[11] none (for A^{Otpy}) or only minor band (for C^{Otpy}) with slower mobility proving a successful complex formation was observed (see Fig. S9 in ESI†). In the case of pex^{rnd16} containing four Otpy-modifications successful complex formation with Ni^{2+} and Fe^{2+} ions, shown by bands with slower mobility, was observed (Fig. 6, lanes 8 and 10). A similar change in mobility was not observed for non-modified DNA mixed with these metal ions (lanes 3 and 5).

Intra-strand *vs.* inter-strand DNA complex

Since, the quantitative complex formation was observed only for DNAs containing four Otpy-modifications, the question arises whether intra-strand or inter-strand complexes are formed. A short and rigid acetylene linker predetermined modified DNA to form inter-strand complexes (Fig. 7A), whereas the long and flexible octadiyne linker enables also metal-templated intra-strand cyclization of Otpy-modified oligonucleotides (Fig. 7B).

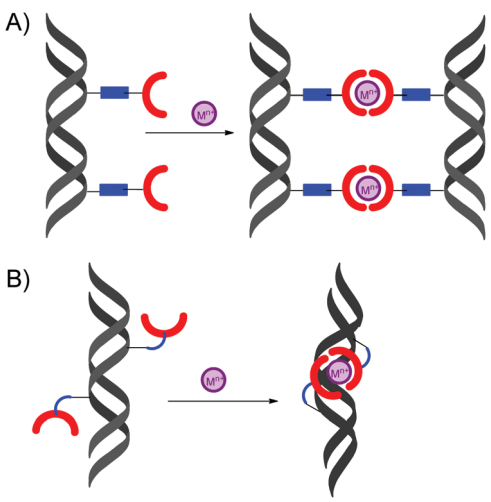


Fig. 7 Schematic representation of: (A) inter-strand, (B) intra-strand DNA complexes.

Therefore, we compared complexations of previously described DNA bearing tpy-modification attached *via* an

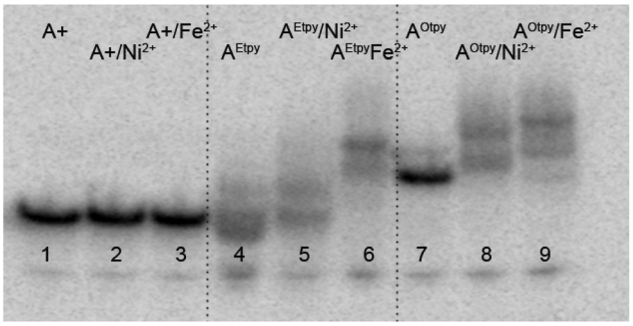


Fig. 8 Non-denaturing gel electrophoresis (8% SB_PAGE) of DNA duplexes in the absence and in the presence of M^{2+} for pex^{rnd16}. 5′-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA (dATP, dTTP, dCTP, dGTP); A+/M^{2+}: unmodified DNA mixed with indicated divalent metals; A^{Etpy}: Etpy-modified DNA (**dA^{Etpy}TP**, dTTP, dCTP, dGTP); A^{Etpy}/M^{2+}: Etpy-modified DNA mixed with indicated metal cations; A^{Otpy}: Otpy-modified DNA (**dA^{Otpy}TP 8b**, dTTP, dCTP, dGTP); A^{Otpy}/M^{2+}: Otpy-modified DNA mixed with indicated metal cations.

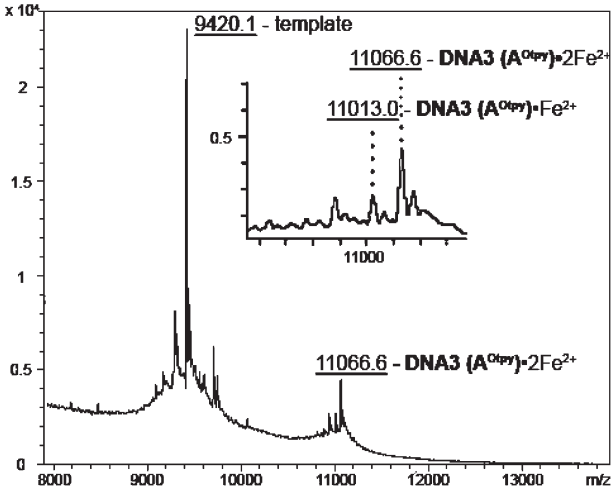


Fig. 9 MALDI-TOF spectra of **DNA3 (A^{Otpy})** mixed with Fe^{2+} (calculated mass: 11 010.7 Da for **DNA3 (A^{Otpy})·1Fe^{2+}** and 11 060.9 Da for **DNA3 (A^{Otpy})·2Fe^{2+}**).

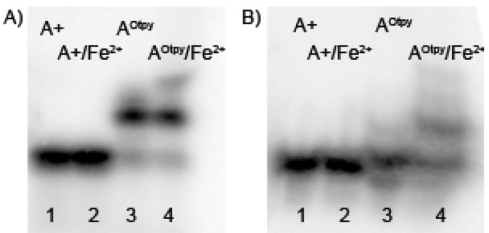


Fig. 10 Non-denaturing gel electrophoresis (8% SB_PAGE) of DNA duplexes in the absence and in the presence of M^{2+} for temp^{compA1}(A) and temp^{comp3gA} (B). 5′-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA (dATP, dTTP, dCTP, dGTP); A+/Fe^{2+}: unmodified DNA mixed with Fe^{2+}; A^{Otpy}: Otpy-modified DNA (**dA^{Otpy}TP 8b**, dTTP, dCTP, dGTP); A^{Otpy}/Fe^{2+}: Otpy-modified DNA mixed with Fe^{2+}.

acetylene linker (made from **dN^{Etpy}TP**)[11] with the complexation of DNA modified by an octadiyne linker (made from **dN^{Otpy}TP**). Since the DNAs containing only one **dN^{Otpy}** did not form metal complexes and **dC^{Etpy}TP** was not a good substrate in multiple incorporations,[11] we focused in our investigation on DNAs prepared by PEX experiment using temp^{rnd16} and dATP (natural DNA), **dA^{Etpy}TP** (modified DNA with acetylene linked oligopyridine units) and **dA^{Otpy}TP 8b** (modified DNA with octadiyne linked oligopyridine units).

First we compared the mobility of these complexes on native polyacrylamide gel (Fig. 8). The Fe^{2+} complex of DNA with acetylene linked oligopyridine tpy-units (lane 6) showed significantly slower mobility in comparison to non-metalated Etpy-modified DNA (lane 4) due to inter-duplex complexation. On the other hand, the Ni^{2+} or Fe^{2+} complexes (lanes 8 and 9) formed from Otpy-modified DNA moved only slightly more slowly compared to non-metalated Otpy-modified DNA (lane 7) suggesting possible formation of (less bulky) intra-strand complexes.

Metal-templated intra-strand cyclization of Otpy-modified oligonucleotide was also confirmed by measurement of MALDI spectra, where **DNA3(A^{Otpy})·Fe^{2+}** and **DNA3(A^{Otpy})·2Fe^{2+}** were successfully detected (Fig. 9), while no non-metalated modified DNA was observed.

To further verify the hypothesis that the DNA containing Otpy-modification(s) allows formation of intra-strand complexes, we have prepared other DNA sequences (PEX-experiments using temp^{compA1} and temp^{comp3gA}) bearing just one Otpy and tested their complexation. In these cases, very weak bands of slower mobility were also observed (Fig. 10). These must be due to inter-strand complexation since no intra-strand complexes could be formed with only one tpy-modification. Some other tested sequences are shown in ESI.† We were unable to find any rationale why some sequences did and some others did not show the formation of these inter-strand complexes. However, since the inter-strand complexes of DNAs bearing a single Otpy modification are either not formed or only very weak and the complexations of DNAs containing four Otpy units with Fe^{2+} and Ni^{2+} are quantitative, it seems that the intra-strand complexes were formed (or even preferred) in some sequences.

The CD spectra of natural and tpy-modified **DNA3** (either non-metalated or metalated) prepared by PEX using temp^{rnd16} were measured in order to verify the possible intra-strand complex formation (Fig. 11). For natural, as well as for tpy-modified DNAs, classical B-structure was observed. While the negative minimum for natural (A+) and Etpy-modifed (A^{Etpy}) DNAs was found at 245 nm, the negative minimum for Otpy-modified (A^{Otpy}) DNA was shifted to 250 nm. A positive maximum was found at 270 nm with a shoulder at 290 nm for tpy-modified DNA (blue and magenta line). This shoulder is more significant after addition of Fe^{2+} ions, while for complexes formed from Otpy-modified DNA (A^{Otpy}/Fe^{2+}) (red line) it is even more obvious than for complexes formed from Etpy-modified DNA (A^{Etpy}/Fe^{2+}) (green line). Although the interpretation of CD-spectra of corresponding DNA complexes might be complicated by formation of diverse structures (inter-strand DNA complexes, products of inter- or intra-strand intercalation), small spectral changes in non-metalated tpy-modified DNA can be explained by diminishing the arrangement of the

DNA double helix caused by the presence of the tpy-modification or by intercalation of tpy-units, while the formation of the band at 290 nm in the DNA complex formed from Otpy-modified DNA and Fe^{2+} ions (A^{Otpy}/Fe^{2+}) can be a result of partial distortion of the B-DNA double helix, required (according to the computational studies) for formation of intra-strand DNA complex **DNA(A^{Otpy})·2Fe^{2+}** (Fig. 11).

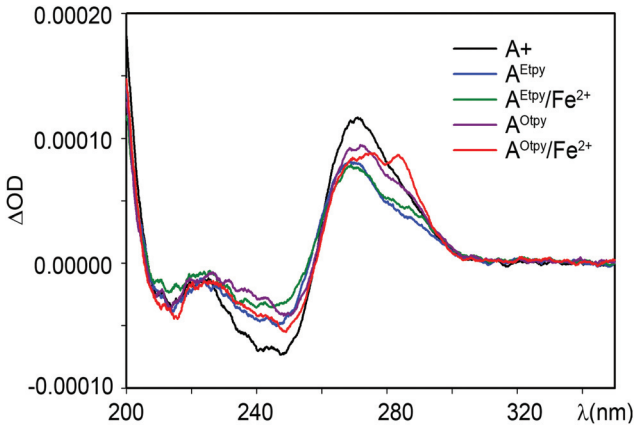


Fig. 11 CD-spectra of natural and tpy-modified DNA duplexes prepared from **DNA3** in the absence or presence of Fe^{2+} ions.

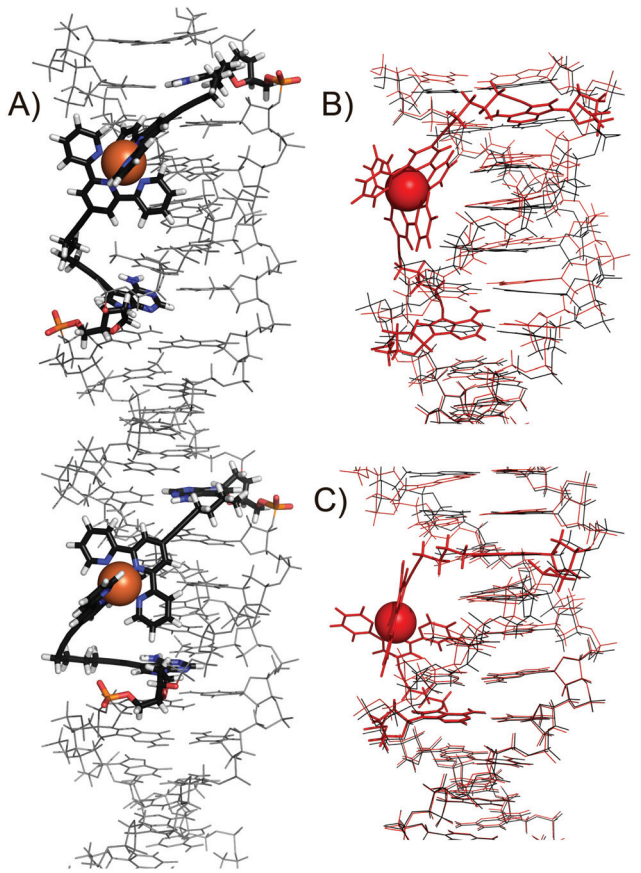


Fig. 12 (A) Detailed view of the QM/MM optimized structure of **DNA3** with 1–2 and 3–4 intra-strand DNA complexes. Comparison of (B) 1–2 and (C) 3–4 complexes (in red) with unmodified DNA (in black).

Further, the possibility of intra-strand complex formation was studied by molecular modelling. In **DNA3** containing four **dA^{Otpy}** units, there are in principle four combinations of intramolecular complexes: 1–2 (separated by 4 base-pairs), 3–4 (separated by 3 bp), 1–3 (separated by 9 bp) and 2–4 (separated by 8 bp). The 1–2 and 3–4 complexes were built and the geometry was successfully minimized by using QM/MM methodology (Fig. 12). The 3–4 complex was by 3.1 kcal mol^{-1} more stable than the 1–2 complex due to better proximity and orientation of the modified nucleotides in the 3–4 complex. The formation of the complex 3–4 also induced smaller geometrical changes in the B-DNA duplex (RMSD 0.269 Å) compared to the 1–2 complex (RMSD 0.662 Å). We did not succeed in building the 1–3 and 2–4 complexes since the distances between modified nucleotides were too large for any complex formation. These calculations clearly confirm the possibility of intra-strand complex formation when the two tpy-modified nucleotides are separated by 3 or 4 bps in the DNA duplex.

Conclusions

Novel **dN^R** bearing oligopyridine ligands attached *via* an octadiyne linker were prepared in a single-step Sonogashira cross-coupling reaction of iodinated nucleosides with the corresponding oligopyridine–octadiyne building blocks. Modified nucleosides **dN^R** were used as starting materials for synthesis of functionalized **dN^RTP**s by triphosphorylation. Modified **dN^RTP**s were shown to be good substrates for DNA polymerases and were incorporated into the DNA by primer extension. Oligopyridine functionalized DNAs, containing either one or four modifications, were tested for post-synthetic complexation with M^{2+} ions. For DNAs containing only one tpy-unit, weak inter-strand complex formation was observed only for some sequences. On the other hand, DNAs containing four tpy-units (**DNA3** and **DNA4**) form quantitatively complexes with Ni^{2+} and Fe^{2+} ions suggesting that intra-strand complexes might be involved. Unfortunately, no hard proof of the intra-strand complexes was found, probably due to reversibility of the complexation[21] and complicated equilibrium of different types of complexes. However, we found some evidence for the possibility of intra-strand complexation from gel electrophoresis, MALDI, CD and QM/MM calculations. Therefore, we can conclude that the attachment of terpyridine ligands to DNA by a flexible octadiyne linker allows formation of intra-strand metal complexes when more tpy-units are present in the same DNA molecule separated by 3 or 4 bps. However, as the inter-strand complex formation is also possible, at least for some sequences, the reversible formation of inter- and intra-strand complexes may complicate future applications of tpy-modified ONs and DNA in self-assembly.

Experimental

All reactions were performed under an argon atmosphere. $POCl_3$ and $PO(OMe)_3$ used for phosphorylation of nucleoside

were distilled before using. Other chemicals were purchased from commercial suppliers and were used as received. Preparative flash chromatography on reverse phase was performed on a Biotage SP1 flash purification system. Semi-preparative HPLC separations were performed on a column packed with 10 μm C18 reversed phase (Phenomenex, Luna C18(2)). NMR spectra were measured on a Bruker Avance 500 (500.0 MHz for ^{1}H, 125.7 MHz for ^{13}C and 202.3 for ^{31}P) or a Bruker Avance II 600 (600.1 MHz for ^{1}H and 150.9 MHz for ^{13}C) in $CDCl_3$ (^{1}H referenced to TMS as an internal standard (δ = 0 ppm); ^{13}C referenced to the solvent signal (δ = 77.0 ppm)), in DMSO-d_6 (^{1}H referenced to the residual solvent signal (δ = 2.50 ppm); ^{13}C referenced to the solvent signal (δ = 39.7 ppm)), or in CD_3OD (^{1}H referenced to the residual solvent signal (δ = 3.31 ppm); ^{13}C referenced to the solvent signal (δ = 49.0 ppm); ^{31}P referenced to H_3PO_4 (δ = 0 ppm) as an external standard). Chemical shifts are given in ppm (δ scale), coupling constants (J) in Hz. Complete assignment of all NMR signals was achieved by use of a combination of H,H-COSY, H,C-HSQC, and H,C-HMBC experiments. Mass spectra were measured on an LCQ classic (Thermo-Finnigan) spectrometer using ESI or Q-Tof Micro (Waters, ESI source, internal calibration with lock-spray). Mass spectra of functionalized DNA were measured by Maldi-TOF, Reflex IV (Bruker) with nitrogen laser. UV/Vis spectra were measured on a Varian CARY 100 Bio spectrophotometer at room temperature. CD spectra were recorded on a Jasco 815 spectropolarimeter (Japan) at room temperature. The optical path length was 0.1 cm and the CD signal was monitored from 200 nm to 350 nm. For each experiment the data are average of 2 scans taken with the time constant of 32 s, a scanning speed of 5 nm min^{-1} with blank subtracted. The CD spectra are expressed in differential absorption ($\Delta OD = A_L - A_R$).

For a numbering scheme for NMR assignment see Fig. S1 in ESI.†

General procedure A: synthesis of ligand building blocks

To an argon-purged flask containing 5-bromo-2,2′-bipyridine (**1a**) or (2,2′:6′,2″-terpyridine-4′-yl) trifluoromethanesulfonate (**1b**) (500 mg), $Pd(PPh_3)_2Cl_2$ (5 mol%) and CuI (5 mol%) were added THF (10 mL), Et_3N (10 equiv.) and 1,7-octadiyne (3 equiv.). The reaction mixture was heated at 75 °C for 3 h. After evaporation of the solvent under reduced pressure, the residue was extracted with three 100 mL portions of $CHCl_3$. Organic phases were combined and dried over $MgSO_4$. The residue was then purified by silica gel chromatography using hexane/ethyl acetate (0%–9%).

4′-(Octa-1‴,7‴-diyn-1‴-yl)-2,2′:6′,2″-terpyridine (3b)

Product **3b** was prepared according to general procedure A from (2,2′:6′,2″-terpyridine-4′-yl) trifluoromethanesulfonate (**1b**). It was isolated as an orange oil in the yield of 75% (331.8 mg).

Mp 69.0–69.5 °C; IR: 3207, 2940, 1581, 1564, 1467, 1391, 1263, 1113 cm^{-1}; ^{1}H NMR (500.0 MHz, $CDCl_3$): 1.73 (m, 2H, HC≡C–CH_2CH_2**CH_2**CH_2–C≡C–tpy); 1.77 (m, 2H, HC≡C–$CH_2CH_2CH_2$**CH_2**–C≡C–tpy); 1.98 (t, 1H, ^4J = 2.7, HC≡C–); 2.28 (td, 2H, J_{vic} = 6.8, ^4J = 2.7, HC≡C–C**H_2**$CH_2CH_2CH_2$–C≡C–tpy); 2.51 (t, 2H, J_{vic} = 6.8, HC≡C–$CH_2CH_2CH_2CH_2$**CH_2**–C≡C–tpy); 7.43 (ddd, 2H, $J_{5′,4′}$ = 7.5, $J_{5′,6′}$ = 5.0, $J_{5′,3′}$ = 1.2, H-5′); 7.97 (ddd, 2H, $J_{4′,3′}$ = 8.0, $J_{4′,5′}$ = 7.5, $J_{4′,6′}$ = 1.8, H-4′); 8.48 (s, 2H, H-3,5); 8.69 (d, 1H, $J_{3′,4′}$ = 8.0, H-3′); 8.78 (ddd, 1H, $J_{6′,5′}$ = 5.0, $J_{6′,4′}$ = 1.8, $J_{6′,3′}$ = 0.9, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$): 17.96 (HC≡C–**CH_2**$CH_2CH_2CH_2$–C≡C–tpy); 19.05 (HC≡C–CH_2**CH_2**CH_2CH_2–C≡C–tpy); 27.30, 27.47 (HC≡C–CH_2**CH_2**CH_2CH_2–C≡C–tpy); 68.67 (H**C**≡C); 79.04 (tpy–**C**≡C); 83.99 (HC≡**C**); 95.87 (tpy–C≡**C**); 121.93 (CH-3′); 123.83 (CH-3,5); 124.34 (CH-5′); 134.64 (C-4); 138.29 (CH-4′); 148.21 (CH-6′); 154.25 (C-2,6); 154.55 (C-2′); MS (ESI): m/z (%) = 3385 (100) [M$^+$ + H]; HRMS-ESI: m/z [M + H]$^+$ calcd for $C_{23}H_{23}N_3$: 338.16517; found: 338.16516; Anal. Calcd for $C_{23}H_{19}N_3$·1/5MeOH: C, 81.04; H, 5.80; N, 12.22. Found: C, 81.42; H, 5.58; N, 11.93.

General procedure B: Sonogashira cross-coupling reaction – synthesis of modified deoxynucleosides

DMF (1 mL) and Et(i-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing nucleoside 5-iodo-2′-deoxycytidine (**dC^I**, **5**) or 7-iodo-7-deaza-2′-deoxyadenosine (**dA^I**, **4**) (50 mg), an octadiyne modified oligopyridine **3a–b** (1.5 equiv.) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and P(Ph-$SO_3Na)_3$ (2.5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of DMF (0.5 mL). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stirred at 75 °C for 2 h. The solvent was then evaporated *in vacuo*. Products were directly purified by flash chromatography on reverse phase using H_2O/MeOH (0% to 100%) as an eluent. Products were recrystallized from the mixture MeOH–H_2O.

7-[8‴″-(2″,2‴:6‴,2‴″-Terpyridin-4‴-yl)octa-1‴″,7‴″-diyn-l‴″-yl]-7-deaza-2′-deoxyadenosine (dA^{Otpy}, 6b)

Product **6b** was prepared according to the general procedure B from **dA^I** (**4**) and **3b**. It was isolated as a brownish powder in the yield of 45% (35.0 mg).

Mp 109–113 °C; IR: 3444, 3185, 2928, 1584, 1566, 1468, 1393, 1305, 1046 cm^{-1}; ^{1}H NMR (600.1 MHz, DMSO-d_6): 1.76 (m, 4H, dapur–C≡C–CH_2C**H_2**C**H_2**CH_2–C≡C–tpy); 2.15 (ddd, 1H, J_{gem} = 13.1, $J_{2′b,1′}$ = 6.0, $J_{2′b,3′}$ = 2.7, H-2′b); 2.46 (ddd, 1H, J_{gem} = 13.1, $J_{2′a,1′}$ = 8.1, $J_{2′a,3′}$ = 5.7, H-2′a); 2.58 (m, 2H, dapur–C≡C–C**H_2**$CH_2CH_2CH_2$–C≡C–tpy); 2.62 (m, 2H, dapur–C≡C–$CH_2CH_2CH_2$C**H_2**–C≡C–tpy); 3.49 (ddd, 1H, J_{gem} = 11.8, $J_{5′b,OH}$ = 6.0, $J_{5′b,4′}$ = 4.4, H-5′b); 3.56 (ddd, 1H, J_{gem} = 11.8, $J_{5′a,OH}$ = 5.3, $J_{5′a,4′}$ = 4.4, H-5′a); 3.81 (td, 1H, $J_{4′,5′}$ = 4.4, $J_{4′,3′}$ = 2.7, H-4′); 4.32 (m, 1H, $J_{3′,2′}$ = 5.7, 2.7, $J_{3′,OH}$ = 4.0, $J_{3′,4′}$ = 2.7, H-3′); 5.07 (dd, 1H, $J_{OH,5′}$ = 6.0, 5.3, OH-5′); 5.26 (d, 1H, $J_{OH,3′}$ = 4.0, OH-3′); 6.46 (dd, 1H, $J_{1′,2′}$ = 8.1, 6.0, H-1′); 7.52 (ddd, 2H, $J_{5‴,4‴}$ = 7.5, $J_{5‴,6‴}$ = 4.7, $J_{5‴,3‴}$ = 1.2, H-5‴); 7.67 (s, 1H, H-6); 8.02 (ddd, 2H, $J_{4‴,3‴}$ = 7.9, $J_{4‴,5‴}$ = 7.5, $J_{4‴,6‴}$ = 1.8, H-4‴); 8.09 (s, 1H, H-2); 8.34 (s, 2H, H-3″,5″); 8.61 (ddd, 2H, $J_{3‴,4‴}$ = 7.9, $J_{3‴,5‴}$ = 1.2, $J_{3‴,6‴}$ = 0.9, H-3‴); 8.72 (ddd, 2H, $J_{6‴,5‴}$ = 4.7, $J_{6‴,4‴}$ = 1.8, $J_{6‴,3‴}$ = 0.9, H-6‴); ^{13}C NMR (150.9 MHz, DMSO-d_6): 18.52 (dapur–C≡C–$CH_2CH_2CH_2$**CH_2**–C≡C–tpy); 18.70 (dapur–C≡C–

C$H_2CH_2CH_2CH_2$–C≡C–tpy); 27.34, 27.70 (dapur–C≡C–CH_2**C**$H_2CH_2CH_2$–C≡C–tpy); 39.93 (CH_2-2′); 62.11 (CH_2-5′); 71.18 (CH-3′); 74.07 (dapur–**C**≡C); 79.10 (tpy–**C**≡C); 83.28 (CH-1′); 87.67 (CH-4′); 92.45 (dapur–C≡**C**); 95.69 (C-5); 96.40 (tpy–C≡**C**); 102.52 (C-4a); 121.06 (CH-3‴); 122.25 (CH-3″,5″); 124.96 (CH-5‴); 125.75 (CH-6); 133.55 (C-4″); 137.77 (CH-4‴); 149.25 (C-7a); 149.64 (CH-6‴); 152.78 (CH-2); 154.51 (C-2″,6″); 155.47 (C-2‴); 157.77 (C-4); MS (ESI): *m*/*z* (%) = 586 (27) [M^+ + H], 608 (100) [M^+ + Na]; HRMS-ESI: *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{32}O_3N_7$: 586.25611; found: 586.25592.

General procedure C: phosphorylation of oligopyridine modified nucleosides (dN^R) – synthesis of modified dN^{R}TPs

Dry trimethyl phosphate (1 mL) was added to an argon-purged flask containing nucleoside analogue **dN^R** (**6a–b** or **7a–b**, 50 mg), cooled to 0 °C on ice followed by the addition of $POCl_3$ (1.5 equiv.). A solution of $(NHBu_3)_2H_2P_2O_7$ (5 equiv., 1 mL) in dry DMF with an addition of Bu_3N (4.5 equiv.) was prepared in a separate flask and cooled down to 0 °C. Like this the prepared solution was then added to the reaction mixture and stirred for 1.5 h and quenched by 2 M TEAB buffer (2 mL). The product was isolated on a DEAE Sephadex column (150 mL) eluting with a gradient 0 to 1.2 M TEAB, evaporated, co-distilled with water (3 times) and re-purified by semi-preparative HPLC on a C18 column using a linear gradient of 0.1 M TEAB (triethylammonium bicarbonate) in H_2O to 0.1 M TEAB in H_2O–MeOH (1 : 1) as an eluent. Several co-distillations with water followed by freeze-drying from water gave the products as brownish powder.

7-[8‴″-(2″,2‴:6‴,2‴″-Terpyridin-4‴-yl)octa-1‴″,7‴″-diyn-l‴″-yl]-7-deaza-2′-deoxyadenosine-5′-O-triphosphate (dA^{Otpy}TP, 8b)

This compound was prepared according to the general procedure C from **dA^{Otpy}** (**6b**) in the yield of 14% (13.2 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.29 (t, 27H, J_{vic} = 7.3, C**H**$_3$CH$_2$N); 1.84 (m, 4H, dapur–C≡C–CH_2C**H**$_2$C**H**$_2$$CH_2$–C≡C–tpy); 2.30 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 5.8, $J_{2'b,3'}$ = 2.9, H-2′b); 2.46 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 8.0, $J_{2'a,3'}$ = 5.7, H-2′a); 2.58 (t, 2H, J_{vic} = 6.8, dapur–C≡C–C**H**$_2$$CH_2CH_2CH_2$–C≡C–tpy); 2.61 (t, 2H, J_{vic} = 6.8, dapur–C≡C–$CH_2CH_2CH_2$C**H**$_2$–C≡C–tpy); 3.18 (q, 18H, J_{vic} = 7.3, CH_3C**H**$_2$N); 4.14 (m, 1H, H-4′); 4.26 (m, 2H, H-5′); 4.60 (dt, 1H, $J_{3',2'}$ = 5.7, 2.9, $J_{3',4'}$ = 2.9, H-3′); 6.57 (dd, 1H, $J_{1',2'}$ = 8.0, 5.8, H-1′); 7.42 (ddd, 2H, $J_{5''',4'''}$ = 7.3, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 0.6, H-5‴); 7.69 (s, 1H, H-6); 7.92 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.3, $J_{4''',6'''}$ = 1.6, H-4‴); 8.26 (s, 3H, H-2, H-3″,5″); 8.52 (bd, 2H, $J_{3''',4'''}$ = 7.9, H-3‴); 8.62 (bd, 2H, $J_{6''',5'''}$ = 4.8, H-6‴); ^{13}C NMR (150.9 MHz, CD_3OD): 9.12 (**C**H_3CH_2N); 19.75 (dapur–C≡C–$CH_2CH_2CH_2CH_2$–C≡C–tpy); 20.12 (dapur–C≡C–**C**H_2–$CH_2CH_2CH_2$–C≡C–tpy); 28.87, 28.93 (dapur–C≡C–CH_2**C**H_2–**C**H_2CH_2–C≡C–tpy); 41.85 (CH_2-2′); 47.44 (CH_3CH_2N); 66.87 (d, $J_{C,P}$ = 5.5, CH_2-5′); 72.56 (CH-3′); 72.74 (dapur–**C**≡C); 80.10 (tpy–**C**≡C); 84.92 (CH-1′); 87.62 (d, $J_{C,P}$ = 8.5, CH-4′); 95.16 (dapur–C≡**C**); 96.79 (tpy–C≡**C**); 99.97 (C-5); 102.80 (C-4a); 122.69 (CH-3‴); 123.95 (CH-3″,5″); 125.55 (CH-5‴); 128.22 (CH-6); 135.51 (C-4″); 138.70 (CH-4‴); 146.66 (CH-2); 148.17 (C-7a); 150.16 (CH-6‴); 153.75 (C-4); 156.56 (C-2″,6″); 156.70 (C-2‴); ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): −21.99 (bdd, *J* = 20.7, 19.3, P_β); −9.56 (d, *J* = 20.7, P_α); −8.71 (d, *J* = 19.3, P_γ); MS (ES$^-$): found *m*/*z*: 824.2 (M − 1), 744.3 (M − PO_3H_2 − 1); HRMS (ES): *m*/*z* calcd for $C_{34}H_{33}O_{12}N_7P_3$: 824.14055; found: 824.13982.

Primer extension, purification and analysis of the PEX products

Synthetic ONs were purchased from Sigma Aldrich (USA). Primer: 5′-CAT GGG CGG CAT GGG-3′ (prim^{rnd}), 5′-CAT GGG CGG CAT CTC-3′ (prim^{comp}); templates: 5′-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3′ (temp^{rnd16}), 5′-**CAG ACC AGC CCT CCC** *GAG ATG CCG CCC ATG*-3′ (temp^{comp3gA}), 5′-**CCC G***CC CAT GCC GCC CAT G*-3′ (temp^{C}), 5′-**CCC T***CC CAT GCC GCC CAT G*-3′ (temp^{A}), **T***CC CAT GCC GCC CAT G*-3′ (temp^{A1}), **G***CC CAT GCC GCC CAT G*-3′ (temp^{C1}), 5′-**T***GA GAT GCC GCC CAT G*-3′ (temp^{compA1}) (segments forming duplex with the primer are in italics, the replicated segments are in bold). Templates used in experiment involving the DBstv magnetoseparation procedure were biotinylated at their 5′ ends. Streptavidine magnetic beads MagPrep P-25 Streptavidine Particles were obtained from Novagen (EMD Chemicals, USA), Pwo DNA polymerase from PeqLab (Germany), DyNAzyme II DNA polymerases from Finnzymes (Finland), KOD XL DNA polymerase from Novagen (EMD Chemicals, USA), Deep Vent DNA polymerases as well as T4 polynukleotide kinase and natural nucleoside triphosphate (dATP, dCTP, dGTP and dCTP) from New England Biolabs (Great Britain) and γ-^{32}P-ATP from Izotop, Institute of Isotopes Co, Ltd. (Hungary).

Primer extension experiment for single incorporation. The reaction mixture (20 μL) contained Pwo polymerase (0.1 U μL^{-1}, 2 μL), dNTPs (either natural or modified, 4 mM, 1 μL), ^{32}P-prelabelled primer at 5′-end (3 μM, 1 μL) and template temp^{A} or temp^{C} (3 μM, 1.5 μL) in 2 μL of corresponding buffer supplied by manufacturer. The reaction mixture was incubated for 30 min at 60 °C.

Primer extension for multiple incorporation. The reaction mixture (20 μL) contained Pwo polymerase (0.1 U μL^{-1}, 2 μL) or DyNAzyme II polymerase (0.2 U μL^{-1}, 1 μL), dNTPs (either natural or modified, 4 mM, 1 μL), ^{32}P-prelabelled primer at 5′-end (3 μM, 1 μL) and temp^{rnd16} (3 μM, 1.5 μL) in 2 μL of corresponding buffer supplied by manufacturer. The reaction mixture was incubated for 30 min at 60 °C.

For magnetoseparation were used unlabelled primers and biotinylated templates.

Primer extension for kinetics study. The reaction mixture (20 μL) contained DNA polymerase: Pwo (0.1 U μL^{-1}, 2 μL), dATP/**dA^{R}TP 8a–b** or dCTP/**dC^{R}TP 9a–b** (4 mM, 1 μL), ^{32}P-prelabelled primer at 5′-end (3 μM, 1 μL) and template temp^{A1} or temp^{C1} (3 μM, 1.5 μL) in 2 μL of corresponding buffer supplied by manufacturer. The reaction mixture was incubated at 60 °C for required time.

Denaturating polyacrylamide gel electrophoresis. The products of the primer extension reaction were mixed with loading buffer (40 μL, 80% [w/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol), heated

5 min at 95 °C and subjected to gel electrophoresis in 12.5% denaturating polyacrylamide gel containing 1 × TBE buffer (pH 8) and 7% urea at 60 W for ~60 min. Gel was dried and visualized by a phosphoimager.

Complexation of **dN^R**s. Complexes of modified nucleoside **dN^R**s (**6a–b** or **7a–b**) with diverse transition metals were prepared by mixing 100 μL of a methanolic solution of the corresponding nucleosides (100 μM) with 100 μL of a methanolic solution of divalent metal ions M^{2+} (50 μM, $Cu(BF_4)_2{\cdot}6H_2O$, $Ni(BF_4)_2{\cdot}6H_2O$, $Zn(BF_4)_2{\cdot}H_2O$, $Fe(BF_4)_2{\cdot}6H_2O$) at room temperature for 10 min.

Complexation of ONs for recording UV-spectra. Double stranded DNAs were prepared by PEX-experiment on a larger scale. The reaction mixture (100 μL) contained Deep Vent polymerase (2 U μL^{-1}, 7.5 μL) or DyNazyme II polymerase (2 U μL^{-1}, 7.5 μL), dNTP (either natural or modified, 4 mM, 15 μL), unlabeled primer (100 μM, 6 μL), and temp^{rnd16} (100 μM, 6 μL) in 10 μL of corresponding buffer supplied by manufacturer. The reaction mixture was incubated for 30 min at 60 °C. PEX-products were purified by NucAway Spin Columns (Ambion), where 50 μL portions of each sample were applied on the top of the column. After collecting all the portions 0.5 equiv. of $Fe(BF_4)_2{\cdot}6H_2O$ to number of modifications (0.24 μL, 10 mM) was added to the corresponding sample and the solution was mixed overnight (25 °C, 550 rpm).

Complexation of ON for gel electrophoresis. Double stranded ONs were prepared by PEX-experiment. The reaction mixture (20 μL) contained DNA polymerase: Pwo (0.1 U μL^{-1}, 2 μL), DyNAzyme II (0.2 U μL^{-1}, 1 μL), dNTP (either natural or modified, 4 mM, 1 μL), ^{32}P-prelabelled primer at 5′-end primer (3 μM, 1 μL), and temp (3 μM, 1.5 μL) in 2 μL of corresponding buffer supplied by manufacturer. The reaction mixture was incubated for 30 min at 60 °C. After addition of 1 μL of $Fe(BF_4)_2{\cdot}6H_2O$ (4 mM), the solution was mixed overnight (25 °C, 550 rpm).

Non-denaturating SB polyacrylamide gel electrophoresis. The products of the primer extension reaction were mixed with loading buffer (4 μL, 40% [w/v] saccharose, 0.2% [w/v] bromophenol blue, 0.2% [w/v] xylene cyanol) subjected to gel electrophoresis in 8% non-denaturating polyacrylamide gel containing 1 × SB buffer (pH 8) and at 600 V for ~3 h at room temperature. Gel was dried and visualized by a phosphoimager.

Molecular modelling and calculations. The structure was built by using the Nucleic Acid Builder in AMBER[22] and PyMOL.[23] The system was neutralized with sodium ions. Molecular mechanics parameters from the ff03 force field were employed. Parameters of Fe^{2+} were taken from ref. 24 and the GAFF parameters were used for the modified residue. We used a subtractive QM/MM scheme.[25] The substructure scheme couples a small QM region treated by the DFT-D[26] level of theory with an MM description of the rest of the system. We used the SVP/B-LYP level of theory for optimization followed by TPSS/TZVP single point calculations. The QM/MM procedure is implemented in a Cuby[27] framework developed in our laboratory. It calls Turbomole[28] for QM and AMBER for the MM calculations. To account for solvation of the whole system, we used the Generalized Born (GB) solvent model at the MM level. The QM part comprised the octadiyne linkers, tetrapyridine ligands and Fe^{2+} ions (*i.e.* 182 atoms).

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