Univerzita Karlova v Praze
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Příprava nukleosidů, nukleotidů a nukleových kyselin nesoucích ligandy bipyridinového typu

Synthesis of nucleosides, nucleotides and nucleic acids bearing bipyridine-type ligands

Disertační práce

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 17.08.2012

Podpis

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List of abbreviations

Ac = acetyl

AFM = atomic force microscopy

AQ = antraquinone

bpy = 2,2'-bipyridine

5bpy = 2,2'-bipyridine-5-yl

6bpy = 2,2'-bipyridine-6-yl

CD = circular dichroism

EDTA = ethylenediaminetetraacetic acid

dATP = 2'-deoxyadenosine triphosphate

DB_{stv} = streptavidine-coated magnetic beads

dCTP = 2'-deoxycytidine triphosphate

dGTP = 2'-deoxyguanosine triphosphate

DMTr = bis-(4-methoxyphenyl)phenylmethyl

dNMP = 2'-deoxyribunucleoside monophosphate

dNTP = 2'-deoxyribunucleoside triphosphate

DMF = dimethylformamide

DMSO = dimethylsulfoxid

DNA = deoxyribonucleic acid

ds = double-stranded

dTTP = 2'-deoxythimidine triphosphate

dpp = bis(2,9-diphenyl)-1,10-phenantroline

Et = ethyl

HPA = 3-hydroxypicolinic acid

Me = methyl

MALDI = matrix-assisted laser desorption/ionization

MLCT = metal-to-ligand charge-transfer

OD = optical density

ON = oligonucleotide

ss = single-stranded

PAGE = polyacrylamide gel electrophoresis

PEX = primer extension experiment

PCR = polymerase chain reaction

Ph = phenyl

i-Pr = propan-2-yl

tpy = 2,2':6',2''-terpyridine

Etpy = 4-ethynyl-2,2'':6',2'-terpyridine

Otpy = 4-(octa-1''',7'''-diyn-1'''-yl)-2,2'':6',2'-terpyridine

PA = picolinic acid

SB = sodium borate buffer

TBE = tris/borate/ethylenediaminetetraacetic acid buffer

TCA = trichloroacetic acid

TEAB = triethylammonium bicarbonate

THF = tetrahydrofurane

TMS = tetramethylsilane

TPPTS = bis(3-sulfonatophenyl)phosphine hydrate, sodium salt

TTF = tetrathiafulvalene

Abstract

An efficient methodology of construction of base-modified nucleosides bearing oligopyridine ligands, based on the Sonogashira or Suzuki cross-coupling reaction of halogenated nucleosides, was developed. This methodology was then successfully employed in construction of base-modified DNA bearing oligopyridine ligands which were studied in post-synthetic complexation with labile transition metals. The first step in construction of modified DNA is the synthesis of deoxynucleoside triphosphate (dNTPs) bearing various metal chelating groups, which are in second step enzymatically incorporated into DNA by primer extension experiment. The first task was the synthesis of dNTPs bearing different oligopyridine ligands, which could be done by aqueous phase cross-coupling reaction with suitable building blocks or by triphosphorylation of oligopyridine-modified deoxynucleosides. Both ways were successfully used. Aqueous phase Sonogashira cross-coupling was used for synthesis of dNTPs bearing oligopyridine ligands attached via short and rigid acetylene tether, while classical triphosphorylation of modified nucleosides was used for construction of dNTPs bearing oligopyridine ligands attached via long and flexible octadiyne linker. Sonogashira cross-coupling reaction was also used for preparation of both types of oligopyridine-modified nucleosides (with acetylene or octadiyne linker), used as model compounds. Oligopyridine-modified dNTPs were tested as substrates for several thermostable DNA polymerases and were successfully incorporated into DNA by primer extension experiments and then tested for post-synthetic complexation. DNA bearing 2,2':6',2"-terpyridine forms stable complexes with Fe^{2+} ions which were detected by polyacrylamide gel electrophoresis and by UV/Vis spectroscopy. While DNA bearing 2,2':6',2"-terpyridine attached via rigid acetylene tether can form only the inter-strand complexes, DNA bearing 2,2':6',2"-terpyridine attached via flexible octadiyne linker can form inter-strand as well intra-strand complexes. Formation of intra-strand complex was clearly showed by faster mobility on gel in comparison to twice as large inter-strand complex formed from DNA bearing 2,2':6',2"-terpyridine attached via acetylene tether. Formation of intra-strand DNA complex was also confirmed by MALDI, CD spectroscopy and modeling.

Abstrakt

Byla vyvinuta efektivní metodika přípravy modifikovaných nukleosidů nesoucích oligopyridinové ligandy založená na Sonogashirově nebo Suzukiho reakci halogenovaných nukleosidů. Tatéž metodika byla úspěšně uplatněna při syntéze modifikované DNA nesoucí oligopyridinové ligandy za účelem studia její post-syntetické komplexace s labilními přechodnými kovy. Deoxynukleosid trifosfáty (dNTPs), syntetizované v prvním kroku a nesoucí různé skupiny chelatující kovy, byly ve druhém kroku pomocí PEX enzymaticky inkorporovány do DNA. Prvním cílem byla syntéza dNTPs nesoucích různé oligopyrinové ligandy, která může být provedena kaplinkem příslušných stavebních bloků ve vodné fázi nebo trifosforylací deoxynukleosidů modifikovaných oligopyridinem. Oba tyto přístupy byly úspěšně použity. dNTPs nesoucí oligopyridinový ligand připojený přes krátký a rigidní acetylenový můstek byly připraveny Sonogashirovým kaplinkem ve vodné fázi. Naproti tomu dNTPs modifikované oligopyridinovým ligandem napojeným dlouhou a flexibilní oktadiynovou spojkou byly syntetizovány klasickou trifosforylací. Sonogashirova reakce byl použit také pro přípravu obou typů nukleosidů (s acetylenovým i oktadiynovým můstkem), které byly použity jako modelové sloučeniny. dNTPs modifikované oligopyridinem byly testovány jako substráty několika termostabilních DNA polymeráz, pomocí PEX byly úspěšně inkorporovány do DNA a poté testovány v post-syntetických komplexačních reakcích. DNA nesoucí 2,2':6',2''-terpyridin tvoří stabilní komplexy s Fe^{2+} ionty, které byly detekovány polyakrylamidovou gelovou elektroforézou a UV/Vis spektroskopií. Zatímco DNA nesoucí 2,2':6',2''-terpyridin připojený přes rigidní acetylenovou spojku může tvořit pouze mezivláknové komplexy, DNA spojená s 2,2':6',2''-terpyridinem přes flexibilní oktadyinový můstek tvoří komplexy jak mezi dvěma řetězci, tak v rámci jednoho řetězce. Vznik komplexu v jednom řetězci byl prokázán jeho rychlejší mobilitou na gelu ve srovnání s dvojnásobně větším mezivláknovým komplexem vytvořeným mezi DNA nesoucí 2,2':6',2''-terpyridin připojený přes acetylenový můstek. Tvorba komplexu v jednom řetězci byla potvrzena také pomocí MALDI, CD spektroskopií a modelování.

1 Introduction

1.1 DNA structure

DNA is a linear polymer build up from similar units consisting of three components: a sugar (deoxyribose), a phosphate, and a base. Whereas the negatively charged backbone, formed from sugars linked by phosphodiester bridges, is constant, the bases vary from one monomer to the next. Two of the bases are derivatives of purine: adenine and guanine; and two of pyrimidine: cytosine and thymine (Figure 1).[1]

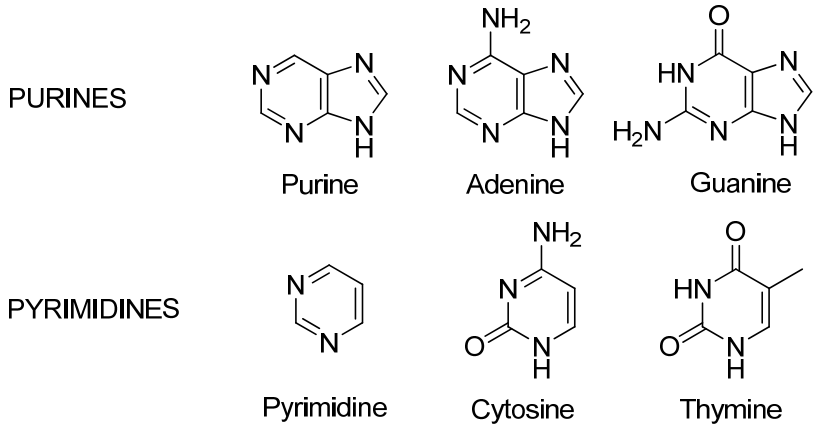


Figure 1. Bases derived from purine and pyrimidine

Double-stranded helical structure was determined by Watson, Crick, Wilkins and Franklin based on X-ray diffraction.[2] Pursuant to diffraction patterns it is known, that bases are placed inside the DNA duplex and are nearly perpendicular to the helix axis, and adjacent bases are separated by 3.4 Å. The helical structure repeats every 34 Å, so there are 10 bases per turn of helix. The diameter of the helix is 20 Å. This regular structure is stabilized by characteristic base paring (Figure 2).

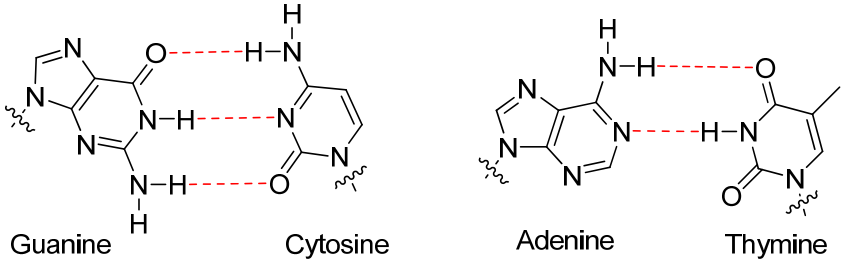


Figure 2. Base pairing proposed by Watson and Crick.

Due to the nanometric dimension, predictable secondary structure, high stability and selective recognition of the complementary strand through the Watson-Crick base pairing, the DNA emerged as unique template for construction and organization of nanostructures and arrays with precisely controlled features.

1.2 DNA-based nanostructures

The reliability of specific DNA sequence to recognize its complementary strand has been used to create new nanometer-scale two- and three-dimensional objects. Seeman's Holliday junction (Figure 3a), which is formed from four strands of DNA (two identical pairs of complementary strands) where double-helical domains meets at a branched point and exchange base pairing partner strands[3], was only the beginning in DNA-nanotechnology. Since then many 2D and 3D DNA structures were prepared (Figure 3). 'Origami method' reported by Rothemund (Figure 3b) was successfully used for formation of discrete objects formed from long DNA strand and small 'staple strand'.[4] Once, the formation of two-dimensional DNA nanostructures was known, formation of three-dimensional DNA structures became the object of interest of many chemists. Investigation of higher ordered assemblies was not the only reason. Synthesis of potential nanocontainers with deliberately controlled pore size, geometry and addressability for guest molecule was much more interesting. DNA nano-cubes[5] (Figure 3c) and boxes[6], polyhedra of different shape and size[7] (Figure 3d) were successfully synthesized as well as three-dimensional origami structures[8] (Figure 3e).

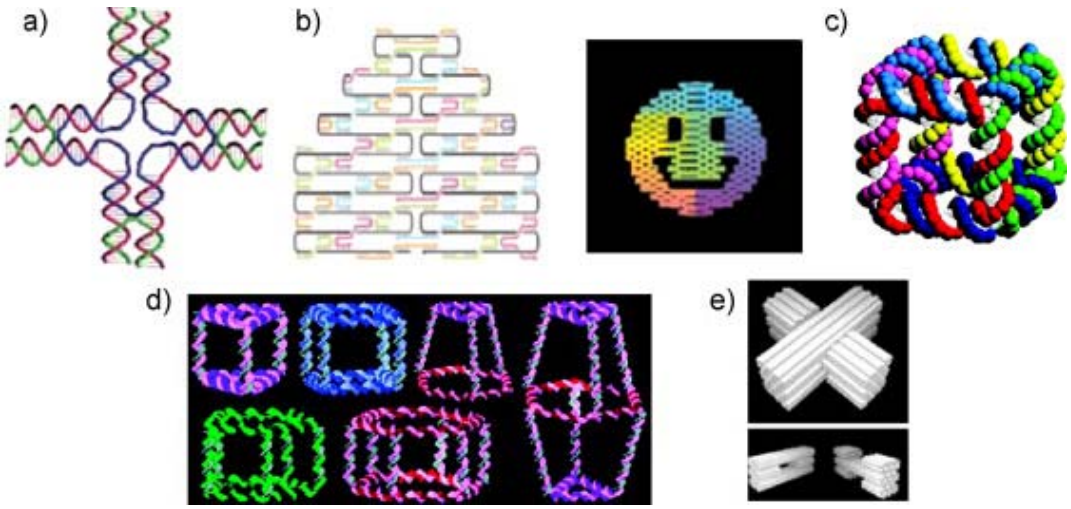


Figure 3. 2D and 3D DNA assemblies: a) Holliday junction, b) 2D origami structure, c) DNA cubes, d) diverse polyhedra[7d], e) 3D DNA origami. Figure panels were reproduced from [13].

1.2.1 Metal-mediated DNA assemblies

Besides the π-π interaction[9], the metal ion-ligand interaction is also an additional binding motif, which enhanced the complexity of DNA-based nanostructures. Incorporation of transition metals into DNA allows us to build the assemblies with otherwise inaccessible geometry and spatial arrangements through the geometries and coordination numbers available to different metals. Metal complexes attached to DNA can also influence the functionality of like this modified macromolecule (enhanced stability[10], redox activity and photoactivity[11] and catalytic properties[12]).

There are three different approaches in the introduction of transition metals into the molecule of DNA: direct metal binding to natural DNA, attachment of previously synthesized metal complex to the DNA or attachment of metal-chelating ligand followed by post-synthetic complexation (Figure 4).[13]

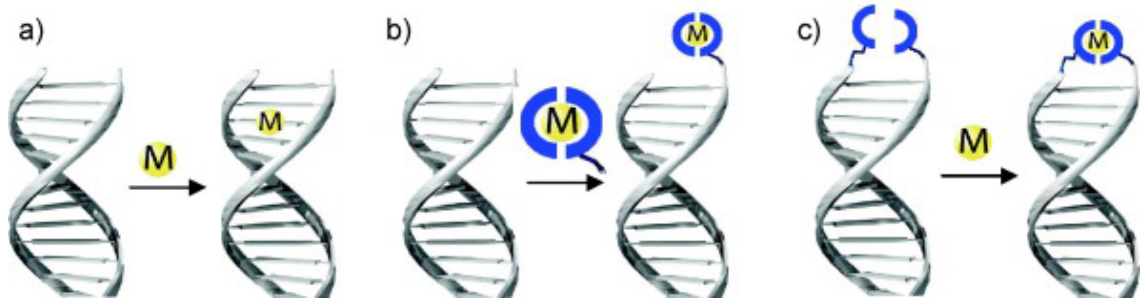


Figure 4. Site-specific incorporation of transition metals into DNA: a) direct metal binding to natural DNA, b) attachment of whole metal complex to DNA, c) attachment of metal-chelating group and post-synthetic complexation.[13] Figure panels were adapted from [13].

DNA-complexes can have the transition metal place inside (artificial metal-base- pairs or intercalators) or outside (metal complex covalently attached to nucleobase, sugar or phosphate) the DNA duplex.

1.2.1.1 Metal-mediated base pairs

One of the most commonly used approaches to form metalated DNA is the replacement of Watson-Crick base pairs with binding of artificial nucleobases with transition metals (non-covalent ligand-metal-ligand bond) in the interior of the DNA duplex. For this purpose, unnatural nucleobases have to be synthesized and incorporated into the DNA sequence. Appropriate combination of the artificial nucleosides with metal ion allows formation of stable metal-mediated base pairs which are geometrically

analogous to H-bonded base pairs. While the structural features of DNA remain unchanged, the chain of metals within the DNA duplex plays an essential role in charge or energy transfer and nanowire formation. Since the metal coordinative bond has higher binding energy than non-covalent hydrogen bond, the metal ions incorporation should stabilize DNA duplex.

The first example of metal-mediated base pair was reported by Shionoya[14], who demonstrated DNA base pairing through the square-planar 2:1 complex formation of *β*-C-nucleoside bearing *o*-phenylenediamine ligand with Pd^{2+} (Figure 5). Incorporation of this complex into oligonucleotide was not initially reported.

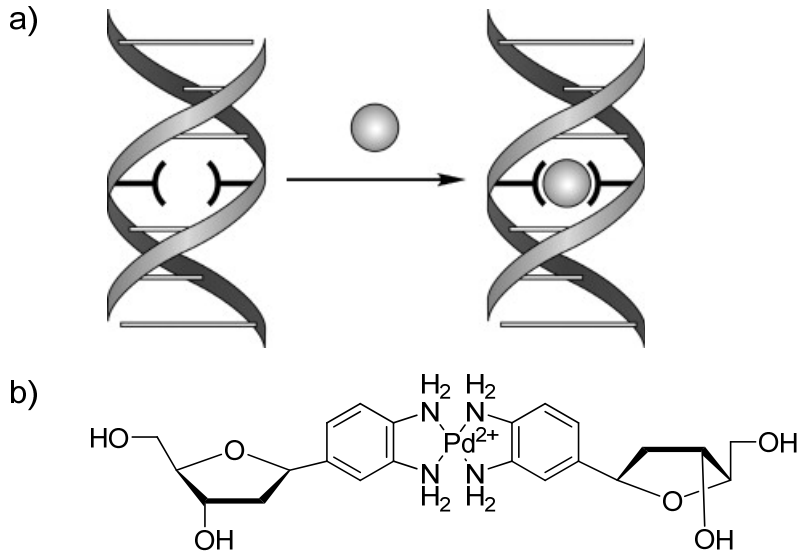


Figure 5. Metal-assisted DNA base pairs: a) schematic representation of metal-induced duplex formation (Figure was reproduced from [15]), b) the first metal-mediated artificial base pair.

Since then many *β*-C- or *β*-N-nucleosides bearing ligands (Figure 6) designed for complexation with diverse transition metals were successfully synthesized and used for metal-assisted DNA base pairing.[16,17]

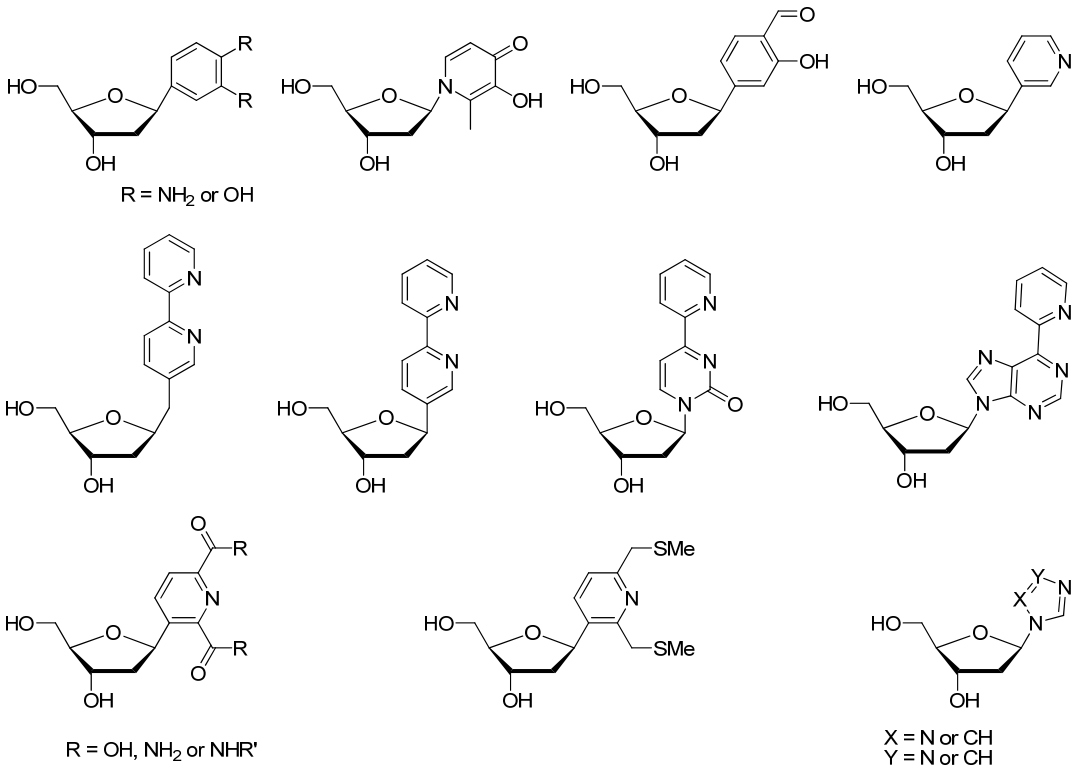


Figure 6. Artificial ligand-type nucleosides.[16,17]

The first metal-mediated base pair inside the DNA duplex was reported by Meggers, Romesberg and Schultz.[16e] Copper-mediated base pair between tridentate ligand pyridine-2,6-dicarboxylate and pyridine nucleoside leads to significant stabilization of DNA duplex. However, when other metal ions (Ce^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pd^{2+} or Pt^{2+}) were used, no duplex stabilization was observed.

Tremendous stabilization was observed when metal-salene base pairs were present in DNA duplex. Unusual stability of metal-salene base pairs resulted from additional cross-linking via ethylenediamine linker (Figure 7).

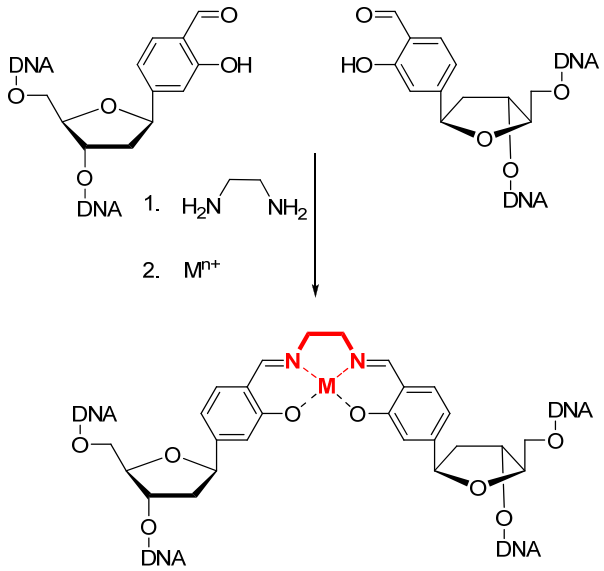


Figure 7. Formation of metal-salene base pairs.

1.2.1.2 DNA modified with metal complex

Some metal-mediated base pairs can cause distortion of the DNA duplex. To obtain a stable structure, artificial bases have to be precisely designed and their size and shape have to fit within the DNA duplex and the π-π stacking of DNA base pairs have to be retained. Less challenging approach in formation of metalated DNA is attachment of previously prepared metal complex to DNA. This methodology is suitable only for incorporation of kinetically stable metal complexes, which resist the conditions of oligonucleotide synthesis. Like this modified DNAs were mostly studied as luminescent labels for biosensors or media for long-range charge transport.

One of the most extensively studied luminescent labels are ruthenium and osmium complexes (Figure 8). Complex can be attached to the nucleobase[18,11a,c,d], to the deoxyribose[19] or to the DNA backbone[20]. Corresponding complex can be incorporated as a phosphoramidite derivative using solid-phase synthesis[11c,d,19,20] or as a particular triphosphate by enzymatic incorporation using DNA polymerases[11a]. For synthesis of Ru-derivatized oligonucleotide a post-modification was also used.[18]

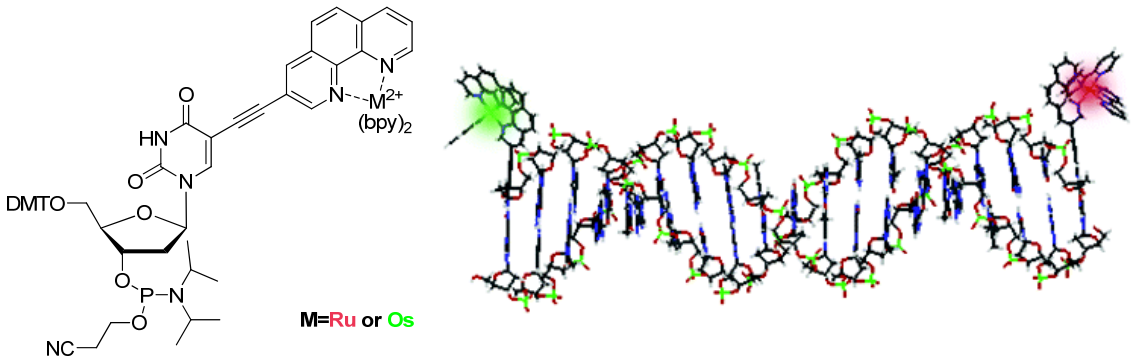


Figure 8. Ru and Os complex as a luminescent label for DNA.[11b-d] Figure was reproduced from [11d].

On the other hand, oligonucleotides modified with Rh-complexes capable of intercalation with DNA duplex were mainly studied as a media for long-range charge transport,[21] resulting for example in oxidative DNA damage[22] (Figure 9).

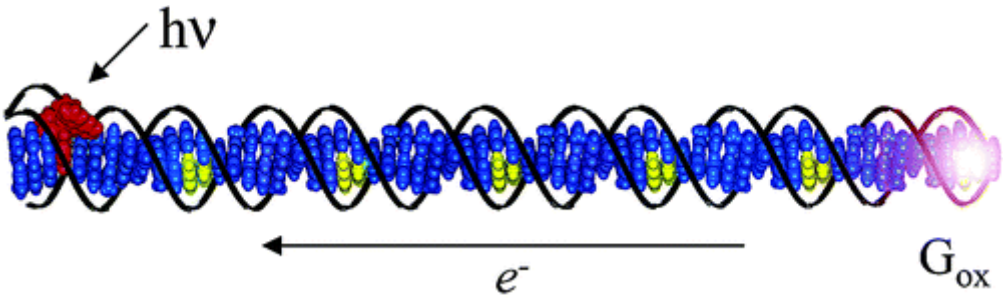


Figure 9. Schematic representation of oxidative damage of each of six guanine doublet sites as a result of photooxidation of rhodium intercalator.[21] Figure was adapted from [21].

For formation of higher ordered structures, mostly Ru-modified DNA complexes were used as building blocks (Figure 10). Synthesis of Ru-bridged DNA hairpin required the preparation of ligand bearing two functional groups, one of which is protected while the other is activated. Sleiman's group developed the synthesis of branched oligonucleotide, in which transition metal acts as the vertex joining two parallel DNA strands[23] (Figure 10a) as well as the synthesis of cyclic DNA formed from short DNA duplexes joined together via transition metals vertices[24] (Figure 10b). Multiarmed metal-centered DNA building blocks (Ru^{2+}-complex with six DNA arms and Ni^{2+}-complex with four DNA arms; Figure 10c,d) as precursors of suplamolecular assemblies were reported by McLaughlin's group.[25]

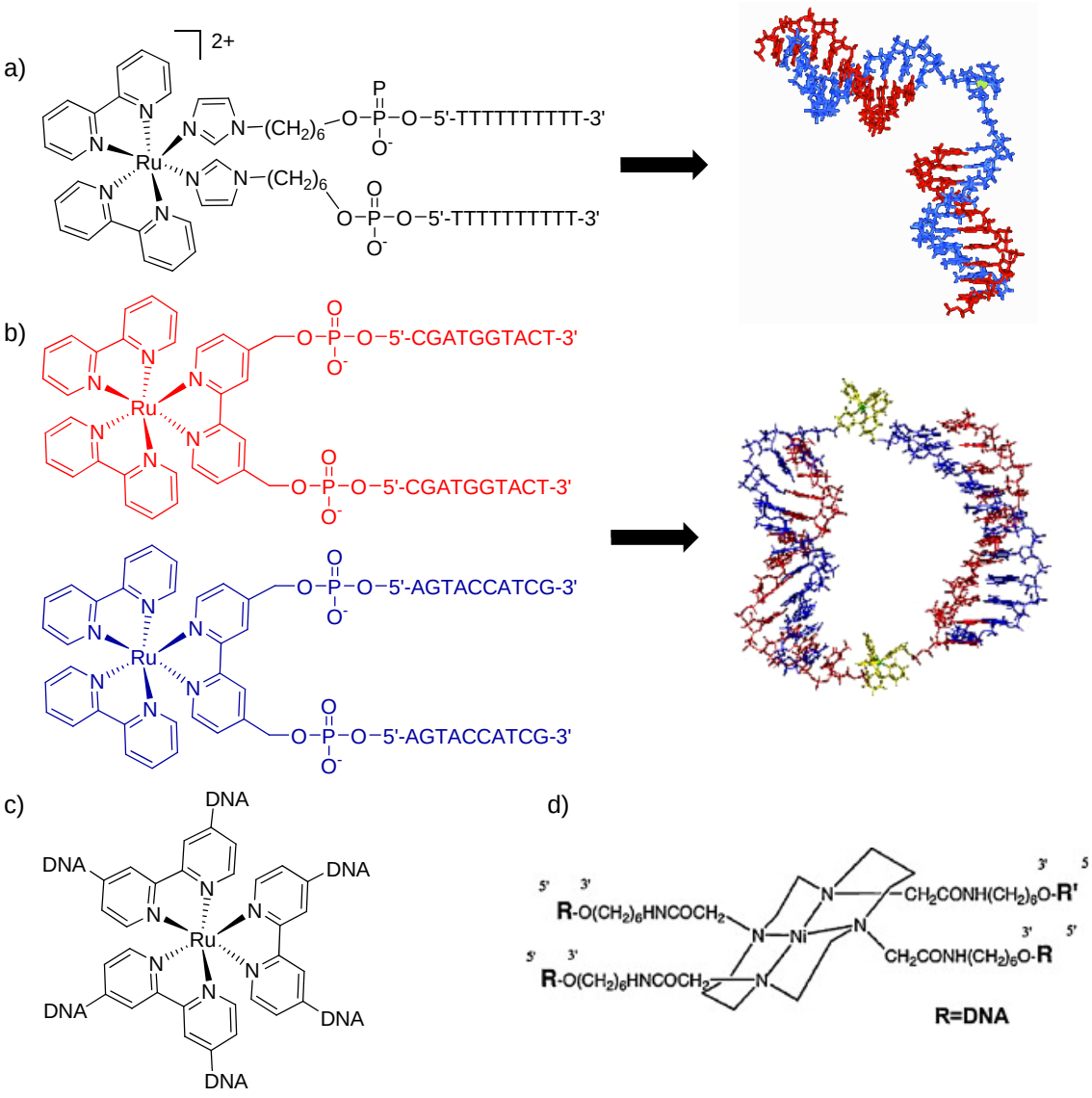


Figure 10. Metal-mediated DNA assemblies: a) metal-induced DNA junction[23] (Figure was adapted from [23]), b) cyclic metal-DNA structure[24] (Figure was reproduced from [13]), c) six-armed metal-centered DNA complex[25a], d) four-armed metal-centered DNA complex[25b] (Figure was adapted from [25b]) .

1.2.1.3 DNA modified with metal chelating ligand and its complexation

For incorporation of kinetically labile metals into the DNA, the post-synthetic complexation of DNA modified with metal chelating group is the most suitable method. Ligands can be easily incorporated into the DNA duplex using solid-phase synthesis or enzymatic incorporation. For this purpose, oligopyridine ligands such as bipyridine, phenantroline and terpyridine, are the most commonly used ligands.

First structure prepared according to this approach was an alternative to the Holiday junction formed by complexation of two DNA strands modified at their ends with terpyridine with Fe^{2+} ions (Figure 11).[26] Three self-complementary branched

oligonucleotides with Fe^{2+}-complex in their vertexes were then successfully used for construction of DNA triangle.

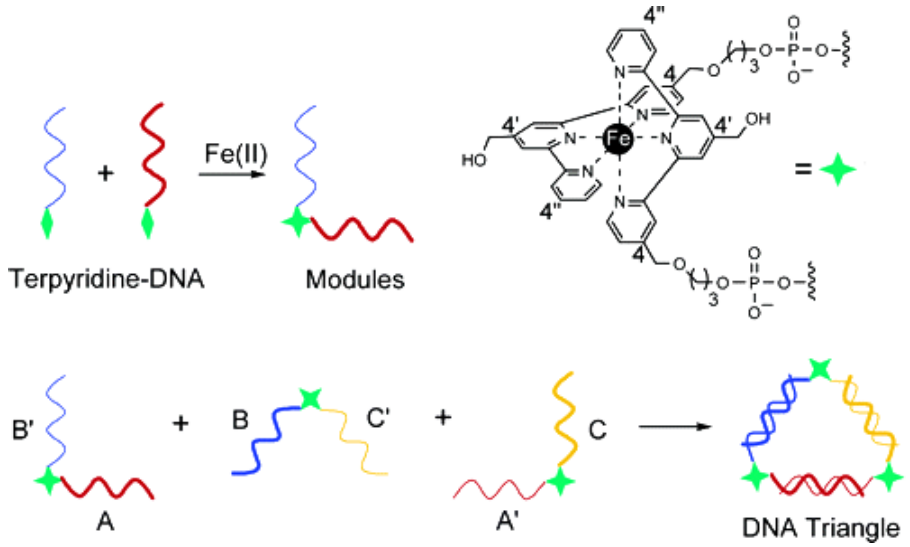


Figure 11. Preparation of metal-mediated DNA junction and its use for formation of DNA triangle.[26] Figure was adapted from [26].

Some metals showed reversible coordination and were used for building of dynamic DNA structures, such is an allosteric regulation of oligonucleotide hybridization of bis(terpyridine)-modified single-stranded DNA by Zn^{2+} (Figure 12).[27] In the absence of metal ion, modified DNA strand was hybridized with complementary strand. Addition of one equivalent of Zn^{2+} assisted the ring closure and off-regulates the hybridization, while excess of Zn^{2+} ion reestablished hybridization. Similar behavior was not observed when Fe^{2+} was used as a metal ion.

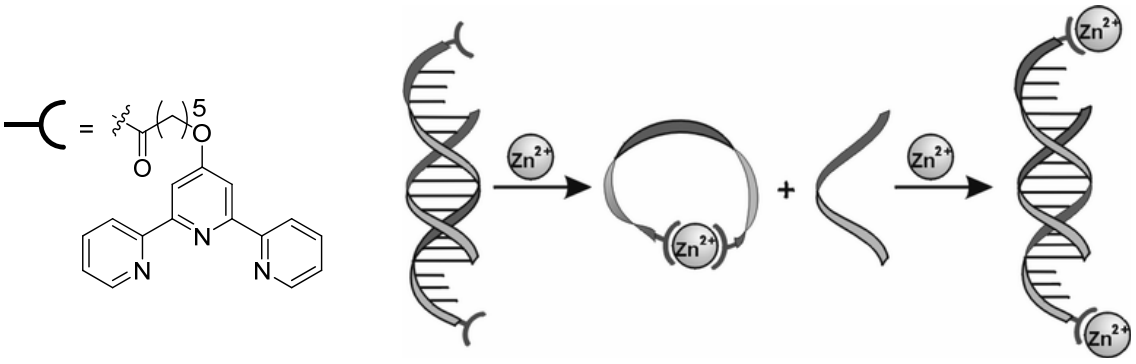


Figure 12. Allosteric control of oligonucleotide hybridization by Zn^{2+}. Figure was reproduced from [27].

Reversible coordination of metal ions was also reported by Wengel's group (Figure 13).[28] In order to affect the stability of DNA duplex, the terpyridine modification was placed in both strands of DNA duplex. Addition of one equivalent of Ni^{2+} resulted in inter-strand complexation of these strands and the complex formation was provided with extraordinary stabilization of DNA duplex (relative to non-metalated DNA duplex), while using an excess of metal ion leads to disassembling of this additional linkage and thermal stability was similar to non-metalated DNA duplex.

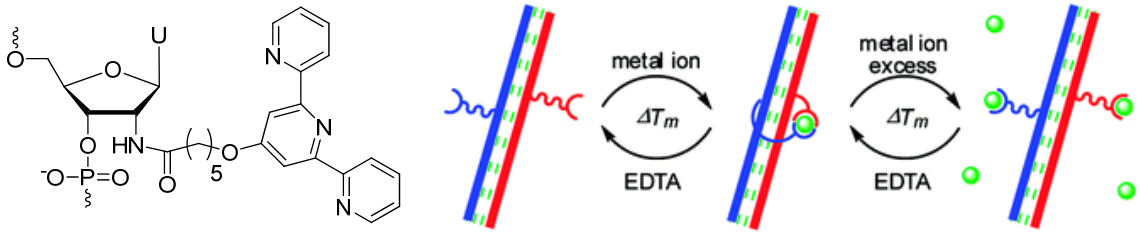


Figure 13. Modulation of DNA duplex stability by reversible coordination of Ni^{2+}.[28] Figure was reproduced from [28].

DNA-templated method to create metal-DNA branch points was reported by Sleiman *et al.*[29] To achieve this purpose, two complementary strands modified with bis(2,9-diphenyl)-1,10-phenantroline (dpp) at their 5' and 3' ends were synthesized. Hybridization and subsequent complexation with Cu^{+}, Cu^{2+} or Ag^{+} resulted in formation of metal-DNA junction characterized by enormous stability. The increase of melting temperature was 21 and 37 °C, respectively, depending on the metal used for complexation. It is interesting that incorporation of kinetically labile metals into the DNA vertices can so dramatically increase the stability of resulting structure. Except enhanced stability, chirality transfer from DNA duplex, which induced helical arrangements of the dpp ligands, was observed. Copper complex formed from this system can be reversibly oxidized and reduced between +1 and +2 oxidation states by adding oxidizing or reducing agent, respectively (Figure 14).

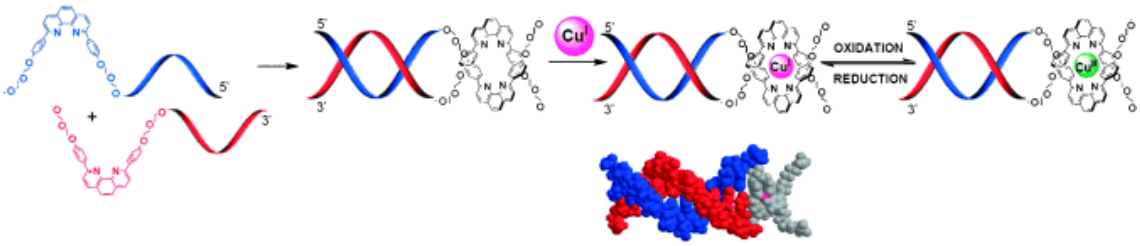


Figure 14. Formation of chiral metal DNA complex and its reversible switching between two redox states.[29] Figure was adapted from [29].

This approach was used also for formation of multinuclear structure, when three building blocks were used as templates for assembling of a DNA triangle. Using two external single-stranded oligonucleotides allows the structural switching of this triangle. The structure can be compress via hybridization with one of these single-strands and release by its re-hybridization with the second one (Figure 15).

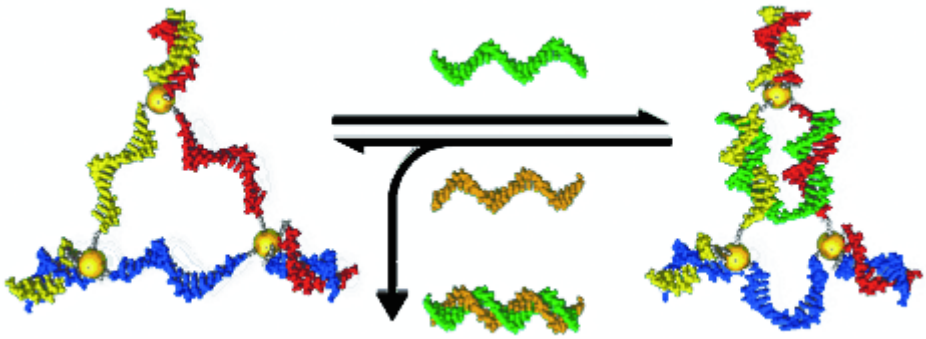


Figure 15. Dynamic switchable metal-mediated DNA triangle.[29] Figure adapted from [29].

Previously described approach allows formation of stable complexes with variety of metals (also with labile and reactive transition metals) due to stabilizing forces (π-π stacking of ligand and bases, metal coordination and ability of ligands to adopt the helicity of DNA duplex). Sleiman's group also achieved the selective incorporation of different transition metals into the DNA in site-specific manner.[30] For this purpose, different DNA-ligand environment had to be synthesized. By incorporation of terpyridine (tpy) and dpp into DNA strands, three different environments were created: **tpy$_2$:DNA**, **tpy:dpp:DNA**, and **dpp$_2$:DNA**. These three different DNA ligand environments showed strong preference for different metals. The **tpy$_2$:DNA** environment shows to be selective for Fe^{2+} ion, while **dpp$_2$:DNA** exhibits preference for Cu^{+} and mixed **tpy:dpp:DNA** prefer complex formation with Cu^{2+} ion (Figure 16).

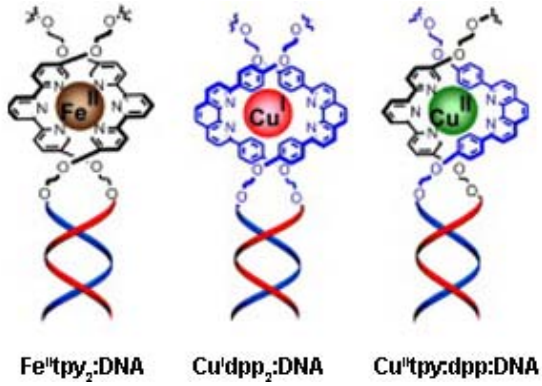


Figure 16. Selective coordination of specific metal ions into different DNA-ligand environments. Figure adapted from [13].

The 'error correction', if metal was placed into 'incorrect' environment, was also reported (Figure 17). For example, addition of Cu^{2+} to **Ag^{I}tpy:dpp:DNA** resulted in the replacement of Ag^{+} by Cu^{2+} as a preferred metal (Figure 17a). Interestingly, copper ion can change its oxidation state when is placed into 'incorrect' environment. Therefore, in **Cu^{I}tpy:dpp:DNA** Cu^{+} is oxidized to Cu^{2+}, which is the most preferred metal, and stable **Cu^{II}tpy:dpp:DNA** complex is clearly formed (Figure 17b). In contrast, Cu^{2+} placed in the **dpp$_2$:DNA** environment, which shows strong selectivity for Cu^{+}, undergoes spontaneous reduction to Cu^{+} (Figure 17c). Reorganization of ligand environment was observed when Fe^{2+} was placed in the **tpy:dpp:DNA** environment. In this case, Fe^{2+} ion is selectively bound to two terpyridine ligands in intermolecular fashion (Figure 17d).

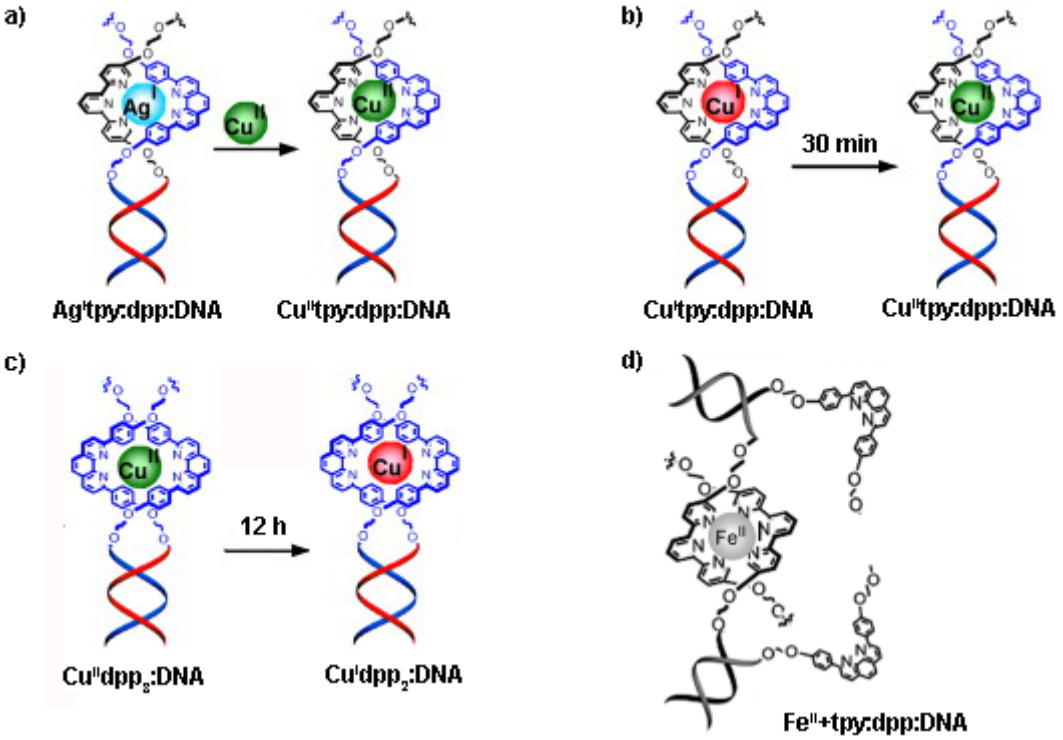


Figure 17. The examples of 'error correction': a) displacement of unfavored metal, b) spontaneous oxidation, c) spontaneous reduction, d) reorganization of coordination site. [30] Figure panels a, b, c were reproduced from [13], while figure d was adapted from [30].

This approach was also used for formation of higher ordered structures. While cyclic triangle (either simple or switchable) was the first reported 2D DNA assembly, the first 3D nanostructure is presented by metal-DNA cages (Figure 18), which are formed from six different oligonucleotides bearing dpp ligand.[31] Each three strands assemble into two triangular structures due to the complementary ending sequences. This assembling brings the dpp ligand in close proximity for further complexation. And these triangles assemble into a DNA prism by hybridization with three linking strands. The prism structure is fixed by hybridization of single-stranded arms with complementary strands. Like this prepared cage contains six dpp-dpp junctions prepared for site-specifically coordination of transition metals.

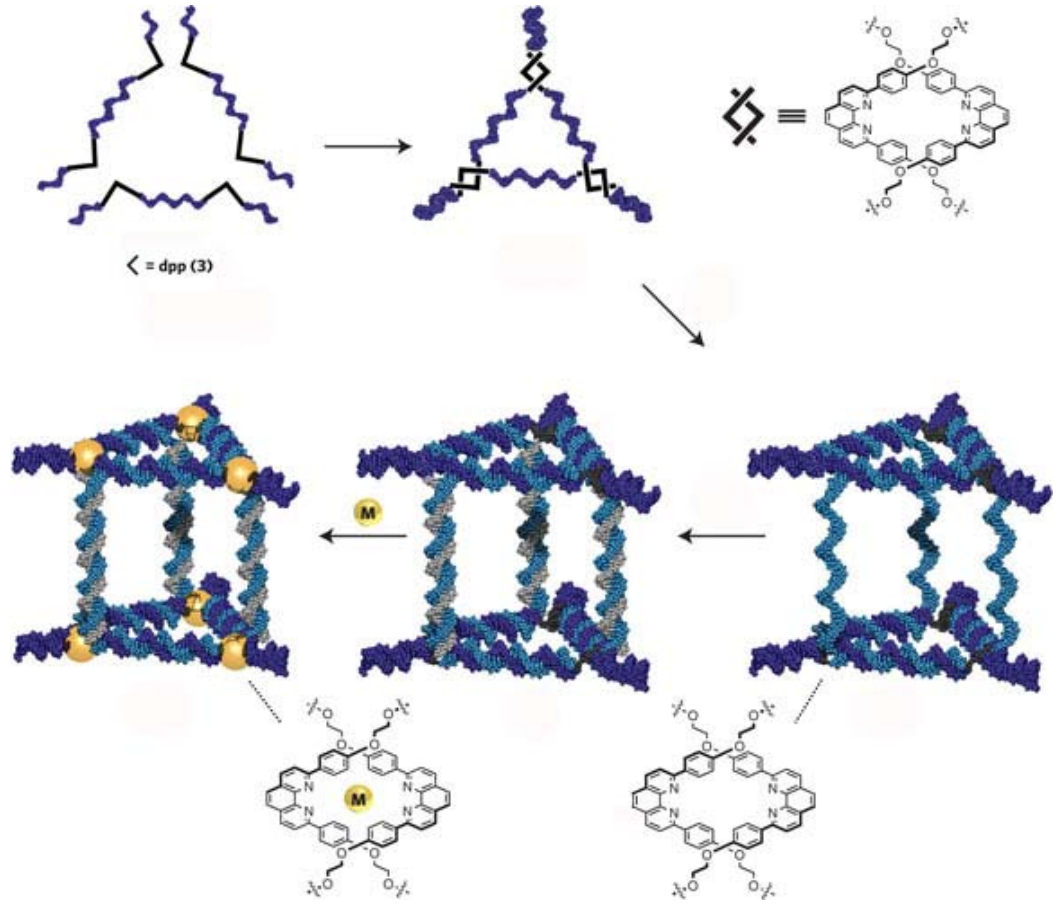


Figure 18. Schematic representation of formation of metal-DNA cage.[31] Figure was adapted from [31].

Self-assembly of metalated nanotubular structures was also successfully published. Sleiman *et al*.[32] first reported formation of four arm DNA junction with metal in its center, formed from two dpp-modified oligonucleotides (Figure 19a). Such metal-DNA junction was then used as building blocks for construction of DNA nanotubular assemblies, where three different four-arm junctions were designed to hybridize and form a cyclic structure. Resulting tubular structure arises from connecting cyclic structures by linking strands (Figure 19b). Formation of tubular structure was shown by atomic force microscopy (AFM) (Figure 19c), while the non-metalated triangles gave under the same conditions only globular structure (Figure 19d).

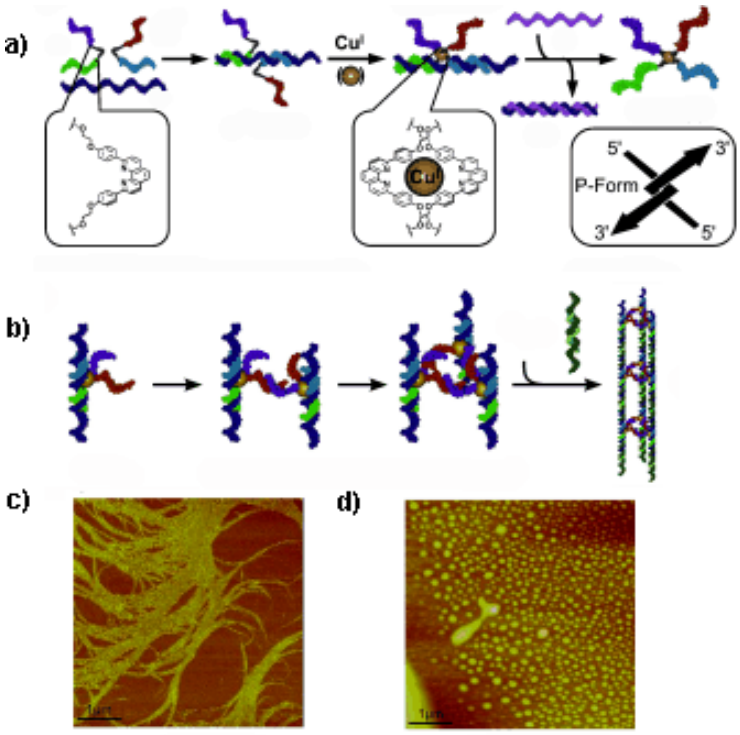


Figure 19. Assembly of a metal-DNA nanotubular structure: a) formation of four-arm metal-DNA junction, b) assembly of metal-DNA nanotube, c) AFM analysis of metal-DNA nanotube, d) AFM analysis of control sample without Cu^{I}. Figure panels were reproduced from [32].

Formation of tubular assemblies induced by metal coordination was published by Stulz *et al*.[33] For this purpose terpyridine-modified DNA was used. Formation of resulting tubular structure was detected by AFM (Figure 20).

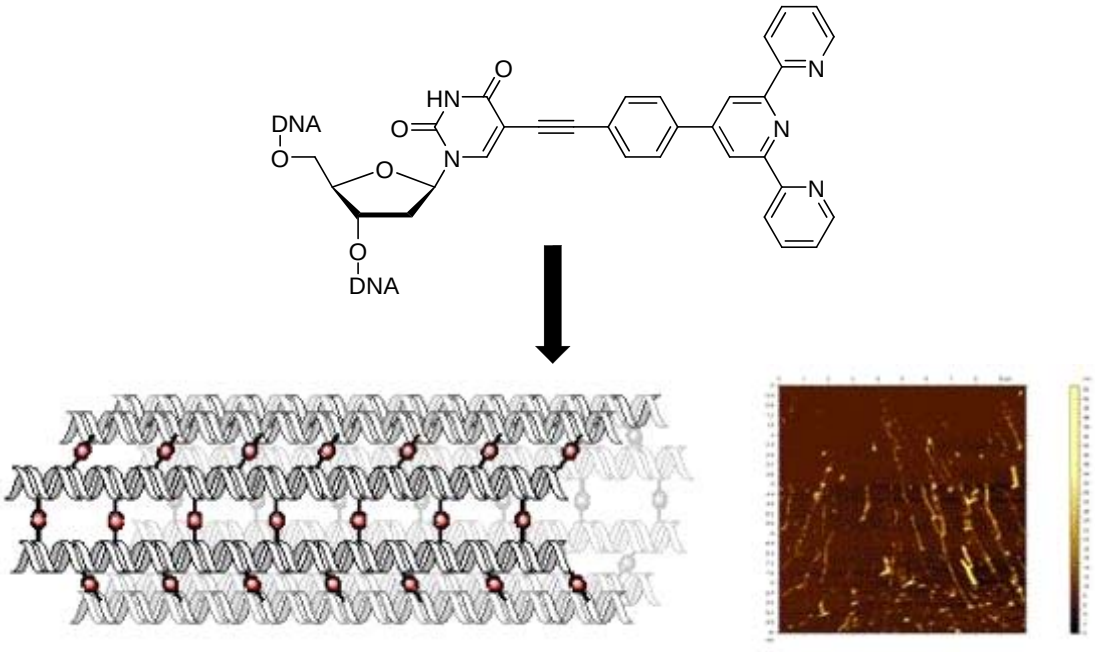


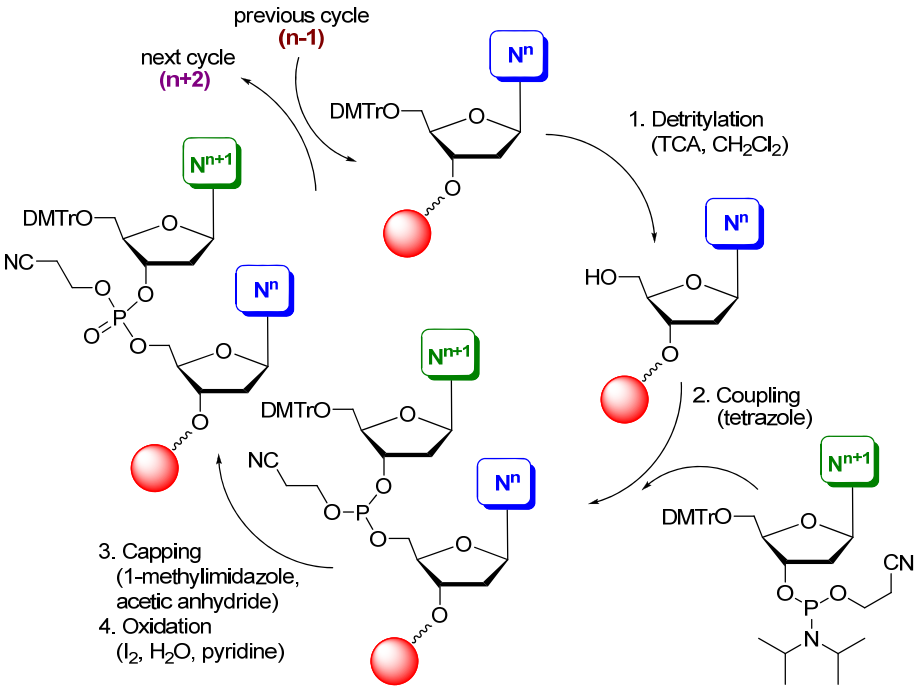
Figure 20. Metal-induced formation of nanotubular assemblies.[33] Figure was reproduced from [33].

1.3 DNA synthesis

To achieve metal-mediated DNA assemblies by any above mentioned approaches, modified DNA has to be synthesized. For this purpose chemical synthesis or enzymatic incorporation can be used and modifications can be attached either to phosphodiester backbone, sugar or nucleobase as it was already mentioned above (see chapter 1.2.1.2).

1.3.1 Chemical synthesis

For the synthesis of short oligonucleotides various approaches can be used. Older methods, such are H-phosphonate[34], phosphodiester[35], phosphotriester[36] or phosphite triester[37] method are used very rarely. Nowadays, the most common method for synthesis of oligonucleotides (even the modified ones) is solid-phase phosphoramidite method[38]. Synthesis of oligonucleotide, proceeds from 5' to 3' end, consist of four basic steps: detritylation of 5'-hydroxyl group, coupling with protected phosphoramidite, capping and oxidation, which are repeated until the oligonucleotide of required length and sequence is synthesized (Scheme 1). Resulting oligonucleotide is purified after deprotection and release from solid support.



Scheme 1. Phosphoramidite method for synthesis of oligonucleotides.

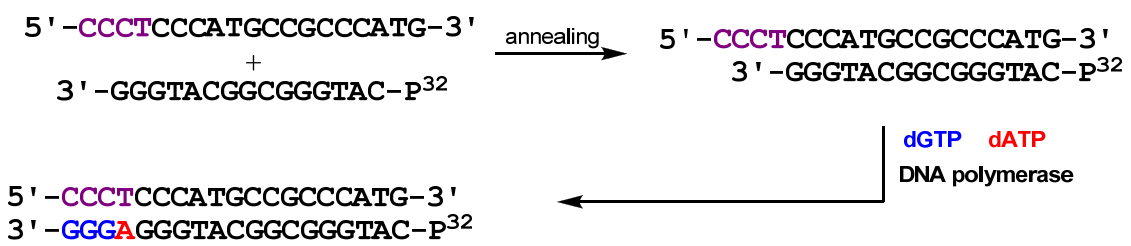
Limitation of this methodology is the requirement of very high yield for each coupling step. If 20-mer oligonucleotide is synthesized and the yield of each coupling step is 99.8%, the total yield will be 96%. But if the yield of each coupling step is 99%, total yield will be only 82% and if the yield of coupling step is only 90% the total yield will drop to 13%. The longer oligonucleotide is synthesized, the more significant decrease in total yield is observed (total yield of synthesis of 100-mer oligonucleotide with 99% coupling yields is only 37%). Therefore, this method is not used for synthesis of oligonucleotides longer then 100 bases.

Using this method for synthesis of modified oligonucleotides has some disadvantages. Starting modified phosphoramidites are available after several steps synthesis and they are quite unstable. But the most limiting factor is that not any modification can be used and the coupling yields of modified phosphoramidites are usually very low. The modification, which is used, has to be compatible with condition required for the synthesis (acidic detritylation, coupling, capping, oxidation and deprotection).

1.3.2 Enzymatic synthesis

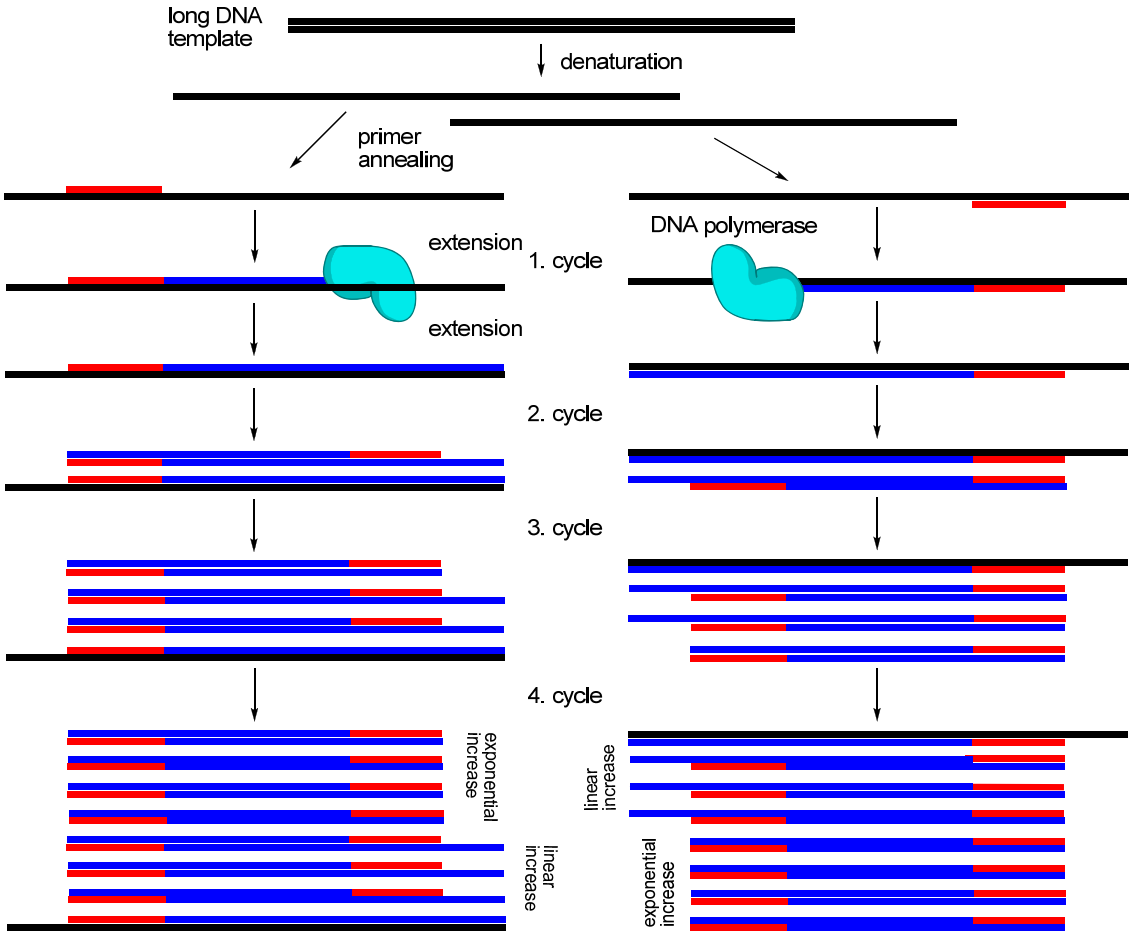
Alternative to the phosphoramidite synthesis of oligonucleotides is enzymatic synthesis using for incorporation of nucleoside triphosphates by DNA polymerases. Due to the high efficiency of each step, this method can be used also for preparation of long DNA sequences. Enzymatic synthesis of DNA involve as primer extension (PEX) as polymerase chain reaction (PCR).

In PEX the radio-labeled primer at its 5'-end is hybridized to the template and then is extended using DNA polymerase which incorporates natural and modified dNTPs according to the template (Scheme 2).



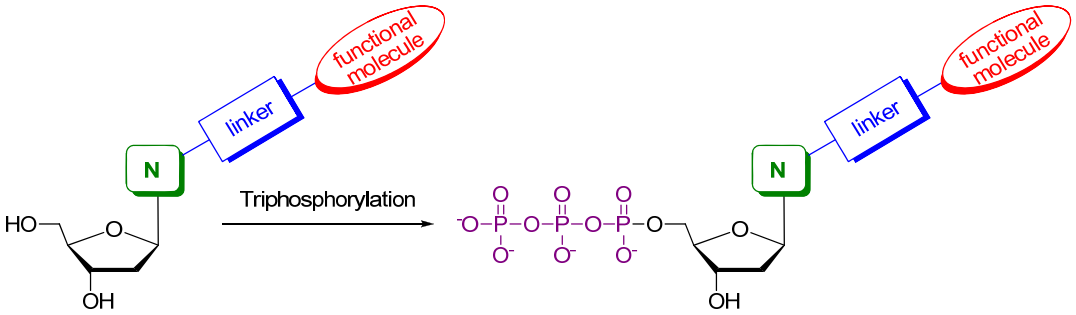
Scheme 2. Primer extension experiment.

PCR consists of several cycles and each cycle consists of three steps (Scheme 3). Each cycle starts by denaturation at 95 °C. While common proteins are irreversible denaturated under these conditions, new portion of polymerase had to be added until the discovery of thermophilic DNA polymerases. The second step is annealing of primers, which is carried usually at 55 °C. Afterwards, extension of primers by incorporation of nucleotides complementary to the template proceeds at 72 °C. PCR is utilized for synthesis of longer DNA in high amount due to the exponential increase of target sequences during this process.



Scheme 3. PCR amplification.

Both methods can be used also for synthesis of modified oligonucleotides, although the PCR is more challenging. The functionalized 2'-deoxyribonucleoside triphosphates ($dN^{R}TPs$), which are incorporated by DNA polymerases[39] in the absence of corresponding natural dNTP, can be easily prepared by triphosphorylation of previously prepared modified nucleosides (Scheme 4).



Scheme 4. Schematic representation of synthesis of modified dNTPs using classical triphosphorylation.

DNA polymerases have quite high tolerance of different functional molecules attached to the nucleobase of dNTPs. 5-substituted pyrimidine- and 7-substituted-7-deaza purine-analogs were shown as good substrates for DNA polymerases[40], while 8-modified purine analogs are not suitable substrates for enzymatic incorporation, due to the steric hindrance[41] (vide infra).

1.3.3 Application of enzymatic incorporation of modified triphosphates

An efficient method for synthesis of functionalized DNA based on the combination of single-step aqueous cross-coupling reaction of halogenated dNTPs with enzymatic incorporation by DNA polymerase developed in our group[42] was used for introducing variety of functional groups into DNA. As it was mentioned above, 8-modified purine analogs are not suitable substrates for DNA polymerase but 8-modified dATPs bearing relatively small substituents as bromine or methyl (Figure 21a) were successfully incorporated into DNA, while phenyl group is already too bulky and therefore is poor substrate for DNA polymerases.[43] Except these small substituents, bulky and hydrophobic groups such are bile acids[44] (Figure 21b) and reactive group as aldehyde[45] (Figure 21c) were also successfully used for functionalization of DNA.

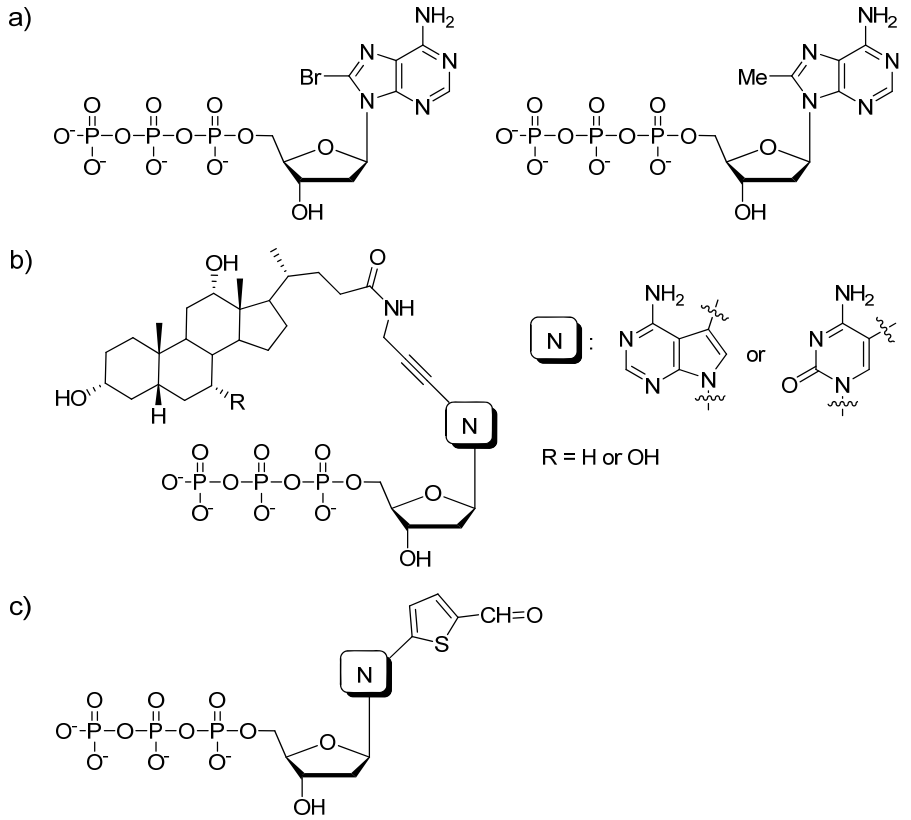


Figure 21. dNTPs modified with diverse functional group: a) 8-substituted dATPs bearing small substituents; b) bile acid-modified dNTPs; c) aldehyde-functionalized dNTPs

DNA bearing reactive groups such is bromine or aldehyde can be use for further post-synthetic modification, thus the aldehyde-functionalized DNA was used for DNA staining via conjugation with arylhydrazines and methodology of reductive amination was later used for conjugation with lysine a lysine-containing tripeptide[46] (Figure 22).

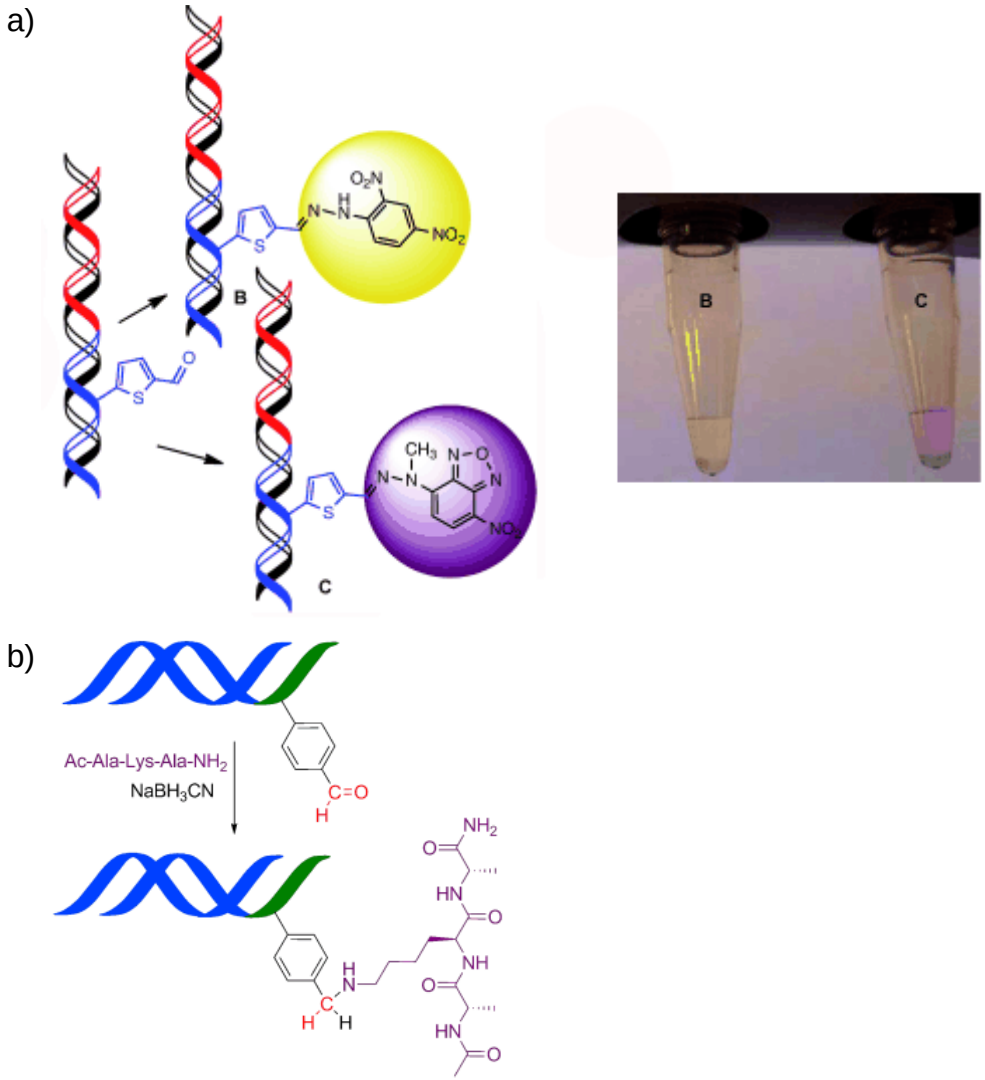


Figure 22. Post-synthetic modification of DNA bearing reactive group: a) conjugation with arylhydrazines used for DNA staining[45] (Figure was adapted from [45]), b) conjugation with peptide (Figure was reproduced from [46]).

Most of the modifications, which were introduced into DNA in our group, were used for labeling of DNA. Recently, synthesis and incorporation of biaryl-substituted dNTPs was published (Figure 23) in order to achieve pH-sensitive dual fluorescent and ^{19}F NMR labeling of DNA.[47]

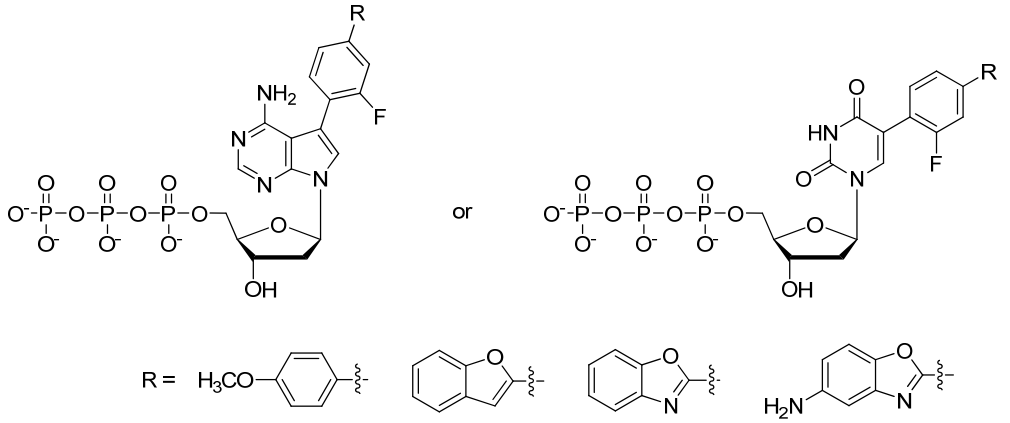


Figure 23. Biaryl-substituted dNTPs used for dual labeling of DNA.

Other modifications were studied as redox labels and used for development of multicolor labeling of DNA. Tetrathiafulvalene (TTF) label could not be used for electrochemical detection, due to its very weak redox signal.[48] Another big disadvantage of this label is an inhibition of DNA polymerases during the incorporation of TTF-modified dNTPs (Figure 24) which prevent the incorporation of more TTF-modifications into the DNA and increasing of redox signal.

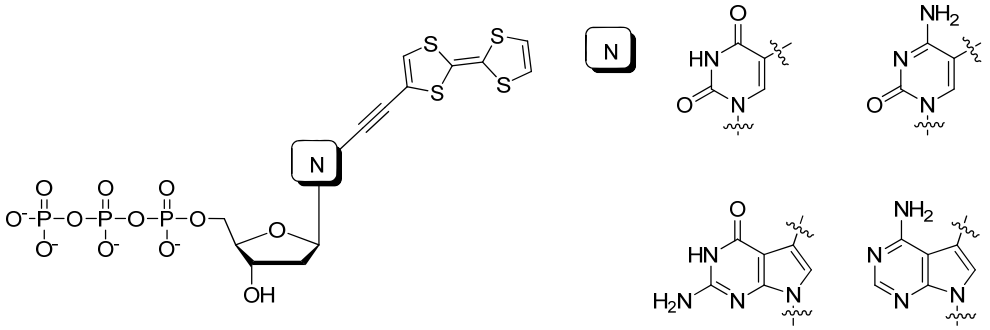


Figure 24. TTF-modified dNTPs.

On the other hand, aminophenyl- and nitrophenyl- modifications (Figure 25) showed to be suitable electrochemical labels, which can be perfectly distinguished if they are incorporated into the same DNA by oxidation (NH_2) or reduction (NO_2).[49]

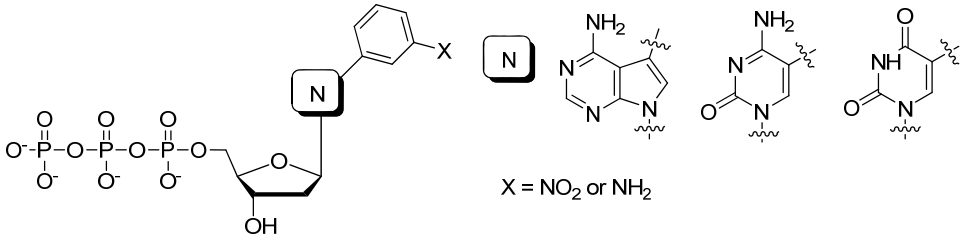


Figure 25. Aminophenyl- and nitrophenyl-labelled dNTPs.

In addition to nitrophenyl tag, antraquinone-labelled (AQ) DNA was prepared by enzymatic incorporation of AQ-labelled dNTPs (Figure 26).[50] Since the reduction of NO_2 is irreversible and produces no oxidation signal which could interfere with that of AQH_2 oxidation and can be easily detected through oxidation of hydroxylamine as a product of reduction of NO_2, both labels can be easily distinguish if they are present in the same DNA and therefore can be used for site-specific multiple labeling.

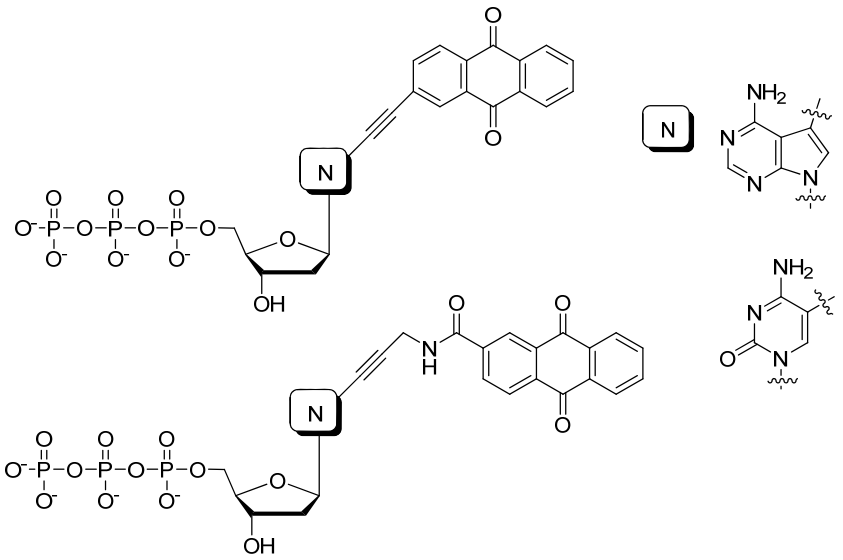
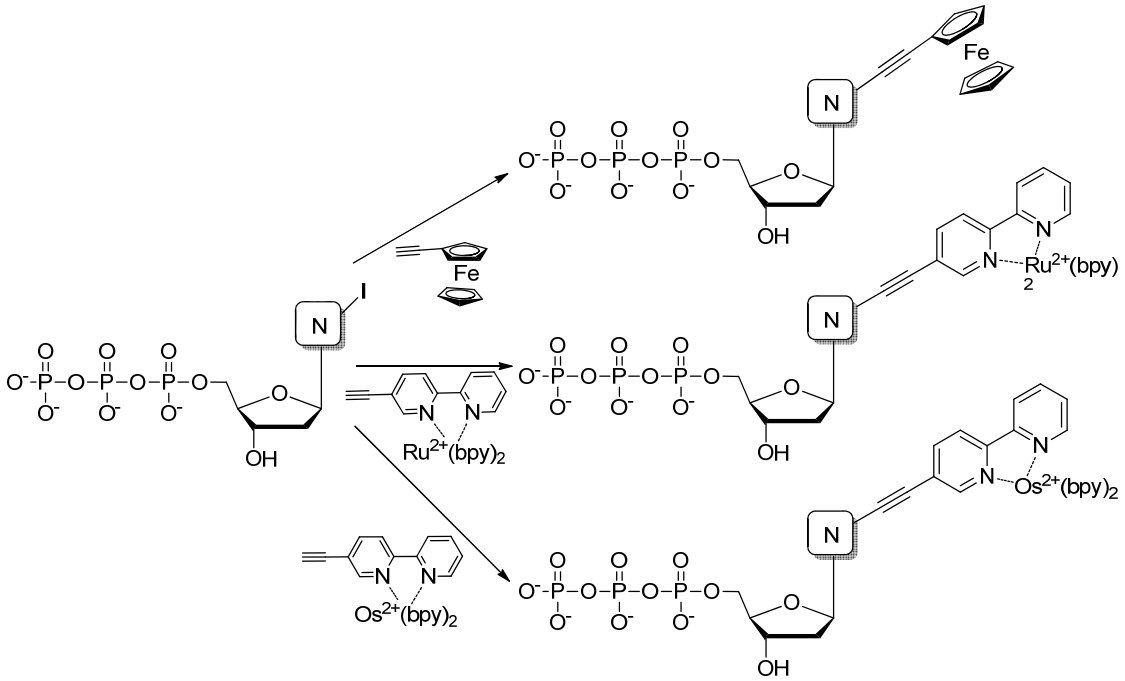


Figure 26. Antraquinone-labelled dNTPs.

Several metal complexes, such are ferocene, $[Ru(bpy)_3]^{2+}$ or $[Os(bpy)_3]^{2+}$ were studied as electrochemical labels.[11a,51] For the synthesis of such modified DNA, straightforward synthesis, based on enzymatic incorporation of metalated dNTPs, was successfully used. Corresponding dNTPs were prepared by single-step aqueous Sonogashira cross-coupling reaction with terminal acetylene derivatives of the complexes (Scheme 5). In the case of Ru- and Os- complexes, direct attachment of whole metal complex was preferred to attachment of metal chelating group followed by post-synthetic complexation, due to the harsh conditions required for the complexation.



Scheme 5. Synthesis of dNTPs bearing metal complexes via aqueous Sonogashira cross-coupling reaction.[11a,51]

As it was mentioned before (see chapters 1.2.1.2 and 1.2.1.3), this approach is not compatible with introducing of labile metals. These can be introduced by post-synthetic complexation of DNA bearing metal-chelating group with corresponding metal ions. And this approach will be more closely discussed in this thesis.

2 Specific aims of the thesis

1. Synthesis of nucleosides and nucleotides bearing oligopyridine ligands attached via rigid (acetylene or phenylene) linker as a model compound.
2. Development of methodology for post-synthetic complexation of nucleoside monophosphates bearing oligopyridine ligands attached via acetylene linker with diverse transition metals.
3. Synthesis of corresponding acetylene linked oligopyridine modified nucleoside triphosphates.
4. Study on enzymatic incorporation of oligopyridine modified $dN^{ER}TPs$ into DNA including simple kinetic studies.
5. Study of post-synthetic complexation of DNA bearing oligopyridine ligands attached via acetylene linker.
6. Synthesis of nucleosides and nucleotides bearing oligopyridine ligands attached via flexible octadiyne linker as a model compound.
7. Development of methodology for post-synthetic complexation of modified nucleosides bearing oligopyridine ligands attached via octadiyne linker with diverse transition metals.
8. Synthesis of corresponding octadiyne linked oligopyridine modified nucleoside triphosphates.
9. Study on enzymatic incorporation of oligopyridine modified $dN^{OR}TPs$ into DNA including simple kinetic studies.
10. Study of post-synthetic complexation of oligopyridine modified DNA bearing oligopyridine ligands attached via octadiyne linker.

Rationale of the specific aims

Oligopyridine ligands are commonly used in coordination chemistry and are widely used for construction of molecular and supramolecular devices. The direct synthesis of purine and 7-deazapurine nucleosides bearing oligopyridine ligands attached via acetylene or phenylene linker from halogenated nucleosides was previously published.[54,55] To extend the methodology of direct functionalization to pyrimidine nucleosides, my first task was to synthesized 2'-deoxyuridine and 2'-deoxycytidine

bearing bipyridine and terpyridine ligands attached via acetylene or phenylene tether. Since such base modification should be compatible also with enzymatic synthesis of modified DNA, my second task was to prepare base-modified nucleoside triphosphates bearing oligopyridine ligands and to incorporate them into DNA. Oligopyridine modified DNAs are widely used in post-synthetic complexation with diverse transition metals, but most of modified oligonucleotides have the modification placed in phosphate backbone via flexible linker. The attachement of modification to the nucleobase allows internal as well as terminal modification and thus formation of more variable metal-mediated DNA assemblies. DNA bearing oligopyridine ligands attached via short and rigid acetylene linker allows only inter-strand side by side complexation. Using long and flexible linker might enable also intra-strand complex formation and therefore analogues bearing oligopyridine ligands attached via acetylene and octadiyne linker were prepared. The DNA, prepared by incorporation of corresponding modified triphosphates, was intended for the post-synthetic complexation studies.

3 Results and discussion

3.1 Oligopyridine analogues with rigid (acetylene) linker

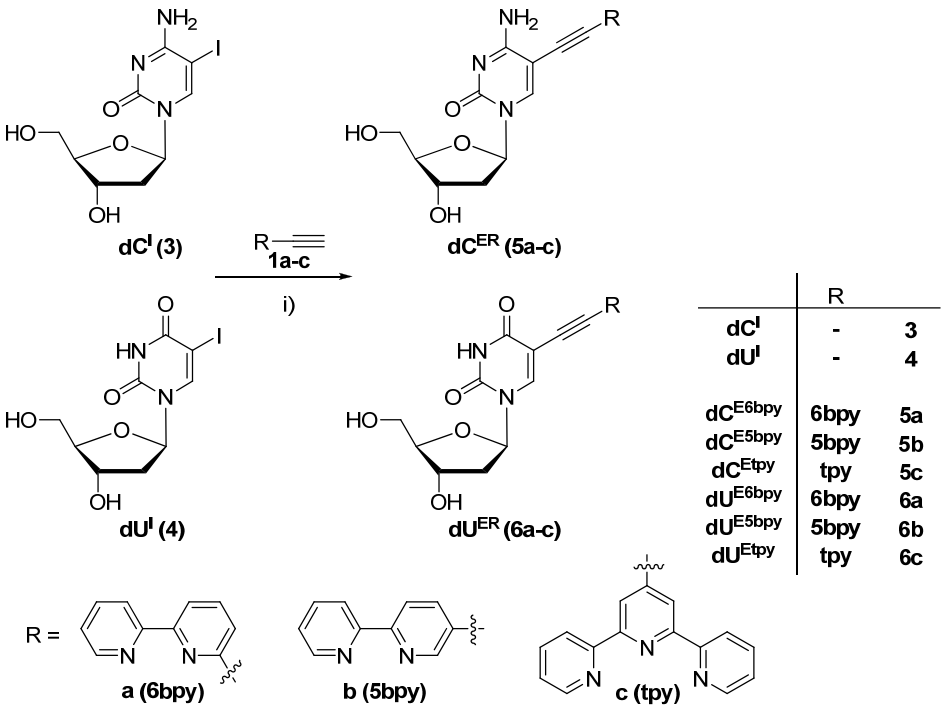
3.1.1 Synthesis of modified nucleosides

For synthesis of modified nucleosides two approaches can be used. The first one is a traditional method, where protection of halogenated nucleosides followed by cross-coupling reaction and subsequent deprotection of modified nucleosides is required. Second approach is direct protection-free approach using hydrophobic catalytic system capable of aqueous-phase cross-coupling reaction developed by Shaughnessy *et al.*[52] Water-soluble tris(3-sulfonatophenyl)phosphine (TPPTS) ligand in combination with $Pd(OAc)_2$ was shown as an efficient and general catalytic system in cross-coupling reactions at mild conditions.[52,53] It was also used for previously published synthesis of 8-substituted 2'-deoxyadenosine nucleosides[54] and 7-substituted 7-deaza-2'-deoxyadenosine nucleosides[55] bearing oligopyridine ligands attached via acetylene or phenylene tether from corresponding unprotected halogenated nucleosides via Sonogashira or Suzuki cross-coupling reaction. Therefore my first task was the synthesis of unprotected 5-modified pyrimidine (2'-deoxyuridine and 2'-deoxycytidine) nucleosides.

Cross-coupling reactions of commercially available 5-iodo-2'-deoxycytidine (**dC^I; 3**) and -deoxyuridine (**dU^I; 4**) with the corresponding oligopyridine building blocks were studied. Reagents of choice for Sonogashira coupling were terminal acetylenes **1a-c**[56] bearing bypiridine (bpy) attached via position 5 or 6 or terpyridine (tpy) ligand, while corresponding 4-(pinacolboronato)phenyl derivatives (**2a-c**) were used for Suzuki-Miyaura coupling. Both types of oligopyridine building blocks **1a-c** and **2a-c** were prepared according to the literature procedure.

The Sonogashira cross-couplings of iodinated pyrimidine nucleosides **dC^I** (**3**) and **dU^I** (**4**) with terminal acetylene **1a-c** (Scheme 6, Table 1) proceeded under previously developed conditions using 5 mol% of $Pd(OAc)_2$, TPPTS, CuI and Hünig's base. The reactions were performed in DMF, which was published as more suitable solvent than mixture water/acetonitrile.[55,57] To reach full conversion, the reaction mixture was heated at 75 °C for 2 hours. The products **dC^{ER}** (**5a-c**) and **dU^{ER}** (**6a-c**) were isolated after the reverse phase column chromatography in the yields of 63-75%. Considering,

that synthesis of target oligopyridine modified nucleosides **dC^{ER}** (**5a-c**) and **dU^{ER}** (**6a-c**) is just a single-step procedure and protection and subsequent deprotection steps were successfully avoided, the yields are satisfactory.



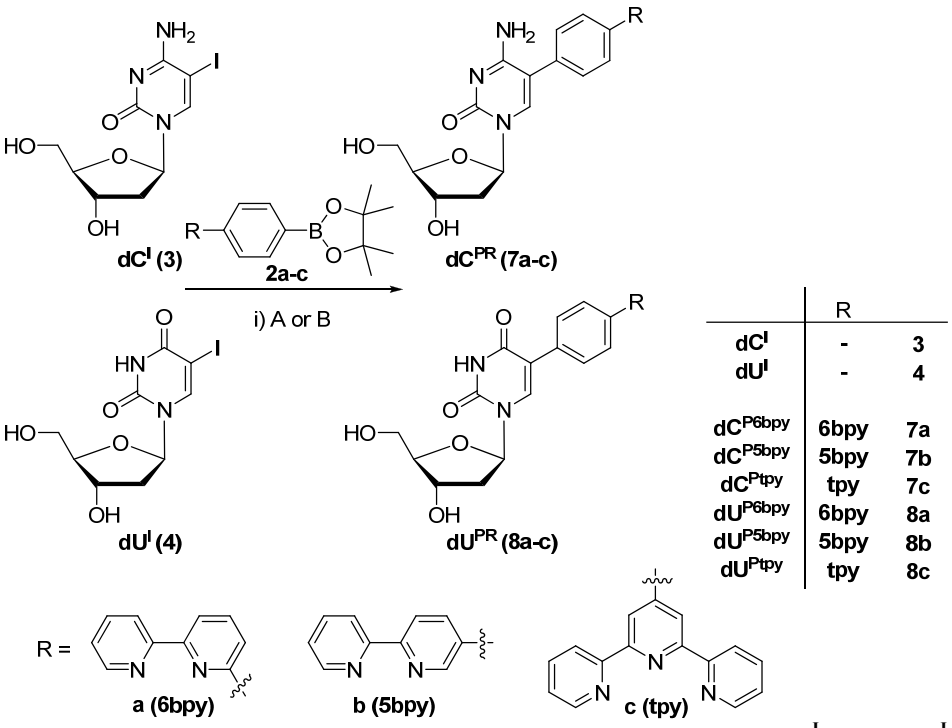
Scheme 6. The Sonogashira cross-coupling reaction of unprotected nucleosides **dC^{I}** (**3**) and **dU^{I}** (**4**) with terminal acetylene **1a-c**. *Reagents and conditions*: i) $Pd(OAc)_2$ (5 mol%), TPPTS (2.5 equiv. to Pd), CuI (10 mol%), Et(*i*-Pr)$_2$N (10 equiv.), DMF, 75 °C, 2 h.

Table 1. Sonogashira cross-coupling reaction of nucleosides **dC^{I}** (**3**) and **dU^{I}** (**4**) with alkynes **1a-c**.

Entry	Nucleoside	Alkyne	Product	Yield (%)
1	**dC^{I} (3)**	**1a**	**dC^{E6bpy} (5a)**	70
2	**dU^{I} (4)**	**1a**	**dU^{E6bpy} (6a)**	75
3	**dC^{I} (3)**	**1b**	**dC^{E5bpy} (5b)**	67
4	**dU^{I} (4)**	**1b**	**dU^{E5bpy} (6b)**	70
5	**dC^{I} (3)**	**1c**	**dC^{Etpy} (5c)**	63
6	**dU^{I} (4)**	**1c**	**dU^{Etpy} (6c)**	67

The second class of target compounds were the corresponding phenylene linked conjugates **dC^{PR}** (**7a-c**) and **dU^{PR}** (**8a-c**). First, the Suzuki-Miyaura cross-coupling reactions of haloganated nucleosides **dC^{I}** (**3**) and **dU^{I}** (**4**) with boronates **2a-c** were performed under previously developed aqueous-phase conditions[54,57,58] using 5 mol% of $Pd(OAc)_2$, TPPTS, and Cs_2CO_3 in the mixture water/acetonitrile (2:1). The reaction mixture was heated at 80 °C (Scheme 7, condition A; Table 2). While the products **dN^{P6bpy}** (**7a** and **8a**) were isolated in acceptable 60-65% yields (entries 1-2), products **dN^{P5bpy}** (**7b** and **8b**) and **dN^{Ptpy}** (**7c** and **8c**) were obtained in very low yields (entries 3-6), due to very slow reaction of boronates **2b** and **2c** and from this resulted

incomplete conversion of starting material. As the prolongation of reaction time did not resulted in full conversion of starting material, the condition of the Suzuki reaction were further optimized.



Scheme 7. Suzuki-Miayaura cross-coupling reaction of unprotected nucleosides **dC^{I}** (**3**) and **dU^{I}** (**4**) with boronates **2a-c**. *Reagents and conditions*: i) A: $Pd(OAc)_2$ (5 mol%), TPPTS (2.5 equiv. to Pd), Cs_2CO_3 (3 equiv.), H_2O/ CH_3CN (2:1), 80 °C; B: $Pd(OAc)_2$ (10 mol%), TPPTS (5 equiv. to Pd), Cs_2CO_3 (3 equiv.), H_2O/ CH_3CN (1:2), 90 °C.

Table 2. The Suzuki-Miyaura cross-coupling reaction of nucleosides **dC^{I}** (**3**) and **dU^{I}** (**4**) with boronates **2a-c** under the condition A.

Entry	Nucleoside	Boronate	Product	Yield (%)
1	**dC^{I} (3)**	**2a**	**dC^{P6bpy} (7a)**	65
2	**dU^{I} (4)**	**2a**	**dU^{P6bpy} (8a)**	60
3	**dC^{I} (3)**	**2b**	**dC^{P5bpy} (7b)**	12
4	**dU^{I} (4)**	**2b**	**dU^{P5bpy} (8b)**	7
5	**dC^{I} (3)**	**2c**	**dC^{Ptpy} (7c)**	28
6	**dU^{I} (4)**	**2c**	**dU^{Ptpy} (8c)**	24

First, several sources of Pd in combination with different ligands successfully used in other Suzuki reactions have been tested for the reaction of **dC^{I}** (**3**) with boronate **2c**. The reactions using $Pd(PPh_3)_4$ or $Pd(OAc)_2$ in combination with Buchwald's ligands (S-Phos,[59] dicyclohexylphosphinobiphenyl[60]) or catalytic system prepared from Na_2PdCl_4 and disulfonated fluorenyldialkylphosphine[61] (Figure 27) using different bases (Cs_2CO_3, NaOH, K_2CO_3, K_3PO_4) did not bring any improvement. The yields varied from 0 to 15%. All results are summarized in Table 3.

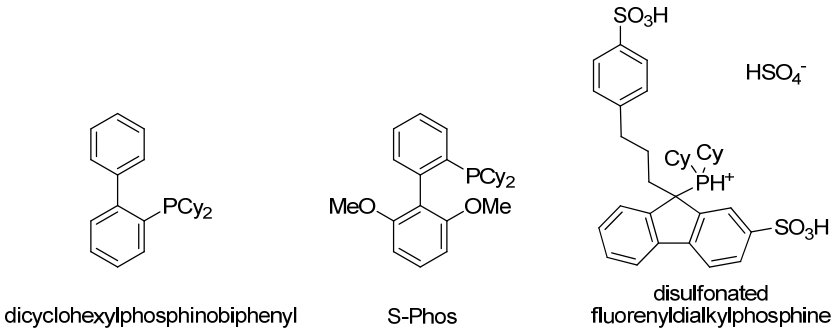


Figure 27. Structure of ligands used for optimization of Suzuki-Miyaura cross-coupling reaction of nucleoside **dC^{I}** (**3**) with boronate **2c**.

Table 3. Optimization of Suzuki-Miyaura cross-coupling reaction of nucleoside **dC^{I}** (**3**) with boronate **2c**.

Catalyst	Ligand	Base	Solvent	Yield
$Pd(PPh_3)_4$	-	NaOH	THF/H_2O/MeOH (2:1:2)	6%
$Pd(PPh_3)_4$	-	NaOH	CH_3CN/H_2O (1:2)	0%
$Pd(PPh_3)_4$	-	Cs_2CO_3	CH_3CN/H_2O (1:2)	15%
$Pd(PPh_3)_4$	-	K_2CO_3	DMF/H_2O (8:1)	0%
$Pd(OAc)_2$	dicyclohexylphosphinobiphenyl	K_3PO_4	Dioxane	0%
$Pd(OAc)_2$	S-Phos	K_2CO_3	CH_3CN/H_2O (1:2)	0%
Na_2PdCl	$PropPhenFluPCy_2DS.H_2SO_4$	K_2CO_3	H_2O/*n*-butanol	12%

Therefore, in further optimization experiments, examined for the reaction of **dC^{I}** (**3**) with boronate **2b,** was used the original catalytic system $Pd(OAc)_2$/TPPTS. Different ratio of reagents and catalyst as well as different reaction conditions was tested. When previously published conditions[54,57,58] were applied, product **dC^{P5bpy}** (**7b**) was observed in very low yields of 12 % (Table 4, entry 1). When higher amount of boronate **2b** (2 equiv.) or base (5 equiv.) was used, product **dC^{P5bpy}** (**7b**) was isolated in yield of 14% or 7%, respectively (Table 4, entries 3 and 6). Changing solvent to DMF did not lead to any product formation (Table 4, entry 2), but when higher catalyst loading or higher temperature in combination with different ratio of solvents was used, the product

dC^{P5bpy} (**7b**) was isolated in somewhat higher yield of 21% (entries 4-5) or 33% (entry 7), respectively. The highest yield of **dC**^{P5bpy} (**7b**), 55 %, was observed, when 10 mol% of the catalyst in combination with higher amount of TPPTS ligand (5 equiv. to Pd), higher temperature (90 °C) and water/acetonitrile (1:2) mixture was used (entry 8). All the results are summarized in the Table 4.

Table 4. Optimization of Suzuki-Miyaura cross-coupling reaction of nucleoside **dC**^{I} (**3**) with boronate **2b**.

Entry	**2b** (equiv.)	$Pd(OAc)_2$ (mol %)	TPPTS (equiv. to Pd)	Cs_2CO_3 (equiv.)	Temperature	Solvent	Yield
1	1.2	5	2.5	3	80 °C	H_2O/MeCN (2:1)	12%
2	1.2	5	2.5	3	80 °C	DMF	0%
3	2	5	2.5	3	80 °C	H_2O/MeCN (2:1)	14%
4	1.2	10	2.5	3	80 °C	H_2O/MeCN (2:1)	21%
5	1.2	5	5	3	80 °C	H_2O/MeCN (2:1)	21%
6	1.2	5	2.5	5	80 °C	H_2O/MeCN (2:1)	7%
7	1.2	5	2.5	3	90 °C	H_2O/MeCN (1:2)	33%
8	1.2	10	5	3	90 °C	H_2O/MeCN (1:2)	55%

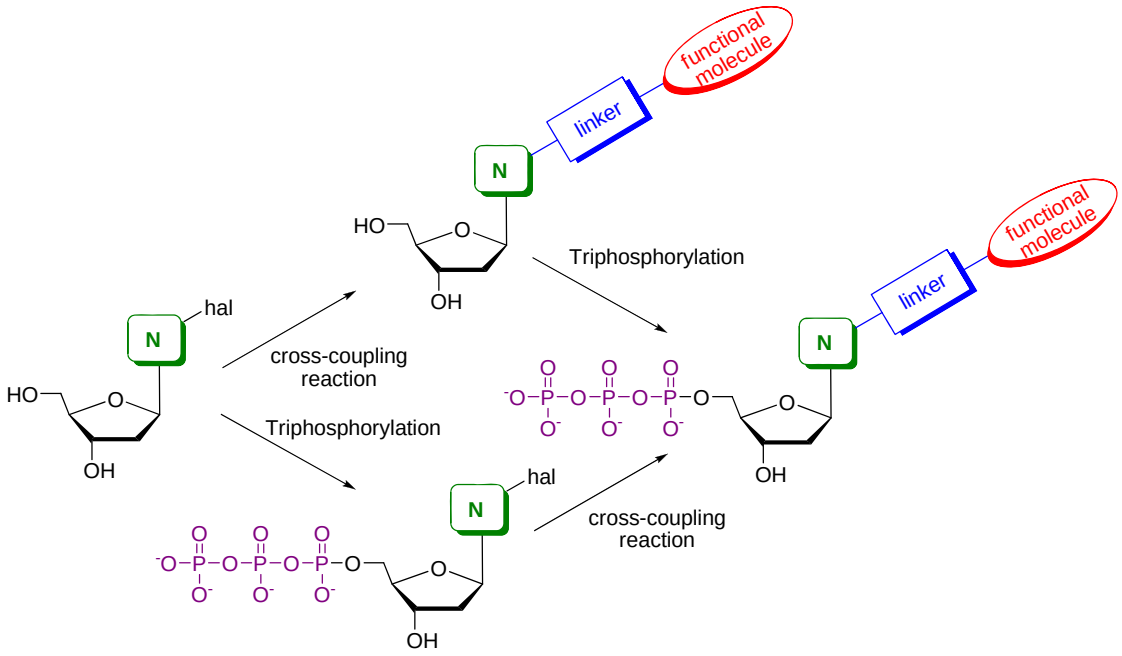
These final optimized conditions were then applied for synthesis of whole series of phenylene linked conjugates **dN**^{PR} (**7a-c** and **8a-c**) (Scheme 7, conditions B; Table 5). In all cases, the yields of modified **dN**^{PR} (**7a-c** and **8a-c**) were substantially improved compared to the reactions where conditions A were applied. While the reaction of 6bpy-boronate **2a** and tpy-boronate **2c** gave full conversion and satisfactory isolated yields of corressponding products **dN**^{P6bpy} (**7a** and **8a**) and **dN**^{Ptpy} (**7c** and **8c**) (70-75%), the conversion of 5bpy-boronate **2b** was incomplete and therefore the products **dN**^{P5bpy} (**7b** and **8b**) were isolated in moderate yields of 55 or 35 %, respectively.

Table 5. The Suzuki-Miyaura cross-coupling reaction of nucleosides **dC^{I}** (**3**) and **dU^{I}** (**4**) with boronates **2a-c** under the conditions B.

Entry	Nucleoside	Boronate	Product	Yield (%)
1	**dC^{I} (3)**	**2a**	**dC^{P6bpy} (7a)**	75
2	**dU^{I} (4)**	**2a**	**dU^{P6bpy} (8a)**	75
3	**dC^{I} (3)**	**2b**	**dC^{P5bpy} (7b)**	55
4	**dU^{I} (4)**	**2b**	**dU^{P5bpy} (8b)**	35
5	**dC^{I} (3)**	**2c**	**dC^{Ptpy} (7c)**	70
6	**dU^{I} (4)**	**2c**	**dU^{Ptpy} (8c)**	70

3.1.2 Synthesis of modified nucleotides and dNTPs

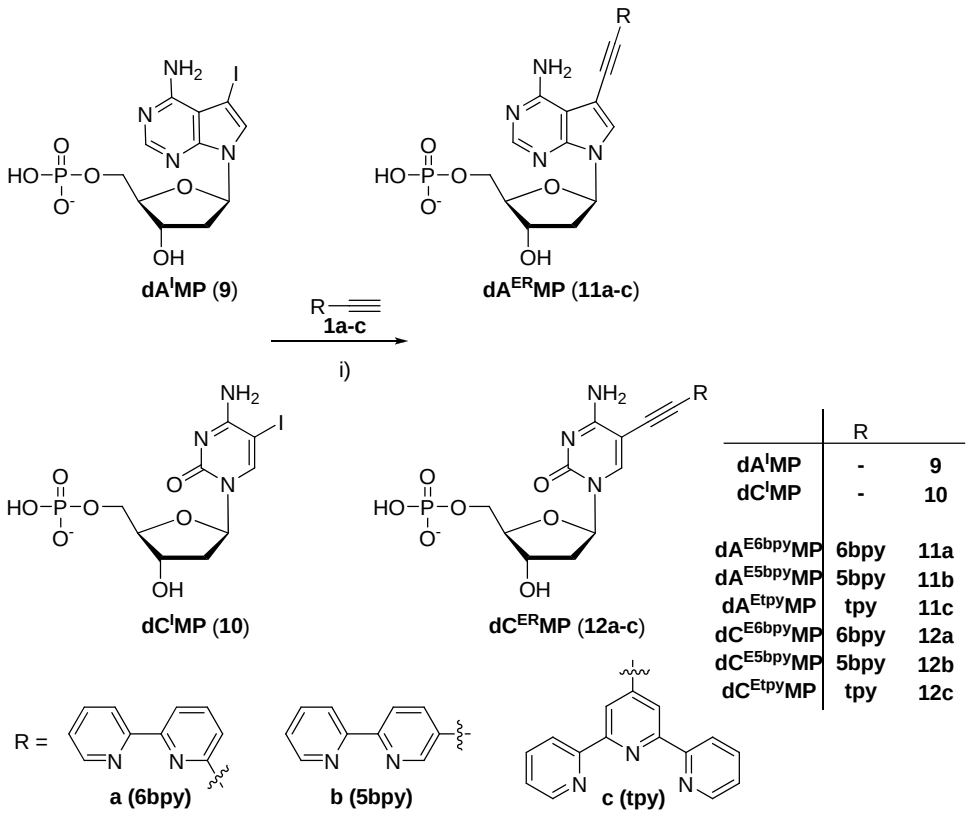
In order to prepare modified oligonucleotides via enzymatic incorporation, oligopyridine ligands had to be attached to the nucleobase in dNTPs via suitable linker (in our case rigid, linear and electron-conjugated acetylene linker). In order to prepare modified dNTPs bearing oligopyridine ligands, classical triphosphorylation of modified nucleosides can be used. But triphosphorylation reaction is very sensitive to substrate and even small changes on a base can cause different reactivity of the substrate and from this resulted difficult optimization of reaction conditions. The second approach for synthesis of modified dNTPs, based on aqueous-phase cross-coupling reactions of halogenated dNTPs, was for first time published by Thoresen *et al.*[62], who describe the synthesis of thymidine triphosphates bearing fluorescein dye via Sonogashira cross-coupling reaction of 5-iodo-2'-deoxy- and 5-iodo-2',3'-dideoxyuridine triphosphate. This two-step approach, consisting of triphosphorylation of halogenated nucleoside followed by cross-coupling reaction, was extended in our group for Sonogashira and Suzuki cross-coupling reactions of 5-iodo-2'-deoxyuridine-, 5-iodo-2'-deoxycytidine- and 7-iodo-7-deaza-2'-deoxyadenosine triphosphates and used for attachement of numbers of different functional groups (see chapter 1.3.3). Both methods for synthesis of modified dNTPs are schematically depicted in Scheme 8. It is very well known, that dNTPs are unstable at high temperature or in acidic conditions, when undergo the hydrolysis to di- and monophosphates. Cross-coupling reactions do not require extremely high temperature and reaction is always stopped after one hour to avoid the hydrolysis of starting and final triphosphates, even if the consumption of starting material is not finished.



Scheme 8. Schematic representation of synthesis of modified dNTPs using cross-coupling reactions.

To develop the synthetic methodology and further complexation of ONs, the chemistry was first performed on model nucleosides monophosphates (dNMPs). The targets of our choice were 7-substituted 7-deaza-2'-deoxyadenosine [**dA^{ER}MP** (**11a-c**)] and 5-substituted 2'-deoxycytidine 5'-monophosphates [**dC^{ER}MP** (**12a-c**)], since the corresponding triphosphates are known as good substrates for DNA polymerases.[39c,40a,63,]

Reaction conditions, employing catalytic system formed from water-soluble TPPTS ligand and $Pd(OAc)_2$, used for synthesis of oligopyridine modified nucleosides were also successfully applied for synthesis of corresponding nucleosides monophosphates and triphosphates in aqueous conditions. Oligopyridine modified nucleotides were prepared by Sonogashira cross-coupling reaction of halogenated nucleoside monophosphates **dA^{I}MP** (**9**) and **dC^{I}MP**[64] (**10**) (prepared according to the literature procedure) with terminal acetylene **1a-c** in the presence of $Pd(OAc)_2$, TPPTS, CuI and Hünig's base in the mixture water/acetonitrile (2:1) at 80 °C for 1.5 h (Scheme 9, Table 6). Desired corresponding products **dA^{E6bpy}MP** (**11a**), **dA^{E5bpy}MP** (**11b**), **dA^{Etpy}MP** (**11c**), **dC^{E6bpy}MP** (**12a**), **dC^{E5bpy}MP** (**12b**), **dC^{Etpy}MP** (**12c**) were easily isolated after the purification on reverse-phase HPLC in good yields (from 47% to 89%, Table 6).



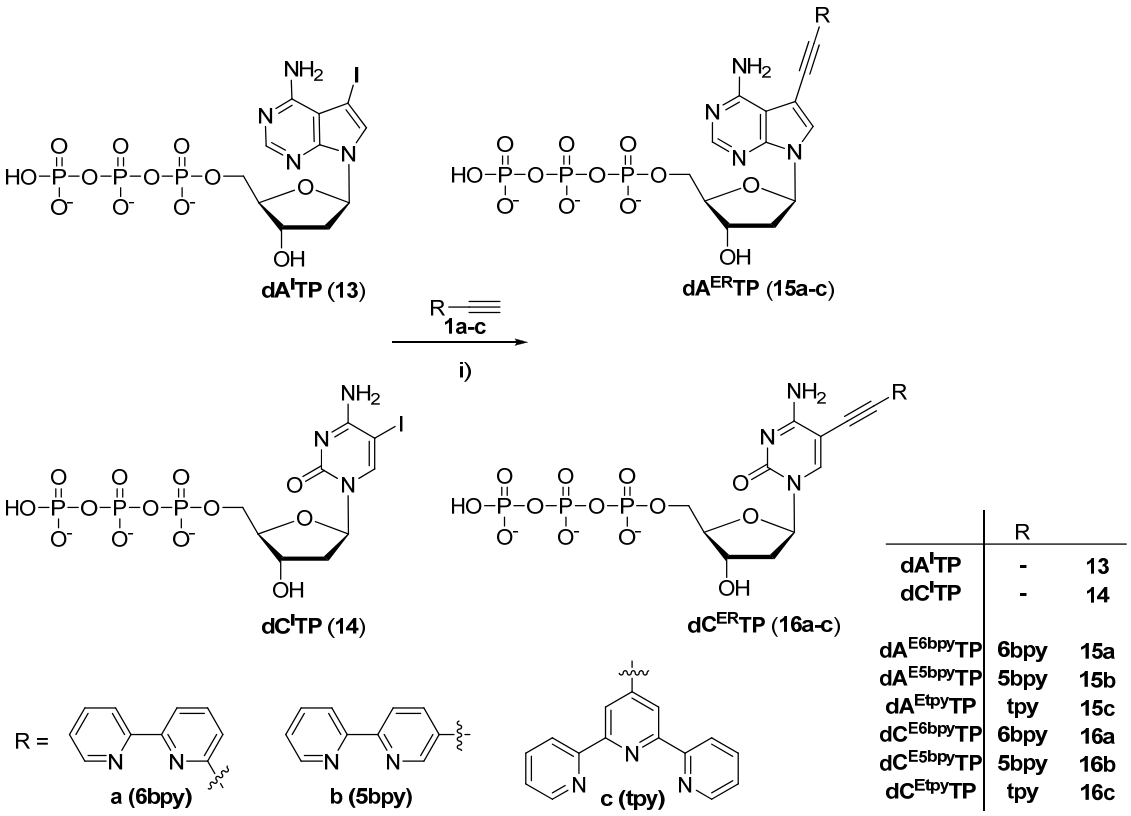
Scheme 9. Synthesis of modified **dN^{ER}MPs** (**11a-c** and **12a-c**). *Reagents and conditions*: i) $Pd(OAc)_2$ (5 mol%), TPPTS (5 equiv. to Pd), CuI (10 mol%), Et(*i*-Pr)$_2$N (10 equiv.), H_2O/CH_3CN (2:1), 80 °C, 1.5 h.

Table 6. Synthesis of modified **dN^{ER}MPs** (**11a-c** and **12a-c**) by Sonogashira cross-coupling reaction.

Entry	Monophosphate	Alkyne	Product	Yield
1	**dA^{I}MP (9)**	**1a**	**dA^{E6bpy}MP (11a)**	52%
2	**dA^{I}MP (9)**	**1b**	**dA^{E5bpy}MP (11b)**	70%
3	**dA^{I}MP (9)**	**1c**	**dA^{Etpy}MP (11c)**	57%
4	**dC^{I}MP (10)**	**1a**	**dC^{E6bpy}MP (12a)**	89%
5	**dC^{I}MP (10)**	**1b**	**dC^{E5bpy}MP (12b)**	89%
6	**dC^{I}MP (10)**	**1c**	**dC^{Etpy}MP (12c)**	47%

Aqueous-phase Sonogashira cross-coupling reaction was then used for direct functionalization of dNTPs in analogy to the modification of dNMPs (Scheme 10, Table 7). Halogenated nucleoside triphosphates **dA^{I}TP** (**13**) and **dC^{I}TP** (**14**) (prepared according to the literature procedure) were heated with terminal acetylene **1a-c** in the presence of $Pd(OAc)_2$, TPPTS, CuI and Hünig's base in the mixture water/acetonitrile (2:1) at 80 °C. As it was mentioned above, to avoid the hydrolysis of starting and final triphosphate, the reaction mixture was heated only for 1 h. The corresponding products **dA^{E6bpy}TP** (**15a**), **dA^{E5bpy}TP** (**15b**), **dA^{Etpy}TP** (**15c**) as well as **dC^{E6bpy}TP** (**16a**), **dC^{E5bpy}TP** (**16b**), **dC^{Etpy}TP** (**16c**) were isolated after the purification on reverse-phase

HPLC in good yields (40-48% for **dA^{ER}TPs** (**15a-c**) and 59-69% for **dC^{ER}TPs** (**16a-c**), Table 7).



Scheme 10. Synthesis of modified **dN^{ER}TPs** (**15a-c** and **16a-c**). *Reagents and conditions*: i) $Pd(OAc)_2$ (5 mol%), TPPTS (5 equiv. to Pd), CuI (10 mol%), Et(*i*-Pr)$_2$N (10 equiv.), H_2O/CH_3CN (2:1), 80 °C, 1 h.

Table 7. Synthesis of modified **dN^{ER}TPs** (**15a-c** and **16a-c**) by Sonogashira cross-coupling reaction.

Entry	Monophosphate	Alkyne	Product	Yield
1	**dA^{I}TP (13)**	**1a**	**dA^{E6bpy}TP (15a)**	42%
2	**dA^{I}TP (13)**	**1b**	**dA^{E5bpy}TP (15b**	48%
3	**dA^{I}TP (13)**	**1c**	**dA^{Etpy}TP (15c)**	40%
4	**dC^{I}TP (14)**	**2a**	**dC^{E6bpy}TP (16a)**	67%
5	**dC^{I}TP (14)**	**2b**	**dC^{E5bpy}TP (16b)**	59%
6	**dC^{I}TP (14)**	**2c**	**dC^{Etpy}TP (16c)**	69%

3.1.3 Incorporation of dN^{ER}TPs into DNA

All the functionalized **dA^{ER}TPs** (**15a-c**) and **dC^{ER}TPs** (**16a-c**) were tested as substrates for several termostable DNA polymerases in primer extension experiment (PEX). Each PEX experiment, analyzed by denaturing polyacrylamide gel electrophoresis (PAGE), was compared with positive control proceeded only with natural dNTPs, and negative control experiment proceeded with natural dNTPs in the absence of one particular dNTP, in order to exclude any miss-incorporation. Single and

multiple incorporations of oligopyridine modified triphosphates were tested. For sequences of primer and templates see Table 8.

Table 8. Primer and templates used for primer extension in chapter 3.1.3[a]

prim^{rnd}	5'-CATGGGCGGCATGGG-3'
temp^{rnd16}	5'-**CTAGCATGAGCTCAGT***CCCATGCCGCCCATG*-3'
temp^{C}	5'-**CCCG***CCCATGCCGCCCATG*-3'
temp^{A}	5'-**CCCT***CCCATGCCGCCCATG*-3'
temp^{A1}	5'-**T***CCCATGCCGCCCATG*-3'
temp^{C1}	5'-**G***CCCATGCCGCCCATG*-3'

[a]In the template (temp) ONs segments that form a duplex with primer are printed in *italics*, the replicated segments are printed in **bold**. For magnetic separation of the extended primer strands, the templates are biotinylated at their 5'-end. The acronyms used in the text for primer products are analogs to those introduces for templates (e.g. the PEX product pex^{rnd16} was synthesized on temp^{rnd16} template).

Single-nucleotide extension experiments were tested separately with each of three **dA^{ER}TPs** (**15a-c**) and **dC^{ER}TPs** (**16a-c**) by using 19-mer templates temp^{A} and temp^{C} (Figure 28). Experiments using DyNAzyme II polymerase leads to some miss-incorporation (Figure 28a, lanes 3 and 9), therefore other DNA polymerases (Pwo, Therminator, 9°Nth, KOD XL) were tested. Experiments using Therminator, 9°Nth or KOD XL polymerases gave the mixture of ONs with different length. Experiments using Pwo polymerase (Figure 28b) were mostly successful to give fully extended products for all of the dNTPs except **dC^{Etpy}TP** (**16c**) (lane 12), which gave the mixture of fully and partially extended ONs. The lack of extension in negative control (A- or C-; lanes 3 and 9) proves that no miss-incorporation occurred. The reaction conditions of the incorporation of **dC^{ER}TPs** (**16a-c**) by Pwo and DyNAzyme II polymerase were further optimized.

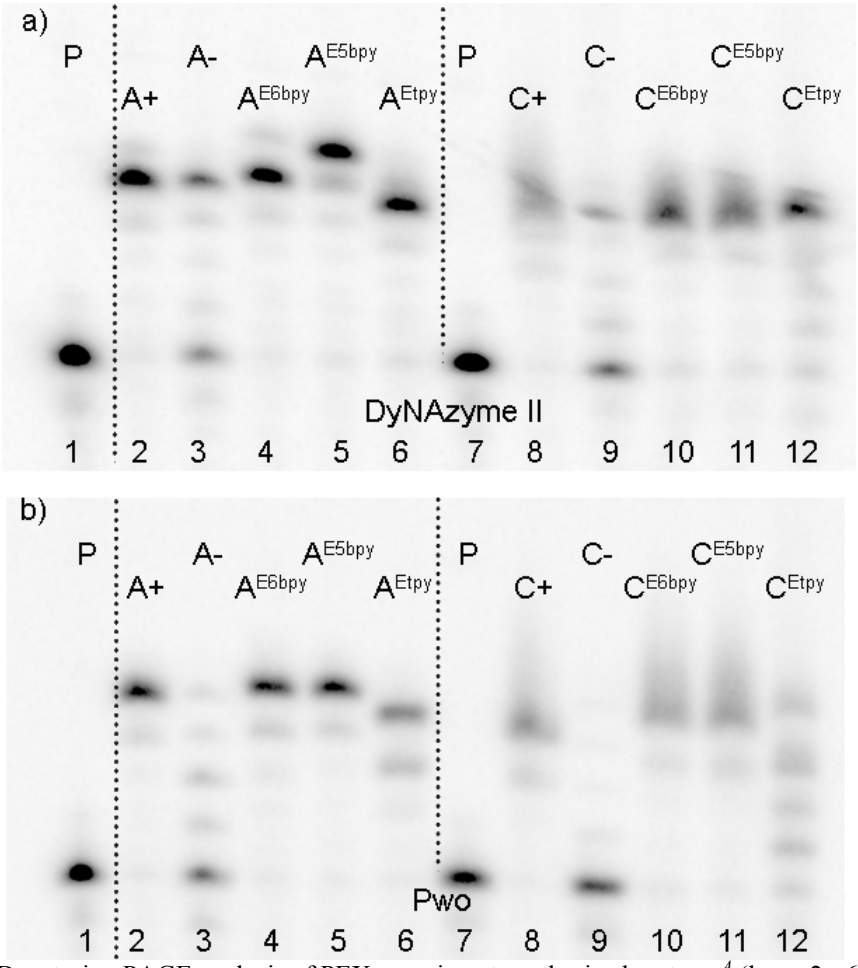


Figure 28. Denaturing PAGE analysis of PEX experiment synthesized on temp^{A} (lanes 2 - 6) and temp^{C} (lanes 7 - 12) with DyNAzyme II (a) or Pwo (b) polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; A+: natural dATP, dGTP; A-: dGTP; A^{E6bpy}: **$dA^{E6bpy}TP$** (**15a**), dGTP; A^{E5bpy}: **$dA^{E5bpy}TP$** (**15b**), dGTP; A^{Etpy}: **$dA^{Etpy}TP$** (**15c**), dGTP; C+: natural dCTP, dGTP; C-: dGTP; C^{E6bpy}: **$dC^{E6bpy}TP$** (**16a**), dGTP; C^{E5bpy}: **$dC^{E5bpy}TP$** (**16b**), dGTP; C^{Etpy}: **$dC^{Etpy}TP$** (**16c**), dGTP.

After the optimization, when different concentrations of DNA polymerases, natural and modified dNTPs were tested, was found, that only fully extended products were observed, when lower concentration of DyNAzyme II polymerase was used in combination with lower concentration of natural dGTPs and shorter reaction time (10 or 15 minutes instead of 30 minutes; Figure 29).

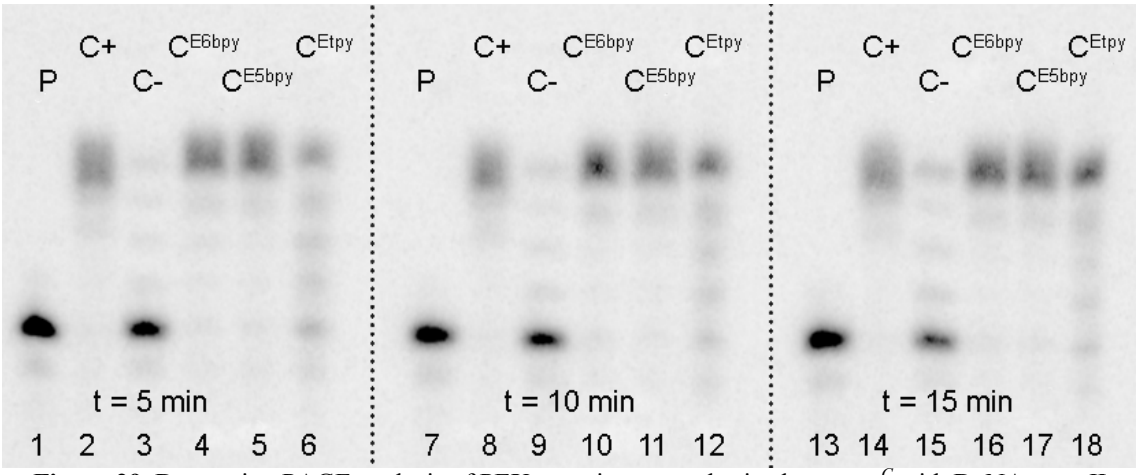


Figure 29. Denaturing PAGE analysis of PEX experiment synthesized on temp^{C} with DyNAzyme II polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs at 60 °C for 5 minutes (lanes 2-6), 10 minutes (lanes 8-12) and 15 minutes (lanes 14-18). P: Primer; C+: natural dCTP, dGTP; C-: dGTP; C^{E6bpy}: **dC^{E6bpy}TP** (**16a**), dGTP; C^{E5bpy}: **dC^{E5bpy}TP** (**16b**), dGTP; C^{Etpy}: **dC^{Etpy}TP** (**16c**), dGTP.

In order to compare the efficiency in incorporation of the oligopyridine-modified dNTPs in comparison with the natural ones, we have performed a simple kinetics study in single-nucleotide PEX experiment. The experiments with two of modified dATPs [**dA^{E5bpy}TP** (**15b**) and **dA^{Etpy}TP** (**15c**)] in comparison with the natural dATP were performed using Pwo polymerase and template temp^{A1} (Figure 30a). The PEX with natural dATP was finished within 1 minute whereas the PEX with **dA^{E5bpy}TP** (**15b**) took 2 minutes and, with the more bulky **dA^{Etpy}TP** (**15c**), even 5 minutes to complete. Same results were observed for incorporation of natural and modified dCTPs. The experiments with **dC^{E5bpy}TP** (**16b**) and **dC^{Etpy}TP** (**16c**) in comparison with the natural dCTP were performed using DyNAzyme II polymerase and template temp^{C1} (Figure 30b). The PEX with natural dCTP was, like in the case of the experiment using natural dATPs, finished within 1 minute whereas the PEX with **dC^{ER}TP** (**16b-c**) took 2 minutes or 5 minutes to complete. This shows that the modified **dN^{ER}TP** are worse substrates for the polymerase. Therefore, the reaction time for multiple incorporations must be prolonged to 30 minutes to ensure full length product formation.

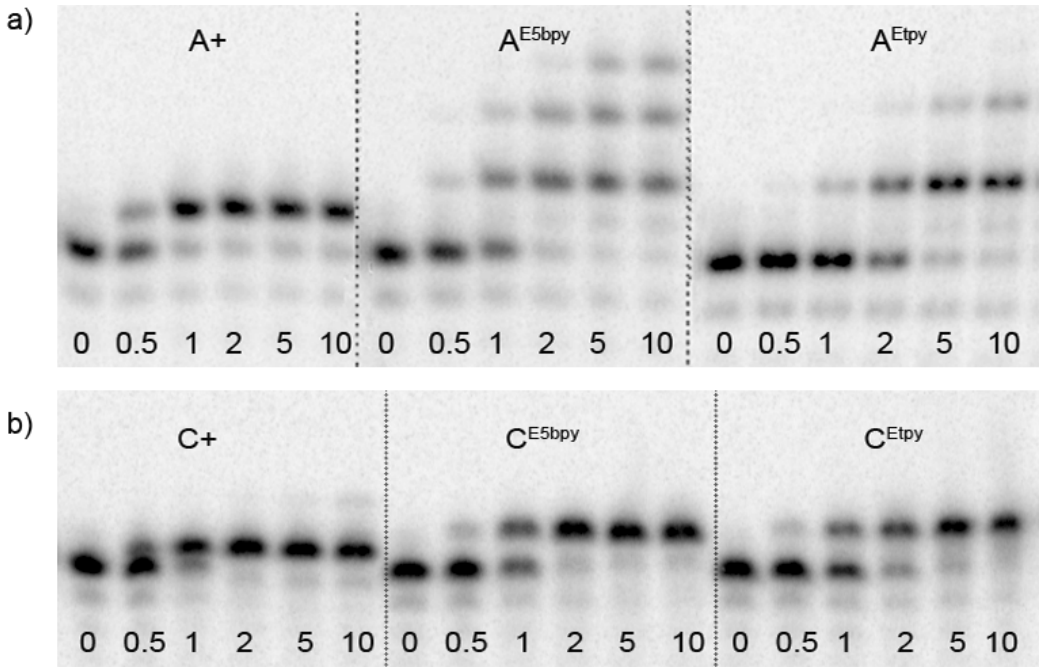


Figure 30. Comparison of the rate of the single-nucleotide PEX: a) with natural (dATP) and modified dNTPs [**dA^{E5bpy}TP** (**15b**) and **dA^{Etpy}TP** (**15c**)] using Pwo polymerase with temp^{A1}, b) with natural C+ (dCTP) and modified dNTPs [**dC^{E5bpy}TP** (**16b**) and **dC^{Etpy}TP** (**16c**)] using DyNAzyme II polymerase with temp^{C1}. The reaction mixture was incubated for time intervals indicated (in minutes), followed by stopping the reaction by addition of PAGE loading buffer and immediate heating.

Multiple incorporations were tested on 31-mer template temp^{rnd16} containing four copies of each of the four modified dNTPs in separate positions. Several polymerases were tested: Pwo, DyNAzyme II, Vent (exo$^-$), Phusion (Figure 31), KOD XL, Therminator, Deep Vent (exo$^-$) and Deep Vent (Figure 32).

The incorporation of **dA^{ER}TPs** (**15a-c**) using Pwo (Figure 31a, lanes 5-7) or Deep Vent (Figure 32b, lanes 15-17) led to fully extended products, while using other enzymes gave the mixture of ONs with different length or incorporation of **dA^{ER}TPs** (**15a-c**) was less feasible and resulted in early termination of PEX. The incorporation of **dC^{ER}TPs** (**16a-c**) using any of above mentioned DNA polymerase gave the mixture of ONs with different length and the reaction conditions were further optimized. Different combinations of various concentration of DNA polymerases and natural or modified dNTPs were tested. It was found, that five time higher concentration of DyNAzyme II DNA polymerase in combination with double concentration of **dC^{ER}TPs** (**16a-c**) was the most efficient in the incorporation of **dC^{ER}TPs** (**16a-c**) (Figure 33), while incorporation of **dC^{E6bpy}TP** (**16a**) and **dC^{E5bpy}TP** (**16b**) gave fully extended products (lanes 4-5). But even after the optimization of conditions, the incorporation of **dC^{Etpy}TP** (**16c**) was less feasible and resulted in early termination of PEX (Figure 33, lane 6).

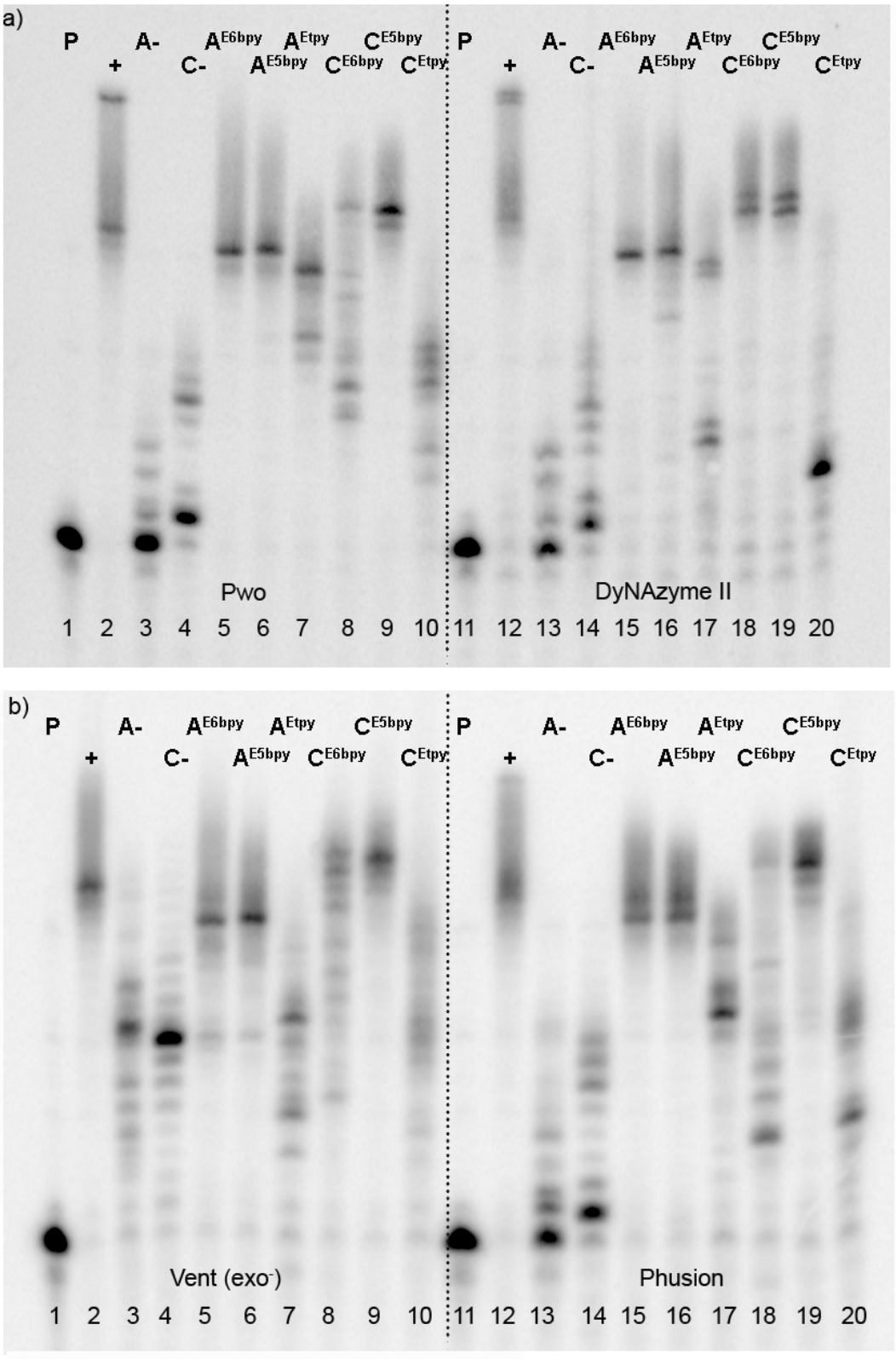


Figure 31. Denaturing PAGE analysis of PEX experiment synthesized on temp^{rnd16} with Pwo and DyNAzyme II polymerases (a) or Vent (exo$^-$) and Phusion polymerase (b). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; +: natural dNTPs; A-: dTTP, dCTP, dGTP; C-:dATP, dTTP, dGTP; A^{E6bpy}: **dA^{E6bpy}TP** (**15a**), dTTP, dCTP, dGTP; A^{E5bpy}: **dA^{E5bpy}TP** (**15b**), dTTP, dCTP, dGTP; A^{Etpy}: **dA^{Etpy}TP** (**15c**), dTTP, dCTP, dGTP; C^{E6bpy}: **dC^{E6bpy}TP** (**16a**), dATP, dTTP, dGTP; C^{E5bpy}: **dC^{E5bpy}TP** (**16b**), dATP, dTTP, dGTP; C^{Etpy}: **dC^{Etpy}TP** (**16c**), dATP, dTTP, dGTP.

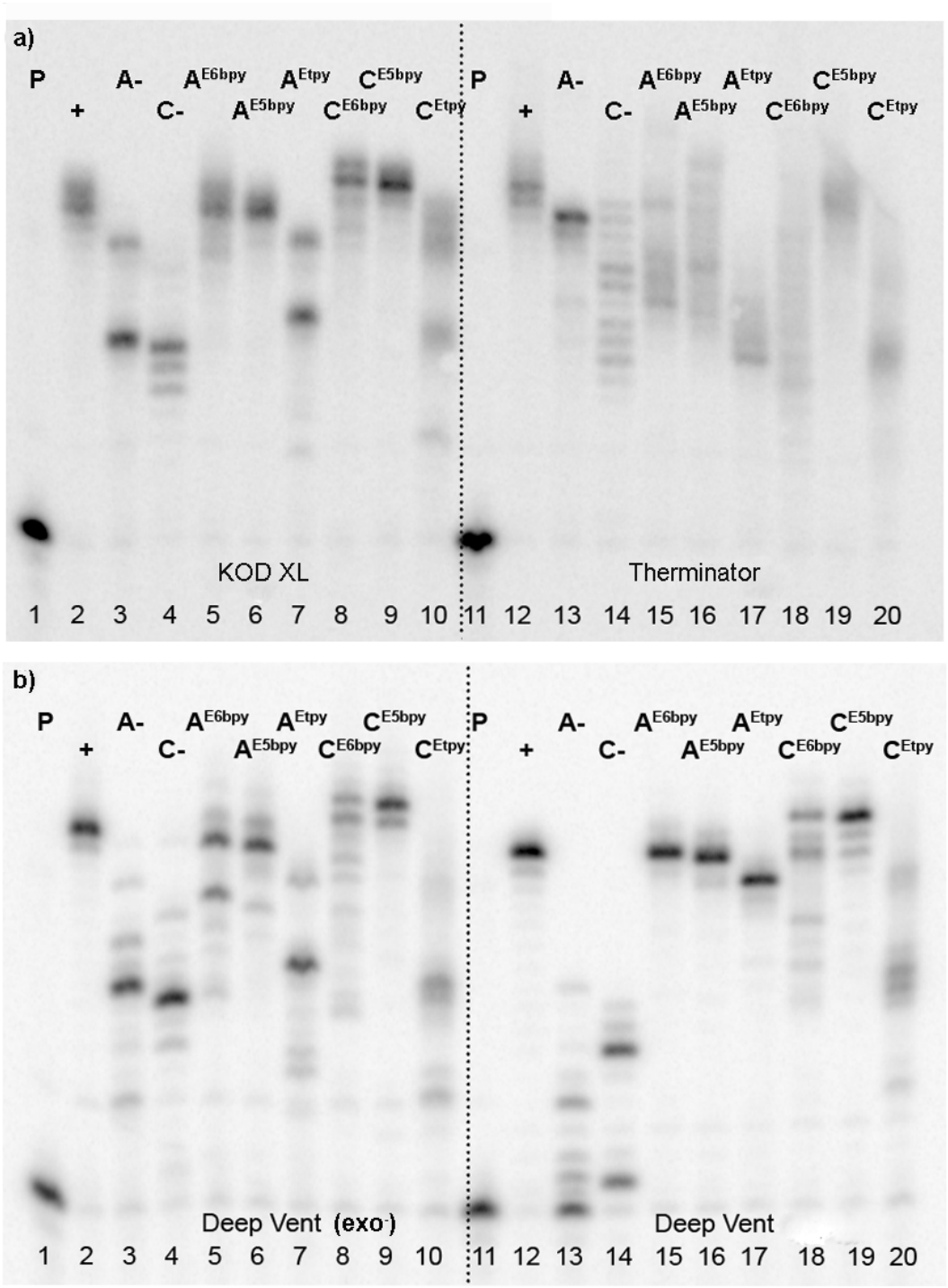


Figure 32. Denaturing PAGE analysis of PEX experiment synthesized on temp^{rnd16} with KOD XL and Therminator polymerases (a) or Deep Vent (exo$^-$) and Deep Vent polymerase (b). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; +: natural dNTPs; A-: dTTP, dCTP, dGTP; C-:dATP, dTTP, dGTP; A^{E6bpy}: **dA^{E6bpy}TP** (**15a**), dTTP, dCTP, dGTP; A^{E5bpy}: **dA^{E5bpy}TP** (**15b**), dTTP, dCTP, dGTP; A^{Etpy}: **dA^{Etpy}TP** (**15c**), dTTP, dCTP, dGTP; C^{E6bpy}: **dC^{E6bpy}TP** (**16a**), dATP, dTTP, dGTP; C^{E5bpy}: **dC^{E5bpy}TP** (**16b**), dATP, dTTP, dGTP; C^{Etpy}: **dC^{Etpy}TP** (**16c**), dATP, dTTP, dGTP.

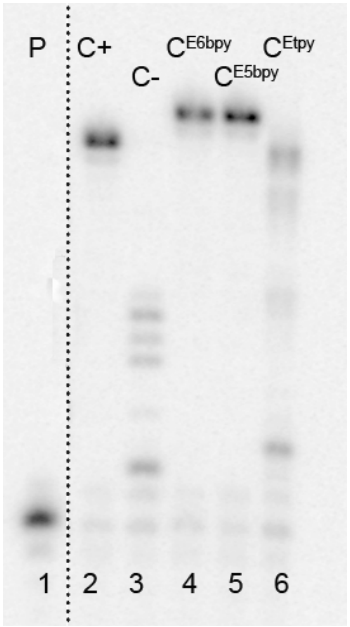


Figure 33. Denaturing PAGE analysis of PEX experiment synthesized on temp^{rnd16} with DyNAzyme II polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; C +: natural dNTPs; C-: dATP, dTTP, dGTP; C^{E6bpy}: **dC^{E6bpy}TP** (**16a**), dATP, dTTP, dGTP; C^{E5bpy}: **dC^{E5bpy}TP** (**16b**), dATP, dTTP, dGTP; C^{Etpy}: **dC^{Etpy}TP** (**16c**), dATP, dTTP, dGTP.

Each spot of PEX-product, even in the positive and negative control experiments, is accompanied by a weak band of one nucleotide shorter product due to 3'-5' exonuclease activity of the enzyme. The products of pex^{rnd16} slightly differ in electrophoretic mobilities on gel, due to the combination of effects of the higher molecular weight and possible secondary structure formation. Therefore, the successful incorporation and full-length products were verified by measurement of MALDI mass spectra of PEX products. Single-stranded DNA, required for measuring MALDI mass spectra, was isolated by simple magnetoseparation protocol.[51] PEX reaction was performed with a 5'-biotynylated template and then the double-stranded product was captured at streptavidine-coated magnetic beads (DB_{stv}). The captured product was purified by repeated magnetoseparation and re-suspension of the beads in the washing medium, to separate the components from reaction mixture such are unconsumed dNTPs, DNA polymerase and unreacted primer or template. Extended primer was then released from DB_{stv} via thermal denaturation of the duplex followed by the measurement of MALDI mass spectra. The correct masses were confirmed for the fully-extended products (Table 9).

Table 9. MALDI-Tof experiment (ss-ON)

Single-stranded PEX product [dNTPs used in PEX reaction]	Calculated mass (Da)	Found mass (Da)
pex^{A} [dATP, dGTP]	5973.0	5974.5
pex^{A} [**dA^{E6bpy}TP (15a)**, dGTP]	6150.2	6154.4
pex^{A} [**dA^{E5bpy}TP (15b),** dGTP]	6150.2	6152.2
pex^{A} [**dA^{Etpy}TP (15c),** dGTP]	6227.3	6228.7
pex^{C} [dCTP; dGTP]	5949.0	5950.3
pex^{C} [**dC^{E6bpy}TP (16a),** dGTP]	6126.2	6128.3
pex^{C} [**dC^{E5bpy}TP (16b),** dGTP]	6126.2	6128.3
pex^{C} [**dC^{Etpy}TP (16c),** dGTP]	6203.3	6204.8
pex^{rnd16} [dATP, dCTP, dTTP, dGTP]	9613.1	9614.5
pex^{rnd16} [**dA^{E6bpy}TP (15a),** dCTP, dTTP, dGTP]	10321.9	10323.7
pex^{rnd16} [**dA^{E5bpy}TP (15b),** dCTP, dTTP, dGTP]	10321.9	10326.5
pex^{rnd16} [**dA^{Etpy}TP (15c),** dCTP, dTTP, dGTP]	10630.3	10633.5
pex^{rnd16} [(dCTP, dATP, dTTP, dGTP]	9613.1	9616.7
pex^{rnd16} [**dC^{E6bpy}TP (16a),** dATP, dTTP, dGTP]	10325.9	10327.5
pex^{rnd16} [**dC^{E5bpy}TP (16b),** dATP, dTTP, dGTP]	10325.9	10329.0

3.1.4 Complexation studies

3.1.4.1 Complexation of oligopyridine modified nucleoside monophosphates

All six modified nucleoside monophosphates [**dN^{ER}MPs** (**11a-c** and **12a-c**)] were tested as model compounds for further complexation studies on oligopyridine modified oligonucleotides. Aqueous solutions of **dN^{ER}MPs** (**11a-c** and **12a-c**) were mixed with 0.5 equiv. of divalent metal such are Cu^{2+}, Zn^{2+}, Ni^{2+} and Fe^{2+}. After incubation for 10 minutes at room temperature, the complex formation was detected by UV/Vis spectroscopy. The spectra were recorded for the non-metalated and metalated monophosphate. The MLCT bands of 6bpy-monophosphates [**dN^{E6bpy}MPs** (**11a** and

12a)] with above mentioned divalent metals are partially overlapping with dominating absorbance at 350 nm due to the presence of bpy ligands (Figure 34).

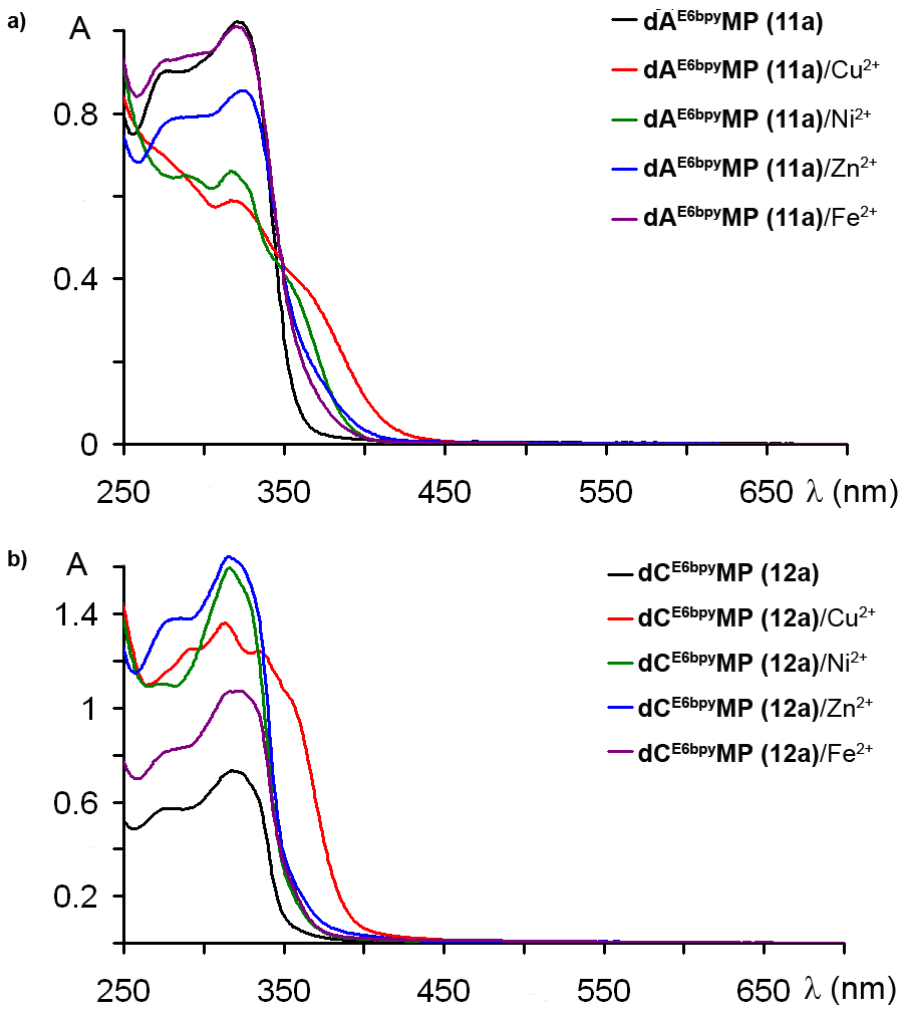


Figure 34. UV/Vis spectra of: a) **dA^{E6bpy}MP** (**11a**), b) **dC^{E6bpy}MP** (**12a**) with divalent metals.

While, MLCT bands of 5bpy-monophosphates [**dN^{E5bpy}MPs** (**11b** and **12b**)] with Cu^{2+}, Ni^{2+} and Zn^{2+} are partially overlapping with dominating absorbance at 350 nm due to the presence of bpy ligands, the complex formed with Fe^{2+} shows small MLCT band at 560 nm[65] (Figure 35).

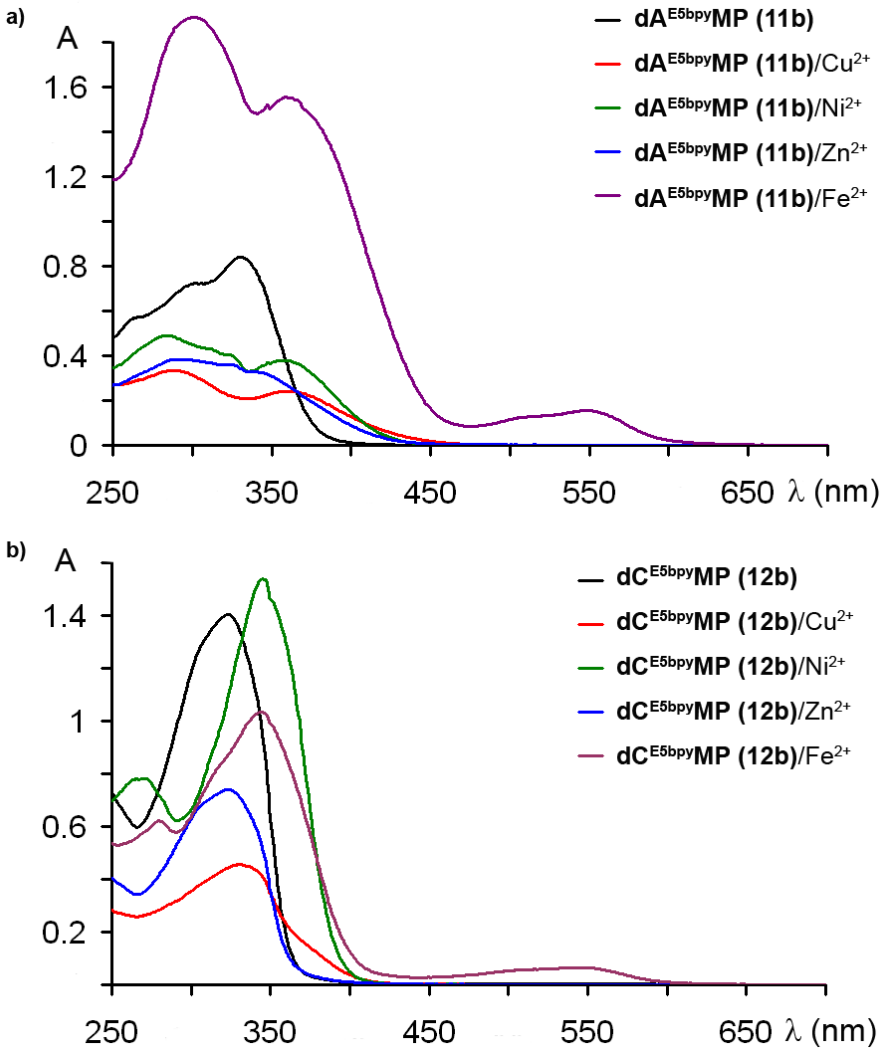


Figure 35. UV/Vis spectra of: a) **dA^{E5bpy}MP** (**11b**), b) **dC^{E5bpy}MP** (**12b**) with divalent metals.

Similar results were observed for the complexes formed by mixing **dN^{Etpy}MP** (**11c** and **12c**) with divalent metals (Figure 36). While, MLCT bands of Cu^{2+}, Ni^{2+} and Zn^{2+} complexes are partially overlaping with dominating absorbance of tpy-modification, MLCT band of Fe^{2+} complex can be very easily detected due to characteristic absorbance at 580 nm (Figure 36, magenta line).

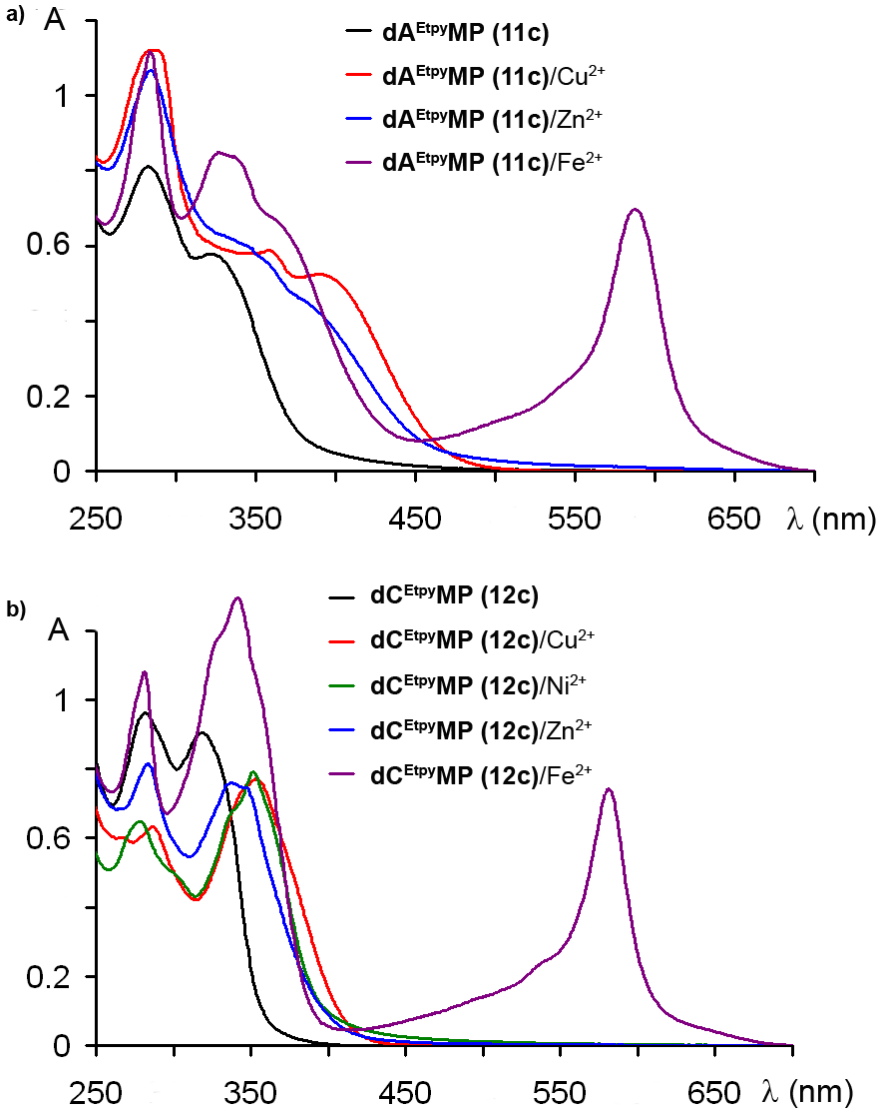


Figure 36. UV/Vis spectra of: a) **dA^{Etpy}MP (11c)**, b) **dC^{Etpy}MP (12c)** with divalent metals.

Due to this easily detectable MLCT band of $Fe(tpy)_2$ complexes, complexation of ONs were proceeded only with tpy-modified oligonucleotides and Fe^{2+} ions.

3.1.4.2 Complexation of oligopyridine-modified DNA

Complex formation of tpy-modified oligonucleotides was detected by native polyacrylamide gel electrophoresis and UV/Vis spectroscopy.

Oligonucleotides used for complexation studies detected by UV/Vis spectroscopy were prepared by PEX on larger scale using Deep Vent polymerase and template temp^{rnd16}, due to the higher concentration of tpy-modification in product. Since the **dC^{Etpy}TP (16c)** was not a good substrate in multiple incorporations, this experiment was performed only with **dA^{Etpy}TP (15c)**. Natural DNA was prepared by incorporation of natural dCTP, dGTP, dTTP and dATP, while the modified ON was prepared by using

dA^{Etpy}TP (**15c**) as surrogates of natural dATP. Before addition of Fe^{2+} ions, the PEX product had to be purified to remove unincorporated **dA^{Etpy}TP** (**15c**). To achieve this goal, the magnetoseparation protocol, which was successfully used for isolation of ss-ONs for MALDI analysis (see chapter 3.1.3), was first tested. After the PEX experiment followed by purification using magnetic beads, the corresponding sample was mixed with 0.5 equiv. of $Fe(BF)_4 \cdot 6H_2O$ per each modification and incubated at room temperature for 3h. Then the UV spectra of tpy-modified ON, both non-metalated and metalated were recorded and compared with UV spectra of natural DNA (Figure 37). The dominant absorbance at *ca.* 260 nm originate from the absorbance of natural nucleotides (black line), small absorbance band at *ca.* 350 nm is due to the presence of tpy modification (red line) while the band at *ca.* 590 is the MLCT band of complex formed by tpy-modified ON with Fe^{2+} (green line). Whereas the MLCT band of $Fe(tpy)_2$ complex was observed in the sample where no Fe^{2+} ions were added (Figure 36, red line), method of separation on magnetic beads could not be used for isolation of tpy-modified ON for complexation experiments. Therefore, other methods of purification were tested.

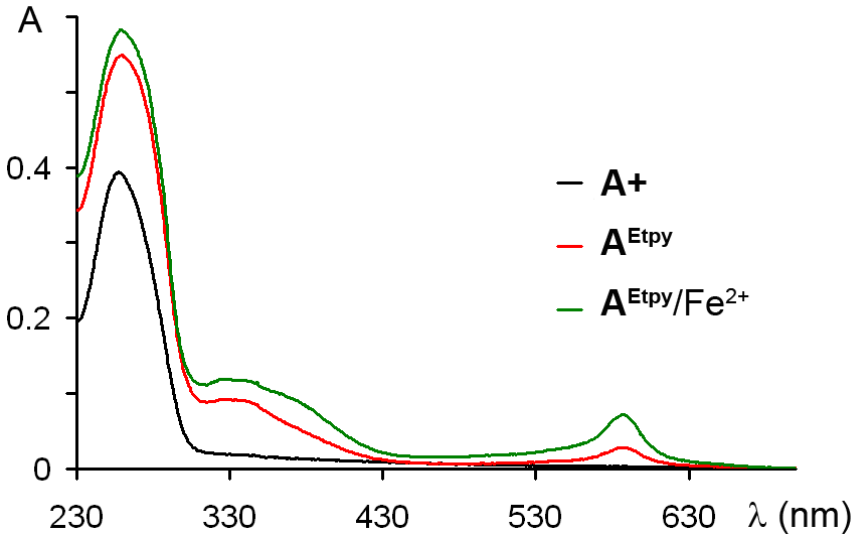


Figure 37. UV/Vis spectra of natural and tpy-modified ss-ON with $Fe(BF_4)_2 \cdot 6H_2O$.

After testing of several commercially available kits, it was successfully selected NucAway Spin Columns (Ambion), which provided sufficiently purified tpy-modified ds-DNA. Like this prepared sample was then mixed with 0.5 equiv. of $Fe(BF)_4 \cdot 6H_2O$ per each modification and after the incubation for several hours at room temperature, the UV/Vis spectra were recorded for non-metalated and metalated DNA duplexes, either natural or modified one (Figure 38). While the MLCT band[30] at *ca.* 590 nm prove

successful complex formation of tpy-modified DNA with Fe^{2+} (blue line), similar band was not observed for the natural DNA mixed with Fe^{2+} (red line).

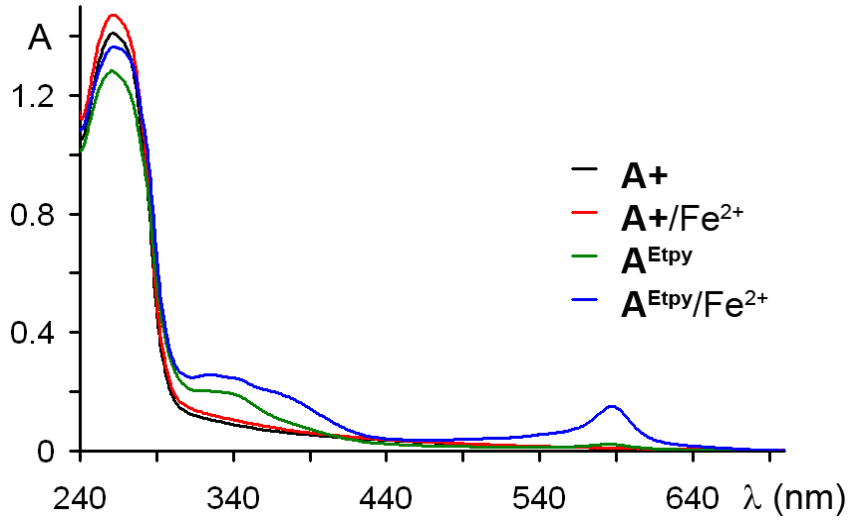


Figure 38. UV/Vis spectra of natural and tpy-modified DNA duplexes with $Fe(BF_4)_2 \cdot 6H_2O$.

For the experiments detected by native polyacrylamide gel electrophoresis, products of monoincorporation, prepared by PEX experiment using $temp^A$ and dATP (natural DNA) or modified **dA^{Etpy}TP** (**15c**) (Figure 39a), or $temp^C$ and dCTP (natural DNA) or **dC^{Etpy}TP** (**16c**) (Figure 39b) as well as products of multiincorporation prepared by PEX experiment using $temp^{rnd16}$ and dATPs (natural DNA) or **dA^{Etpy}TP** (**15c**) (Figure 40), were tested. In these experiments, non-metalated and metalated oligonucleotides, either natural or modified ones, were compared. Different reaction conditions were tested. Successful complex formation was observed, when PEX products were directly, without previous purification, mixed with 1 equiv. of $Fe(BF_4)_2 \cdot 6H_2O$ or $FeCl_2$ (calculated to the amount of **dN^{Etpy}TP** (**15c** or **16c**) in PEX experiment) and incubated at room temperature for 3 h. Complex formation of pex^A and pex^C, containing only one tpy-modification, with Fe^{2+} ions is clearly shown by bands with slower mobility (Figure 39, lanes 4 and 8), while no change in mobility was observed for natural DNA mixed with Fe^{2+} ions (Figure 39, lanes 2 and 6).

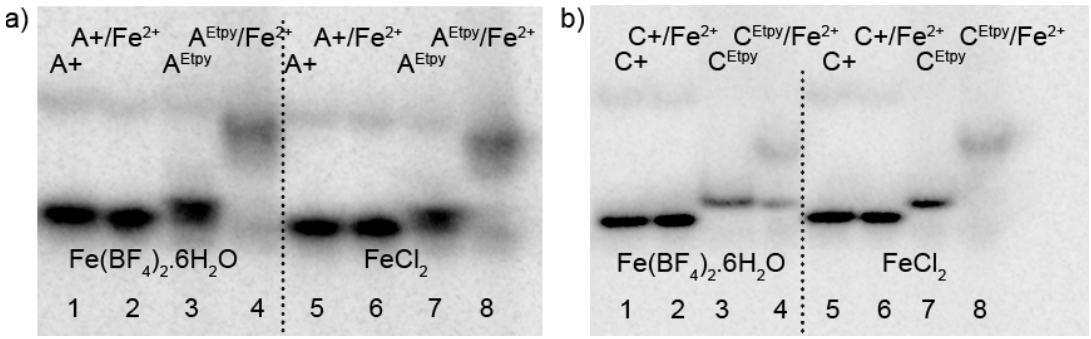


Figure 39. Non-denaturing gel electrophoresis (8% SB_PAGE) of Etpy-modified DNA duplexes in the absence and in the presence of Fe^{2+} for pex^{A} (a) or pex^{C} (b). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dGTP]; A+/Fe^{2+}: unmodified DNA mixed with Fe^{2+}; A^{Etpy}: Etpy-modified DNA [**$dA^{Etpy}TP$** (**15c**), dGTP]; A^{Etpy}/Fe^{2+}: Etpy-modified DNA mixed with Fe^{2+}; C+: unmodified DNA [dCTP, dGTP]; C+/Fe^{2+}: unmodified DNA mixed with Fe^{2+}; C^{Etpy}: Etpy-modified DNA [**$dC^{Etpy}TP$** (**16c**), dGTP]; C^{Etpy}/Fe^{2+}: Etpy-modified DNA mixed with Fe^{2+}.

Similar results were shown also for pex^{rnd16} containing four tpy-modification (Figure 40), where complex formation is clearly shown by bands with slower mobility (lanes 4 and 8), while no change in mobility was observed for natural DNA mixed with Fe^{2+} ions (lanes 2 and 6). Here the bands are more smeared since mixture of possible products is formed.

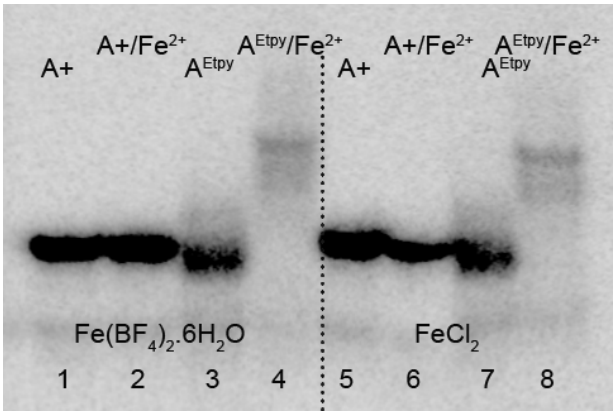
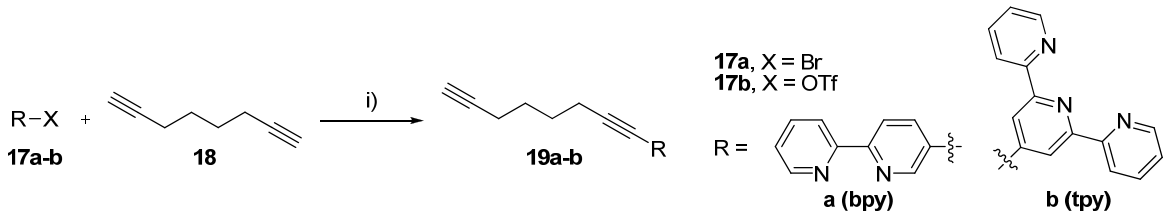


Figure 40. Non-denaturing gel electrophoresis (8% SB_PAGE) of Etpy-modified DNA duplexes in the absence and in the presence of Fe^{2+} for pex^{rnd16}. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dTTP, dCTP, dGTP]; A+/Fe^{2+}: unmodified DNA mixed with Fe^{2+}; A^{Etpy}: Etpy-modified DNA [**$dA^{Etpy}TP$** (**15c**), dTTP, dCTP, dGTP]; A^{Etpy}/Fe^{2+}: Etpy-modified DNA mixed with Fe^{2+}.

3.2 Oligopyridine analogues with flexible (octadiyne) linker

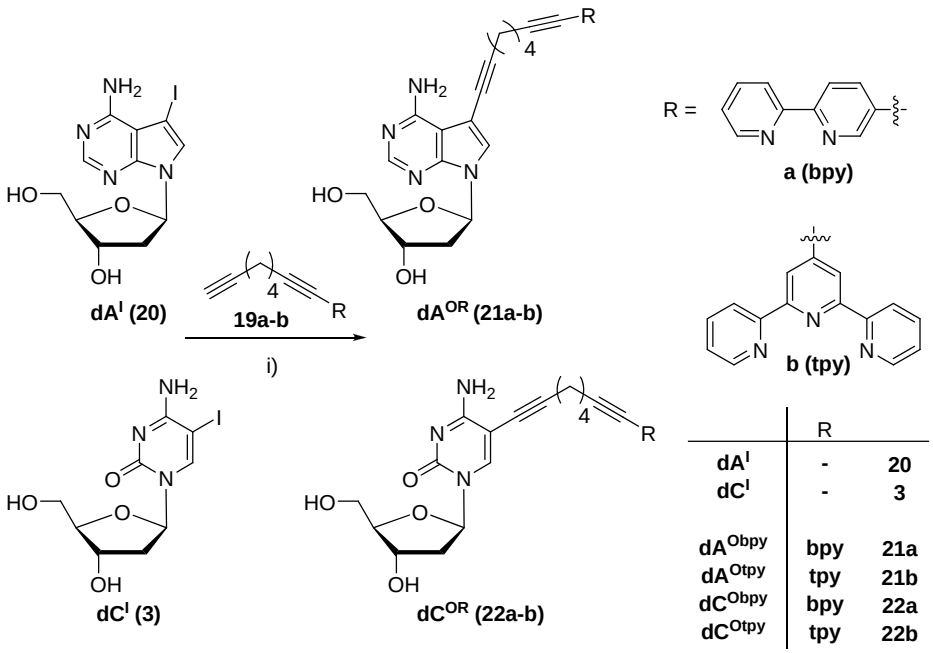
3.2.1 Synthesis of modified nucleosides and dNTPs

In order to prepare functionalized nucleosides bearing oligopyridine ligands attached via long and flexible octadiyne linker, suitable ligand building blocks had to be first prepared. The building blocks were prepared via simple Sonogashira cross-coupling reaction of activated oligopyridine **17a-b**, prepared according to the literature procedure, with an 3 equiv. of 1,7-octadiyne (**18**) in the presence of $Pd(PPh_3)_2Cl_2$, CuI and Et_3N (Scheme 11). The reaction mixture was heated at 70 °C for 3 h. Desired products were observed in good yields 66% for **19a** or 75% for **19b**.



Scheme 11. Synthesis of ligand building blocks: i) $Pd(PPh_3)_2Cl_2$ (5 mol%), CuI (5 mol%) and Et_3N (10 equiv.), THF, 70 °C, 3h.

The Sonogashira cross-coupling reaction was also used for attachment of above describe building blocks to position 5 of 2'-deoxycytidine and to position 7 of 7-deaza-2'-deoxyadenosine. The Pd-catalyzed Cu-mediated cross-coupling reaction of 5-iodo-2'-deoxycytidine [**dC^I** (**3**)] or 7-iodo-7-deaza-2'-deoxyadenosine [**dA^I** (**20**)] with oligopyridine building blocks **19a-b** were performed in the presence of 5 mol% of $Pd(OAc)_2$, TPPTS (2.5 equiv. to Pd), CuI (10 mol%) and Hünig's base in DMF in analogy to previously developed procedure (Scheme 12, Table 10). All the reactions proceeded at 75 °C to reach full conversion in 2 h. Desired products were purified by flash chromatography on reverse phase and after the crystallization were isolated as brownish solid in acceptable yields shown in Table 10. Taking into consideration that attachment of non-polar building blocks **19a-b** to polar nucleoside in single-step reaction without use of protecting groups, the yields were satisfactory.

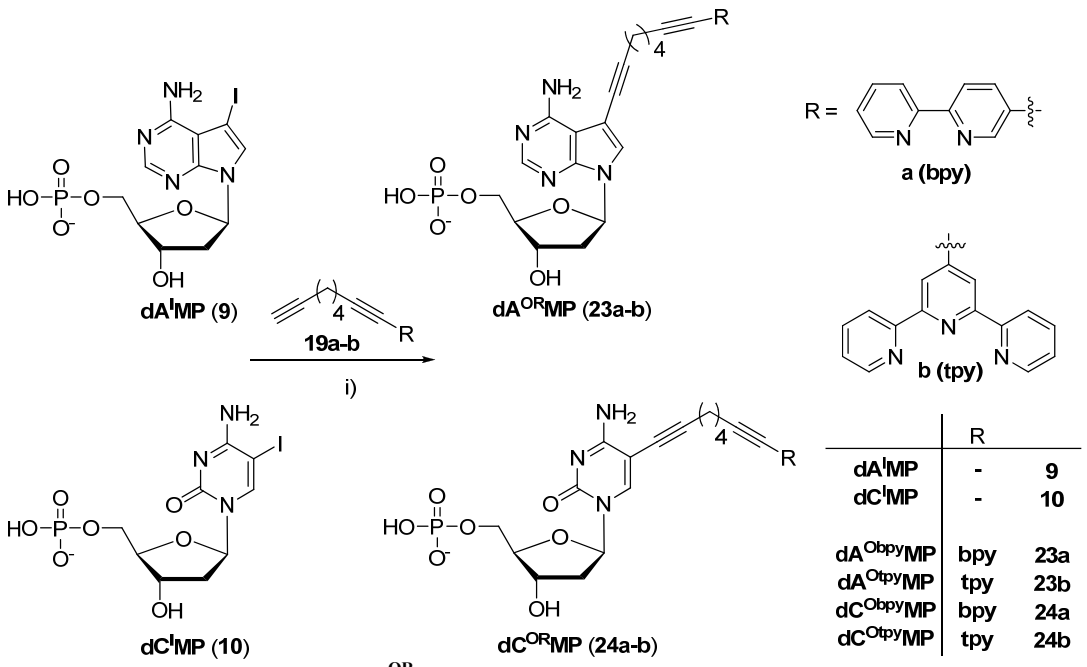


Scheme 12. Synthesis of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**). *Reagents and conditions*: i) $Pd(OAc)_2$ (5 mol%), TPPTS (2.5 equiv. to Pd), CuI (10 mol%), Et (*i*-Pr)$_2$N (10 equiv.), DMF, 75 °C, 2 h.

Table 10. The Sonogashira cross-coupling reaction for synthesis of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**).

Entry	Nucleoside	Building block	Product	Yield
1	**dA^{I} (20)**	**19a**	**dA^{Obpy} (21a)**	46%
2	**dA^{I} (20)**	**19b**	**dA^{Otpy} (21b)**	45%
3	**dC^{I} (3)**	**19a**	**dC^{Obpy} (22a)**	38%
4	**dC^{I} (3)**	**19b**	**dC^{Otpy} (22b)**	72%

To develop the synthetic methodology for synthesis of modified **dN^{OR}TPs** (**25a-b** and **26a-b**), the chemistry was first performed on model nucleoside monophosphates **dN^{OR}MPs** (**23a-b** and **24a-b**). Aqueous phase Sonogashira cross-coupling reaction, successfully used for synthesis of **dN^{ER}MPs** (**11a-c** and **12a-c**) (see chapter 3.1.2), could not be used in this case due to low solubility of oligopyridine building blocks **19a-b** in the mixture water/acetonitrile (2:1) and from that resulting poor yields of **dN^{OR}MPs** (**23a** and **24a-b**, Scheme 13, Table 11).



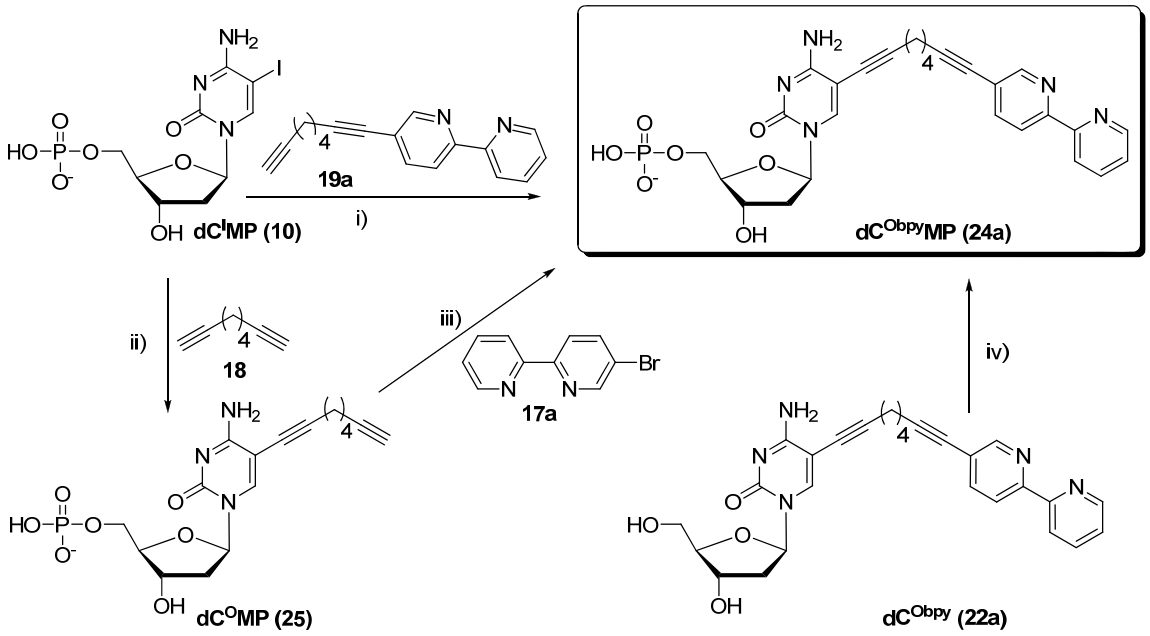
Scheme 13. Synthesis of modified **dN^{OR}MPs** (**23a-b** and **24a-b**) via Sonogashira cross-coupling reaction. *Reagents and conditions*: i) $Pd(OAc)_2$ (5 mol%), TPPTS (5 equiv. to Pd), CuI (10 mol%), Et(*i*-Pr)$_2$N (10 equiv.), H_2O/CH_3CN (2:1), 80 °C, 1.5 h.

Table 11. The Sonogashira cross-coupling reaction for synthesis of modified nucleoside monophosphates **dN^{OR}MPs** (**23a-b** and **24a-b**).

Entry	Monophosphate	Building block	Product	Yield
1	**dA^{I}MP (9)**	**19a**	**dA^{Obpy}MP (23a)**	23%
2	**dA^{I}MP (9)**	**19b**	**dA^{Otpy}MP (23b)**	-
3	**dC^{I}MP (10)**	**19a**	**dC^{Obpy}MP (24a)**	11%
4	**dC^{I}MP (10)**	**19b**	**dC^{Otpy}MP (24b)**	11%

Synthesis of **dN^{OR}MPs** (**23a-b** and **24a-b**) was further optimized only for the preparation of **dC^{Obpy}MP** (**24a**). Different synthetic approaches were tested (Scheme 14, Table 12). Aqueous-phase Sonogashira cross-coupling reaction performed in the mixture CH_3CN/H_2O (1:2) gave **dC^{Obpy}MP** (**24a**) in the yield of 12% (Table 11, entry 3). Changing the solvent to DMF/H_2O (4:1) did not bring any improvement and **dC^{Obpy}MP** (**24a**) was isolated in the yield of 11% (Table 12, entry 1). Therefore the synthetic approach was changed and cytidine monophosphate bearing octadiyne substituent **dC^{O}MP** (**25**) was first prepared using aqueous Sonogashira cross-coupling reaction. The **dC^{O}MP** (**25**) was isolated after the HPLC purification on reverse-phase in the yield of 49%. The Sonogashira reaction was used for the preparation of **dC^{Obpy}MP** (**24a**) via attachment of bpy-ligand **17a**. Desired product was isolated after HPLC purification in the yield lower than 1% (Table 12, entry 2). The third approach in the synthesis of **dC^{Obpy}MP** (**24a**) consists of phosphorylation of already modified

nucleosides **dC^{Obpy}** (**22a**). In this case product **dC^{Obpy}MP** (**24a**) was isolated in the yield of 31% (Table 12, entry 3).



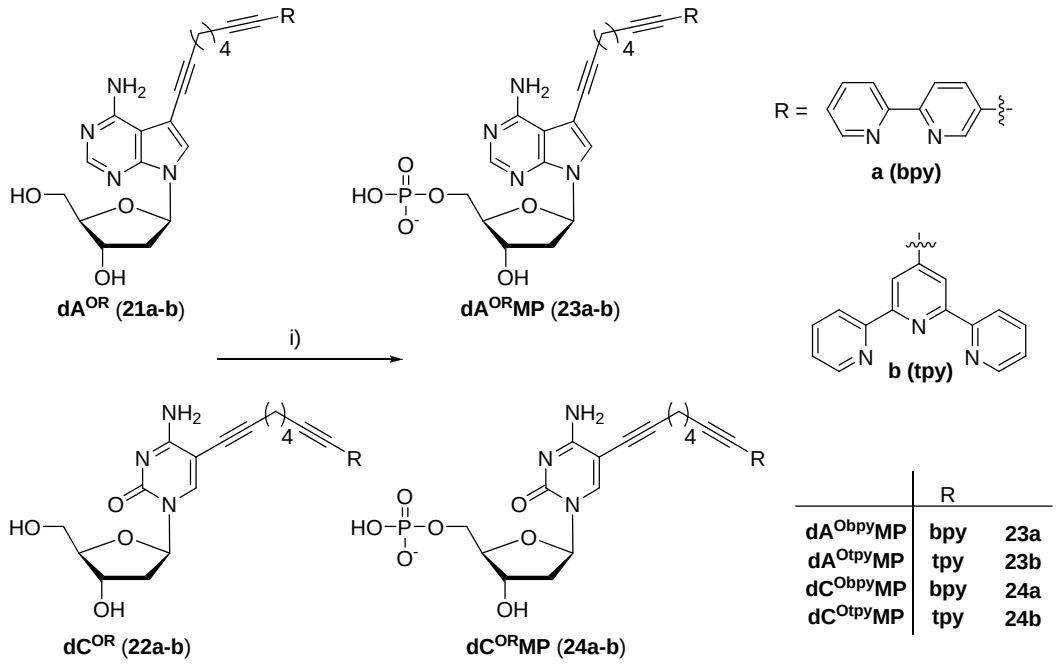
Scheme 14. Synthesis of **dC^{Obpy}MP** (**24a**)**.** *Reagents and conditions:* i) $Pd(OAc)_2$ (5 mol%), TPPTS (5 equiv. to Pd), CuI (10 mol%), Et(*i*-Pr)$_2$N (10 equiv.), H_2O/DMF (1:4), 80 °C, 4.5 h; ii) $Pd(OAc)_2$ (5 mol%), TPPTS (5 equiv. to Pd), CuI (10 mol%), Et_3N (10 equiv.), H_2O/CH_3CN (1:2), 80 °C, 1.5 h; iii) $Pd(OAc)_2$ (5 mol%), TPPTS (5 equiv. to Pd), CuI (10 mol%), Et(*i*-Pr)$_2$N (10 equiv.), H_2O/DMF (1:4), 80 °C, 1.5 h; iv) 1. $PO(OMe)_3$, $POCl_3$ (1.3 equiv.), 0 °C, 1.5 h; 2. 2M TEAB.

Table 12. Synthesis of **dC^{Obpy}MP** (**24a**).

Entry	Starting Material	Ligand	Yield
1	**dC^{I}MP (10)**	**19a**	11%
2	**dC^{O}MP (25)**	**17a**	<1%
3	**dC^{Obpy} (22a)**	**-**	31%

Phosphorylation of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**), was then applied for the synthesis of whole series of modified nucleoside monophosphates **dN^{OR}MP** (**23a-b** and **24a-b,** Scheme 15). The solution of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**) in $PO(OMe)_3$ was stirred with $POCl_3$ at 0 °C for 1 h and then the reaction was quenched by addition of 2 ml of 2M TEAB. The corresponding products **dA^{Obpy}MP** (**23a**) and **dC^{OR}MP** (**24a-b**) were isolated after HPLC column chromatography in the yield shown in Table 13. Even though the yields of bipyridinyl modified nucleoside monophosphates **dN^{Obpy}MP** (**23a** and **24a**) were significantly improved compare to case, when modified **dN^{OR}MP** (**23a-b** and **24a-b**) were prepared from halogenated monophosphates **dN^{I}MP** (**9** and **10**) using Sonogashira cross-coupling reaction (Scheme 13, Table 11), the yield of terpyridinyl functionalized

cytidine monophosphate **dC^{Otpy}MP** (**24b**) remained unchanged. Terpyridynyl modified 7-deaza-adenosine monophosphate **dA^{Otpy}MP** (**23b**) was not successfully isolated, due to the problematic purification.

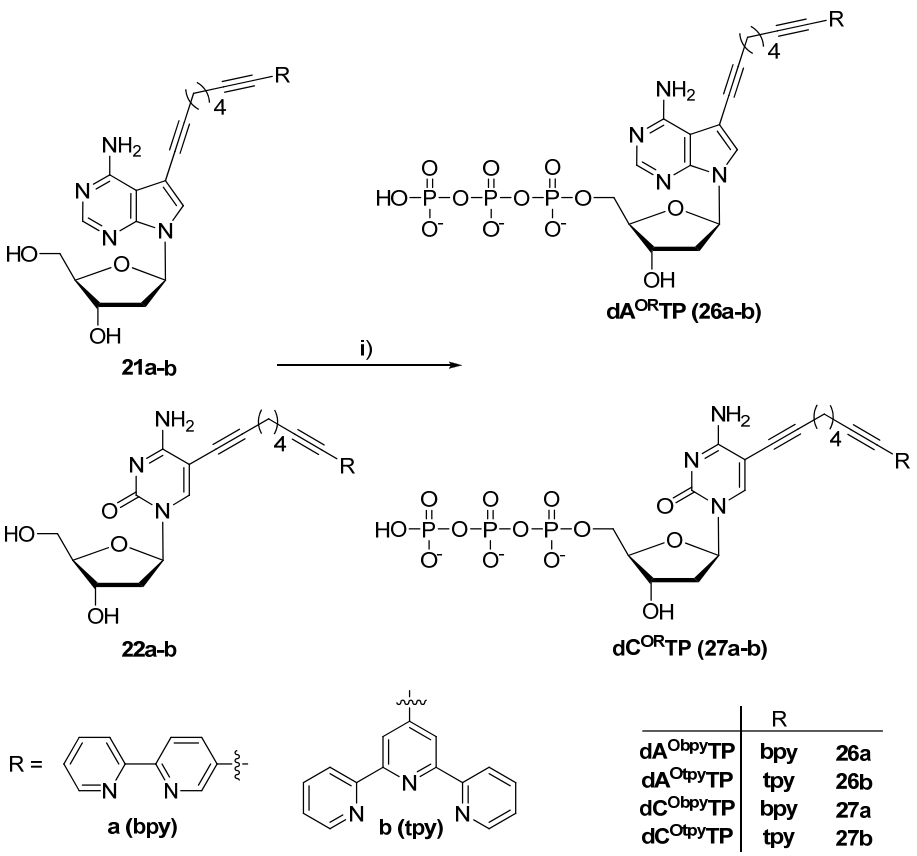


Scheme 15. Synthesis of modified **dN^{OR}MPs** (**23a-b** and **24a-b**) via phosphorylation of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**). *Reagents and conditions:* 1. $PO(OMe)_3$, $POCl_3$ (1.3 equiv.), 0 °C, 1 h; 2. 2M TEAB.

Table 13. Synthesis of modified nucleosides monophosphates **dN^{OR}MP**s (**23a-b** and **24a-b**) via phosphorylation of modified nucleosides **dN^{OR}**s (**21a-b** and **22a-b**).

Entry	Nucleoside	dNTPs	Yield
1	**dA^{Obpy} (21a)**	**dA^{Obpy}MP (23a)**	30%
2	**dA^{Otpy} (21b)**	**dA^{Otpy}MP (23b)**	-
3	**dC^{Obpy} (22a)**	**dC^{Obpy}MP (24a)**	48%
4	**dC^{Otpy} (22b)**	**dC^{Otpy}MP (24b)**	12%

Classical triphosphorylation of modified nucleosides[66] was then successfully used also for synthesis of modified **dN^{OR}TP**s (**26a-b** and **27a-b**, Scheme 16). The solution of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**) in $PO(OMe)_3$ was stirred with $POCl_3$ at 0 °C for 1 h. The solution of tributylammoniumpyrophosphate in DMF with an addition of Bu_3N was prepared in separate flask, cooled down to 0 °C, injected into the reaction mixture which was then stirred at 0 °C for another 1.5 h. After this time, the reaction was quenched by addition of 2 ml of 2M TEAB. Desired products were isolated in the yields shown in Table 14.



Scheme 16. Synthesis of modified **dN^{OR}TP**s (**26a-b** and **27a-b**). *Reagents and conditions:* 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. 2M TEAB.

Table 14. Synthesis of modified **dN^{OR}TPs** (**26a-b** and **27a-b**) by triphosphorylation of modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**).

Entry	Nucleoside	dNTPs	Yield
1	**dA^{Obpy} (21a)**	**dA^{Obpy}TP (26a)**	35%
2	**dA^{Otpy} (21b)**	**dA^{Otpy}TP (26b)**	14%
3	**dC^{Obpy} (22a)**	**dC^{Obpy}TP (27a)**	39%
4	**dC^{Otpy} (22b)**	**dC^{Otpy}TP (27b)**	31%

Low yields are the consequence of difficult isolation of modified **dN^{OR}TPs** (**26a-b** and **27a-b**), due to their amphiphilic character. Two-step purification, using first Sephadex DEAE purification followed by semi-preparative HPLC column chromatography, had to be used to obtain pure products.

3.2.2 Incorporation of dN^{OR}TPs into DNA

Based on previous experience (see chapter 3.1.3) all functionalized **dA^{OR}TPs** (**26a-b**) and **dC^{OR}TPs** (**27a-b**) were tested in single and multiple incorporation as substrates for four different termostable DNA polymerases (Pwo, DyNAzyme II, KOD XL, Deep Vent) in PEX experiment. Each PEX experiment, compared with

positive and negative control, was again analyzed by polyacrylamide gel electrophoresis. For sequences of primer and templates see Table 15.

Table 15. Primer and templates used for primer extension in chapter 3.2.2[a]

prim^{rnd}	5'-CATGGGCGGCATGGG-3'
temp^{rnd16}	5'-**CTAGCATGAGCTCAGT***CCCATGCCGCCCATG*-3'
temp^{C}	5'-**CCCG***CCCATGCCGCCCATG*-3'
temp^{A}	5'-**CCCT***CCCATGCCGCCCATG*-3'
temp^{A1}	5'-**T***CCCATGCCGCCCATG*-3'
temp^{C1}	5'-**G***CCCATGCCGCCCATG*-3'

[a]In the template (temp) ONs segments that form a duplex with primer are printed in *italics*, the replicated segments are printed in **bold**. For magnetic separation of the extended primer strands, the templates are biotinylated at their 5'-end. The acronyms used in the text for primer products are analogs to those introduces fro templates (e.g. the PEX product pex^{rnd16} was synthesized on temp^{rnd16} *template*).

Single nucleotide extension experiments were tested separately with all modified **dN^{OR}TPs** (**26a-b** and **27a-b**) by using 19-mer templates temp^{A} and temp^{C} (Figure 41). While experiments using Pwo polymerase (Figure 41a, lanes 2-9) were successful to give fully extended products for all of the dNTPs and the lack of extension in negative control (A- and C-; lanes 3 and 7) prove that no miss-incorporation occurred, experiments using other DNA polymerases (Figure 41a, lanes 10-17 for DyNAzyme II polymerase; Figure 41b for KOD XL and Deep Vent polymerases) 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.

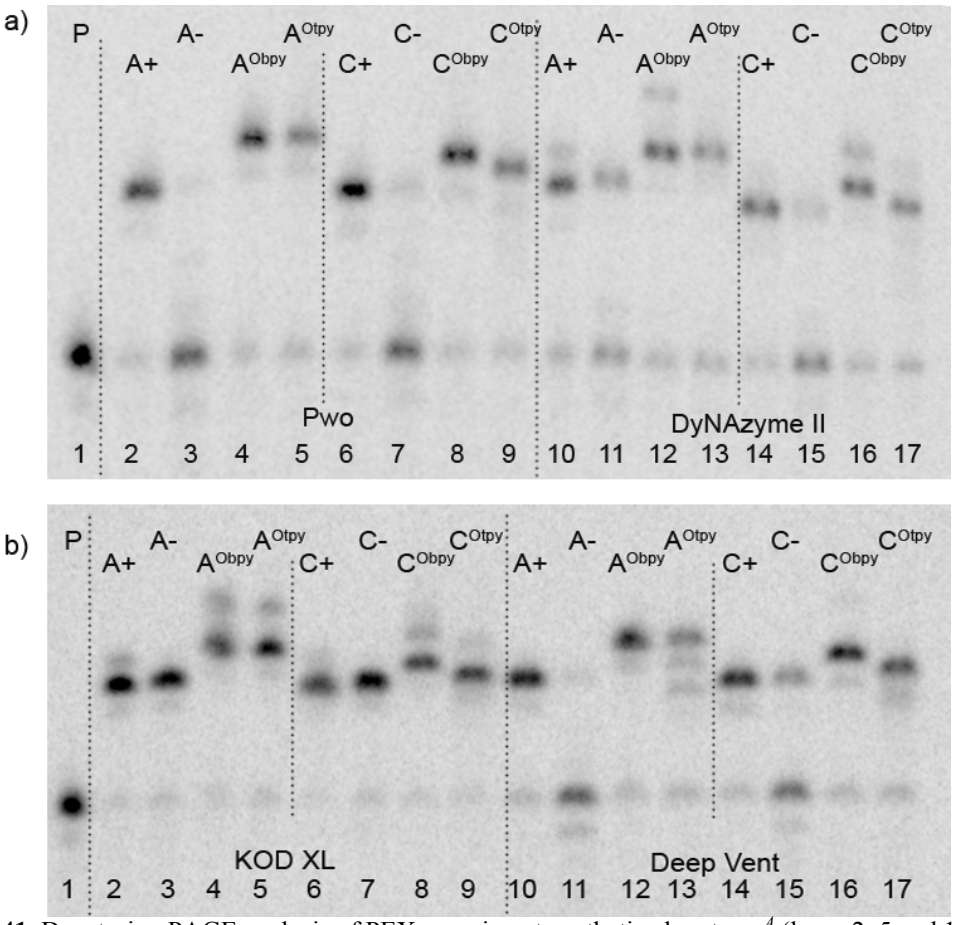


Figure 41. Denaturing PAGE analysis of PEX experiment synthetized on temp^{A} (lanes 2–5 and 10-13) and temp^{C} (lanes 6–9 and 14-17) with Pwo and DyNAzyme II (a) or with KOD XL and Deep Vent (b) polymerases. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; A+: natural dATP, dGTP; A-: dGTP; A^{Obpy}: **dA^{Obpy}TP** (**26a**), dGTP; A^{Otpy}: **dA^{Otpy}TP** (**26b**), dGTP; C+: natural dCTP, dGTP; C-: dGTP; C^{Obpy}: **dC^{Obpy}TP** (**27a**), dGTP; C^{Otpy}: **dC^{Otpy}TP** (**27b**), dGTP.

Kinetics studies, comparing efficiency in incorporation of natural and modified dNTPs, were peformed also for incorporation of **dN^{OR}TPs** (**26a-b** and **27a-b**). The experiments were performed using Pwo polymerase and temp^{A1} template for experiments with natural and modified dATPs (Figure 42a), whereas the temp^{C1} template was used for experiments with natural and modified dCTPs (Figure 42b). The PEX experiments with natural dNTPs were finished within 1 minute, whereas the PEX with **dC^{OR}TP** (**27a-b**) took 2 minutes and the PEX with **dA^{OR}TP** (**26a-b**) took 5 minutes to complete. Reaction time for multiple incorporations was like in the case of incorporation of **dN^{ER}TPs** (**15a-c** and **16a-c**; see chapter 3.1.3) prolonged to 30 minutes, to ensure full length product formation.

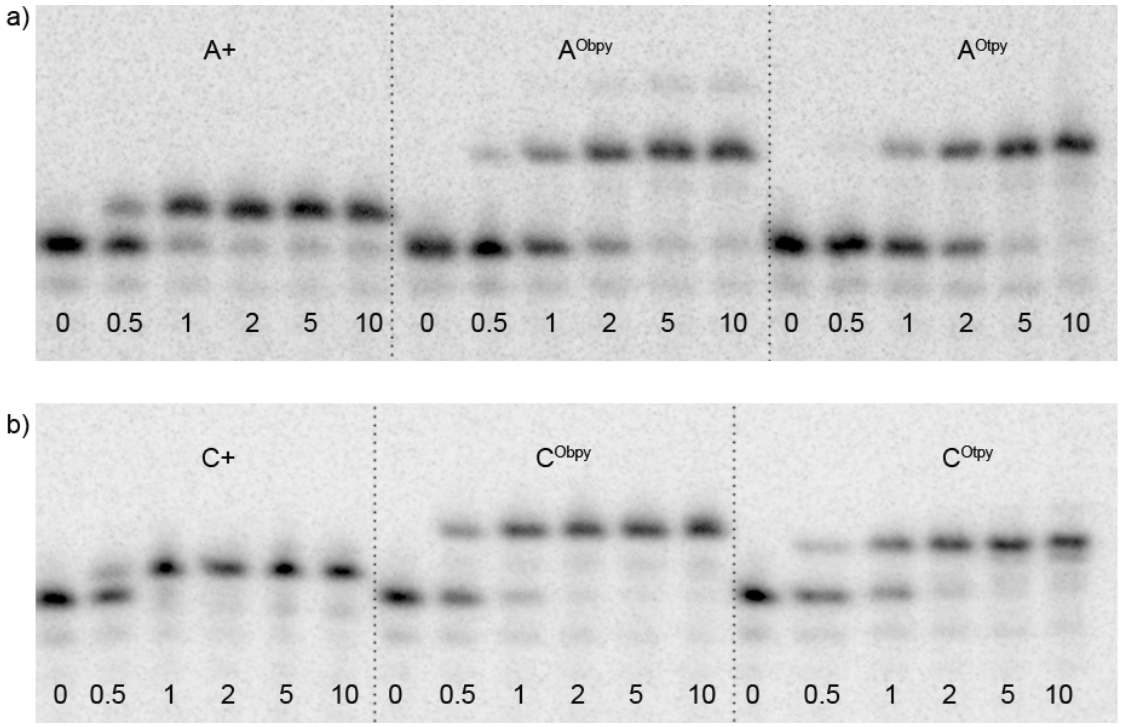


Figure 42. Comparison of the rate of the single-nucleotide PEX using Pwo polymerase with: a) natural dATP and modified **dA^{Obpy}TP** (**26a**) and **dA^{Otpy}TP** (**26b**) nucleotides and temp^{A1}, b) natural dCTP and modified **dC^{Obpy}TP** (**27a**) and **dC^{Otpy}TP** (**27b**) nucleotides and temp^{C1}. The reaction mixture was incubated for time intervals indicated (in minutes), followed by stopping the reaction by addition of PAGE loading buffer and immediate heating.

Multiple incorporations using 31-mer template temp^{rnd16}, requiring incorporation of four modified dNTPs in separate positions, were also tested (Figure 43). For incorporations of modified **dA^{OR}TPs** (**26a-b**) into the ONs Pwo (Figure 43a, lanes 5-6), KOD XL and Deep Vent (Figure 43b, lanes 5-6 and 12-13) polymerases can be used. Using above mentioned DNA polymerases lead to formation of fully extended products. Incorporation using DyNAzyme II polymerase was less feasible for incorporation of **dA^{Otpy}TP** (**26b**) and resulted in early termination of PEX (Figure 43a, lane 13). On the other hand, DyNAzyme II polymerase is the only suitable polymerase for incorporation of modified **dC^{OR}TP** (**27a-b**) (Figure 43a, lanes 14-15). Experiments using other polymerases lead to the mixture of ONs with different length (Figure 43, lanes 7-8 and 14-15).

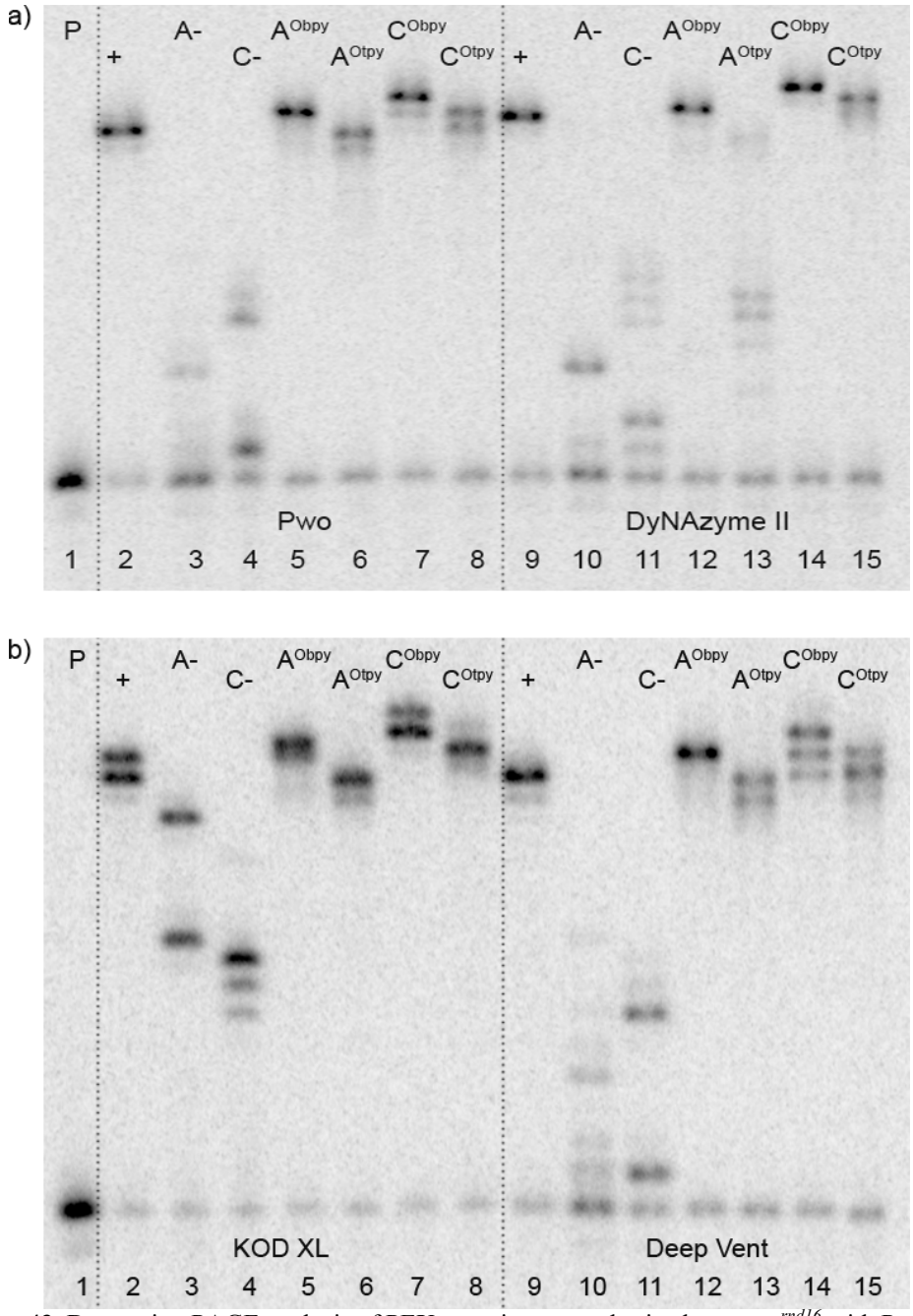


Figure 43. Denaturing PAGE analysis of PEX experiment synthesized on temp^{rnd16} with Pwo and DyNAzyme II polymerases (a) or KOD XL and Deep Vent (b) polymerase. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs. P: Primer; +: natural dNTPs; A-: dTTP, dCTP, dGTP; C-: dATP, dTTP, dGTP; A^{Obpy}: **$dA^{Obpy}TP$** (**26a**), dTTP, dCTP, dGTP; A^{Otpy}: **$dA^{Otpy}P$** (**26b**), dTTP, dCTP, dGTP; C^{Obpy}: **$dC^{Obpy}TP$** (**27a**), dATP, dTTP, dGTP; C^{Otpy}: **$dC^{Otpy}TP$** (**27b**), dATP, dTTP, dGTP.

Difference in electrophoretic mobility on gel between natural and modified ONs is even more significant than it was in the case of incorporation of **$dN^{ER}TPs$** (**15a-c** and **16a-c**; see chapter 3.1.3), due to the higher molecular weight of the modification. Therefore, verification of full-length products formation by measurement of MALDI mass spectroscopy of PEX products was necessary. Magnetoseparation protocol

(described in the chapter 3.1.3) was used and correct masses were confirmed for all products (Table 16).

Table 16. MALDI-Tof experiment (ss-ON)

Single-stranded PEX product [dNTPs used in PEX reaction]	Calculated mass (Da)	Found mass (Da)
pex^{A} [dATP, dGTP]	5973.0	5976.3
pex^{A} [**dA^{Obpy}TP (26a),** dGTP]	6230.3	6231.6
pex^{A} [(**dA^{Otpy}TP (26b),** dGTP]	6307.4	6310.0
pex^{C} [dCTP, dGTP]	5949.0	5948.2
pex^{C} [**dC^{Obpy}TP (27a),** dGTP]	6207.3	6208.8
pex^{C} [**dC^{Otpy}TP (27b),** dGTP]	6284.4	6286.0
pex^{rnd16} [dATP, dCTP, dTTP, dGTP]	9617.3	9616.5
pex^{rnd16} [**dA^{Obpy}TP (26a),** dCTP, dTTP, dGTP]	10646.6	10648.6
pex^{rnd16} [**dA^{Otpy}TP (26b),** dCTP, dTTP, dGTP]	10954.9	10956.1
pex^{rnd16} [dCTP, dATP, dTTP, dGTP]	9617.3	9618.3
pex^{rnd16} [**dC^{Obpy}TP (27a),** dATP, dTTP, dGTP]	10650.6	10651.7
pex^{rnd16} [**dC^{Otpy}TP (27b),** dATP, dTTP, dGTP]	10598.9	10959.8

3.2.3 Complexation studies

3.2.3.1 Complexation of modified nucleosides dN^{OR}

Due to the difficulties with the preparation and isolation of modified nucleoside monophosphates **dN^{OR}MPs** (**23a-b** and **24a-b**), easily obtainable modified nucleosides **dN^{OR}** (**21a-b** and **22a-b**) were tested as a model compound in complexation studies. Methanolic solutions of **dN^{OR}** (**21a-b** and **22a-b**) were mixed with 0.5 equiv. of divalent metal such as 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 (Figure 44, Figure 45).

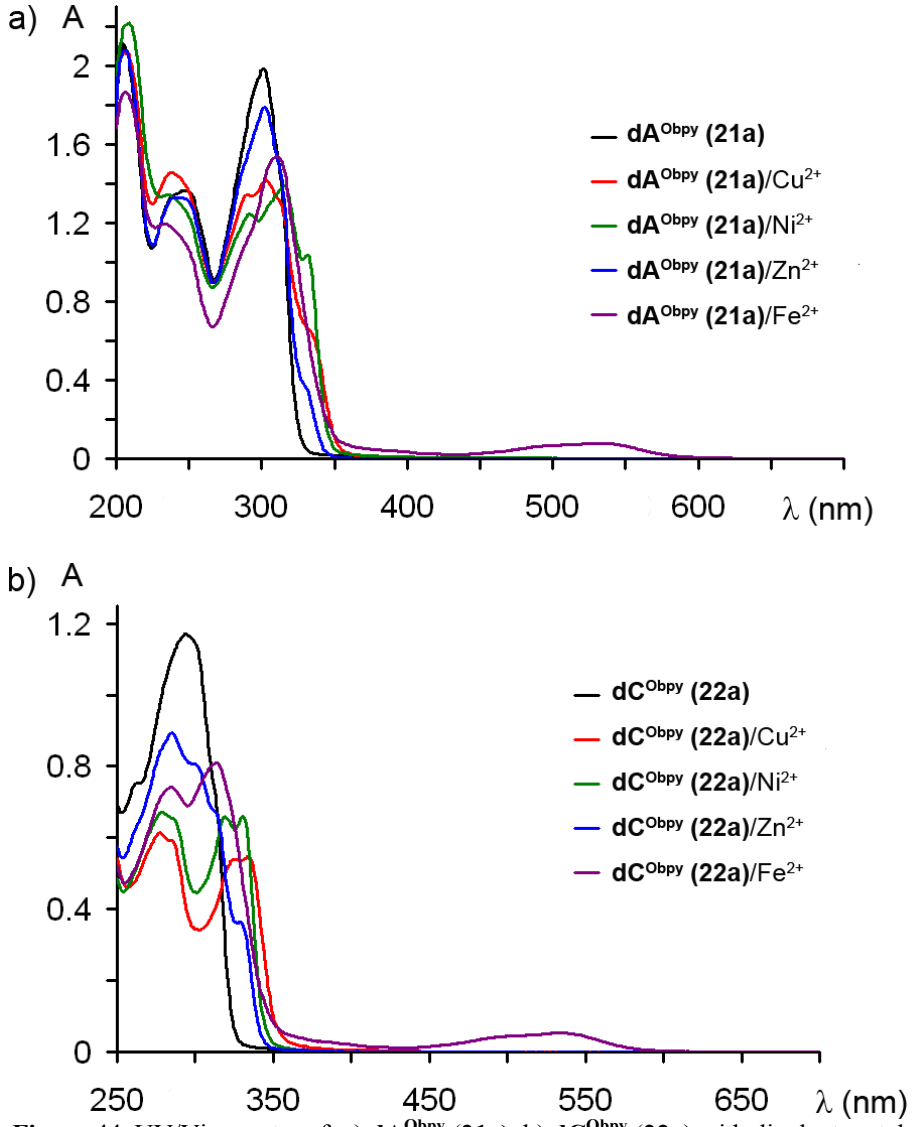


Figure 44. UV/Vis spectra of: a) **dA^{Obpy} (21a)**, b) **dC^{Obpy} (22a)** with divalent metals.

Although the MLCT bands of metal complexes of **dN^{Obpy}** (**21a** and **22a**) are overlapping with dominating absorbance due to the presence of bpy-modification (Figure 44), MLCT bands of all metal complexes of **dN^{Otpy} (21b** and **22b)** were easily detected (Figure 45), due to the fact, that oligopyridine unit is not conjugated with the nucleobase the dominating absorbance is shifted to lower wavelength, unlike the case when oligopyridine unit was attached to the nucleobase via acetylene linker (Figure 36, page 55).

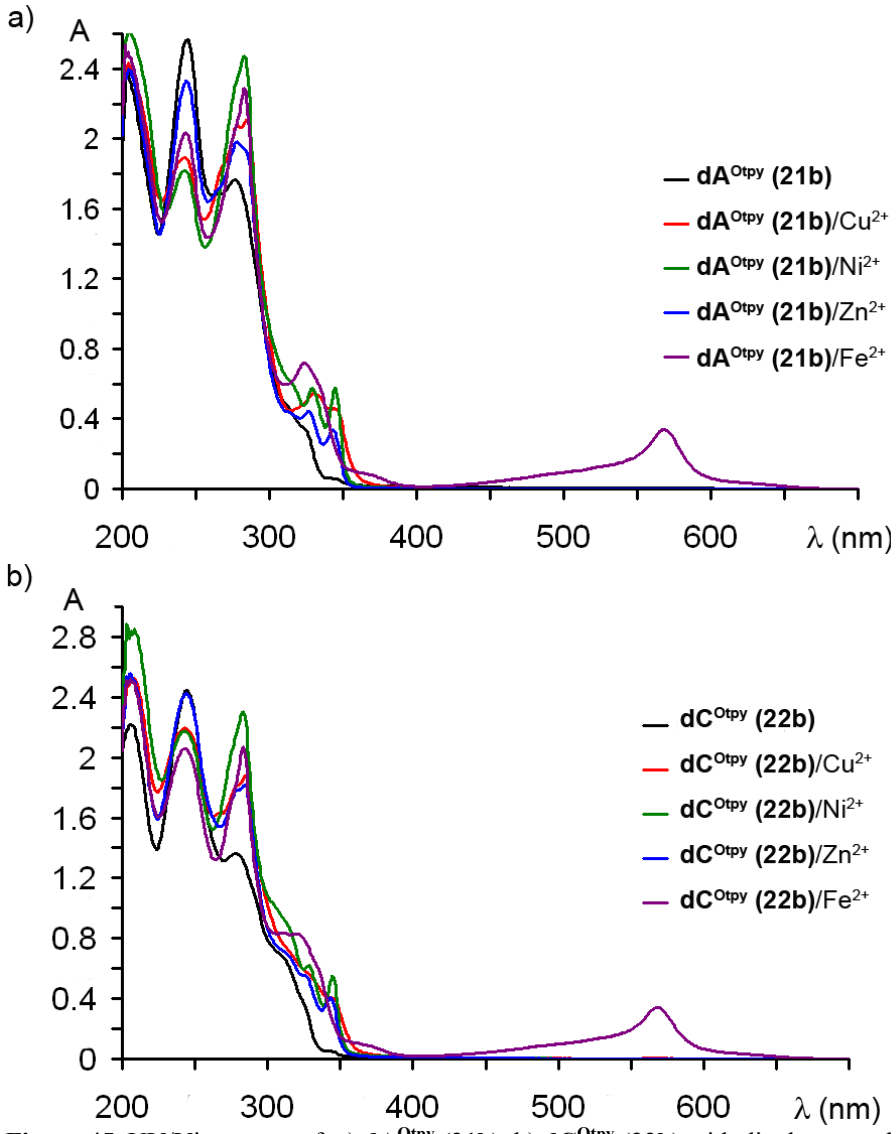


Figure 45. UV/Vis spectra of: a) **dA^{Otpy} (21b)**, b) **dC^{Otpy} (22b)** with divalent metals.

Due to the fact, that MLCT bands of all complexes formed with **dN^{Otpy}** (**21b** and **22b**) were successfully observed, complexation with oligopyridine modified DNAs were performed with all above mentioned metals.

3.2.3.2 Complexation of DNA modified with oligopyridine attached via octadiyne linker

Similarly as in the previous case (see chapter 3.1.4.2), the complex formation of modified DNA was detected by native polyacrylamide gel and UV/Vis spectroscopy.

For complexation studies, detected by native polyacrylamide gel electrophoresis, products of monoincorporation as well as products of multiple incorporations were tested. While pex^{A} were prepared by using temp^{A} (designed for incorporation of only one modified dATP) and by incorporation of dATP (natural DNA) and **dA^{Obpy}TP** (**26a**)

or **dA^{Otpy}TP** (**26b**) (modified DNA), pex^{C} were prepared by using temp^{C} (designed for incorporation of only one modified dCTP) and by incorporation of dCTP (natural DNA) and **dC^{Obpy}TP** (**27a**) or **dC^{Otpy}TP** (**27b**) (modified DNA) using Pwo polymerase. 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^{OR}TP** (**26a-b** and **27a-b**) used in PEX experiment) and incubated at room temperature overnight. Neither Obpy-modified (Figure 46) nor Otpy-modified DNA (Figure 47) duplexes formed complexes with any of used metals. Complex formation would result in a band with slower mobility in comparison to the band of non-metalated DNA duplex (lanes 1 or 6), but it was not observed for any of the metalated DNA duplexes, either natural (lanes 2-5), or modified ones (lanes 7-10), despite of longer time of incubation compared to the time required for complex formation of acetylene linked derivatives (see chapter 3.1.4.2).

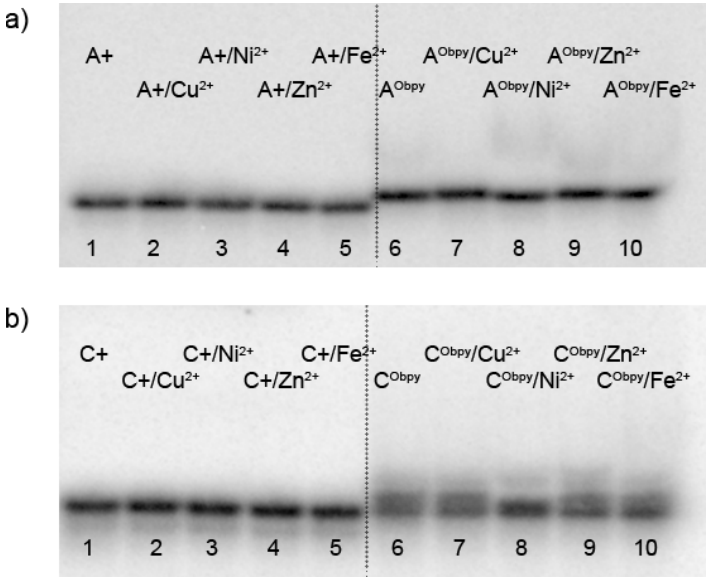


Figure 46. Non-denaturing gel electrophoresis (8% SB_PAGE) of Obpy-modified DNA duplexes in the absence and in the presence of M^{2+} for pex^{A} (a) and pex^{C} (b). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dGTP]; A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Obpy}: Obpy-modified DNA [**dA^{Obpy}TP** (**26a**), dGTP]; A^{Obpy}/M^{2+}: Obpy-modified DNA mixed with corresponding divalent metals; C+: unmodified DNA [dCTP, dGTP]; C+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; C^{Obpy}: Obpy-modified DNA [**dC^{Obpy}TP** (**27a**), dGTP]; C^{Obpy}/M^{2+}: Obpy-modified DNA mixed with corresponding divalent metals

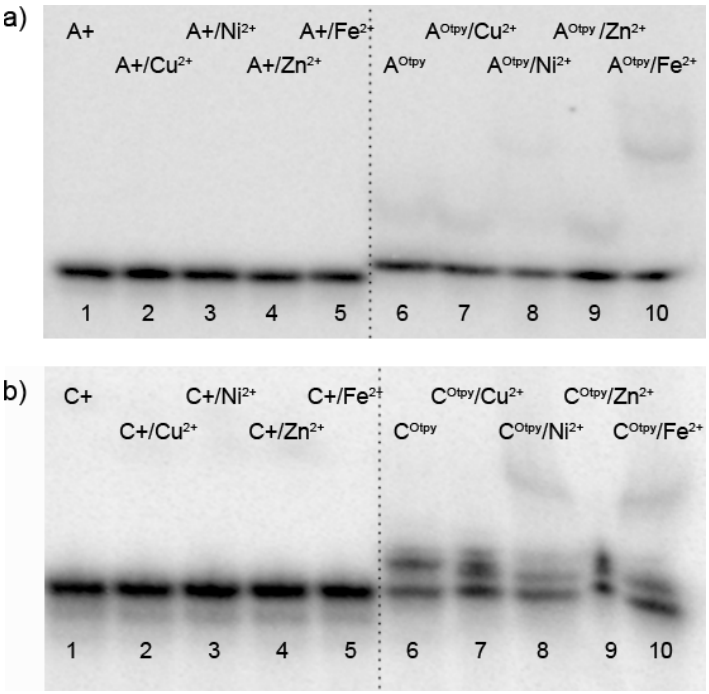


Figure 47. Non-denaturing gel electrophoresis (8% SB_PAGE) of Otpy-modified DNA duplexes in the absence and in the presence of M^{2+} for pex^{A} (a) and pex^{C} (b). 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dGTP]; A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Otpy}: Otpy-modified DNA [**$dA^{Otpy}TP$** (**26b**), dGTP]; A^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals; C+: unmodified DNA [dCTP, dGTP]; C+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; C^{Otpy}: Otpy-modified DNA [**$dC^{Otpy}TP$** (**27b**), dGTP]; C^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals.

In the case of products of multiple incorporations prepared by using $temp^{rnd16}$ (designed for incorporation of four modified dNTPs) and by incorporation of dATP (natural DNA) and **$dA^{Obpy}TP$** (**26a**) or **$dA^{Otpy}TP$** (**26b**) (modified DNA) using Pwo polymerase, or by incorporation of dCTP (natural DNA) and **$dC^{Obpy}TP$** (**27a**) or **$dC^{Otpy}TP$** (**27b**) using DyNAzyme II polymerase, successful complex formation (shown by bands with slower mobility) was observed only for Otpy-modified DNA duplexes with Ni^{2+} and Fe^{2+} ions (Figure 49, lanes 8 and 10). Similar change in mobility was not observed for natural DNA mixed with these metal ions (lanes 3 and 5). For Obpy-modified DNA duplexes, none (for C^{Obpy}) or only minor band (for A^{Obpy}/Ni^{2+} and $A^{Obpy}Fe^{2+}$) with slower mobility, proving successful complex formation with corresponding metal ions, was observed (Figure 48).

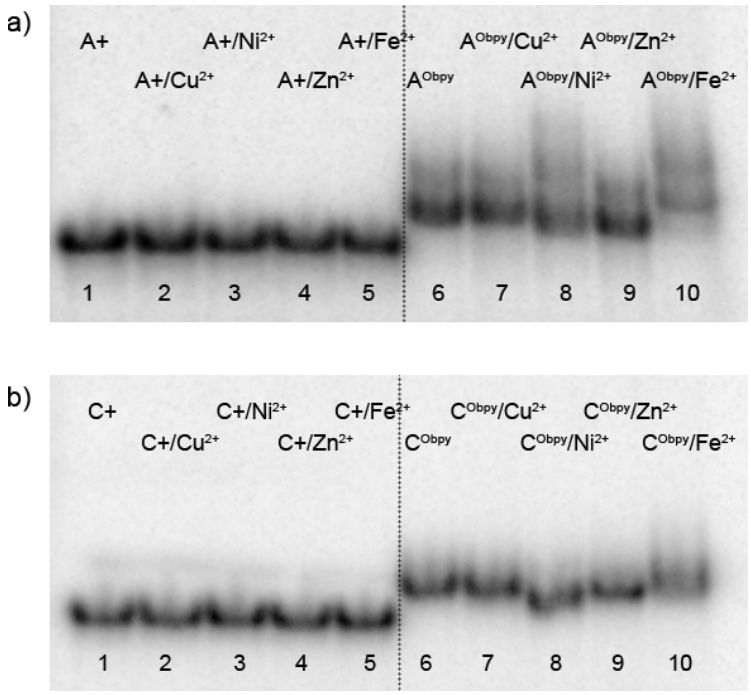


Figure 48. Non-denaturing gel electrophoresis (8% SB_PAGE) of Obpy-modified DNA duplexes in the absence and in the presence of M^{2+} for pex*rnd16*. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dTTP, dCTP, dGTP]; A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Obpy}: Obpy-modified DNA [**dA^{Obpy}TP** (**26a**), dTTP, dCTP, dGTP]; A^{Obpy}/M^{2+}: Obpy-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^{Obpy}: Obpy-modified DNA [dATP, dTTP, **dC^{Obpy}TP** (**27a**), dGTP]; C^{Obpy}/M^{2+}: Obpy-modified DNA mixed with corresponding divalent metals.

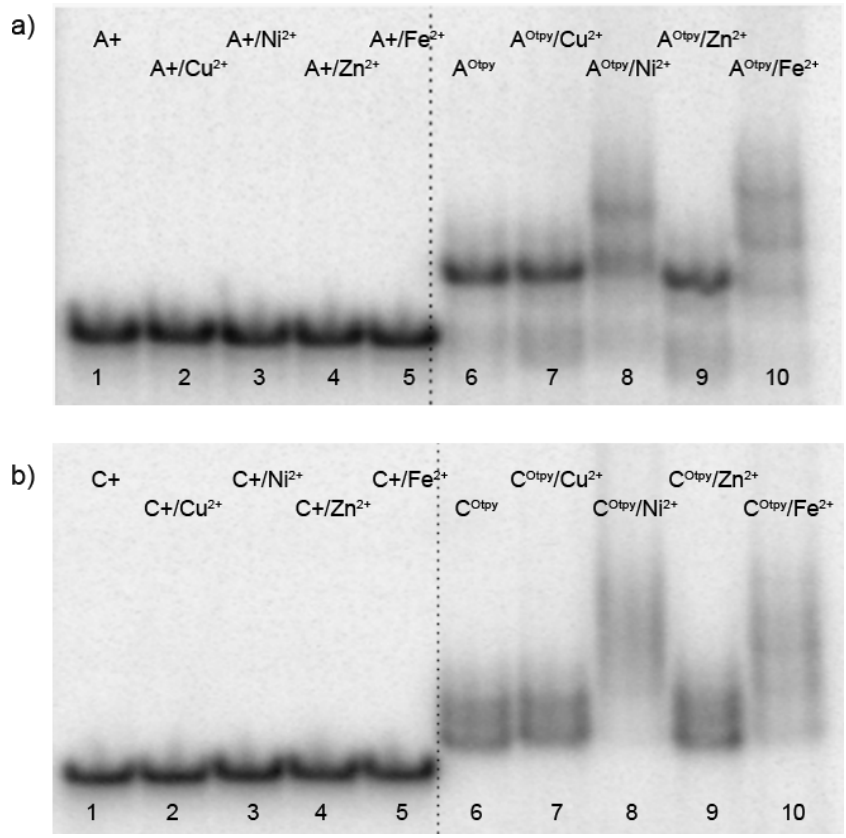


Figure 49. Non-denaturing gel electrophoresis (8% SB_PAGE) of Otpy-modified DNA duplexes in the absence and in the presence of M^{2+} for pex*rnd16*. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dTTP, dCTP, dGTP]; A+/M^{2+}: unmodified DNA mixed with corresponding divalent metals; A^{Otpy}: Otpy-modified DNA [**dA^{Otpy}TP** (**26b**), 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** (**27b**), dGTP]; C^{Otpy}/M^{2+}: Otpy-modified DNA mixed with corresponding divalent metals.

Successful complex formation of pex*rnd16* was also confirmed by measurement of UV/Vis spectra. The pex*rnd16* products were prepared on larger scale by using Deep Vent [for incorporation of **dA^{Otpy}TP** (**26b**)] or DyNAzyme II polymerases [for incorporation of **dC^{Otpy}TP** (**27b**)]. Natural DNA was prepared by incorporation of natural dNTPs, while modified DNAs were prepared by using **dA^{Otpy}TP** (**26b**) as surrogates of natural dATP in one case or **dC^{Otpy}TP** (**27b**) as surrogates of natural dCTP in the second case. After the PEX reaction, PEX products were purified from unincorporated **dN^{Otpy}TPs** (**26b** or **27b**) and then 0.5 equiv of M^{2+} ions per each modification was added to the solution of natural or modified DNA. The mixture was incubated overnight at room temperature and then UV/Vis spectra of non-metalated and metalated DNA duplexes (either natural or modified one) were recorded. The dominant absorbance at ca. 260 nm originates from the absorbance of natural nucleotides (Figure 50, 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 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 as 580 nm (magenta line).

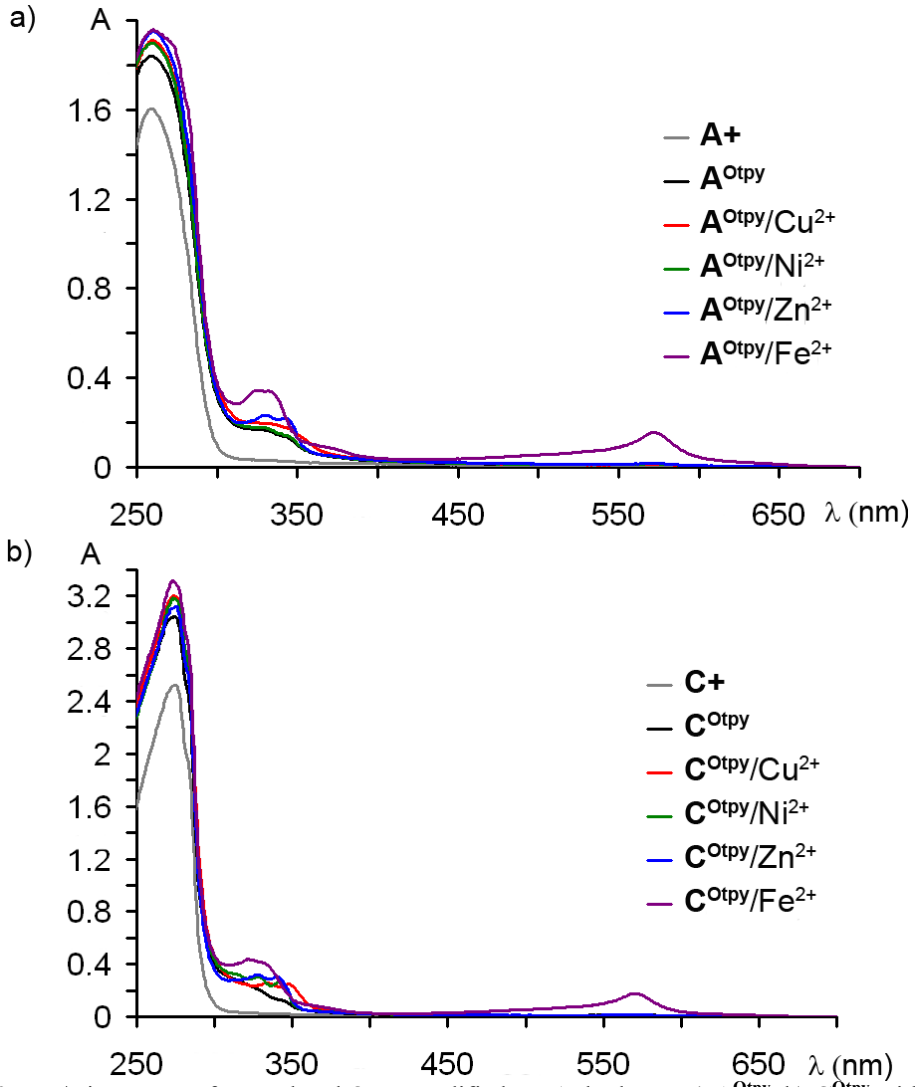


Figure 50. UV/Vis spectra of natural and Otpy-modified DNA duplexes: a) $\mathbf{A^{Otpy}}$, b) $\mathbf{C^{Otpy}}$ with divalent metals.

Similar MLCT bands were not observed for natural DNA duplex in the presence of the corresponding metal cations (Figure 51).

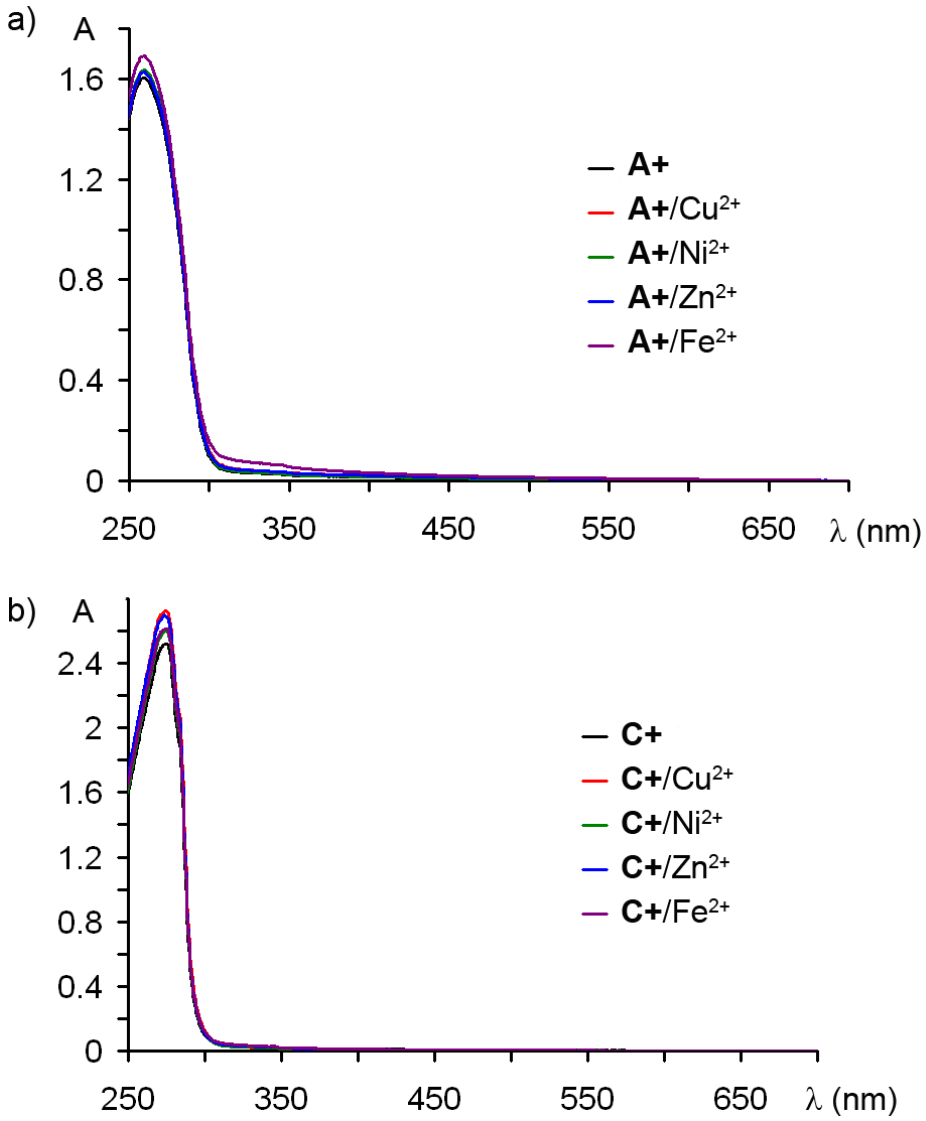


Figure 51. UV/Vis spectra of natural DNA duplexes: a) **A**+, b) **C**+ with divalent metals.

3.3 DNA complexes with acetylene linker vs. DNA complexes with octadiyne linker

Short and rigid acetylene linker pre-determinated modified DNA to form only inter-strand complexes (Figure 52a), while long and flexible octadiyne linker might also enable formation of intra-strand complexes (Figure 52b).

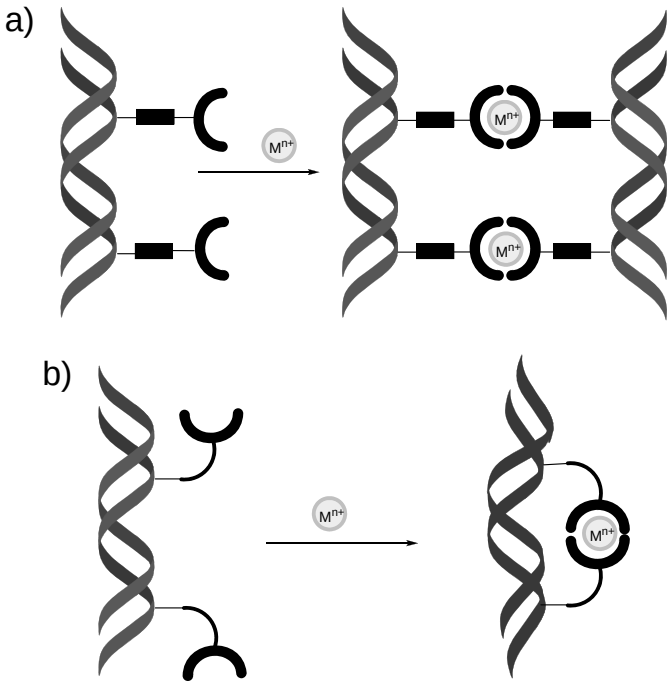


Figure 52. Schematic representation of: a) inter-strand, b) intra-strand DNA complexes.

While formation of metal complexes of Etpy-modified DNA were observed for duplexes modified with one as with four Etpy-modifications (see chapter 3.1.3), Otpy-modified DNA formed complex only in the case, when four Otpy-modifications were placed in the macromolecule (see chapter 3.2.2). Since no complex formation was observed for DNA containing only one Otpy-modification, the question if intra-strand or inter-strand complex is formed arises.

The possible formation of intra-strand DNA complex was proved by computational studies (Figure 53) carried out by Dr. Jindřich Fanfrlík. The structure was build and minimized by the Nucleic Acid Builder in AMBER[67]. The system was neutralized with sodium ions. The generalized Born implicit solvent model was used. Molecular mechanics parameters from ff03 force field were employed. Parameters of $(tpy)_2Fe$ were taken from the literature[68] and the GAFF parameters were used for the modified residue.

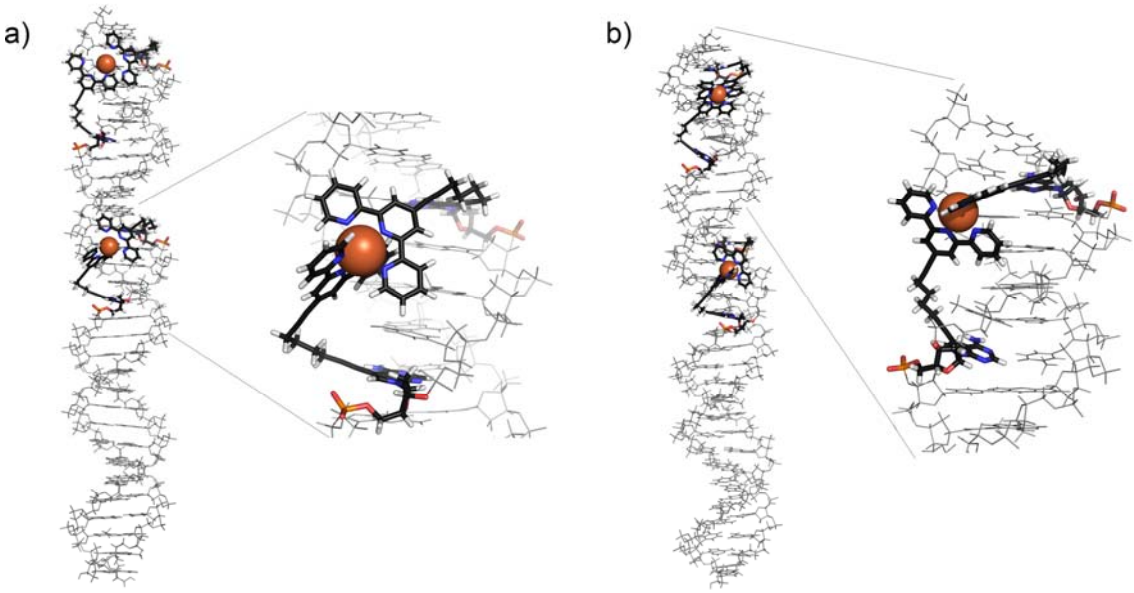


Figure 53. The calculated structure of possible intra-strand DNA complex.

If the B-type double helix of DNA remained unaffected during this calculation, only one $(tpy)_2Fe$ complex (when two modified A^{Otpy} units are separated by three natural nucleotides) can be successfully formed (Figure 53a), while the second pair of tpy-modifications (separated by four natural nucleotides) is too distant from each other to adopt the octahedral geometry of $(tpy)_2Fe$ complex. On the other hand, formation of two $(tpy)_2Fe$ complexes must involve rotation of a modified nucleotide and from that resulting bending of DNA double helix (Figure 53b).

Due to different types of behavior of Etpy-modified and Otpy-modified DNA during the complexation studies, the corresponding complexes were compared. Since the ONs containing only one Otpy-modification do not form metal complexes (see chapter 3.2.2) and **dC^{Etpy}TP** (**16c**) was not a good substrate in multiple incorporations (see chapter 3.1.3), we focused in our investigation on DNA prepared by PEX experiment using temp^{rnd16} and dATP (natural DNA), **dA^{Etpy}TP** (**16c**) (modified DNA with acetylene linked oligopyridine units) and **dA^{Otpy}TP** (**26b**) (modified DNA with octadiyne linked oligopyridine units).

First we compared the mobility of these complexes on native polyacrylamide gel (Figure 54). The complex of Etpy-modified DNA with Fe^{2+} ions show slower mobility (lane 6) in comparison to non-metalated Etpy-modified DNA (lane 4) due to its double DNA chain length, than it is in the case of intra-strand DNA complex formed from Otpy-modified DNA (lane 9) in comparison to non-metalated Otpy-modified DNA (lane 7).

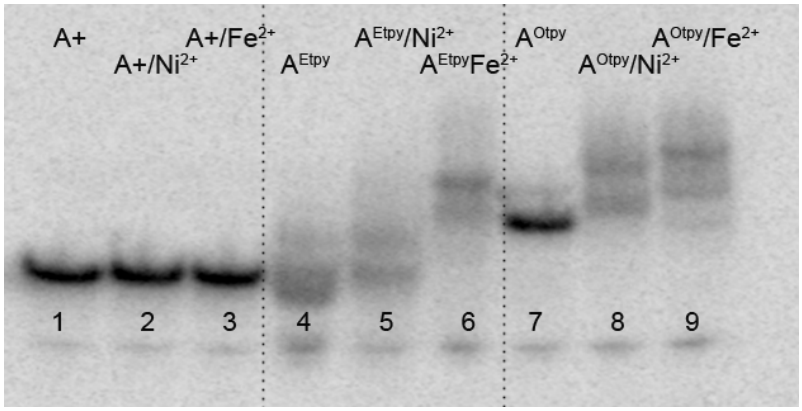


Figure 54. Non-denaturing gel electrophoresis (8% SB_PAGE) of tpy-modified DNA duplexes in the absence and in the presence of M^{2+} for pex^{rnd16}. 5'-^{32}P-end labelled primer-template was incubated with different combinations of natural and functionalized dNTPs: A+: unmodified DNA [dATP, dTTP, dCTP, dGTP]; A+/M^{2+}: unmodified DNA mixed with indicated divalent metals; A^{Etpy}: Etpy-modified DNA [**dA^{Etpy}TP** (**16c**), dTTP, dCTP, dGTP]; A^{Etpy}/M^{2+}: Etpy-modified DNA mixed with indicated divalent metals; A^{Otpy}: Otpy-modified DNA [**dA^{Otpy}TP** (**26b**), dTTP, dCTP, dGTP]; A^{Otpy}/M^{2+}: Otpy-modified DNA mixed with indicated divalent metals.

The possible formation of intra-strand complexes of Otpy-modified DNA prepared by PEX experiment using temp^{rnd16} was also confirmed by measurement of MALDI-Tof spectra (Figure 55). For this experiment, natural and Otpy-modified DNA prepared by incorporation of dATP or **dA^{Otpy}TP** (**26b**) by using Deep Vent polymerase were prepared. PEX products were purified from unincorporated dNTPs and then Otpy-modified DNA was divided into two parts one of which was mixed with 1 equiv. of $Fe(BF_4)_2 \cdot 6H_2O$ (calculated to the number of modification in DNA). Measurement of MALDI spectra proved successful formation of full length products in the case of natural DNA - **DNA (A+)** (Figure 55a) as well as in the case of Otpy-modified DNA - **DNA (A^{Otpy})** (Figure 55b), where the masses of the template and complementary strand were observed. For metalated DNA, formation of two complexes **DNA (A^{Otpy})·1Fe^{2+}** and **DNA (A^{Otpy})·2Fe^{2+}** was detected, while no non-metalated modified DNA was observed (Figure 55c). Calculated and observed masses are shown in Table 17, where are dedicated only the masses of synthesized strand, while the mass of the template is the same for all of the PEX products (calculated mass of temp^{rnd16}: 9421 Da). Althought the formation of inter-strand DNA complex (calculated mass: 21956.4 Da) was not observed in MALDI, its presence can not be exluded, because of the difficult ionization of high molecular weight oligonucleotides and from this resulting complicated detection.

Table 17. MALDI-Tof experimend (ds DNA).

Double-stranded PEX products [dNTPs used in PEX reaction]	Calculated mass (Da)	Found mass (Da)
DNA (A+) [dATP, dCTP, dGTP, dTTP]	9613.6	9618.3
DNA (A^{Otpy}) [**$dA^{Otpy}TP$ (26b),** dCTP, dGTP, dTTP]	10951.3	10957.6
DNA (A^{Otpy})·1Fe^{2+} [**$dA^{Otpy}TP$ (26b),** dCTP, dGTP, dTTP]	11005.1	11013.0
DNA (A^{Otpy})·2Fe^{2+} [**$dA^{Otpy}TP$ (26b),** dCTP, dGTP, dTTP]	11060.9	11066.6

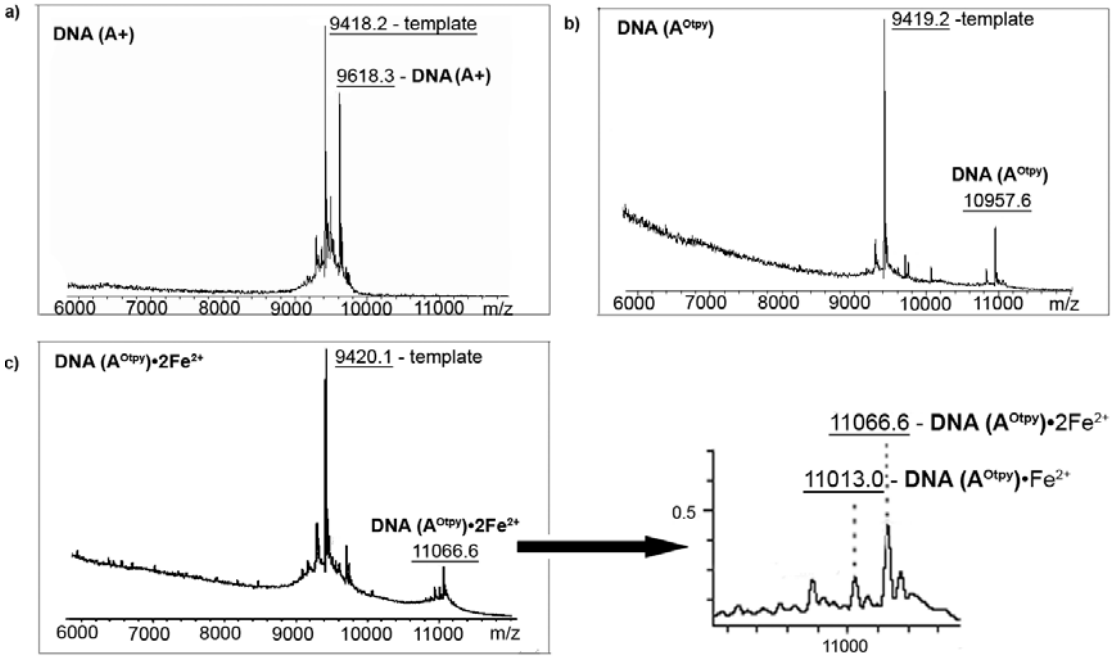


Figure 55. MALDI-Tof spectra of: a) natural DNA - **DNA (A+)** (calculated mass: 9613.6 Da), b) Otpy-modified DNA - **DNA (A^{Otpy})** (calculated mass: 10951.3 Da), c) Otpy-modified DNA – **DNA (A^{Otpy})·xFe^{2+}** (calculated mass: 11005.1 Da for **DNA (A^{Otpy})·1Fe^{2+}** and 11060.9 Da for **DNA (A^{Otpy})·2Fe^{2+}**).

The CD-spectra of natural and tpy-modified DNA (either non-metalated or metalated) prepared by PEX using $temp^{rnd16}$ (Figure 56), were measured and interpreted by Dr. Lucie Bednárová. For natural as well as for tpy-modified DNAs standard B-type conformation was observed. While the negative minimum for natural (A+) and Etpy-modifed (A^{Etpy}) DNAs was found at 245 nm, negative minimum for Otpy-modified (A^{Otpy}) DNA was shifted to 250 nm. 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 complex 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 the 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 or by diminishing of the arrangement of DNA double helix caused by the presence of the tpy-modification or by intercalation of tpy-unit, 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+}**.

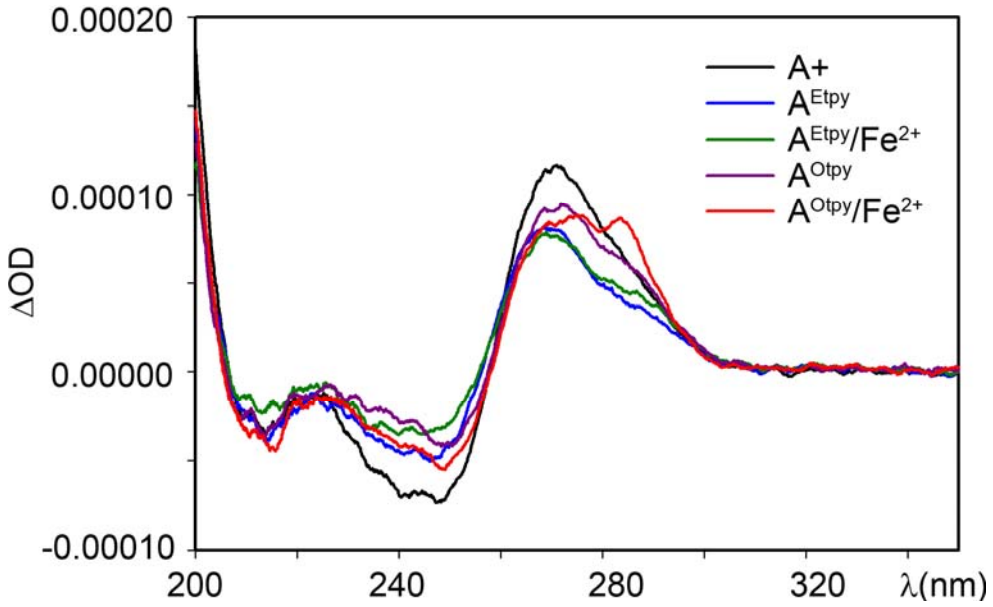


Figure 56. CD-spectra of natural and tpy-modified DNA duplexes prepared from temp^{rnd16} in the absence or presence of Fe^{2+} ions.

Although the possible formation of intra-strand DNA complex was confirmed by PAGE, MALDI, CD spectroscopy and modelling, the formation of diverse inter-strand DNA complex is certainly also possible and their characterization is not feasible. Most likely, these Otpy-modified DNA duplexes in presence of Fe^{2+} ions form dynamic mixtures of complexes (both intra- and inter-strand) and aggregates which makes their application in supramolecular chemistry rather limited.

4 Conclusion

Nucleosides, bearing oligopyridine ligands attached via short and rigid (acetylene or phenylene) or long and flexible (octadiyne) linker, were synthesized. For this purpose, Sonogashira or Suzuki-Miyaura cross-coupling reactions of unprotected nucleosides with oligopyridine-linked acetylenes or boronates in the presence of $Pd(OAc)_2$/TPPTS catalytic system in DMF or in the mixture water/acetonitrile, were successfully used. Although, Sonogashira cross-couplings proceeded under standard condition, reaction conditions of Suzuki cross-coupling had to be optimized. While aqueous-phase Sonogashira cross-coupling reaction was also successfully used for synthesis of nucleotides bearing oligopyridine ligands attached via acetylene tether, nucleotides bearing oligopyridine ligands attached via octadiyne linker had to be prepared by phosphorylation of corresponding oligopyridine-modified nucleosides.

Oligopyridine-modified nucleoside or nucleotides were tested as model compounds for further complexation studies on oligopyridine-modified DNA. Terpyridine modified nucleosides/nucleotides, bearing the modification attached via aoctadiyne/acetylene linker, clearly formed complexes with Fe^{2+} ions, which can be easily detected by UV/Vis spectroscopy due to its characteristic MLCT band at 580 nm.

Two different strategies can be used for the synthesis of modified nucleoside triphosphates: (i) single-step aqueous-phase Sonogashira cross-coupling of halogenated nucleoside triphosphate or (ii) triphosphorylation of corresponding modified nucleosides. Both approaches were utilized. Like in the synthesis of nucleosides monophosphates, aqueous phase Sonogashira cross-coupling was applied for synthesis of nucleoside triphosphates bearing oligopyridine modification attached via acetylene linker, while classical triphosphorylation of modified nucleosides had to be used for synthesis of nucleoside triphosphates bearing oligopyridine modification attached via octadiyne tether, due to the low solubility of corresponding building blocks in the mixture water/acetonitrile and from this resulting low yields.

All oligopyridine-modified nucleosides triphosphates were incorporated into DNA by primer extension experiment. Single and multiple incorporations by using several DNA polymerases were tested. While nucleoside triphosphates bearing oligopyridine ligands attached via octadiyne linker were successfully incorporated in all tested sequences, incorporation of nucleoside triphosphates bearing oligopyridine ligands attached via short acetylene tether was more demanding and has to be optimized. Even

after these optimizations, incorporation of terpyridine-modified dCTP was less feasible and resulted in early termination of primer extension experiment.

Functionalized DNA was tested in complexation studies. Most stable complexes were formed by mixing terpyridine-modified DNA with Fe^{2+} ions and complex formation was detected by polyacrylamide gel electrophoresis and by UV/Vis spectroscopy. While DNA bearing terpyridine unit attached via acetylene linker can formed only inter-strand complex, due to the shortness and rigidity of the linker, DNA bearing terpyridine unit attached via octadiyne tether is able to form as inter-strand as intra-strand metal complexes. Possible formation of intra-strand DNA complex was first proved by computional calculation and then detected by native polyacrylamide gel, MALDI spectra and circular dichroism spectroscopy. However the fact, that formation of inter-strand complexes can be accompanied by formation of inter-strand DNA complexes, may complicate applications in self-assembly.

5 List of publications

1. Kalachova, L.; Pohl, R.; Hocek, M.: "Synthesis of 2'-Deoxyuridine and 2'-Deoxycytidine Nucleosides Bearing Bipyridine and Terpyridine Ligands at Position 5" *Synthesis* **2009**, 105-112.

2. Vrábel, M.; Horáková, P.; Pivoňková, H.; Kalachova, L.; Černocká, H.; Cahová, H.; Pohl, R.; Šebest, P.; Havran, L.; Hocek, M.; Fojta, M.: "Base-Modified DNA Labeled by $[Ru(bpy)_3]^{2+}$ and $[Os(bpy)_3]^{2+}$ Complexes: Construction by Polymerase Incorporation of Modified Nucleoside Triphosphates, Electrochemical and Luminescent Properties, and Applications" *Chem. Eur. J.* **2009**, *15*, 1144-1154.

3. Kalachova, L.; Pohl, R.; Hocek, M.: "Synthesis of nucleoside mono- and triphosphates bearing oligopyridine ligands, their incorporation into DNA and complexation with transition metals" *Org. Biomol. Chem.* **2012**, *10*, 49-55.

4. Kalachova, L.; Pohl, R.; Fanfrlík, J.; Bednárová, L. and Hocek, M.: "Synthesis of nucleosides and dNTPs bearing oligopyridine ligands linked through octadiyne tether, their incorporation into DNA and complexation with transition metal ions" *Org. Biomol. Chem.* (under revision)

6 Experimental section

General

Oligopyridinyl acetylene or boronates, halogenated mohophosphates[45,64] and halogenated triphosphates[42,49] were prepared according to the literature procedures. $POCl_3$ 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. All reactions were performed under argon atmosphere. Preparative flash chromatography on reverse phase was performed on Biotage SP1 flash purification system. Semi-preparative HPLC separations were performed on column packed with 10 μm C18 reversed phase (Phenomenex, Luna C18(2)). NMR spectra were measured on a Bruker Avance 400 (400 MHz for ^{1}H, and 100.6 MHz for ^{13}C), Bruker Avance 500 (500.0 MHz for ^{1}H, 125.7 MHz for ^{13}C and 202.3 for ^{31}P) or 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)), in D_2O (referenced to dioxane as internal standard, $_{\delta}H$ = 3.75 ppm, $_{\delta}C$ = 69.3 ppm, standard for ^{31}P NMR was external H_3PO_4) 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 LCQ classic (Thermo-Finnigan) spectrometer using ESI or Q-Tof Micro (Waters, ESI source, internal calibration with lockspray). Mass spectra of functionalized DNA were measured by Maldi-TOF, Reflex IV (Bruker) with nitrogen laser. UV/Vis spectra were measured on Varian CARY 100 Bio spectrophotometer at room temperature. CD spectra were measured on Jasco 815. Melting points were determined on a Kofler block.

Fot numbering scheme for NMR assignment see Figure 57.

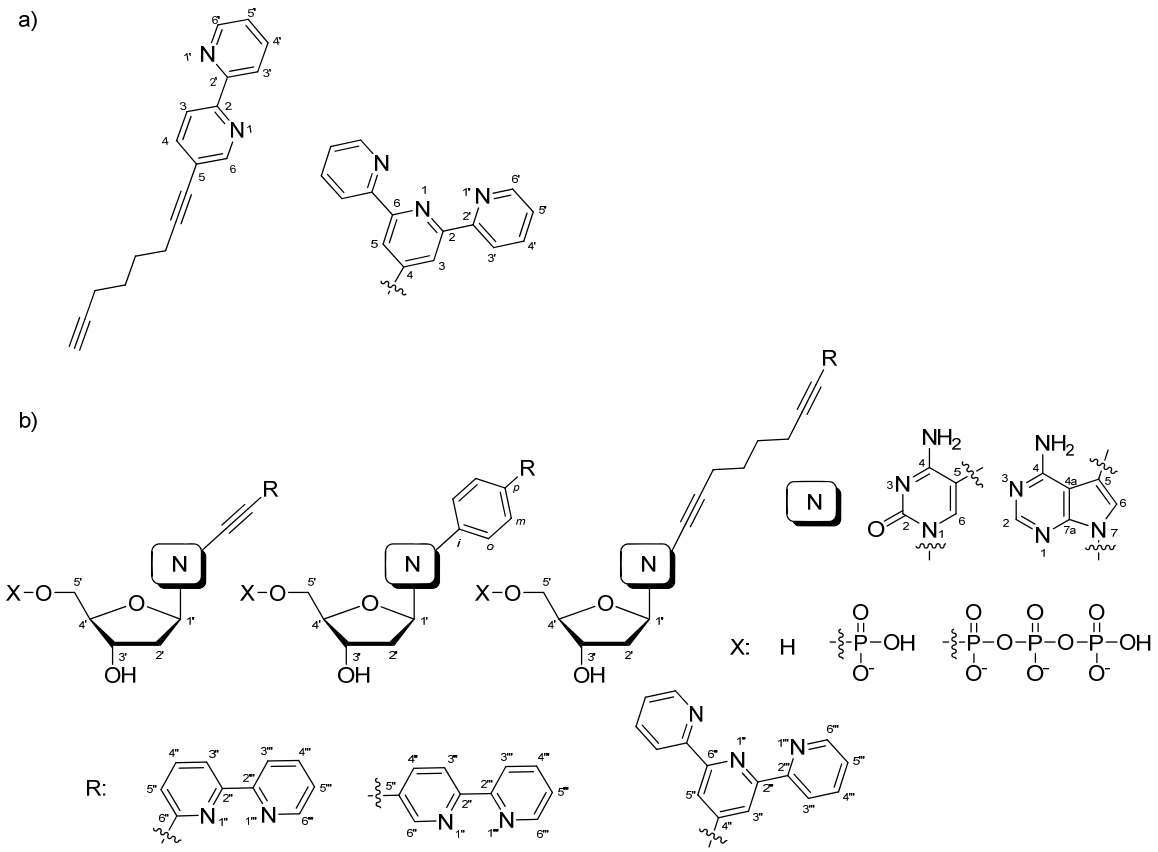


Figure 57. Numbering scheme for NMR assignment for: a) ligand building blocks; b) modified nucleosides, nucleoties and dNTPs

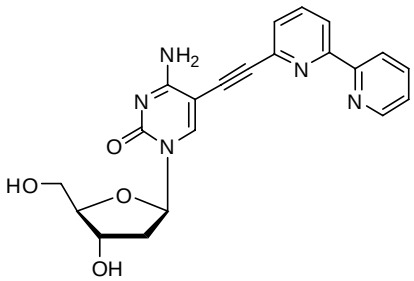
6.1 Oligopyridine analogues with rigid linker

6.1.1 Synthesis of modified nucleosides, nucleotides and nucleoside triphosphates

General procedure I: Sonogashira cross-coupling reaction – synthesis of modified dN^{ER}

DMF (1 ml) and Et(*i*-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing nucleoside **dC^{I} (3)** or **dU^{I} (4)** (44 mg), an alkyne **1a-c** (1.5 equiv.) and CuI (10 mol%). In a separate flask, Pd(OAc)$_2$ (5 mol%) and TPPTS (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 stired at 75 °C for 2 h. The solvent was evaporated in vacuo. Products were directly purified by flash chromotagraphy on reverse phase using H_2O/MeOH (0% to 100%) as an eluent.

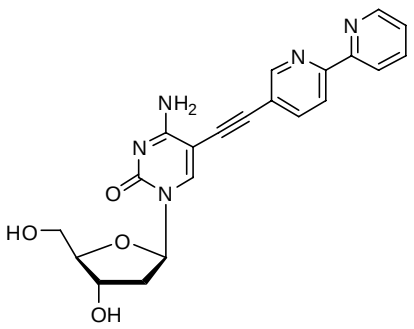
5-[(2'',2'''-bipyridin-6''-yl)ethynyl]-2'-deoxycytidine (dC^{E6bpy}, 5a)



Prepared according to the general procedure I from **dC^{I} (3)** and bipyridinyl acetylene **1a**. It was isolated as a brown powder in the yield of 70% (35.4 mg) which was then crystallized for the purpose of elemental analysis from the mixture MeOH/H_2O.

^{1}H NMR (500 MHz, DMSO-d_6): 2.07 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 6.1, H-2'b); 2.19 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.7, H-2'a); 3.59 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.1, $J_{5'b,4'}$ = 3.7, H-5'b); 3.67 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.1, $J_{5'a,4'}$ = 3.5, H-5'a); 3.82 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.5, $J_{4',3'}$ = 3.3, H-4'); 4.24 (dddd, 1H, $J_{3',2'}$ = 6.1, 3.7, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.3, H-3'); 5.17 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5'); 5.26 (d, 1H, $J_{3',OH}$ = 4.3, OH-3'); 6.15 (dd, 1H, $J_{1',2'}$ = 7.0, 6.1, H-1'); 7.17 (bs, 1H, NH$_a$**H$_b$**); 7.49 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.87 (dd, 1H, $J_{5'',4''}$ = 7.7, $J_{5'',3''}$ = 1.1, H-5''); 7.90 (bs, 1H, N**H$_a$**H$_b$); 7.97 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.00 (dd, 1H, $J_{4'',3''}$ = 8.0, $J_{4'',5''}$ = 7.7, H-4''); 8.36 (dd, 1H, $J_{3'',4''}$ = 8.0, $J_{3'',5''}$ = 1.1, H-3''); 8.38 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 1.0, H-3'''); 8.43 (s, 1H, H-6); 8.71 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 1.0, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 41.10 (CH_2-2'); 61.13 (CH_2-5'); 70.21 (CH-3'); 81.62 (C5-**C**≡C-C6''); 85.76 (CH-1'); 87.75 (CH-4'); 88.79 (C-5); 93.86 (C5-C≡**C**-C6''); 120.10 (CH-3''); 120.90 (CH-3'''); 124.79 (CH-5'''); 128.05 (CH-5''); 137.67 (CH-4'''); 137.95 (CH-4''); 142.42 (C-6''); 146.22 (CH-6); 149.61 (CH-6'''); 153.53 (C-2); 154.72 (C-2'''); 155.70 (C-2''); 164.05 (C-4); MS (ESI): *m/z* (%) = 428 (15) [M^+ + Na], 833 (100) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M^+ + H] calcd for $C_{21}H_{20}O_4N_5$: 406.1510; foud: 406.1514; IR (KBr): 3392, 1645, 1500, 1094, 780 cm^{-1}; Mp 139-146°C.

5-[(2'',2'''-bipyridin-5''-yl)ethynyl]-2'-deoxycytidine (dC^{E5bpy}, 5b)

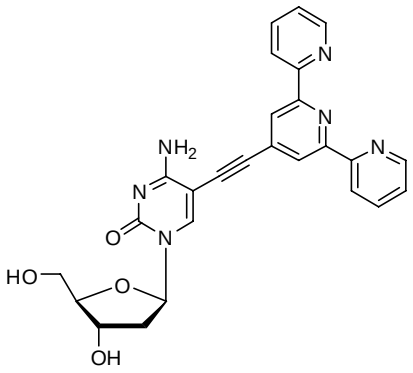


Prepared according to the general procedure I from **dC^{I} (3)** and bipyridinyl alkyne **1b**. It was isolated as a slightly yellow powder in the yield of 67% (33.8 mg), which was then crystallized for the purpose of elemental analysis from the mixture EtOH/H_2O.

^{1}H NMR (500 MHz, DMSO-d_6): 2.05 (dt, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.4, H-2'b); 2.20 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.9, H-2'a); 3.60 and 3.68 (2 × bdt, 2H, J_{gem} = 11.9, $J_{5',OH}$ = $J_{5',4'}$

= 3.5, H-5'); 3.82 (q, 1H, $J_{4',5'} = J_{4',3'} = 3.5$, H-4'); 4.25 (bm, 1H, $J_{3',2'} = 6.4, 3.9$, $J_{3',OH} = 4.3$, $J_{3',4'} = 3.5$, H-3'); 5.17 (bt, 1H, $J_{OH,5'} = 3.5$, OH-5'); 5.26 (bd, 1H, $J_{3',OH} = 4.3$, OH-3'); 6.13 (dd, 1H, $J_{1',2'} = 6.4, 6.1$, H-1'); 7.21 (bs, 1H, $NH_a\mathbf{H_b}$); 7.47 (ddd, 1H, $J_{5''',4'''} = 7.5$, $J_{5''',6'''} = 4.8$, $J_{5''',3'''} = 1.2$, H-5'''); 7.85 (bs, 1H, $N\mathbf{H_a}H_b$); 7.97 (ddd, 1H, $J_{4''',3'''} = 7.9$, $J_{4''',5'''} = 7.5$, $J_{4''',6'''} = 1.8$, H-4'''); 8.14 (dd, 1H, $J_{4'',3''} = 8.3$, $J_{4'',6''} = 2.1$, H-4''); 8.40 (ddd, 1H, $J_{3''',4'''} = 7.9$, $J_{3''',5'''} = 1.2$, $J_{3''',6'''} = 1.0$, H-3'''); 8.41 (dd, 1H, $J_{3'',4''} = 8.4$, $J_{3'',6''} = 0.9$, H-3''); 8.42 (s, 1H, H-6); 8.70 (ddd, 1H, $J_{6''',5'''} = 4.8$, $J_{6''',4'''} = 1.8$, $J_{6''',3'''} = 1.0$, H-6'''); 8.88 (dd, 1H, $J_{6'',4''} = 2.1$, $J_{6'',3''} = 0.9$, H-6''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 41.12 (CH_2-2'); 61.06 (CH_2-5'); 70.09 (CH-3'); 85.77 (CH-1'); 86.36 (C5-**C**≡C-C5''); 87.70 (CH-4'); 89.22 (C-5); 91.18 (C5-C≡**C**-C5''); 120.05 (CH-3''); 120.09 (C-5''); 121.00 (CH-3'''); 124.71 (CH-5'''); 137.68 (CH-4'''); 139.58 (CH-4''); 145.79 (CH-6); 149.68 (CH-6'''); 151.46 (CH-6''); 153.57 (C-2); 154.11 (C-2''); 154.69 (C-2'''); 163.93 (C-4); MS (ESI): *m/z* (%) = 405 (3) [M^+], 428 (16) [M^+ + Na], 833 (100) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M^+ + H] calcd for $C_{21}H_{20}O_4N_5$: 406.1510; found: 406.1512; Anal. Calcd for $C_{21}H_{19}O_4N_5$. 1/2H_2O: C, 60.86; H, 4.86; N, 16.90; Found C, 61.13; H, 4.67; N, 16.60; IR (KBr): 3448, 1656, 1634, 1501, 1462, 1101, 794, 781, 742 cm^{-1}; Mp over 300°C.

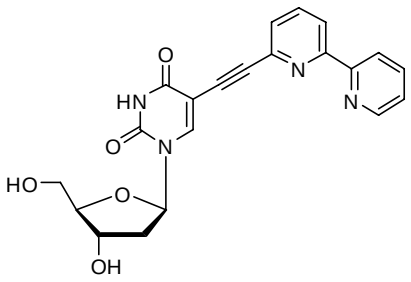
5-[(2'',2'''':6'',2'''-terpyridin-4''-yl)ethynyl]-2'-deoxycytidine (dC^{Etpy}, 5c)



Prepared according to the general procedure I from **dC^{I} (3)** and terpyridinyl acetylene **1c**. It was isolated as a slightly brownish powder in the yield of 63% (37.9 mg) which was then crystallized for the purpose of elemental analysis from the mixture DMSO/H_2O. ^{1}H NMR (500 MHz, DMSO-d_6): 2.09 (dt, 1H, J_{gem} = 13.1, $J_{2'b,1'} = J_{2'b,3'} = 6.4$, H-2'b); 2.21 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'} = 5.9$, $J_{2'a,3'} = 3.6$, H-2'a); 3.62 (ddd, 1H, $J_{gem} = 11.9$, $J_{5'b,OH} = 4.9$, $J_{5'b,4'} = 3.9$, H-5'b); 3.70 (ddd, 1H, $J_{gem} = 11.9$, $J_{5'a,OH} = 5.4$, $J_{5'a,4'} = 3.7$, H-5'a); 3.83 (ddd, 1H, $J_{4',5'} = 3.9, 3.7$, $J_{4',3'} = 3.1$, H-4'); 4.26 (m, 1H, $J_{3',2'} = 6.4, 3.6$, $J_{3',OH} = 4.3$, $J_{3',4'} = 3.1$, H-3'); 5.19 (dd, 1H, $J_{OH,5'} = 5.4, 4.9$, OH-5'); 5.24 (d, 1H, $J_{3',OH} = 4.3$, OH-3'); 6.15 (dd, 1H, $J_{1',2'} = 6.4, 5.9$, H-1'); 7.52 (bs, 1H, $NH_a\mathbf{H_b}$); 7.53 (ddd, 2H, $J_{5''',4'''} = 7.5$, $J_{5''',6'''} = 4.7$, $J_{5''',3'''} = 1.2$, H-5'''); 7.82 (bs, 1H, $N\mathbf{H_a}H_b$); 8.03 (ddd, 2H, $J_{4''',3'''} = 8.0$, $J_{4''',5'''} = 7.5$, $J_{4''',6'''} = 1.8$, H-4'''); 8.49 (s, 1H, H-6); 8.62 (s, 2H, H-3'',5''); 8.63 (ddd, 2H, $J_{3''',4'''} = 8.0$, $J_{3''',5'''} = 1.2$, $J_{3''',6'''} = 0.9$, H-3'''); 8.74 (ddd, 2H, $J_{6''',5'''} = 4.7$, $J_{6''',4'''} = 1.8$, $J_{6''',3'''} = 0.9$, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 41.02 (CH_2-2'); 61.09 (CH_2-5'); 70.10 (CH-3'); 85.78 (CH-1');

87.27 (C5-**C**≡C-C4"); 87.75 (CH-4'); 88.85 (C-5); 92.15 (C5-C≡**C**-C4"); 121.10 (CH-3'"); 121.99 (CH-3",5"); 124.89 (CH-5'"); 133.13 (C-4"); 137.74 (CH-4'"); 146.85 (CH-6); 149.51 (CH-6'"); 153.52 (C-2); 154.78 (C-2'"); 155.30 (C-2",6"); 163.93 (C-4); MS (ESI): *m/z* (%) = 483 (37) [M^+ + H], 505 (100) [M^+ + Na], 982 (86) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{26}H_{23}O_4N_6$: 483.1775; found: 483.1780; IR (KBr): 3422, 2214, 1645, 1602, 1583, 1567, 1502, 1468, 1393, 1264, 1097, 792 cm^{-1}; Mp 284-295°C.

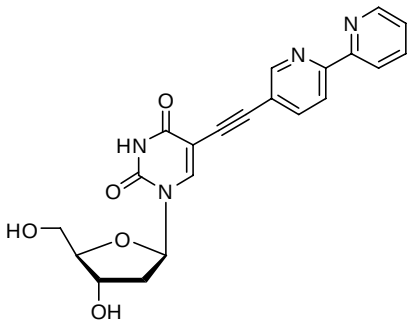
5-[(2'',2'''-bipyridin-6''-yl)ethynyl]-2'-deoxyuridine (dU^{E6bpy}, 6a)



Prepared according to general procedure I from **dU^{I} (4)** and bipyridinyl acetylene **1a**. Product was isolated as a yellow powder in the yield of 75% (37.9 mg) and crystallized for the purpose of elemental analysis from the mixture MeOH/H_2O.

^{1}H NMR (500 MHz, DMSO-d_6): 2.17 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.9, H-2'b); 2.21 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.9, $J_{2'a,3'}$ = 5.9, H-2'a); 3.60 (ddd, 1H, J_{gem} = 12.1, $J_{5'b,OH}$ = 4.5, $J_{5'b,4'}$ = 3.6, H-5'b); 3.68 (ddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 4.8, $J_{5'a,4'}$ = 3.6, H-5'a); 3.83 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 3.0, H-4'); 4.27 (m, 1H, $J_{3',2'}$ = 5.9, 3.9, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.0, H-3'); 5.21 (bdd, 1H, $J_{OH,5'}$ = 4.8, 4.5, OH-5'); 5.30 (bd, 1H, $J_{3',OH}$ = 4.3, OH-3'); 6.14 (dd, 1H, $J_{1',2'}$ = 6.9, 6.2, H-1'); 7.49 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.61 (dd, 1H, $J_{5'',4''}$ = 7.7, $J_{5'',3''}$ = 1.0, H-5''); 7.97 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 7.99 (dd, 1H, $J_{4'',3''}$ = 8.0, $J_{4'',5''}$ = 7.7, H-4''); 8.36 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 1.0, H-3'''); 8.37 (dd, 1H, $J_{3'',4''}$ = 8.0, $J_{3'',5''}$ = 1.0, H-3''); 8.50 (s, 1H, H-6); 8.70 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 1.0, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.48 (CH_2-2'); 61.02 (CH_2-5'); 70.11 (CH-3'); 82.52 (C5-**C**≡C-C6"); 85.24 (CH-1'); 87.86 (CH-4'); 91.76 (C5-C≡**C**-C6"); 97.51 (C-5); 120.27 (CH-3"); 120.95 (CH-3'"); 124.83 (CH-5'"); 127.66 (CH-5"); 137.70 (CH-4'"); 138.25 (CH-4"); 142.24 (C-6"); 145.31 (CH-6); 149.62 (CH-6'"); 149.68 (C-2); 154.67 (C-2'"); 155.97 (C-2"); 161.70 (C-4).; MS (ESI): *m/z* (%) = 406 (9) [M^+], 429 (100) [M^+ + Na], 835 (21) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{21}H_{19}O_4N_5$: 407.1350; found: 407.1357; Anal. Calcd for $C_{21}H_{18}O_4N_5$. $1H_2O$: C, 59.43; H, 4.75; N, 13.20. Found: C, 59.73; H, 4.67; N, 13.12.; IR (KBr): 3424, 3061, 1699, 1626, 1454, 1429, 1274, 1094, 1056, 778 cm^{-1}; Mp 129-136°C.

5-[(2'',2'''-bipyridin-5''-yl)ethynyl]-2'-deoxyuridine (dU^{E5bpy}, 6b)



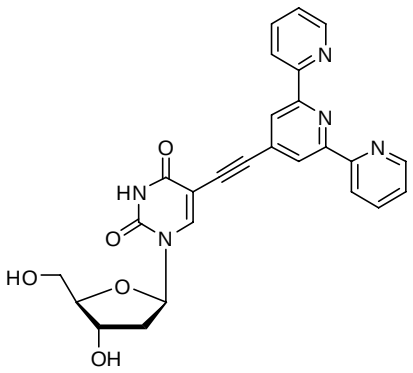
Prepared according to general procedure I from **dU^{I} (4)** and bipyridinyl alkyne **1b** and isolated as a yellowish solid in the yield of 70% (35 mg) and crystallized for the purpose of elemental analysis from the mixture $CHCl_3$/MeOH/heptane.

^{1}H NMR (500 MHz, DMSO-d_6): 2.16 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 4.3, H-2'b); 2.20 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.8, $J_{2'a,3'}$ = 5.8, H-2'a); 3.60 (bddd, 1H, J_{gem} = 12.1, $J_{5'b,OH}$ = 4.0, $J_{5'b,4'}$ = 3.5, H-5'b); 3.68 (bddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 4.4, $J_{5'a,4'}$ = 3.5, H-5'a); 3.82 (td, 1H, $J_{4',5'}$ = 3.5, $J_{4',3'}$ = 3.2, H-4'); 4.27 (bm, 1H, $J_{3',2'}$ = 5.8, 4.3, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 3.2, H-3'); 5.23 (bdd, 1H, $J_{OH,5'}$ = 4.4, 4.0, OH-5'); 5.31 (bd, 1H, $J_{3',OH}$ = 4.2, OH-3'); 6.13 (dd, 1H, $J_{1',2'}$ = 6.8, 6.3, H-1'); 7.48 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.97 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.03 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 2.2, H-4''); 8.39 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3'''); 8.42 (dd, 1H, $J_{3'',4''}$ = 8.3, $J_{3'',6''}$ = 1.0, H-3''); 8.50 (s, 1H, H-6); 8.71 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); 8.76 (dd, 1H, $J_{6'',4''}$ = 2.2, $J_{6'',3''}$ = 1.0, H-6''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.51 (CH_2-2'); 60.99 (CH_2-5'); 70.06 (CH-3'); 85.23 (CH-1'); 87.17 (C5-**C**≡C-C5''); 87.84 (CH-4'); 89.04 (C5-C≡**C**-C5'');. 97.85 (C-5); 119.94 (C-5''); 120.34 (CH-3''); 121.05 (CH-3'''); 124.80 (CH-5'''); 137.73 (CH-4'''); 139.68 (CH-4''); 144.97 (CH-6); 149.66 (C-2); 149.73 (CH-6'''); 151.32 (CH-6''); 154.39 (C-2''); 154.60 (C-2'''); 161.61 (C-4); MS (ESI): *m/z* (%) = 407 (17) [M^+ + H], 429 (19) [M^+ + Na], 835 (21) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{21}H_{19}O_5N_4$: 407.1350; found: 407.1358; Anal. Calcd for $C_{21}H_{18}O_5N_4$. $3H_2O$: C, 54.78; H, 5.25; N, 12.17. Found: C, 54.72; H, 5.19; N, 12.04; IR (KBr): 3419, 3056, 2974, 1716, 1697, 1624, 1458, 1306, 1274, 1092, 1051, 795, 747, cm^{-1}; Mp 134-139°C.

5-[(2'',2'''':6'',2'''-terpyridin-4''-yl)ethynyl]-2'-deoxyuridine (dU^{Etpy}, 6c)

Prepared according to the general procedure I from **dU^{I} (4)** and terpyridinyl acetylene **1c** and isolated as a yellowish powder in the yield of 67% (40 mg) and crystallized for the purpose of elemental analysis from the mixture MeOH/H_2O.

^{1}H NMR (600 MHz, DMSO-d_6): 2.18 (dDD, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 4.0, H-2'b); 2.23 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 6.8, $J_{2'a,3'}$ = 5.8, H-2'a); 3.62 (ddd, 1H, J_{gem} =



12.0, $J_{5'b,OH}$ = 4.6, $J_{5'b,4'}$ = 3.7, H-5'b); 3.70 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 5.1, $J_{5'a,4'}$ = 3.5, H-5'a); 3.84 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.5, $J_{4',3'}$ = 3.2, H-4'); 4.28 (m, 1H, $J_{3',2'}$ = 5.8, 4.0, $J_{3',OH}$ = 4.4, $J_{3',4'}$ = 3.2, H-3'); 5.29 (dd, 1H, $J_{OH,5'}$ = 5.1, 4.6, OH-5'); 5.33 (d, 1H, $J_{3',OH}$ = 4.4, OH-3'); 6.14 (dd, 1H, $J_{1',2'}$ = 6.8, 6.2, H-1'); 7.53 (ddd, 2H, $J_{5''',4'''}$ = 7.4, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 8.03 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.4, $J_{4''',6'''}$ = 1.8, H-4'''); 8.41 (s, 2H, H-3",5"); 8.58 (s, 1H, H-6); 8.63 (ddd, 2H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 1.0, H-3'''); 8.74 (ddd, 2H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 1.0, H-6'''); 11.83 (bs, 1H, NH); ^{13}C NMR (151 MHz, DMSO-d_6): 40.49 (CH_2-2'); 61.02 (CH_2-5'); 70.09 (CH-3'); 85.34 (CH-1'); 87.93 (CH-4'); 88.10 (C5-**C**≡C-C4"); 90.20 (C5-C≡**C**-C4"); 97.40 (C-5); 121.15 (CH-3'''); 121.66 (CH-3",5"); 125.13 (CH-5'''); 132.87 (C-4"); 137.90 (CH-4'''); 145.79 (CH-6); 149.70 (C-2); 149.74 (CH-6'''); 154.48 (C-2'''); 155.62 (C-2",6"); 161.68 (C-4); MS (ESI): *m/z* (%) = 483 (57) [M^+], 506 (100) [M^+ + Na], 9895 (77) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{26}H_{22}O_5N_5$: 484.1615; found: 484.1614; Anal. Calcd for $C_{26}H_{21}O_5N_5.1/2H_2O$: C, 63.41; H, 4.5; N, 14.22. Found: C, 63.49; H, 4.36; N, 14.07; IR (KBr): 3405, 2923, 2228, 1704, 1586, 1568, 1462, 1396, 1282, 1054, 790 cm^{-1}; Mp 234-242°C.

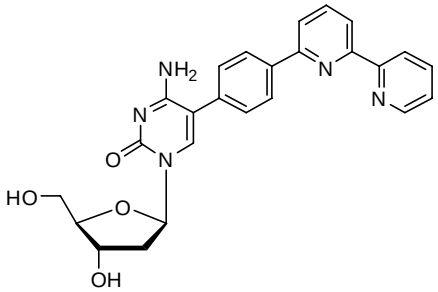
General Procerude II: Suzuki-Miyaura cross-coupling reaction – synthesis of modified dN^{ER}

Conditions A: A mixture of H_2O/CH_3CN (2:1) (1 ml) was added to an argon-purged flask containing nucleoside **dC^I (3)** or **dU^I (4)** (44 mg), a boronate **2a-c** (1.2 equiv.) and Cs_2CO_3 (3 equiv.). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (2.5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of H_2O/CH_3CN (2:1) (0.5 ml). The mixture of catalyst was then injected to the reaction mixture and the reaction mixture was heated at 80 °C for appropriate time. The solvent was evaporated in vacuo. Products **dN^{P6bpy} (7a, 8a), dN^{P5bpy} (7b, 8b)** and **dU^{Ptpy} (8c)** were directly purified by flash chromotagraphy on reverse phase using H_2O/MeOH (0% to 100%) as an eluent. Product **dC^{Ptpy} (7c)** was purified by column chromatography on silica gel using $CHCl_3$/MeOH (10% to 100%) as an eluent. Products were crystallized from the mixture MeOH/H_2O if is not written different.

Conditions B: A mixture of H_2O/CH_3CN (1:2) (1 ml) was added to an argon-purged flask containing nucleoside **3** or **4** (44 mg), a boronate **2a-c** (1.2 equiv.) and Cs_2CO_3

(3 equiv.). In a separate flask, $Pd(OAc)_2$ (10 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of H_2O/CH_3CN (1:2) (0.5 ml). The mixture of catalyst was then injected to the reaction mixture and the reaction mixture was heated at 90 °C until complete consumption of the strating material. Reaction was monitored by TLC. The solvent was evaporated in vacuo. Products **dN^{P6bpy} (7a, 8a), dN^{P5bpy} (7b, 8b)** and **dU^{Ptpy} (8c)** were directly purified by flash chromotagraphy on reverse phase using H_2O/MeOH (0% to 100%) as an eluent. Product **dC^{Ptpy} (7c)** was purified by column chromatography on silica gel using $CHCl_3$/MeOH (10% to 100%) as an eluent. Products were crystallized from the mixture MeOH/H_2O if is not written different.

5-[*p*-(2'',2'''-bipyridin-6''-yl)phenyl]-2'-deoxycytidine (dC^{P6bpy}, 7a)



Prepared according to the general procedure II from **dC^{I} (3)** and bipyridinyl boronate **2a**.

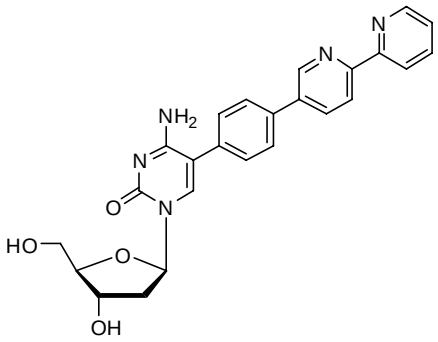
Conditions A: Reaction was heated for 2 h and product was then isolated as a white powder in the yield of 65% (37.1 mg).

Conditions B: Reaction mixture was heated for 1 h and **7a** was isolated as a white powder in the yield of 75% (42.8 mg).

^{1}H NMR (500 MHz, DMSO-d_6): 2.11 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 6.9, $J_{2'b,3'}$ = 6.0, H-2'b); 2.18 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.7, $J_{2'a,3'}$ = 3.7, H-2'a); 3.54 and 3.60 (2 × ddd, 2H, J_{gem} = 11.8, $J_{5',OH}$ = 4.8, $J_{5',4'}$ = 3.6, H-5'); 3.80 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 3.1, H-4'); 4.25 (m, 1H, $J_{3',2'}$ = 6.0, 3.7, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 3.1, H-3'); 5.02 (t, 1H, $J_{OH,5'}$ = 4.8, OH-5'); 5.24 (bd, 1H, $J_{3',OH}$ = 4.2, OH-3'); 6.23 (dd, 1H, $J_{1',2'}$ = 6.9, 6.2, H-1'); 6.54 and 7.42 (2 × bs, 2H, NH_2); 7.49 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.51 (m, 2H, H-*o*-phenylene); 7.99 (s, 1H, H-6); 8.01 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.07 (dd, 1H, $J_{4'',5''}$ = 7.9, $J_{4'',3''}$ = 6.8, H-4''); 8.08 (dd, 1H, $J_{5'',4''}$ = 7.9, $J_{5'',3''}$ = 1.9, H-5''); 8.30 (m, 2H, H-*m*-phenylene); 8.36 (dd, 1H, $J_{3'',4''}$ = 6.8, $J_{3'',5''}$ = 1.9, H-3''); 8.59 (ddd, 1H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3'''); 8.72 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.90 (CH_2-2'); 61.24 (CH_2-5'); 70.35 (CH-3'); 85.37 (CH-1'); 87.51 (CH-4'); 107.45 (C-5); 119.40 (CH-3''); 120.60 (CH-5''); 120.85 (CH-3'''); 124.57 (CH-5'''); 127.41 (CH-*m*-phenylene); 129.42 (CH-*o*-phenylene); 135.10 (C-*i*-phenylene); 137.64 (CH-4'''); 137.69 (C-*p*-phenylene); 138.77 (CH-4''); 140.51 (CH-6); 149.54 (CH-6'''); 154.65 (C-

2); 155.24 and 155.27 (C-2",6"); 155.50 (C-2'''); 163.49 (C-4); MS (ESI): *m/z* (%) = 457 (20) [M^+], 915 (100) [$2M^+$], 937 (87) [$2M^+$ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{25}H_{24}O_4N_5$: 458.1823; found: 458.1831; Anal. Calcd for $C_{25}H_{23}O_4N_5$. 1/3H_2O: C, 64.78; H, 5.15; N, 15.11. Found: C, 64.97; H, 5.04; N, 14.88; IR (KBr): 3462, 3370, 1649, 1599, 1581, 1470, 1456, 1429, 1097, 778 cm^{-1}; Mp over 300°C.

5-[*p*-(2'',2'''-bipyridin-5''-yl)phenyl]-2'-deoxycytidine (dC^{P5bpy}, 7b)



Prepared according to the genral procedure II from **dC^{I} (3)** and bipyridinyl boronate **2b**.

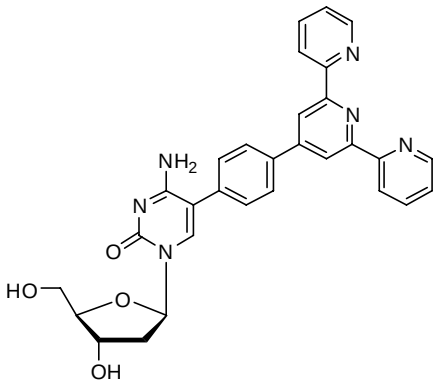
Conditions A: Reaction mixture was heated for 6 h. Even if the starting material was not fully converted, the product **7b** was isolated as a white powder in the yield of 12% (7 mg).

Conditions B: Reaction mixture was heated for 3 h. Product was isolated as a white powder in the yield of 55% (31 mg).

^{1}H NMR (500 MHz, DMSO-d_6): 2.11 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.0, H-2'b); 2.17 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.8, H-2'a); 3.53 and 3.59 (2 × dd, 2H, J_{gem} = 11.8, $J_{5',4'}$ = 3.5, H-5'); 3.79 (q, 1H, $J_{4',5'}$ = $J_{4',3'}$ = 3.5, H-4'); 4.24 (ddd, 1H, $J_{3',2'}$ = 6.0, 3.8, $J_{3',4'}$ = 3.5, H-3'); 5.01 (bs, 1H, OH-5'); 5.23 (bs, 1H, OH-3'); 6.23 (dd, 1H, $J_{1',2'}$ = 7.1, 6.1, H-1'); 6.54 and 7.44 (2 × bs, 2H, NH_2); 7.48 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.50 (m, 2H, H-*o*-phenylene); 7.89 (m, 2H, H-*m*-phenylene); 7.95 (s, 1H, H-6); 7.98 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.29 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 2.4, H-4''); 8.44 (ddd, 1H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3'''); 8.50 (dd, 1H, $J_{3'',4''}$ = 8.3, $J_{3'',6''}$ = 0.9, H-3''); 8.72 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); 9.07 (dd, 1H, $J_{6'',4''}$ = 2.4, $J_{6'',3''}$ = 0.9, H-6''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.82 (CH_2-2'); 61.23 (CH_2-5'); 70.35 (CH-3'); 85.31 (CH-1'); 87.48 (CH-4'); 107.30 (C-5); 120.62 (CH-3'''); 120.76 (CH-3''); 124.45 (CH-5'''); 127.48 (CH-*m*-phenylene); 129.80 (CH-*o*-phenylene); 134.29 (C-*i*-phenylene); 135.21 (CH-4''); 135.29 (C-5''); 135.82 (C-*p*-phenylene); 137.58 (CH-4'''); 140.48 (CH-6); 147.38 (CH-6''); 149.62 (CH-6'''); 154.43 (C-2''); 154.60 (C-2); 155.07 (C-2'''); 163.48 (C-4); MS (ESI): *m/z* (%) = 457 (48) [M^+], 480 (100) [M^+ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{25}H_{24}O_4N_5$: 458.1823; found: 458.1821; Anal. Calcd for $C_{25}H_{23}O_4N_5$: C, 65.63; H, 5.07; N, 15.31. Found: C, 65.34; H, 5.09; N, 15.05; IR (KBr):

3389, 3176, 3068, 2932, 1661, 1631, 1503, 1459, 1263, 1196, 1082, 798, 750 cm^{-1}; Mp over 300°C.

5-[*p*-(2'',2'''':6'',2'''-terpyridin-4''-yl)phenyl]-2'-deoxycytidine (dC^{Ptpy}, 7c)



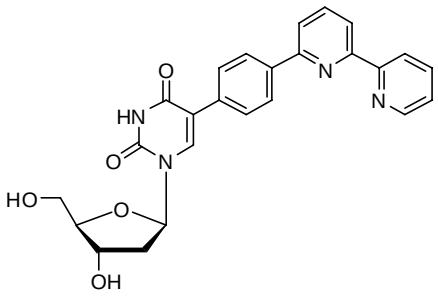
Prepared according to the general procedure II from **dC^{I} (3)** and terpyridinyl boronate **2c** and after chromatography was crystallized from the mixture DMSO/H_2O.

Conditions A: Reaction mixture was heated for 5 h. Even if the starting material was not fully consumed, product **7c** was isolated as a white solid in the yield of 28% (18.7 mg).

Conditions B: Reaction mixture was heated for 3 h. Product **7c** was isolated as a white solid in the yield of 70% (47 mg).

^{1}H NMR (500 MHz, DMSO-d_6): 2.11 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.7, $J_{2'b,3'}$ = 6.0, H-2'b); 2.19 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 4.0, H-2'a); 3.55 and 3.62 (2 × ddd, 2 × 1H, J_{gem} = 11.9, $J_{5',OH}$ = 5.0, $J_{5',4'}$ = 3.7, H-5'); 3.80 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 2.8, H-4'); 4.26 (m, 1H, $J_{3',2'}$ = 6.0, 4.0, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 2.8, H-3'); 5.02 (t, 1H, $J_{OH,5'}$ = 5.0, OH-5'); 5.22 (d, 1H, $J_{3',OH}$ = 4.2, OH-3'); 6.25 (dd, 1H, $J_{1',2'}$ = 6.7, 6.3, H-1'); 6.56 and 7.44 (2 × bs, 2 × 1H, NH_2); 7.54 (ddd, 2H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.1, H-5'''); 7.57 (m, 2H, H-*o*-phenylene); 7.99 (m, 2H, H-*m*-phenylene); 8.04 (s, 1H, H-6); 8.05 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.69 (ddd, 2H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.1, $J_{3''',6'''}$ = 0.9, H-3'''); 8.76 (s, 2H, H-3'',5''); 8.78 (ddd, 2H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.93 (CH_2-2'); 61.15 (CH_2-5'); 70.25 (CH-3'); 85.36 (CH-1'); 87.46 (CH-4'); 107.13 (C-5); 117.98 (CH-3'',5''); 121.16 (CH-3'''); 124.81 (CH-5'''); 127.68 (CH-*m*-phenylene); 129.91 (CH-*o*-phenylene); 135.45 (C-*i*-phenylene); 136.60 (C-*p*-phenylene); 137.73 (CH-4'''); 140.68 (CH-6); 149.24 (C-4''); 149.61 (CH-6'''); 154.57 (C-2); 155.11 (C-2'''); 155.99 (C-2'',6''); 163.41 (C-4); MS (ESI): *m/z* (%) = 534 (3) [M^+], 535 (20) [M^+ + H], 557 (100) [M^+ + Na]; HRMS-ESI: *m/z* [M^+ + H] calcd for $C_{30}H_{27}O_4N_5$: 353.2088; found: 353.2090; IR (KBr): 3464, 3382, 2919, 2852, 1648, 1602, 1587, 1566, 1468, 1392, 1268, 1096, 1063, 1040, 788, 744, 517 cm^{-1}; Mp over 300°C.

5-[*p*-(2'',2'''-bipyridin-6''-yl)phenyl]-2'-deoxyuridine (dU^{P6bpy}, 8a)



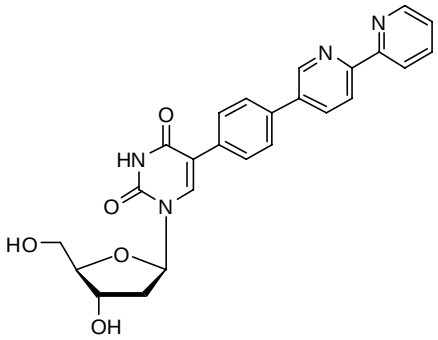
Prepared according to the general procedure II from **dU^{I} (4)** and bypiridinyl boronate **2a**.

Conditions A: The reaction mixture was heated for 2 h and then product was isolated as a white solid in the yield of 60% (34 mg).

Conditions B: The reaction mixture was heated for 1 h and product was then isolated as a white compound in the yield of 75% (43 mg).

^{1}H NMR (500 MHz, DMSO-d_6): 2.19 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.4, $J_{2'b,3'}$ = 3.8, H-2'b); 2.28 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 6.9, $J_{2'a,3'}$ = 6.0, H-2'a); 3.62 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 4.7, $J_{5'b,4'}$ = 3.3, H-5'b); 3.67 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.3, H-5'a); 3.85 (td, 1H, $J_{4',5'}$ = 3.3, $J_{4',3'}$ = 2.9, H-4'); 4.32 (m, 1H, $J_{3',2'}$ = 6.0, 3.8, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 2.9, H-3'); 5.21 (dd, 1H, $J_{OH,5'}$ = 5.0, 4.7, OH-5'); 5.30 (bd, 1H, $J_{3',OH}$ = 4.3, OH-3'); 6.26 (dd, 1H, $J_{1',2'}$ = 6.9, 6.4, H-1'); 7.49 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.76 (m, 2H, H-*o*-phenylene); 8.01 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.9, H-4'''); 8.04 (dd, 1H, $J_{4'',5''}$ = 7.9, $J_{4'',3''}$ = 7.2, H-4''); 8.07 (dd, 1H, $J_{5'',4''}$ = 7.9, $J_{5'',3''}$ = 1.5, H-5''); 8.25 (m, 2H, H-*m*-phenylene); 8.35 (dd, 1H, $J_{3'',4''}$ = 7.2, $J_{3'',5''}$ = 1.5, H-3''); 8.37 (s, 1H, H-6); 8.60 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 1.0, H-3'''); 8.72 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.9, $J_{6''',3'''}$ = 1.0, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.42 (CH_2-2'); 61.14 (CH_2-5'); 70.37 (CH-3'); 84.81 (CH-1'); 87.75 (CH-4'); 113.01 (C-5); 119.29 (CH-3''); 120.54 (CH-5''); 120.87 (CH-3'''); 124.56 (CH-5'''); 126.57 (CH-*m*-phenylene); 128.33 (CH-*o*-phenylene); 134.40 (C-*i*-phenylene); 137.23 (C-*p*-phenylene); 137.63 (CH-4'''); 138.54 (CH-6); 138.70 (CH-4''); 149.53 (CH-6'''); 150.10 (C-2); 155.19 and 155.27 (C-2'',6''); 155.50 (C-2'''); 162.31 (C-4); MS (ESI): *m/z* (%) = 459 (100) [M^+ + H], 481 (33) [M^++Na]; HRMS-ESI: *m/z* [M^+ + H] calcd for $C_{25}H_{23}O_5N_4$: 459.1663; found: 459.1660; Anal. Calcd for $C_{25}H_{22}O_5N_4$. 3/2H_2O: C, 61.85; H, 5.19; N, 11.54. Found: C, 61.90; H, 5.04; N, 11.35; IR (KBr): 3385, 3185, 3059, 1707, 1581, 1457, 1434, 1288, 1093, 783, 599 cm^{-1}; Mp 145-150°C.

5-[*p*-(2'',2'''-bipyridin-5''-yl)phenyl]-2'-deoxyuridine (dU^{P5bpy}, 8b)



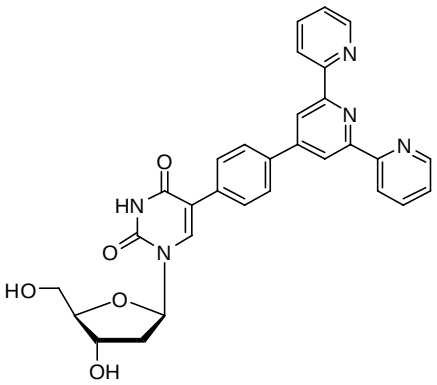
Prepared acoording to the general procedure II from **dU^{I} (4)** and bipyridinyl boronate **2b**.

Conditions A: The reaction mixture was heated for 6 h and even if the starting material was not fully consumed, product was then isolated as a white compound in the yield of 7% (4 mg).

Conditions B: The reaction mixture was heated for 3 h. Product was then isolated as a white compound in the yield of 35% (20 mg).

^{1}H NMR (500 MHz, DMSO-d_6): 2.19 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.4, $J_{2'b,3'}$ = 3.7, H-2'b); 2.29 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 6.9, $J_{2'a,3'}$ = 6.0, H-2'a); 3.61 and 3.66 (2 × bddd, 2H, J_{gem} = 11.8, $J_{5',OH}$ = 4.4, $J_{5',4'}$ = 3.3, H-5'); 3.84 (td, 1H, $J_{4',5'}$ = 3.3, $J_{4',3'}$ = 3.1, H-4'); 4.32 (bm, 1H, $J_{3',2'}$ = 6.0, 3.7, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 3.1, H-3'); 5.18 (bt, 1H, $J_{OH,5'}$ = 4.4, OH-5'); 5.29 (bd, 1H, $J_{3',OH}$ = 4.2, OH-3'); 6.26 (dd, 1H, $J_{1',2'}$ = 6.9, 6.4, H-1'); 7.47 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.73 (m, 2H, H-*o*-phenylene); 7.83 (m, 2H, H-*m*-phenylene); 7.97 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.27 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 2.4, H-4''); 8.32 (s, 1H, H-6); 8.43 (ddd, 1H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3'''); 8.48 (dd, 1H, $J_{3'',4''}$ = 8.3, $J_{3'',6''}$ = 0.9, H-3''); 8.72 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); 9.05 (dd, 1H, $J_{6'',4''}$ = 2.4, $J_{6'',3''}$ = 0.9, H-6''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.32 (CH_2-2'); 61.15 (CH_2-5'); 70.37 (CH-3'); 84.78 (CH-1'); 87.74 (CH-4'); 112.94 (C-5); 120.60 (CH-3'''); 120.69 (CH-3''); 124.40 (CH-5'''); 126.69 (CH-*m*-phenylene); 128.73 (CH-*o*-phenylene); 133.52 (C-*i*-phenylene); 135.13 (CH-4''); 135.39 (C-5''); 135.44 (C-*p*-phenylene); 137.56 (CH-4'''); 138.43 (CH-6); 147.39 (CH-6''); 149.59 (CH-6'''); 150.08 (C-2); 154.32 (C-2''); 155.11 (C-2'''); 162.29 (C-4); MS (ESI): *m/z* (%) = 459 (100) [M^+ + H], 481 (96) [M^+ + Na]; HRMS-ESI: *m/z* [M^+ + H] calcd for $C_{25}H_{23}O_5N_4$: 459.1663; found: 459.1661; Anal. Calcd for $C_{25}H_{22}O_5N_4$. $1H_2O$: C, 63.02; H, 5.08; N, 11.76. Found: C, 63.32; H, 4.92; N, 11.64; IR (KBr): 3427, 2923, 1694, 1589, 1464, 1289, 1265, 1088, 873, 796, 751, 603, 463 cm^{-1}; Mp 188-193°C

5-[*p*-(2'',2'''':6'',2'''-terpyridin-4''-yl)phenyl]-2'-deoxyuridine (8c)



Prepared according to the general procedure II from **dU^{I} (4)** and terpyridinyl boronate **2c**.

Conditions A: The reaction mixture was heated for 7 h and even if the starting material was not fully consumed, the product was then isolated as a brownish compound in the yield of 24% (16 mg).

Conditions B: The reaction mixture was heated for 3 h. Product was isolated as a brownish solid in the yield of 70% (47 mg).

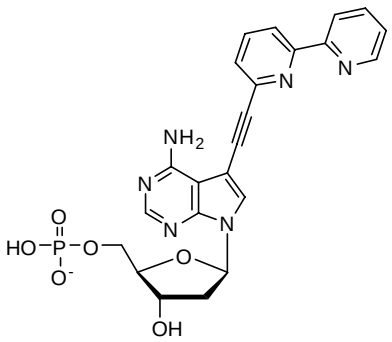
^{1}H NMR (500 MHz, DMSO-d_6): 2.20 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 4.0, H-2'b); 2.29 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 6.7, $J_{2'a,3'}$ = 6.0, H-2'a); 3.63 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 4.3, $J_{5'b,4'}$ = 3.3, H-5'b); 3.70 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 4.3, $J_{5'a,4'}$ = 3.1, H-5'a); 3.86 (dt, 1H, $J_{4',5'}$ = 3.3, 3.1, $J_{4',3'}$ = 3.1, H-4'); 4.33 (m, 1H, $J_{3',2'}$ = 6.0, 4.0, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 3.1, H-3'); 5.24 (bt, 1H, $J_{OH,5'}$ = 4.3, OH-5'); 5.30 (bd, 1H, $J_{3',OH}$ = 4.2, OH-3'); 6.26 (dd, 1H, $J_{1',2'}$ = 6.7, 6.3, H-1'); 7.54 (ddd, 2H, $J_{5''',4'''}$ = 7.4, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.1, H-5'''); 7.82 (m, 2H, H-*o*-phenylene); 7.94 (m, 2H, H-*m*-phenylene); 8.05 (s, 1H, H-6); 8.05 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.4, $J_{4''',6'''}$ = 1.8, H-4'''); 8.68 (ddd, 2H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.1, $J_{3''',6'''}$ = 0.9, H-3'''); 8.74 (s, 2H, H-3'',5''); 8.77 (ddd, 2H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.49 (CH_2-2'); 61.06 (CH_2-5'); 70.26 (CH-3'); 84.87 (CH-1'); 87.71 (CH-4'); 112.69 (C-5); 117.89 (CH-3'',5''); 121.15 (CH-3'''); 124.76 (CH-5'''); 126.84 (CH-*m*-phenylene); 128.80 (CH-*o*-phenylene); 134.76 (C-*i*-phenylene); 136.17 (C-*p*-phenylene); 137.69 (CH-4'''); 138.80 (CH-6); 149.25 (C-4''); 149.57 (CH-6'''); 150.05 (C-2); 155.14 (C-2'''); 155.92 (C-2'',6''); 162.26 (C-4); MS (ESI): *m/z* (%) = 536 (96) [M^+ + H], 558 (100) [M^+ + Na]; HRMS-ESI: *m/z* [M^+ + H] calcd for $C_{30}H_{26}O_5N_5$: 536.1928; found: 536.1925; IR (KBr): 3426, 3055, 2925, 1687, 1583, 1467, 1390, 1292, 1096, 840, 791, 749, 659, 595 cm^{-1}; Mp 240-249°C.

General Procedure III: Sonogashira cross-coupling reaction – synthesis of modified dN^{ER}MPs

Mixture CH_3CN/H_2O (1:2) (1.5 ml) and Et(*i*-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing halogenated nucleoside monophosphate **dA^{I}MP (9)** or **dC^{I}MP (10)** (60 mg), an alkyne **1a-c** (1.5 equiv.) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged

with argon followed by addition of CH_3CN/H_2O (1:2) (0.5 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80 °C for 1.5 h. The solvent was evaporated in vacuo. Products were purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB (triethylammonium bicarbonate) in H_2O to 0.1M TEAB in H_2O/MeOH (1:1) as an eluent. Several co-distillations with water and conversion to sodium salt form (Dowex 50 in Na^+ cycle) followed by freeze-drying from water, gave the products as brownish or yellowish powder.

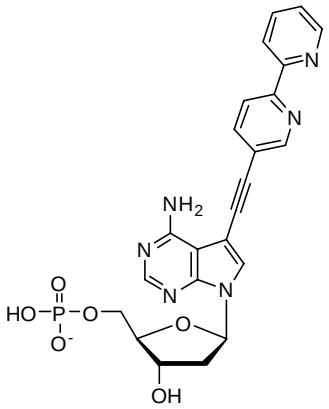
7-[(2'',2'''-bipyridin-6''-yl)ethynyl]-7-deaza-2'-deoxyadenosine 5'-O-monophosphate (dA^{E6bpy}MP, 11a)



This compound was prepared according to the general procedure III from **dA^{I}MP (9)** and bipyridinyl acetylene **1a** in the yield of 52% (34.6 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 2.37 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6, H-2'b); 2.65 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 8.2, $J_{2'a,3'}$ = 5.6, H-2'a); 4.03 (dt, 1H, J_{gem} = 11.0, $J_{H,P}$ = $J_{5'b,4'}$ = 4.6, H-5'b); 4.09 (ddd, 1H, J_{gem} = 11.0, $J_{H,P}$ = 5.8, $J_{5'a,4'}$ = 3.3, H-5'a); 4.13 (m, 1H, H-4'); 4.66 (dt, 1H, $J_{3',2'}$ = 5.6, 2.6, $J_{3',4'}$ = 2.6, H-3'); 6.70 (dd, 1H, $J_{1',2'}$ = 8.2, 6.0, H-1'); 7.47 (ddd, 1H, $J_{5''',4'''}$ = 7.6, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 0.8, H-5'''); 7.68 (d, 1H, $J_{5'',4''}$ = 7.6, H-5''); 7.96 (dd, 1H, $J_{4'',3''}$ = 7.9, $J_{4'',5''}$ = 7.6, H-4''); 7.98 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.6, $J_{4''',6'''}$ = 1.8, H-4'''); 8.05 (s, 1H, H-6); 8.16 (s, 1H, H-2); 8.26 (d, 1H, $J_{3'',4''}$ = 7.9, H-3''); 8.34 (d, 1H, $J_{3''',4'''}$ = 7.9, H-3'''); 8.67 (bd, 1H, $J_{6''',5'''}$ = 4.8, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 41.48 (CH_2-2'); 66.11 (d, $J_{C,P}$ = 4.9, CH_2-5'); 73.29 (CH-3'); 84.35 (C6''-C≡**C**-C5); 84.92 (CH-1′); 87.79 (d, $J_{C,P}$ = 8.8, CH-4′); 92.03 (C6''-**C**≡C-C5); 96.63 (C-5); 103.91 (C-4a); 121.68 (CH-3''); 122.87 (CH-3'''); 125.61 (CH-5'''); 128.11 (CH-5''); 129.26 (CH-6); 138.88 (CH-4'''); 139.10 (CH-4''); 144.23 (C-6''); 150.36 (CH-6'''); 150.87 (C-7a); 153.71 (CH-2); 156.57 (C-2'''); 157.60 (C-2''); 159.08 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.99; MS (ES^-): found m/z: 507.2(M), 508.2 (M+H); HRMS (ES^-): m/z calcd for $C_{23}H_{20}O_6N_6P$: 507.1187; found: 507.1189.

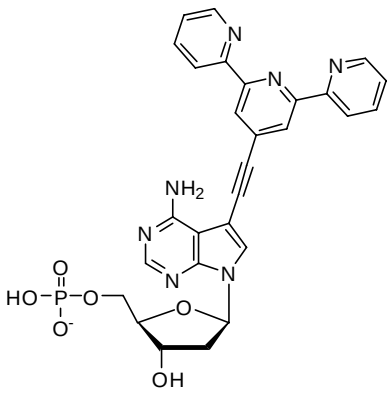
7-[(2'',2'''-bipyridin-5''-yl)ethynyl]-7-deaza-2'-deoxyadenosine 5'-O-monophosphate (dA^{E5bpy}MP, 11b)



This compound was prepared according to the general procedure III from **dA^{I}MP (9)** and bipyridinyl acetylene **1b** in the yield of 70% (46.6 mg).

^{1}H NMR (500.0 MHz, CD_3OD): 2.37 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 2.9, H-2'b); 2.65 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ = 5.9, H-2'a); 3.99 (dt, 1H, J_{gem} = 10.8, $J_{H,P}$ = $J_{5'b,4'}$ = 4.8, H-5'b); 4.06 (ddd, 1H, J_{gem} = 10.8, $J_{H,P}$ = 5.4, $J_{5'a,4'}$ = 4.0, H-5'a); 4.11 (m, 1H, H-4'); 4.69 (dt, 1H, $J_{3',2'}$ = 5.9, 2.9, $J_{3',4'}$ = 2.9, H-3'); 6.67 (dd, 1H, $J_{1',2'}$ = 7.8, 6.1, H-1'); 7.44 (dd, 1H, $J_{5''',4'''}$ = 6.9, $J_{5''',6'''}$ = 4.2, H-5'''); 7.94 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 6.9, $J_{4''',6'''}$ = 1.5, H-4'''); 7.99 (s, 1H, H-6); 8.07 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 1.8, H-4''); 8.15 (s, 1H, H-2); 8.35 (d, 1H, $J_{3'',4''}$ = 8.2, H-3''); 8.38 (d, 1H, $J_{3''',4'''}$ = 8.0, H-3'''); 8.66 (d, 1H, $J_{6''',5'''}$ = 4.2, H-6'''); 8.81 (bs, 1H, H-6''); ^{13}C NMR (125.7 MHz, CD_3OD): 41.15 (CH_2-2'); 65.70 (d, $J_{C,P}$ = 3.7, CH_2-5'); 73.27 (CH-3'); 84.72 (CH-1'); 87.97 (d, $J_{C,P}$ = 8.3, CH-4'); 88.18 (C5''-C≡**C**-C5); 89.38 (C5''-**C**≡C-C5); 96.78 (C-5); 103.88 (C-4a); 121.75 (CH-3''); 121.98 (C-5''); 122.76 (CH-3'''); 125.47 (CH-5'''); 128.81 (CH-6); 138.72 (CH-4'''); 140.48 (CH-4''); 150.36 (CH-6'''); 150.76 (C-7a); 152.29 (CH-6''); 153.61 (CH-2); 155.66 (C-2''); 156.50 (C-2'''); 159.08 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 5.04; MS (ES^-): found m/z: 507.2(M), 508.2 (M+H); HRMS (ES^-): m/z calcd for $C_{23}H_{20}O_6N_6P$: 507.11874; found: 507.11839.

7-[(2'',2'''':6'',2'''-terpyridin-4''-yl)ethynyl]-7-deaza-2'-deoxyadenosine 5'-O-monophosphate (dA^{Etpy}MP, 11c)

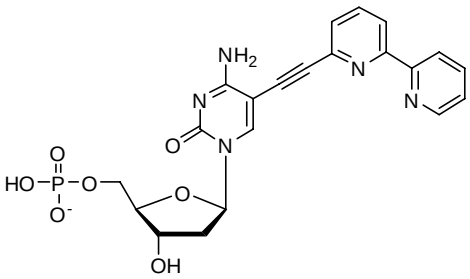


This compound was prepared according to the general procedure III from **dA^{I}MP (9)** and terpyridinyl acetylene **1c** in the yield of 57% (43.5 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 2.37 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 5.9, $J_{2'b,3'}$ = 2.3, H-2'b); 2.65 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 8.5, $J_{2'a,3'}$ = 5.7, H-2'a); 4.05 (dt, 1H, J_{gem} = 10.8, $J_{H,P}$ = $J_{5'b,4'}$ = 4.8, H-5'b); 4.10 (ddd, 1H, J_{gem} = 10.8, $J_{H,P}$ = 5.3, $J_{5'a,4'}$ = 3.2, H-5'a); 4.14 (m, 1H, H-4'); 4.66 (dt, 1H, $J_{3',2'}$ = 5.7, 2.3, $J_{3',4'}$ = 2.3, H-3'); 6.68 (dd, 1H, $J_{1',2'}$ = 8.5, 5.9, H-1'); 7.45

(dd, 2H, $J_{5''',4'''}$ = 7.3, $J_{5''',6'''}$ = 4.6, H-5'''); 7.97 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.3, $J_{4''',6'''}$ = 1.4, H-4'''); 8.03 (s, 1H, H-6); 8.16 (s, 1H, H-2); 8.43 (s, 2H, H-3",5"); 8.59 (d, 2H, $J_{3''',4'''}$ = 7.9, H-3'''); 8.66 (d, 2H, $J_{6''', 5'''}$ = 4.6, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 41.44 (CH_2-2'); 66.23 (d, $J_{C,P}$ = 4.8, CH_2-5'); 73.29 (CH-3'); 85.00 (CH-1'); 87.73 (d, $J_{C,P}$ = 8.8, CH-4'); 88.63 (C4"-C≡**C**-C5); 90.81 (C4"-**C**≡C-C5); 96.66 (C-5); 103.92 (C-4a); 122.78 (CH-3'''); 123.15 (CH-3",5"); 125.63 (CH-5'''); 129.46 (CH-6); 134.58 (C-4"); 138.72 (CH-4'''); 150.23 (CH-6'''); 150.85 (C-7a); 153.57 (CH-2); 156.60 (C-2'''); 156.97 (C-2",C6"); 158.97 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.44. MS (ES-): found m/z: 584.2(M), 585.2 (M+H), 586.2 (M+2H); HRMS (ES-+): m/z calcd for $C_{28}H_{23}O_6N_7P$: 584.1453; found: 584.1454.

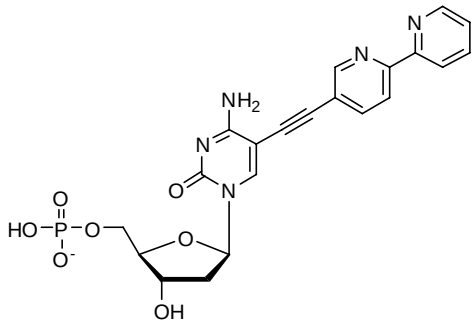
5-[(2'',2'''-bipyridin-6''-yl)ethynyl]-2'-deoxycytidine 5'-O-monophosphate (dC^{E6bpy}MP, 12a)



This compound was prepared according to the general procedure III from **dC^{I}MP (10)** and bipyridinyl acetylene **1a** in the yield of 89% (59.5 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 2.26 (ddd, 1H, J_{gem} = 13.7, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.2, H-2'b); 2.41 (ddd, 1H, J_{gem} = 13.7, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 3.6, H-2'a); 4.05 (dt, 1H, J_{gem} = 10.4, $J_{H,P}$ = $J_{5'b,4'}$ = 4.4, H-5'b); 4.10 (m, 1H, H-4'); 4.12 (ddd, 1H, J_{gem} = 10.4, $J_{H,P}$ = 6.0, $J_{5'a,4'}$ = 3.6, H-5'a); 4.56 (dt, 1H, $J_{3',2'}$ = 6.2, 3.6, $J_{3',4'}$ = 3.6, H-3'); 6.27 (dd, 1H, $J_{1',2'}$ = 7.1, 6.0, H-1'); 7.49 (dd, 1H, $J_{5''',4'''}$ = 7.3, $J_{5''',6'''}$ = 4.8, H-5'''); 7.88 (d, 1H, $J_{5'',4''}$ = 7.7, H-5"); 7.99 (m, 2H, H-4",4'''); 8.23 (d, 1H, $J_{3'',4''}$ = 7.9, H-3"); 8.27 (d, 1H, $J_{3''',4'''}$ = 7.9, H-3'''); 8.45 (s, 1H, H-6); 8.70 (d, 1H, $J_{6''',5'''}$ = 4.8, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 41.66 (CH_2-2'); 65.10 (d, $J_{C,P}$ = 4.5, CH_2-5'); 72.41 (CH-3'); 82.78 (C6"-C≡**C**-C5); 87.88 (CH-1'); 88.36 (d, $J_{C,P}$ = 8.4, CH-4'); 91.71 (C-5); 94.66 (C6"-**C**≡C-C5); 122.26 (CH-3"); 122.90 (CH-3'''); 125.64 (CH-5'''); 128.81 (CH-5"); 138.95 (CH-4'''); 139.44 (CH-4"); 143.86 (C-6"); 147.22 (CH-6); 150.57 (CH-6'''); 156.50 (C-2'''); 156.68 (C-2); 157.58 (C-2"); 166.26 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 5.06; MS (ES^-): found *m/z*: 484.2 (M), 485.2 (M+H), 486.2 (M+2H); HRMS (ES^-): *m/z* calcd for $C_{21}H_{19}O_7N_5P$: 484.1028; found: 484.1028.

5-[(2'',2'''-bipyridin-5''-yl)ethynyl]-2'-deoxycytidine 5'-O-monophosphate (dC^{E5bpy}MP, 12b)

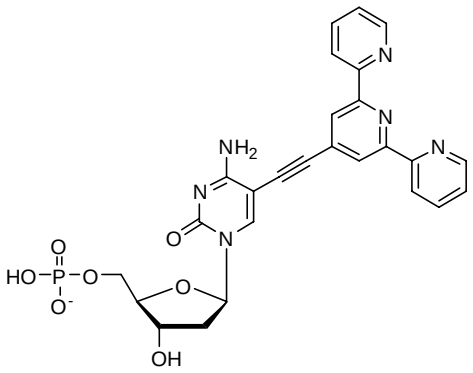


This compound was prepared according to the general procedure III from **dC^{I}MP (10)** and bipyridinyl acetylene **1b**[56] in the yield of 89% (59.5 mg).

^{1}H NMR (499.8 MHz, CD_3OD): 2.24 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.3, H-2'b); 2.41 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.9, $J_{2'a,3'}$ = 3.1, H-2'a); 4.11 (m, 3H, H-4',5'); 4.53 (m, 1H, H-3'); 6.30 (dd, 1H, $J_{1',2'}$ = 7.1, 5.9, H-1'); 7.44 (dd, 1H, $J_{5''',4'''}$ = 6.9, $J_{5''',6'''}$ = 4.2, H-5'''); 7.94 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 6.9, $J_{4''',6'''}$ = 1.3, H-4'''); 8.18 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 1.6, H-4''); 8.33 (d, 1H, $J_{3'',4''}$ = 8.3, H-3''); 8.36 (d, 1H, $J_{3''',4'''}$ = 7.9, H-3'''); 8.42 (s, 1H, H-6); 8.66 (bd, 1H, $J_{6''',5'''}$ = 4.2, H-6'''); 8.89 (bs, 1H, H-6''); ^{13}C NMR (125.7 MHz, CD_3OD): 42.00 (CH_2-2'); 65.64 (d, $J_{C,P}$ = 4.6, CH_2-5'); 72.50 (CH-3'); 85.73 (C5''-C≡**C**-C5); 88.01 (CH-1'); 88.11 (d, $J_{C,P}$ = 8.7, CH-4'); 92.51 (C-5); 92.79 (C5''-**C**≡C-C5); 121.63 (CH-3''); 121.70 (C-5''); 122.78 (CH-3'''); 125.45 (CH-5'''); 138.72 (CH-4'''); 141.00 (CH-4''); 146.59 (CH-6); 150.36 (CH-6'''); 152.66 (CH-6''); 155.69 (C-2''); 156.55 (C-2'''); 156.69 (C-2); 165.94 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.67. MS (ES^-): found *m/z*: 484.2 (M), 485.2 (M+H), 486.2 (M+2H); HRMS (ES^-): *m/z* calcd for $C_{21}H_{19}O_7N_5P$: 484.10276; found: 484.10237.

5-[(2'',2'''':6'',2'''-terpyridin-4''-yl)ethynyl]-2'-deoxycytidine 5'-O-monophosphate (dC^{Etpy}MP, 12c)

This compound was prepared according to the general procedure III from **dC^{I}MP (10)** and terpyridinyl acetylene **1c** in the yield of 47% (36.2 mg).



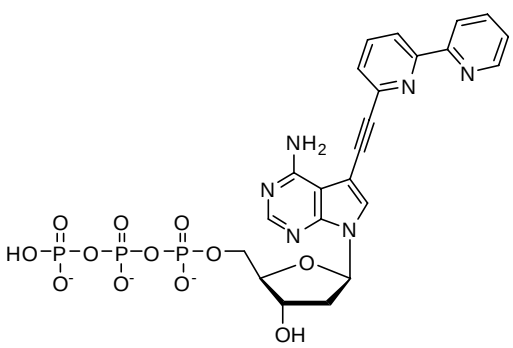
^{11}H NMR (500.0 MHz, CD_3OD): 2.26 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.1, H-2'b); 2.44 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.8, $J_{2'a,3'}$ = 3.0, H-2'a); 4.14 (m, 3H, H-4',5'); 4.54 (dt, 1H, $J_{3',2'}$ = 6.1, 3.0, $J_{3',4'}$ = 3.0, H-3'); 6.29 (dd, 1H, $J_{1',2'}$ = 7.3, 5.8, H-1'); 7.50 (ddd, 2H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.9, $J_{5''',3'''}$ = 0.9, H-5'''); 8.02 (ddd, 2H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.46 (s, 1H, H-6); 8.54 (s, 2H, H-3'',5''); 8.59 (bd, 2H, $J_{3''',4'''}$ = 8.0, H-3'''); 8.70 (bd, 2H, $J_{6''',5'''}$ = 4.9, H-6'''); ^{13}C NMR (125.7 MHz,

CD_3OD): 42.03 (CH_2-2'); 65.78 (d, $J_{C,P}$ = 5.1, CH_2-5'); 72.58 (CH-3'); 86.54 (C4"-C≡**C**-C5); 88.17 (d, $J_{C,P}$ = 8.8, CH-4'); 88.26 (CH-1'); 91.91 (C-5); 93.81 (C4"-**C**≡C-C5); 123.09 (CH-3'"); 123.84 (CH-3",5"); 125.71 (CH-5'"); 134.54 (C-4"); 139.07 (CH-4'"); 147.38 (CH-6); 150.11 (CH-6'"); 156.51 (C-2); 156.54 (C-2'"); 156.75 (C-2",C6"); 165.88 (C-4); ^{31}P NMR (202.3 MHz, CD_3OD): 2.44; MS (ES^-): found m/z: 561.2(M), 562.2 (M+H), 563.2 (M+2H); HRMS (ES^-): m/z calcd for $C_{26}H_{22}O_7N_6P$: 561.1293; found: 561.1292.

General procedure IV: Sonogashira cross-coupling reaction – synthesis of modified dN^{ER}TPs

Mixture CH_3CN/H_2O (1:2) (1.5 ml) and Et(i-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing halogenated nucleoside triphosphate **dA^{I}TP (13)** or **dC^{I}TP (14)** (60 mg), an alkyne **1a-c** (1.5 equiv. for dC^{I}TP or 2 equiv. for dA^{I}TP) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of CH_3CN/H_2O (1:2) (0.5 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80 °C for 1 h. The solvent was evaporated in vacuo. Products were purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB in H_2O to 0.1M TEAB in H_2O/MeOH (1:1) as an eluent. Several co-distillations with water and conversion to sodium salt form (Dowex 50 in Na^+ cycle) followed by freeze-drying from water, gave the products as white or yellow powder.

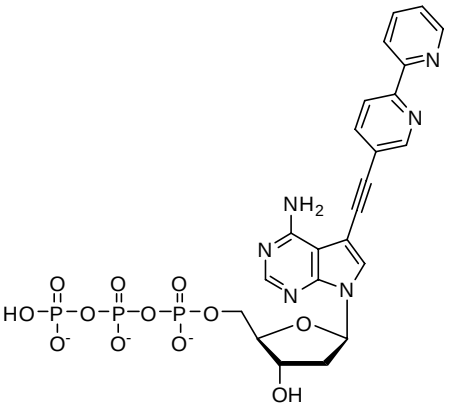
7-[(2'',2'''-bipyridin-6''-yl)ethynyl]-7-deaza-2'-deoxyadenosine 5'-O-triphosphate (dA^{E6bpy}TP, 15a).



This compound was prepared according to the general procedure IV from **dA^{I}TP (13)** and bipyridinyl acetylene **1a** in the yield of 42% (27.1 mg) ^{1}H NMR (499.8 MHz, D_2O, ref$_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.35 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 3.2, H-2'b); 2.49 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.1, H-2'a); 4.14 (m, 2H, H-5'); 4.19 (m, 1H, H-4'); 4.68 (dt, 1H, $J_{3',2'}$ = 6.1, 3.2, $J_{3',4'}$ = 3.2, H-3'); 6.19 (dd, 1H, $J_{1',2'}$ = 7.9, 6.1, H-1'); 7.25 (bdd, 1H, $J_{5''',4'''}$ = 7.7, $J_{5''',6'''}$ =

4.2, H-5'''); 7.29 (bd, 1H, $J_{5'',4''}$ = 7.7, H-5''); 7.52 (s, 1H, H-6); 7.57 (bd, 1H, $J_{3'',4''}$ = 7.7, H-3''); 7.65 (bt, 1H, $J_{4''',3'''} = J_{4''',5'''}$ = 7.7, H-4'''); 7.71 (bt, 1H, $J_{4'',3''} = J_{4'',5''}$ = 7.7, H-4''); 7.75 (bd, 1H, $J_{3''',4'''}$ = 7.7, H-3'''); 7.81 (s, 1H, H-2); 8.30 (bd, 1H, $J_{6''',5'''}$ = 4.2, H-6'''); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.20 (CH_2-2'); 68.32 (d, $J_{C,P}$ = 5.6, CH_2-5'); 73.72 (CH-3'); 85.53 (C6''-C≡**C**-C5); 85.56 (CH-1'); 87.81 (d, $J_{C,P}$ = 8.7, CH-4'); 93.87 (C6''-**C**≡C-C5); 98.48 (C-5); 104.92 (C-4a); 122.92 (CH-3''); 124.26 (CH-3'''); 127.25 (CH-5'''); 130.16 (CH-5''); 130.26 (CH-6); 140.76 (CH-4''); 141.02 (CH-4'''); 144.49 (C-6''); 150.52 (C-7a); 150.76 (CH-6'''); 153.73 (CH-2); 155.78 (C-2'''); 156.86 (C-2''); 158.75 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.86 (bdd, J = 19.2, 18.9, P_β); -9.60 (d, J = 19.2, P_α); -7.40 (bd, J = 18.9, P_γ); MS (ES^-): found *m/z*: 667.0 (M-1), 587.1 (M–PO_3H_2–1); HRMS (ES^-): *m/z* calcd for $C_{23}H_{22}O_{12}N_6P_3$: 667.0514; found: 667.0504.

7-[(2',2'''-bipyridin-5''-yl)ethynyl]-7-deaza-2'-deoxyadenosine 5'-O-triphosphate (dA^{E5bpy}TP, 15b)

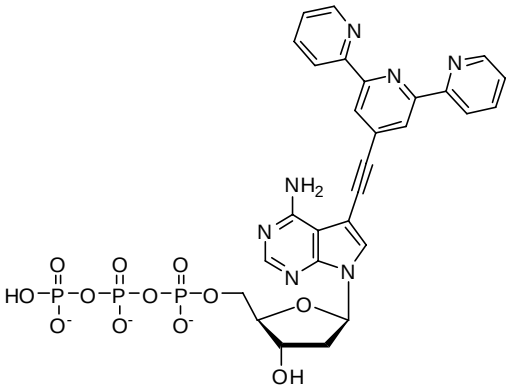


This compound was prepared according to the general procedure IV from **dA^{I}TP (13)** and bipyridinyl acetylene **1b** in the yield of 48% (31.0 mg).

^{1}H NMR 499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.42 (ddd, 1H, J_{gem} = 13.7, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.9, H-2'b); 2.59 (ddd, 1H, J_{gem} = 13.7, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ = 6.4, H-2'a); 4.17 (m, 2H, H-5'); 4.22 (m, 1H, H-4'); 4.72 (dt, 1H, $J_{3',2'}$ = 6.4, 2.9, $J_{3',4'}$ = 2.9, H-3'); 6.15 (dd, 1H, $J_{1',2'}$ = 7.8, 6.0, H-1'); 7.48 (dd, 1H, $J_{5''',4'''}$ = 7.2, $J_{5''',6'''}$ = 4.6, H-5'''); 7.41 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.2, $J_{4''',6'''}$ = 1.2, H-4'''); 7.45 (s, 1H, H-6); 7.52 (d, 1H, $J_{3''',4'''}$ = 8.0, H-3'''); 7.62 (dd, 1H, $J_{4'',3''}$ = 8.3, $J_{4'',6''}$ = 1.7, H-4''); 7.67 (d, 1H, $J_{3'',4''}$ = 8.3, H-3''); 7.78 (s, 1H, H-2); 8.03 (d, 1H, $J_{6''',5'''}$ = 4.6, H-6'''); 8.16 (bs, 1H, H-6''); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.25 (CH_2-2'); 68.42 (d, $J_{C,P}$ = 6.0, CH_2-5'); 73.83 (CH-3'); 85.50 (CH-1'); 87.83 (d, $J_{C,P}$ = 8.7, CH-4'); 89.25 (C5''-C≡**C**-C5); 91.61 (C5''-**C**≡C-C5); 98.74 (C-5); 104.82 (C-4a); 122.62 (C-5''); 123.31 (CH-3''); 123.71 (CH-3'''); 126.72 (CH-5'''); 129.11 (CH-6); 140.39 (CH-4'''); 142.02 (CH-4''); 150.45 (C-7a); 150.52 (CH-6'''); 152.62 (CH-6''); 153.97 (CH-2);

154.67 (C-2"); 155.18 (C-2'''); 159.01 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.84 (dd, *J* = 19.2, 18.9, P_β); -9.61 (d, *J* = 19.2, P_α); -7.29 (d, *J* = 18.9, P_γ); MS (ES^-): found *m/z*: 667.0 (M-1), 587.2 (M–PO_3H_2–1); HRMS (ES^-): *m/z* calcd for $C_{23}H_{22}O_{12}N_6P_3$: 667.0514; found: 667.0512.

7-[(2'',2'''':6'',2'''-terpyridin-4''-yl)ethynyl]-7-deaza-2'-deoxyadenosine 5'-O-triphosphate (dA^{Etpy}TP, 15c)

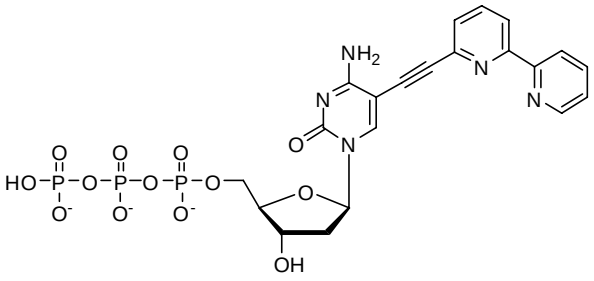


This compound was prepared according to the general procedure IV from **dA^{I}TP (13)** and terpyridinyl acetylene **1c** in the yield of 40% (28.5 mg).

^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.34, 2.44 (2 × bm, 2 × 1H, H-2'); 4.10 (bm, 2H, H-5'); 4.20 (bm, 1H, H-4'); 4.61 (bm, 1H, H-3'); 6.06 (bm, 1H, H-1'); 6.77 (bm, 2H, H-5'''); 7.06 (bm, 2H, H-3'''); 7.18 (bs, 1H, H-6); 7.32 (bm, 2H, H-4'''); 7.72 (bs, 4H, H-3'',5'', H-6'''); 7.76 (bs, 1H, H-2); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 40.72 (CH_2-2'); 68.47 (d, $J_{C,P}$ = 4.7, CH_2-5'); 73.66 (CH-3'); 85.38 (CH-1'); 87.65 (d, $J_{C,P}$ = 8.6, CH-4'); 89.66 (C4"-C≡**C**-C5); 92.89 (C4"-**C**≡C-C5); 97.94 (C-5); 104.84 (C-4a); 123.36 (CH-3'''); 124.08 (CH-3'',5''); 126.96 (CH-5'''); 129.67 (CH-6); 134.80 (C-4''); 140.73 (CH-4'''); 150.08 (CH-6'''); 150.37 (C-7a); 154.14 (CH-2); 154.74 (C-2'''); 155.68 (C-2'',6''); 158.77 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.65 (bt, *J* = 18.7, 19.3, P_β); -9.82 (d, *J* = 18.7, P_α); -6.49 (b, P_γ); MS (ES^-): found *m/z*: 766.0 (M-1), 664.1 (M–PO_3H_2–1); HRMS (ES^-): *m/z* calcd for $C_{28}H_{25}O_{12}N_7P_3$: 744.0780; found: 744.0767.

5-[(2'',2'''-bipyridin-6''-yl)ethynyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{E6bpy}TP, 16a)

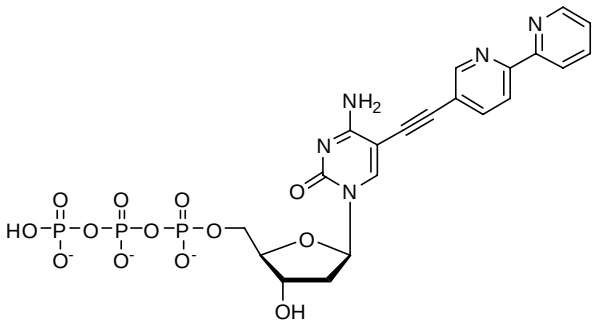
This compound was prepared according to the general procedure IV from **dC^{I}TP (14)** and bipyridinyl acetylene **1a** in the yield of 67% (43.4 mg).



^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.22 (dt, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.8, H-2'b); 2.42 (ddd, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 4.1, H-2'a); 4.23 (m, 3H, H-4',5'); 4.57 (dt, 1H, $J_{3',2'}$ = 6.8, 4.1, $J_{3',4'}$ = 4.1, H-3'); 6.11 (dd, 1H, $J_{1',2'}$ = 6.8, 6.3, H-1'); 7.45 (bdd, 1H, $J_{5''',4'''}$ = 7.8, $J_{5''',6'''}$ = 4.2, H-5'''); 7.57 (bd, 1H, $J_{5'',4''}$ = 7.8, H-5''); 7.92 (bt, 1H, $J_{4'',3''}$ = $J_{4'',5''}$ = 7.8, H-4''); 7.95 (m, 2H, H-3'', H-4'''); 8.02 (bd, 1H, $J_{3''',4'''}$ = 7.8, H-3'''); 8.03 (s, 1H, H-6); 8.54 (bd, 1H, $J_{6''',5'''}$ = 4.2, H-6'''); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.93 (CH_2-2'); 67.92 (d, $J_{C,P}$ = 5.6, CH_2-5'); 72.90 (CH-3'); 83.58 (C6''-C≡**C**-C5); 88.16 (d, $J_{C,P}$ = 8.7, CH-4'); 89.11 (CH-1'); 94.06 (C-5); 96.48 (C6''-**C**≡C-C5); 124.25 (CH-3''); 124.88 (CH-3'''); 127.45 (CH-5'''); 130.46 (CH-5''); 141.35, 141.49 (CH-4'', CH-4'''); 144.23 (C-6''); 148.11 (CH-6); 151.53 (CH-6'''); 156.84 (C-2'''); 158.03 (C-2''); 158.21 (C-2); 167.00 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -21.06 (t, J = 19.7, P_β); -10.10 (d, J = 19.6, P_α); -6.74 (bd, J = 19.6, P_γ); MS (ES^-): found *m/z*: 644.0 (M-1), 564.1 (M–PO_3H_2–1); HRMS (ES^-): *m/z* calcd for $C_{21}H_{21}O_{13}N_5P_3$: 644.0354; found: 644.0348.

5-[(2'',2'''-bipyridin-5''-yl)ethynyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{E5bpy}TP, 16b)

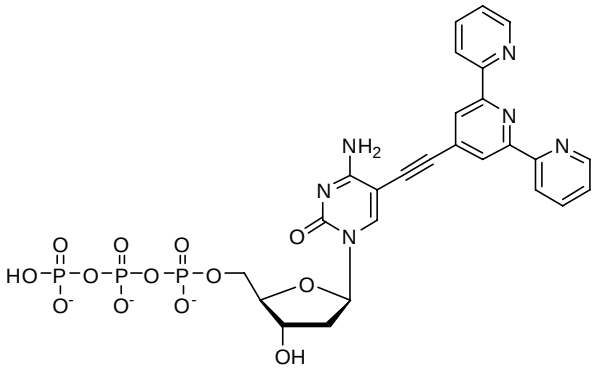
This compound was prepared according to the general procedure IV from **dC^{I}TP (14)** and bipyridinyl acetylene **1b** in the yield of 59% (38.2 mg).



^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.25 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 7.4, $J_{2'b,3'}$ = 6.3, H-2'b); 2.44 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 4.0, H-2'a); 4.25 (m, 3H, H-4',5'); 4.61 (dt, 1H, $J_{3',2'}$ = 6.3, 4.0, $J_{3',4'}$ = 4.0, H-3'); 6.10 (dd, 1H, $J_{1',2'}$ = 7.4, 6.1, H-1'); 7.28 (dd, 1H, $J_{5''',4'''}$ = 7.0, $J_{5''',6'''}$ = 4.6, H-5'''); 7.78 (dd, 1H, $J_{4''',3'''}$ = 7.5, $J_{4''',5'''}$ = 7.0, H-4'''); 7.82 (d, 1H, $J_{4'',3''}$ = 7.9, H-4''); 7.84 (s, 1H, H-6); 7.93 (d, 1H, $J_{3''',4'''}$

= 7.5, H-3'''); 7.95 (d, 1H, $J_{3'',4''}$ = 7.9, H-3''); 8.39 (bs, 1H, H-6''); 8.42 (d, 1H, $J_{6''',5'''}$ = 4.6, H-6'''); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.88 (CH_2-2'); 68.04 (d, $J_{C,P}$ = 5.3, CH_2-5'); 73.03 (CH-3'); 86.72 (C5''-C≡**C**-C5); 88.17 (d, $J_{C,P}$ = 8.6, CH-4'); 89.05 (CH-1'); 94.42 (C-5); 94.75 (C5''-**C**≡C-C5); 122.21 (C-5''); 124.04 (CH-3''); 124.75 (CH-3'''); 127.04 (CH-5'''); 141.09 (CH-4'''); 142.81 (CH-4''); 147.31 (CH-6); 151.61 (CH-6''); 153.30 (CH-6'''); 156.06 (C-2''); 156.46 (C-2'''); 158.04 (C-2); 166.39 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.78 (t, J = 19.2, P_β); -10.01 (d, J = 19.2, P_α); -6.51 (d, J = 19.2, P_γ); MS (ES^-): found *m/z*: 644.0 (M-1), 666.0 (M+Na); HRMS (ES^-): *m/z* calcd for $C_{21}H_{21}O_{13}N_5P_3$: 644.0354; found: 644.0354.

5-[(2'',2'''':6'',2'''-terpyridin-4''-yl)ethynyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{Etpy}TP, 16c)



This compound was prepared according to the general procedure IV from **dC^{I}TP (14)** and terpyridinyl acetylene **1c** in the yield of 69% (49.5 mg).

^{1}H NMR (499.8 MHz, D_2O, $ref_{dioxane}$ = 3.75 ppm, pD = 7.1, phosphate buffer): 2.31, 2.48 (2 × bm, 2 × 1H, H-2'); 4.24 (bm, 2H, H-5'); 4.27 (bm, 1H, H-4'); 4.61 (bm, 1H, H-3'); 6.09 (bm, 1H, H-1'); 7.12 (bm, 2H, H-5'''); 7.35 (bm, 2H, H-3'''); 7.66 (bs, 1H, H-6); 7.68 (bm, 2H, H-4'''); 8.02 (bs, 2H, H-3'',5''); 8.07 (bm, 2H, H-6'''); ^{13}C NMR (125.7 MHz, D_2O, $ref_{dioxane}$ = 69.3 ppm, pD = 7.1, phosphate buffer): 41.47 (CH_2-2'); 68.28 (CH_2-5'); 73.33 (CH-3'); 87.58 (C4''-C≡**C**-C5); 88.08 (d, $J_{C,P}$ = 9.2, CH-4'); 89.44 (CH-1'); 93.66 (C-5); 95.91 (C4''-**C**≡C-C5); 124.26 (CH-3'''); 124.76 (CH-3'',5'''); 127.33 (CH-5'''); 134.80 (C-4''); 141.40 (CH-4'''); 148.33 (CH-6); 150.87 (CH-6'''); 155.34 (C-2'''); 156.43 (C-2'',6''); 157.89 (C-2); 166.14 (C-4); ^{31}P NMR (202.3 MHz, D_2O, $ref_{phosphate\ buffer}$ = 2.35 ppm, pD = 7.1): -20.78 (bdd, J = 20.1, 19.3, P_β); -8.91 (d, J = 19.3, P_α); -6.88 (bd, J = 20.1, P_γ); MS (ES^-): found *m/z*: 721.0 (M-1), 743.0 (M+Na); HRMS (ES^-): *m/z* calcd for $C_{26}H_{24}O_{13}N_6P_3$: 721.0620; found: 721.0595.

6.1.2 Synthesis and analysis of PEX products

Synthetic ONs were purchased from Sigma Aldrich (USA). Primer: 5'-CAT GGG CGG CAT GGG-3' (prim^{rnd}); templates: 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3'(temp^{rnd16}), 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}) (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 were obtained from Sigma Aldrich (MagSelect, USA) or Novagen (MagPrep, USA), Pwo DNA polymerase from PeqLab (Germany), DyNAzyme II and Phusion DNA polymerases from Finnzymes (Finland), KOD XL DNA polymerase from Novagen, Vent (exo$^-$), Deep Vent, Deep Vent (exo$^-$) and Therminator DNA polymerases as well as T4 polynukleotide kinase and natural nucleoside triphosphates (dATP, dCTP, dGTP and dCTTP) from New England Biolabs (Great Britain) and γ-^{2}P-TP from Izotop, Institute of isotopes Co, Ltd. (Hungary).

Primer extension experiment

The reaction mixture (20 µl) contained DNA polymerase: Pwo (0.1 U/µl, 2 µl), DyNAzyme II (0.2 U/µl, 1 µl), Vent (exo-) (0.2 U/µl, 1 µl), Phusion (0.2 U/µl, 1 µl), KOD XL (0.25 U/µl, 0.8 µl), Therminator (0.2 U/µl, 1 µl), Deep Vent (exo-) (0.2 U/µl, 1 µl), Deep Vent (0.2 U/µl, 1 µl), dNTPs (either natural or modified, 4 mM, 1 µl), ^{32}P-prelabelled primer at 5'-end (3 µM, 1 µl) and template (3 µM, 1.5 µl) in 2 µl of corresponding buffer suplied by manufacturer Reaction mixture was incubated for 30 min at 60 °C.

Optimized conditions for primer extension experiment for single incorporation of dC^{ER}TPs (16a-c) by using DyNAzyme II: The reaction mixture (20 µl) contained DyNAzyme II polymerase (0.1 U/µl, 1 µl), natural dNTPs (4 mM, 0.5 µl) and modified **dN^{ER}TPs** (**16a-c**) (4 mM, 1 µl), ^{32}P-prelabelled primer at 5'-end (3 µM, 1 µl) and template temp^{C} (3 µM, 1.5 µl) in 2 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 10 or 15 min at 60 °C.

Optimized conditions for primer extension for multiple incorporation of dC^{ER}TPs (16a-c) by using DyNAzyme II: The reaction mixture (20 µl) contained DyNAzyme II polymerase (1 U/ µl, 1 µl), natural dNTPs (4 mM, 1 µl) and modified **dN^{ER}TPs** (**16a-c**)

(4 mM, 2 µl), ^{32}P-prelabelled primer at 5'-end (3 µM, 1 µl) and template temp^{rnd16} (3 µM, 1.5 µl) in 2 µl of corresponding buffer suplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C.

For magnetoseparation unlabelled primers and biotinylated templates were used.

Primer extension for kinetics study

The reaction mixture (20 µl) contained DNA polymerase: Pwo (0.1 U/µl, 2 µl) or DyNAzyme II (0.1 U/µl, 1 µl), dATP/**dA^{ER}TP (15b-c)** or dCTP/**dC^{ER}TP (16b-c)** (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 suplied by manufacturer. 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, 20mM EDTA, 0.025% [w/v] bromphenole 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 1xTBE buffer (pH 8) and 7% urea at 60 W for ~ 60 min. Gel was dried and visualized by phosphoimager.

MALDI-TOF experiment

A mixture of 3-hydroxypicolinic acid (HPA)/picolinic acid (PA)/ammonium tartrate in ration 8/1/1 in 50% acetonitrile was used as matrix for MALDI-TOF measurement. Then 2 µl of the matrix and 1 µl of the sample were mixed on MTP 384 polished steel target by use of anchor-chip desk. The crystallized spots were washed once by 0.1% formic acid and once by water. The acceleration tension in reflectron mode was 19.5 kV and range of measurement 3–13 kDa. The found differences of 2-9 Da for 6 KDa DNA and 3-12 Da for 10 kDa DNA are still within the experimental error (ca 0.1%) of the low resolution machine also considering the very small amounts of DNA produced by PEX.

Isolation of single-strand oligonucleotides: The reaction mixture (200 µl) contained DNA polymerase: Pwo (1 U/µl, 10 µl) or DyNAzyme II (2 U/µl, 10 µl), dNTPs (either natural or modified, 4mM, 10 µl), unlabeled primer (10 µM, 40 µl, 5'-CAT GGG CGG CAT GGG-3') and biotinylated template temp^{A}-bio (10 µM, 40 µl, 5'-**CCC T***CC CAT GCC GCC CAT G*-3), temp^{C}-bio (10 µM, 40 µl, 5'-**CCC G***CC CAT GCC GCC CAT G*-

3) or temp^{rnd16}-bio (10 μM, 40 μl, 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3') in 20 μl of corresponding buffer suplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C. The separation on magnetic beads (50 μl, Sigma –Aldrich or Novagen) was caried out according to standard techniques:
50 μl of the MagSelect^{TM} SA Streptavidin Particles stock solution from Sigma Aldrich) or MagPrep® P-25 (Streptavidin Particles stock solution from Novagen) was first washed three times by 200 μl of buffer (0.3M NaCl, 10mM TRIS, pH=7.4) and then the reaction mixture containing 0.3 μl of 2.5M NaCl was added. To capture the double-stranded DNA to streptavidine coated magnetic beads, the suspension was incubates at 20 °C (30 min, 1200 rpm). Captured product was three time re-suspended in 500 μl of PBS solution (0.14M NaCl, 3 mM KCl, 4mM sodium phosphate, pH=7.4), three time in 500 μl of buffer (0.3M NaCl, 10mM TRIS, pH=7.4) and two times in 400 μl of PCR ultra water (Top-Bio) and purified by repeated magnetoseparation using a magnetoseparator (Dynal, Norway). Single-strand oligonucleotides were released, after the addition of 100 μl of PCR ultra water (Top-Bio), by incubation at 75 °C (2 min, 900 rpm).

6.1.3 Complexation studies

Complexation of dN^{ER}MPs (11a-c and 12a-c)
Complexes of modified nucleoside monophosphates **dN^{ER}MPs (11a-c** and **12a-c)** with diverse transition metals were prepared by mixing 100 μl of aqueous solution corresponding monophosphate (100 μM) with 100 μl 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 ON for recording UV-spectra
Double stranded ONs were prepared by PEX-experiment on larger scale. The reaction mixture (200 μl) contained Deep Vent polymerase (2 U/μl, 15 μl), dNTP (either natural or modified, 4 mM, 30 μl), unlabeled primer (100 μM, 12 μl), and temp^{rnd16} (100 μM, 12 μl) in 20 μl of corresponding buffer supplied by manufacturer. 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 1 equiv. of $Fe(BF_4)_2{\cdot}6H_2O$ to number of modification

(0.24 µl, 10 mM) was added and the solution was mixed for 3 h (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, 2 µl), DyNAzyme II (0.1 U/µl, 1 µl), dNTP (either natural or modified, 4 mM, 1 µl), ^{32}P-prelabelled primer at 5'-end primer (3 µM, 1 µl), and template (3 µM, 1.5 µl) in 2 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C. For incorporation of **dC^{tpy}TP** using Dynazyme II polymerase and temp^{C} was used 0.5 µl of natural dNTPs (4 mM) and the mixture was incubated only for 15 min. After addition of 1 µl of Fe^{2+} (4mM, $Fe(BF_4)_2 \cdot 6H_2O$ or $FeCl_2$) the solution was mixed for 3 h (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] bromphenol blue, 0.2% [w/v] xylene cyanol) subjected to gel electrophoresis in 8% non-denaturating polyacrylamide gel containing 1xSB buffer (pH 8) and at 500 V for ~ 3 h at room temperature. Gel was dried and visualized by phosphoimager.

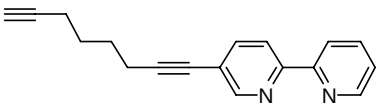
6.2 Oligopyridine analogues with flexible linker

6.2.1 Synthesis of modified nucleosides, nucleotides and nucleosides triphosphates

General procedure V: Synthesis of ligand building blocks

To an argone-purged flask containing 5-bromo-2,2'-bipyridine (**17a**) or (2,2':6',2''-terpyridine-4'-yl) trifluoromethanesulfonate (**17b**) (500mg), $Pd(PPh_3)_2Cl_2$ (5 mol%) and CuI (5 mol%) were added THF (10 ml), Et_3N (10 equiv.) and 1,7-octadiyne (3 equiv.). Reaction mixture was heated at 75 °C for 3 h. After evaporation of solvent under reduced pressure, the residue was extracted with three 100 ml portion 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 %).

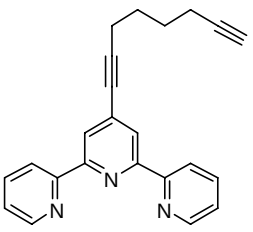
5-(octa-1'',7''-diyn-1''-yl)-2,2'-bipyridine (19a)



Product **19a** was prepared according to general procedure V from 5-bromo-2,2'-bipyridine **(17a)**. It was isolated as orange oil in the yield of 66% (365.3 mg).

^{1}H NMR (500.0 MHz, $CDCl_3$): 1.72 (m, 2H, HC≡C-CH_2C**H$_2$**CH_2CH_2-C≡C-C5); 1.78 (m, 2H, HC≡C-CH_2CH_2C**H$_2$**CH_2-C≡C-C5); 1.98 (t, 1H, ^4J = 2.7, HC≡C-); 2.27 (td, 2H, J_{vic} = 6.8, ^4J = 2.7, HC≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-C5); 2.50 (t, 2H, J_{vic} = 6.9, HC≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-5); 7.40 (ddd, 1H, $J_{5',4'}$ = 7.5, $J_{5',6'}$ = 5.0, $J_{5',3'}$ = 1.2, H-5'); 7.84 (dd, 1H, $J_{4,3}$ = 8.3, $J_{4,6}$ = 2.1, H-4); 7.93 (ddd, 1H, $J_{4',3'}$ = 8.1, $J_{4',5'}$ = 7.5, $J_{4',6'}$ = 1.8, H-4'); 8.49 (m, 2H, H-3,3'); 8.69 (dd, 1H, $J_{6,4}$ = 2.1, $J_{6,3}$ = 0.9, H-6); 8.72 (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, $CDCl_3$): 17.97 (HC≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C5); 19.12 (HC≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C5); 27.41, 27.56 (HC≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C5); 68.64 (H**C**≡C); 77.79 (C5-**C**≡C); 83.96 (HC≡**C**); 95.15 (C5-C≡**C**); 120.95 (CH-3); 121.70 (C-5); 122.05 (CH-3'); 124.16 (CH-5'); 138.50 (CH-4'); 139.80 (CH-4); 147.88 (CH-6'); 151.62 (CH-6); 152.27 (C-2); 154.32 (C-2'); MS (ESI): *m/z* (%) = 261.1 (100) [M^+ + H], 283.1 (30) [M^+ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{18}H_{17}N_2$: 261.13863; found: 261.13813; Anal. Calcd for $C_{18}H_{16}N_2$·1/6MeOH: C, 82.13; H, 6.32; N, 10.54. Found: C, 82.36; H, 6.09; N, 10.23; IR: 3288, 2941, 1585, 1542, 1455, 1432, 1365 cm^{-1}; Mp 36.0 – 38.5 °C.

4-(octa-1''',7'''-diyn-1'''-yl)- 2,2'':6,2'-terpyridine (19b)



Product **19b** was prepared according to general procedure V from (2,2':6',2''-terpyridine-4'-yl) trifluoromethanesulfonate **(17b)**. It was isolated as orange oil in the yield of 75% (331.8 mg).

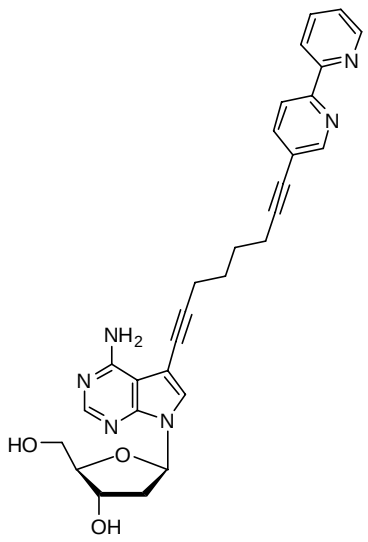
^{1}H NMR (500.0 MHz, $CDCl_3$): 1.73 (m, 2H, HC≡C-CH_2C**H$_2$**CH_2CH_2-C≡C-4); 1.77 (m, 2H, HC≡C-CH_2CH_2C**H$_2$**CH_2-C≡C-C4); 1.98 (t, 1H, ^4J = 2.7, HC≡C-); 2.28 (td, 2H, J_{vic} = 6.8, ^4J = 2.7, HC≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-C4); 2.51 (t, 2H, J_{vic} = 6.8, HC≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-C4); 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-**C**$H_2CH_2CH_2CH_2$-C≡C-C4); 19.05 (HC≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C4); 27.30, 27.47

(HC≡C-CH_2**C**H_2**C**$H_2$$CH_2$-C≡C-C4); 68.67 (H**C**≡C); 79.04 (C4-**C**≡C); 83.99 (HC≡**C**); 95.87 (C4-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 $C_{23}H_{19}N_3 \cdot 1/5MeOH$: C, 81.04; H, 5.80; N, 12.22. Found: C, 81.42; H, 5.58; N, 11.93; IR: 3207, 2940, 1581, 1564, 1467, 1391, 1263, 1113 cm^{-1}; Mp 69.0 – 69.5 °C.

General procedure VI: Sonogashira cross-coupling reaction - synthesis of modified dN^{OR}s

DMF (1 ml) and Et(*i*-Pr)$_2$N (0.25 ml, 10 equiv.) were added to an argon-purged flask containing nucleoside 5-iodo-2'-deoxycytidine (**dC^I, 3**) or 7-iodo-7deaza-2'-deoxyadenosine (**dA^I, 20**) (50 mg), an octadiyne modified oligopyridine **19a-b** (1.5 equiv.) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (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 stired at 75 °C for 2 h. The solvent was evaporated in vacuo. Products were directly purified by flash chromotagraphy 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'''-bipyridin-5''-yl)octa-1'''',7''''-diyn-1''''-yl)-7-deaza-2'-deoxyadenosine (dA^{Obpy}, 21a)

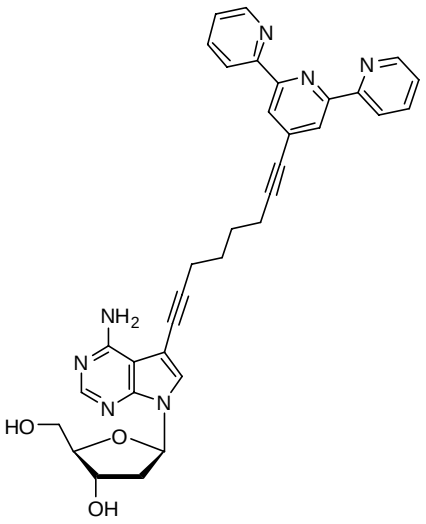


Product **21a** was prepared according to general procedure VI from **dA^I (20)** and **19a**. It was isolated as a brownish powder in the yield of 46% (31.1 mg).

^{1}H NMR (600.1 MHz, DMSO-d_6): 1.74 (m, 4H, C5-C≡C-CH_2C**H_2CH_2**CH_2-C≡C-C5''); 2.16 (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, $J_{2'a,1'}$ = 8.2, $J_{2'a,3'}$ = 5.7, H-2'a); 2.56 (m, 2H, C5-C≡C-C**H_2**$CH_2CH_2CH_2$-C≡C-C5''); 2.57 (m, 2H, C5-C≡C-$CH_2CH_2CH_2$C**H_2**-C≡C-C5''); 3.50 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.8, $J_{5'b,4'}$ = 4.4, H-5'b); 3.75 (ddd, 1H, J_{gem} =

11.7, $J_{5'a,OH}$ = 5.4, $J_{5'a,4'}$ = 4.4, H-5'a); 3.81 (td, 1H, $J_{4',5'}$ = 4.4, $J_{4',3'}$ = 2.5, H-4'); 4.30 (m, 1H, $J_{3',2'}$ = 5.7, 2.7, $J_{3',OH}$ = 4.1, $J_{3',4'}$ = 2.5, H-3'); 5.05 (dd, 1H, $J_{OH,5'}$ = 5.8, 5.4, OH-5'); 5.24 (d, 1H, $J_{OH,3'}$ = 4.1, OH-3'); 6.47 (dd, 1H, $J_{1',2'}$ = 8.2, 6.0, H-1'); 6.63 (bs, 2H, NH_2); 7.46 (ddd, 1H, $J_{5''',4'''}$ = 7.4, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.66 (s, 1H, H-6); 7.94 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.2, H-4''); 7.95 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.10 (s, 1H, H-2); 8.35 (dd, 1H, $J_{3'',4''}$ = 8.2, $J_{3'',6''}$ = 0.8, H-3''); 8.36 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.8, H-3'''); 8.690 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.8, H-6'''); 8.691 (dd, 1H, $J_{6'',4''}$ = 2.2, $J_{6'',3''}$ = 0.8, H-6''); ^{13}C NMR (150.9 MHz, DMSO-d_6): 18.56 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C5''); 18.66 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C5''); 27.49, 27.67 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C5''); 39.95 (CH_2-2'); 62.07 (CH_2-5'); 71.14 (CH-3'); 74.03 (C5-**C**≡C); 77.97 (C5''-**C**≡C); 83.27 (CH-1'); 87.64 (CH-4'); 95.56 (C5-C≡**C**); 95.27 (C5''-C≡**C**); 95.63 (C-5); 102.48 (C-4a); 120.10 (CH-3''); 120.54 (C-5''); 120.82 (CH-3'''); 124.55 (CH-5'''); 125.67 (CH-6); 137.56 (CH-4'''); 139.77 (CH-4''); 149.21 (C-7a); 149.58 (CH-6'''); 151.46 (CH-6''); 152.74 (CH-2); 153.89 (C-2''); 154.66 (C-2'''); 157.74 (C-4); MS (ESI): *m/z* (%) = 509.1 (52) [M^++H], 531.1 (100) [M^+ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{29}H_{29}O_3N_6$: 509.22957; found: 509.22965; IR: 3398, 3324. 2934, 1632, 1572, 1456, 1295, 1200, 1087, 1036 cm^{-1}; Mp 157 – 163 °C.

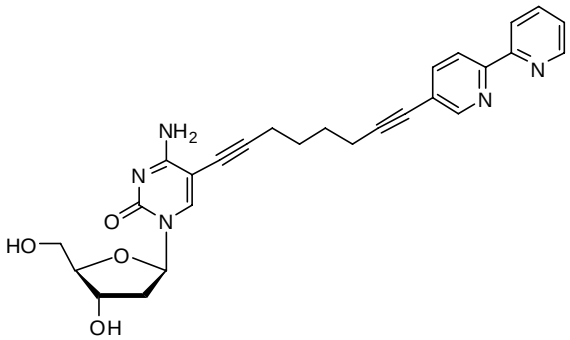
7-[8''''-(2'',2''':6'',2'''-terpyridin-4''-yl)octa-1'''',7''''-diyn-1''''-yl]-7-deaza-2'-deoxyadenosine (dA^{Otpy}, 21b)



Product **21b** was prepared according to the general procedure VI from **dA^{I} (20)** and **19b**. It was isolated as a brownish powder in the yield of 45% (35.0 mg). ^{1}H NMR (600.1 MHz, DMSO-d_6): 1.76 (m, 4H, C5-C≡C-CH_2C**H_2**C**H_2**CH_2-C≡C-C4''); 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, C5-C≡C-C**H_2**$CH_2CH_2CH_2$-C≡C-C4''); 2.62 (m, 2H, C5-C≡C-$CH_2CH_2CH_2$C**H_2**-C≡C-C4''); 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 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C4"); 18.70 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C4"); 27.34, 27.70 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C4"); 39.93 (CH_2-2'); 62.11 (CH_2-5'); 71.18 (CH-3′); 74.07 (C5-**C**≡C); 79.10 (C4"-**C**≡C); 83.28 (CH-1'); 87.67 (CH-4'); 92.45 (C5-C≡**C**); 95.69 (C-5); 96.40 (C4"-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; IR: 3444, 3185, 2928, 1584, 1566, 1468, 1393, 1305, 1046 cm^{-1}; Mp 109 - 113 °C.

5-[8''''-(2'',2'''-bipyridin-5''-yl)-octa-1'''',7''''-diyn-1''''-yl)-2'-deoxycytidine (dC^{Obpy}, 22a)

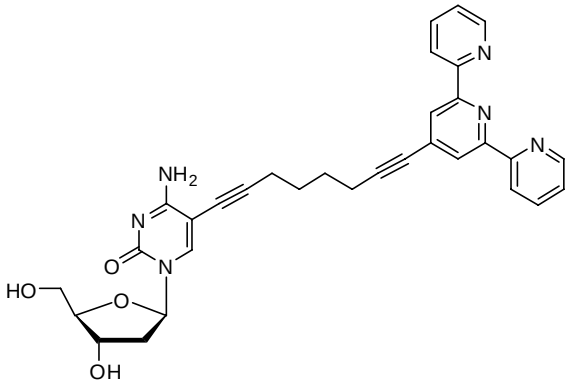


Product **22a** was prepared according to the general precedure VI from **dC^{I} (3)** and **19a**. It was isolated as a brownish powder in the yield of 38% (26.1 mg).

^{1}H NMR (600.1 MHz, DMSO-d_6): 1.70 (m, 4H, C5-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-C5"); 1.98 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.2, $J_{2'b,3'}$ = 6.1, H-2'b); 2.12 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 3.4, H-2'a); 2.48 (m, 2H, C5-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-C"); 2.55 (m, 2H, C5-C≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-C5"); 3.55, 3.61 (2 × ddd, 2 × 1H, J_{gem} = 11.9, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.7, H-5'); 3.78 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 3.4, H-4'); 4.20 (ddt, 1H, $J_{3',2'}$ = 6.1, 3.4, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.4, H-3'); 5.07 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5'); 5.21 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3'); 6.11 (dd, 1H, $J_{1',2'}$ = 7.2, 6.0, H-1'); 6.77 (bs, 1H, NH_a**H$_b$**); 7.46 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.70 (bs, 1H, N**H$_a$**H_b); 7.946 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 1.8, H-4");

7.753 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.09 (s, 1H, H-6); 8.36 (dd, 1H, $J_{3'',4''}$ = 8.2, $J_{3'',6''}$ = 0.8, H-3''); 8.37 (ddd, 1H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.8, H-3'''); 8.690 (dd, 1H, $J_{6'',4''}$ = 1.8, $J_{6'',3''}$ = 0.8, H-6''); 8.693 (ddd, 1H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.8, H-6'''); ^{13}C NMR (150.9 MHz, DMSO-d_6): 18.59 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C5''); 18.85 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C5''); 27.49, 27.53 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C5''); 40.93 (CH_2-2'); 61.24 (CH_2-5'); 73.36 (CH-3'); 72.47 (C5-**C**≡C); 77.98 (C5''-**C**≡C); 85.43 (CH-1'); 87.60 (CH-4'); 90.57 (C-5); 95.41 (C5''-C≡**C**); 95.56 (C5-C≡**C**); 120.16 (CH-3''); 120.60 (C-5''); 120.86 (CH-3'''); 124.63 (CH-5'''); 137.64 (CH-4'''); 139.83 (CH-4''); 143.77 (CH-6); 149.64 (CH-6''); 151.51 (CH-6'''); 153.71 (C-2); 153.92 (C-2''); 154.68 (C-2'''); 164.62 (C-4); MS (ESI): *m/z* (%) = 485 (10) [M^+ + H], 508 (100) [M^+ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{27}H_{28}O_4N_5$: 486.21358; found: 486.21341; Anal. Calcd for $C_{27}H_{27}O_4N_5$: C, 66.79; H, 5.61; N, 14.42. Found: C, 66.71; H, 5.52; N, 14.13; IR: 3414, 3185, 3092, 2935, 1632, 1456, 1106 cm^{-1}; Mp 187 – 193 °C.

5-[8''''-(2'',2''':6'',2'''-terpyridin-4''-yl)octa-1'''',7''''-diyn-1''''-yl]-2'-deoxycytidine (dC^{Otpy}, 22b)



Product **22b** was prepared according to the general procedure VI from **dC^{I} (3)** and **19b**. It was isolated as a brownish powder in the yield of 72% (57.4 mg).

^{1}H NMR (600.1 MHz, DMSO-d_6): 1.73 (m, 4H, C5-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-C4''); 1.97 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 7.2, $J_{2'b,3'}$ = 6.2, H-2'b); 2.11 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.4, H-2'a); 2.50 (m, 2H, C5-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-C4''); 2.60 (m, 2H, C5-C≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-C4''); 3.54, 3.60 (2 × ddd, 2 × 1H, J_{gem} = 11.9, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.7, H-5'); 3.77 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 3.4, H-4'); 4.19 (ddt, 1H, $J_{3',2'}$ = 6.2, 3.4, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 3.4, H-3'); 5.06 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5'); 5.20 (d, 1H, $J_{OH,3'}$ = 4.2, OH-3'); 6.11 (dd, 1H, $J_{1',2'}$ = 7.2, 6.1, H-1'); 6.78 (bs, 1H, NH_a**H$_b$**); 7.52 (ddd, 2H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.7, $J_{5''',3'''}$ = 1.2, H-5'''); 7.70 (bs, 1H, N**H$_a$**H_b); 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-6);

8.34 (s, 2H, H-3",5"); 8.61 (ddd, 2H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 1.0, H-3'''); 8.72 (ddd, 2H, $J_{6''',5'''}$ = 4.7, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 1.0, H-6'''); ^{13}C NMR (150.9 MHz, DMSO-d_6): 18.52 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C4"); 18.85 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C4"); 27.37, 27.48 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C4"); 40.91 (CH_2-2'); 61.25 (CH_2-5'); 70.37 (CH-3'); 72.50 (C5-**C**≡C); 79.03 (C4"-**C**≡C); 85.43 (CH-1'); 87.59 (CH-4'); 90.58 (C-5); 95.55 (C5-C≡**C**); 96.50 (C4"-C≡**C**); 121.06 (CH-3'''); 122.25 (CH-3",5"); 124.98 (CH-5'''); 133.56 (C-4"); 137.77 (CH-4'''); 143.81 (CH-6); 149.65 (CH-6'''); 153.72 (C-2); 154.51 (C-2",6"); 155.48 (C-2'''); 164.62 (C-4).MS (ESI): *m/z* (%) = 563 (5) [M^+ + H], 585 (100) [M^+ + Na]; HRMS-ESI: *m/z* [M + H]$^+$ calcd for $C_{32}H_{31}O_4N_6$: 563.24013; found: 563.23995; IR: 3438, 3320, 2940, 1643, 1582, 1467, 1048 cm^{-1}; Mp 155 - 169 °C.

General procedure VII: Sonogashira cross-coupling reaction – Synthesis of modified dN^{OR}MPs

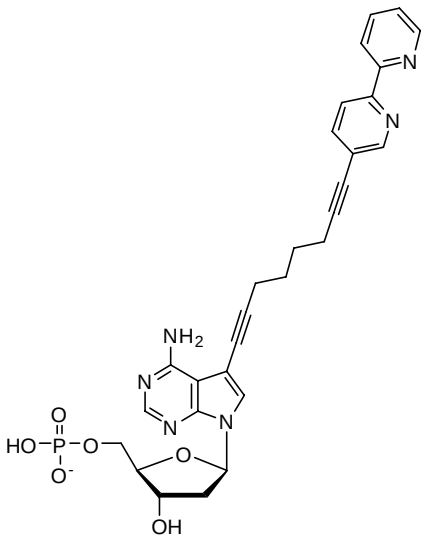
Mixture CH_3CN/H_2O (1:2) (1 ml) and Et(*i*-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing halogenated nucleoside monophosphate **dA^{I}MP (9)** or **dC^{I}MP (10)** (30 mg), an building blocks **19a-b** (1.5 equiv.) and CuI (10 mol%). In a separate flask, $Pd(OAc)_2$ (5 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of CH_3CN/H_2O (1:2) (0.5 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80 °C for 1.5 h. The solvent was evaporated in vacuo. Products were purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB in H_2O to 0.1M 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 or greenish powder.

General procedure VIII: Phosphorylation of modified nucleosides – Synthesis of modified dN^{OR}MPs

Dry trimethyl phosphate (1.5 ml) was added to an argon-purged flask containing nucleoside analogue **dN^{OR}** (**21a-b** or **22a-b**, 50 mg), cooled to 0 °C on ice followed by the addition of $POCl_3$ (1.3 equiv.). Reaction mixture was stirred at 0 °C for 1 h. and then quenched by 2M TEAB buffer (2 ml). The product was purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB in H_2O to 0.1M 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 or greenish powder.

7-[8''''-(2'',2'''-bipyridin-5''-yl)octa-1'''',7''''-diyn-1''''-yl)-7-deaza-2'-deoxyadenosine 5'-O-monophosphate (dA^{Obpy}MP, 23a).

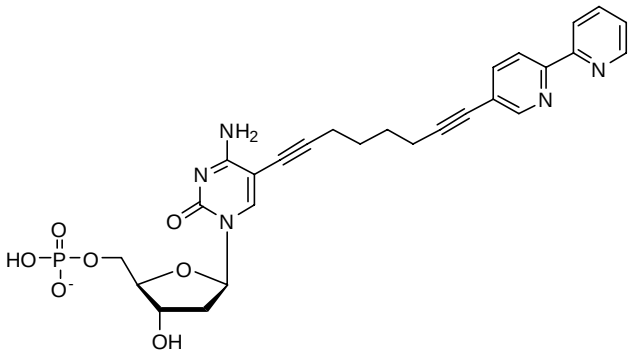


a) This compound was prepared according to the general procedure VII from **dA^{I}MP (9)** and a building block **19a** in the yield of 23% (10.1 mg)

b) This compound was prepared according to the general procedure VIII from **dA^{Obpy} (21a)** in the yield of 30% (20.4 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.29 (t, 9H, J_{vic} = 7.3, C**H$_3$**CH$_2$N); 1.82 (m, 4H, C5-C≡C-CH$_2$C**H$_2$**C**H$_2$**CH$_2$-C≡C-C5''); 2.31 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.7, H-2'b); 2.57 (m, 5H, H-2′a, C5-C≡C-C**H$_2$**CH$_2$CH$_2$C**H$_2$**-C≡C-C5''); 3.17 (q, 6H, J_{vic} = 7.3, CH$_3$C**H$_2$**N); 4.03 (ddd, 1H, J_{gem} = 11.0, $J_{H,P}$ = 5.5, $J_{5'b,4'}$ = 4.4, H-5'b); 4.06 (ddd, 1H, J_{gem} = 11.0, $J_{H,P}$ = 6.0, $J_{5'a,4'}$ = 3.6, H-5'a); 4.09 (m, 1H, H-4'); 4.59 (dt, 1H, $J_{3',2'}$ = 6.0, 2.7, $J_{3',4'}$ = 2.7, H-3'); 6.61 (dd, 1H, $J_{1',2'}$ = 8.2, 5.9, H-1'); 7.42 (ddd, 1H, $J_{5''',4'''}$ = 7.6, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.67 (s, 1H, H-6); 7.87 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.2, H-4''); 7.92 (ddd, 1H, $J_{4''',3'''}$ = 8.1, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.08 (s, 1H, H-2); 8.25 (dd, 1H, $J_{3'',4''}$ = 8.2, $J_{3'',6''}$ = 0.9, H-3''); 8.31 (ddd, 1H, $J_{3''',4'''}$ = 8.1, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.8, H-3'''); 8.62 (dd, 1H, $J_{6'',4''}$ = 2.2, $J_{6'',3''}$ = 0.8, H-6''); 8.63 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 9.15 (**C**H$_3$CH$_2$N); 19.74, 19.87 (C5-C≡C-**C**H$_2$CH$_2$CH$_2$**C**H$_2$-C≡C-C5''); 28.93, 29.05 (C5-C≡C-CH$_2$**C**H$_2$**C**H$_2$CH$_2$-C≡C-C5''); 41.26 (CH$_2$-2'); 47.71 (CH$_3$**C**H$_2$N); 66.26 (d, $J_{C,P}$ = 5.2, CH$_2$-5'); 73.04 (CH-3'); 74.20 (C5-**C**≡C); 78.62 (C5''-**C**≡C); 84.68 (CH-1'); 87.50 (d, $J_{C,P}$ = 8.5, CH-4'); 94.95 (C5-C≡**C**); 95.83 (C5''-C≡**C**); 98.94 (C-5); 103.86 (C-4a); 121.73 (CH-3''); 122.68 (CH-3'''); 122.71 (C-5''); 125.37 (CH-5'''); 127.15 (CH-6); 138.73 (CH-4'''); 140.82 (CH-4''); 149.71 (C-7a); 150.27 (CH-2); 151.32 (CH-6''); 152.59 (CH-6'''); 155.24 (C-2''); 156.52 (C-4); 157.65 (C-2'''); ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): 2.30; MS (ES$^-$): found *m/z*: 587.4 (M), 588.4 (M+H), 589.4 (M+2H); HRMS (ES$^-$): *m/z* calcd for $C_{29}H_{28}O_6N_6P$: 587.18134; found: 587.18105.

5-[8''''-(2'',2'''-bipyridin-5''-yl)-octa-1'''',7''''-diyn-1''''-yl)-2'-deoxycytidine 5'-O-monophosphate (dC^{Obpy}MP, 24a)



a) This compound was prepared according to the general procedure VII from **dC^{I}MP (10)** and a building block **19a** in the yield of 11% (4.6 mg)

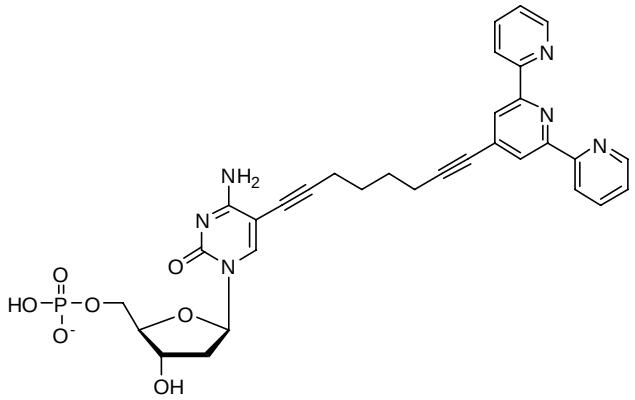
b) This compound was prepared according to the general procedure VIII from **dC^{Obpy} (22a)** in the yield of 48% (33.0 mg).

c) This compound was prepared by Sohogashira cross-coupling from **dC^{O}MP (25)**. Mixture DMF/H_2O (4:1) (2 ml) and Et(*i*-Pr)$_2$N (10 equiv.) were added to an argon-purged flask containing **dC^{O}MP (25)** (30 mg), bipyridyl bromid **17a** (1.5 equiv.) and CuI (10 mol%). In a separate flask, Pd(OAc)$_2$ (5 mol%) and TPPTS (5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of DMF/H_2O (4:1) (0.5 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80 °C for 1.5 h. The solvent was evaporated in vacuo. Products were purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB in H_2O to 0.1M 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 or greenish powder in the yield lower than 1%.

^{1}H NMR (600.1 MHz, CD_3OD): 1.30 (t, 9H, J_{vic} = 7.3, C**H$_3$**CH$_2$N); 1.80 (m, 4H, C5-C≡C-CH$_2$C**H$_2$**C**H$_2$**CH$_2$-C≡C-C5''); 2.16 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.9, $J_{2'b,3'}$ = 6.1, H-2'b); 2.36 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.8, $J_{2'a,3'}$ = 2.8, H-2'a); 2.54 (t, 2H, J_{vic} = 6.8, C5-C≡C-C**H$_2$**CH$_2$CH$_2$CH$_2$-C≡C-C5''); 2.56 (t, 2H, J_{vic} = 6.7, C5-C≡C-CH$_2$CH$_2$CH$_2$C**H$_2$**-C≡C-C5''); 3.19 (q, 6H, J_{vic} = 7.3, CH$_3$C**H$_2$**N); 4.00-4.10 (m, 3H, H-4',5'); 4.47 (dt, 1H, $J_{3',2'}$ = 6.1, 2.8, $J_{3',4'}$ = 2.8, H-3'); 6.24 (dd, 1H, $J_{1',2'}$ = 7.8, 5.7, H-1'); 7.43 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.89 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.1, H-4''); 7.94 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.07 (s, 1H, H-6); 8.28 (dd, 1H, $J_{3'',4''}$ = 8.2, $J_{3'',6''}$ = 0.9, H-3''); 8.34 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.8, H-3'''); 8.63 (dd, 1H, $J_{6'',4''}$ = 2.1, $J_{6'',3''}$ = 0.9, H-6''); 8.64 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.8, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 9.17 (**C**H$_3$CH$_2$N); 19.71 (C5-C≡C-CH$_2$CH$_2$CH$_2$**C**H$_2$-C≡C-C5''); 19.89 (C5-C≡C-

C$H_2CH_2CH_2CH_2$-C≡C-C5"); 28.81, 28.95 (C5-C≡C-CH_2**C**H_2**C**$H_2CH_2CH_2$-C≡C-C5"); 41.66 (CH_2-2'); 47.74 (CH_3**C**H_2N); 65.87 (d, $J_{C,P}$ = 5.0, CH_2-5'); 71.97 (C5-**C**≡C); 72.64 (CH-3'); 78.51 (C5"-**C**≡C); 87.82 (CH-1'); 87.89 (d, $J_{C,P}$ = 8.6, CH-4'); 93.83 (C-5); 96.00 (C5"-C≡**C**); 97.62 (C5-C≡**C**); 121.76 (CH-3"); 122.70 (CH-3'''); 122.80 (C-5"); 125.39 (CH-5'''); 138.78 (CH-4'''); 140.84 (CH-4"); 144.78 (CH-6); 150.26 (CH-6"); 152.60 (CH-6'''); 155.21 (C-2"); 156.25 (C-2); 156.53 (C-2'''); 166.22 (C-4); $^{31}P\{^{1}H\}$ NMR (202.3 MHz, CD_3OD): 2.28; MS (ES^-): found *m/z*: 564.4 (M), 565.4 (M+H), 566.4 (M+2H); HRMS (ES^-): *m/z* calcd for $C_{27}H_{27}O_7N_5P$: 564.16536; found: 564.16519.

5-[8''''-(2'',2''':6'',2'''-terpyridin-4''-yl)octa-1'''',7''''-diyn-1''''-yl]-2'-deoxycytidine 5'-O-monophosphate (dC^{Otpy}MP, 24b)



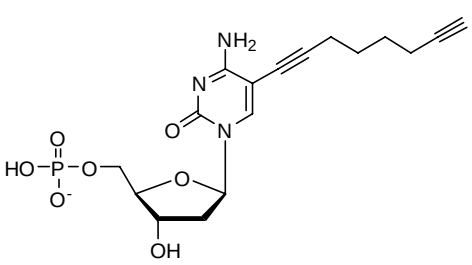
a) This compound was prepared according to the general procedure VII from **dC^{I}MP (10)** and a building block **19b** in the yield of 11% (5.1 mg)

b) This compound was prepared according to the general procedure VIII from **dC^{Otpy} (22b)** in the yield of 12% (7.9 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.30 (t, 9H, J_{vic} = 7.3, C**H$_3$**CH_2N); 1.85 (m, 4H, C5-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-C4"); 2.15 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.8, $J_{2'b,3'}$ = 6.0, H-2'b); 2.34 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.7, $J_{2'a,3'}$ = 2.8, H-2'a); 2.57 (m, 2H, C5-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-C4"); 2.61 (m, 2H, C5-C≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-C4"); 3.19 (q, 6H, J_{vic} = 7.3, CH_3C**H$_2$**N); 4.00-4.10 (m, 3H, H-4',5'); 4.46 (dt, 1H, $J_{3',2'}$ = 6.0, 2.8, $J_{3',4'}$ = 2.8, H-3'); 6.22 (dd, 1H, $J_{1',2'}$ = 7.8, 5.7, H-1'); 7.47 (ddd, 2H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.985 (ddd, 2H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.07 (s, 1H, H-6); 8.30 (s, 2H, H-3",5"); 8.59 (ddd, 2H, $J_{3''',4'''}$ = 7.9, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3'''); 8.67 (ddd, 2H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 9.17 (**C**H_3CH_2N); 19.69 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C4"); 19.90 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C4"); 28.78, 28.82 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C4"); 41.66 (CH_2-2'); 47.77 (CH_3**C**H_2N); 65.85 (d, $J_{C,P}$ = 5.1, CH_2-5'); 72.02 (C5-**C**≡C); 72.63 (CH-3'); 80.01 (C4"-**C**≡C); 87.83 (CH-1'); 87.89 (d,

$J_{C,P}$ = 8.6, CH-4'); 93.85 (C-5); 96.83 (C4''-C≡**C**); 97.64 (C5-C≡**C**); 122.81 (CH-3'''); 123.94 (CH-3'',5''); 125.65 (CH-5'''); 135.63 (C-4''); 138.86 (CH-4'''); 144.80 (CH-6); 150.19 (CH-6'''); 156.18 (C-2); 156.63 (C-2'',6''); 156.79 (C-2'''); 166.17 (C-4); $^{31}P\{^{1}H\}$ NMR (202.3 MHz, CD_3OD): 2.29; MS (ES^-): found *m/z*: 641.2 (M), 462.2 (M+H), 443.2 (M+2H); HRMS (ES^-): *m/z* calcd for $C_{32}H_{30}O_7N_6P$: 641.19191; found: 641.19178.

5-(octa-1'',7''-diyn-1''-yl)-2'-deoxycytidine 5'-O-monophosphate (dC^{O}MP, 25)



Mixture CH_3CN/H_2O (2:1) (4 ml) and Et_3N (0.2 ml, 1.3 mmol, 10 equiv.) were added to an argon-purged flask containing **dC^{I}MP (10)** (60 mg, 0.12 mmol), 1,7-octadiyne (50 µl, 0.38 mmol, 3 equiv.) and CuI (2.4 mg, 12.58 µmol, 10 mol%). In a separate flask, $Pd(OAc)_2$ (1.4 mg, 6.29 µmol, 5 mol%) and TPPTS (17.8 mg, 31.45 µmol, 5 equiv. to Pd) were combined, evacuated and purged with argon followed by addition of CH_3CN/H_2O (2:1) (1 ml). The mixture of catalyst was then injected into the reaction mixture and the reaction mixture was stired at 80 °C for 1.5 h. The solvent was evaporated in vacuo. Products were purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB in H_2O to 0.1M 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 or greenish powder in the yield of 49% (31.4 mg).

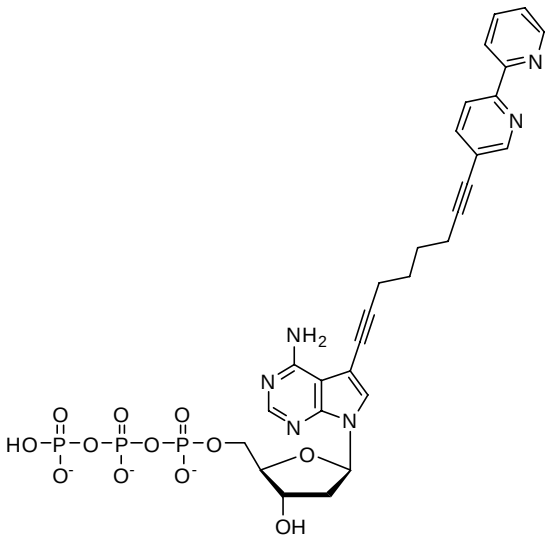
^{1}H NMR (499.8 MHz, CD_3OD): 1.31 (t, 9H, J_{vic} = 7.3, C**H$_3$**CH_2N); 1.65 (m, 2H, C5-C≡C-CH_2CH_2C**H$_2$**CH_2-C≡CH); 1.73 (m, 2H, C5-C≡C-CH_2C**H$_2$**CH_2CH_2-C≡CH); 2.16 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.9, $J_{2'b,3'}$ = 6.0, H-2'b); 2.22 (m, 1H, HC≡C); 2.24 (m, 2H, C5-C≡C-$CH_2CH_2CH_2C$**H$_2$**-C≡CH); 2.36 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.7, $J_{2'a,3'}$ = 2.7, H-2'a); 2.48 (t, 2H, J_{vic} = 7.0, C5-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡CH); 3.20 (q, 6H, J_{vic} = 7.3, CH_3C**H$_2$**N); 4.03 (dt, 1H, J_{gem} = 11.9, $J_{H,P}$ = $J_{5'b,4'}$ = 5.0, H-5'b); 4.08 (m, 2H, H-4',5'a); 4.47 (dt, 1H, $J_{3',2'}$ = 6.6, 2.7, $J_{3',4'}$ = 2.7, H-3'); 6.25 (dd, 1H, $J_{1',2'}$ = 7.9, 5.7, H-1'); 8.05 (s, 1H, H-6); ^{13}C NMR (125.7 MHz, CD_3OD): 9.09 (**C**H_3CH_2N); 18.57 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡CH); 19.81 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡CH); 28.59 (C5-C≡C-CH_2**C**$H_2CH_2CH_2$-C≡CH); 28.95 (C5-C≡C-CH_2CH_2**C**H_2CH_2-C≡CH); 41.64 (CH_2-

2′); 47.74 (CH_3**C**H_2N); 65.88 (d, $J_{C,P}$ = 5.1, CH_2-5′); 69.79 (H**C**≡C); 71.82 (C5-**C**≡C); 72.66 (CH-3'); 84.76 (**C**≡CH); 87.84 (CH-1'); 87.88 (d, $J_{C,P}$ = 8.7, CH-4'); 93.84 (C-5); 97.66 (C5-C≡**C**); 144.74 (CH-6); 156.17 (C-2); 166.17 (C-4); $^{31}P\{^1H\}$ NMR (202.3 MHz, CD_3OD): 2.28; MS (ES^-): found *m/z*: 410.2 (M), 411.2 (M+H), 412.2 (M+2H); HRMS (ES^-): *m/z* calcd for $C_{17}H_{21}O_7N_3P$: 410.11226; found: 410.11231.

General procedure IX: Phosphorylation of modified nucleosides – Synthesis of modified dN^{OR}TPs

Dry trimethyl phosphate (1 ml) was added to an argon-purged flask containing nucleoside analogue **dN^{OR}** (**21a-b** or **22a-b**, 50 mg), cooled to 0 °C on ice followed by the addition of $POCl_3$ (1.5 equiv.) and the rection mixture was stirred at 0 °C for 1 h. 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 separate flask and cooled down to 0 °C. Like this prepared solution was added to the reaction mixture and stirred for 1.5 h and quenched by 2M TEAB buffer (2 ml). The product was isolated on DEAE Sephadex column (150 ml) eluting with a gradient 0 to 1.2M TEAB, evaporated, co-distilled with water (3 times) and re-purified by semi-preparative HPLC on C18 column using linear gradient of 0.1M TEAB in H_2O to 0.1M 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'''-bipyridin-5''-yl)octa-1'''',7''''-diyn-1''''-yl)-7-deaza-2'-deoxadenosine 5'-O-triphosphate (dA^{Obpy}TP, 26a).



This compound was prepared according to the general procedure IX from **dA^{Obpy} (21a)** in the yield of 35% (36.2 mg).

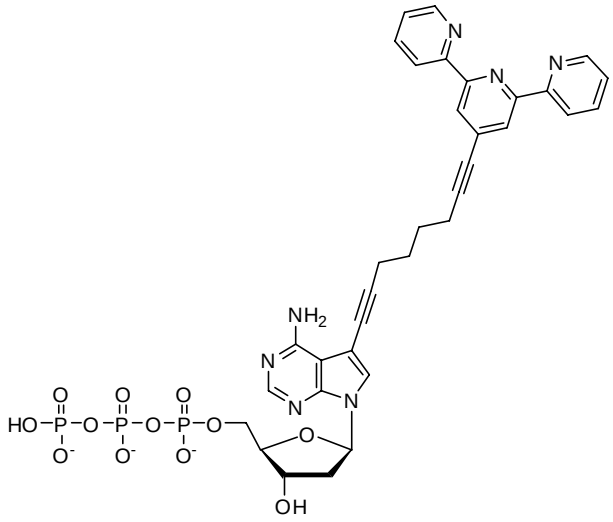
^1H NMR (600.1 MHz, CD_3OD): 1.29 (t, 27H, J_{vic} = 7.3, C**H$_3$**CH_2N); 1.81 (m, 4H, C5-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-C5''); 2.32 (ddd, 1H, J_{gem} = 13.5, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 3.1, H-2'b); 2.54 (ddd, 1H, J_{gem} = 13.5, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ = 6.0, H-2'a); 2.26 (t, 2H, J_{vic} = 6.6, C5-C≡C-C**H$_2$**CH_2CH_2CH_2-C≡C-

C5''); 2.28 (t, 2H, J_{vic} = 6.6, C5-C≡C-$CH_2CH_2CH_2C\mathbf{H_2}$-C≡C-C5''); 3.17 (q, 18H, J_{vic} = 7.3, $CH_3C\mathbf{H_2}N$); 4.12 (tdd, 1H, $J_{4',5'}$ = 4.4, $J_{4',3'}$ = 3.1, $J_{H,P}$ = 1.0, H-4'); 4.21 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 5.4, $J_{5'b,4'}$ = 4.4, H-5'b); 4.26 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.8, $J_{5'a,4'}$ = 4.4, H-5'a); 4.65 (dt, 1H, $J_{3',2'}$ = 6.0, 3.1, $J_{3',4'}$ = 3.1, H-3'); 6.61 (dd, 1H, $J_{1',2'}$ = 7.8, 6.1, H-1'); 7.41 (ddd, 1H, $J_{5''',4'''}$ = 7.5, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.2, H-5'''); 7.68 (s, 1H, H-6); 7.88 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.1, H-4''); 7.92 (ddd, 1H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.5, $J_{4''',6'''}$ = 1.8, H-4'''); 8.20 (s, 1H, H-2); 8.26 (dd, 1H, $J_{3'',4''}$ = 8.3, $J_{3'',6''}$ = 0.8, H-3''); 8.32 (ddd, 1H, $J_{3''',4'''}$ = 8.0, $J_{3''',5'''}$ = 1.2, $J_{3''',6'''}$ = 0.9, H-3'''); 8.62 (dd, 1H, $J_{6'',4''}$ = 2.1, $J_{6'',3''}$ = 0.8, H-6''); 8.63 (ddd, 1H, $J_{6''',5'''}$ = 4.8, $J_{6''',4'''}$ = 1.8, $J_{6''',3'''}$ = 0.9, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 9.09 ($\mathbf{C}H_3CH_2N$); 19.75 (C5-C≡C-$CH_2CH_2CH_2\mathbf{C}H_2$-C≡C-C5''); 19.96 (C5-C≡C-$\mathbf{C}H_2CH_2CH_2CH_2$-C≡C-C5''); 28.98 (C5-C≡C-$CH_2\mathbf{C}H_2\mathbf{C}H_2CH_2$-C≡C-C5''); 41.31 ($CH_2$-2'); 47.32 ($CH_3\mathbf{C}H_2N$); 66.97 (d, $J_{C,P}$ = 5.7, CH_2-5'); 72.63 (CH-3'); 73.57 (C5-**C**≡C); 78.59 (C5''-**C**≡C); 84.68 (CH-1'); 87.46 (d, $J_{C,P}$ = 8.8, CH-4'); 94.37 (C5-C≡**C**); 95.93 (C5''-C≡**C**); 99.27 (C-5); 103.48 (C-4a); 121.72 (CH-3''); 122.66 (CH-3'''); 122.73 (C-5''); 125.35 (CH-5'''); 127.47 (CH-6); 138.68 (CH-4'''); 140.82 (CH-4''); 149.21 (C-7a); 149.49 (CH-2); 150.30 (CH-6''); 152.61 (CH-6'''); 155.28 (C-2''); 156.00 (C-4); 156.56 (C-2'''); ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): -22.23 (t, J = 20.7, P_β); -9.70 (d, J = 20.7, P_α); -8.91 (d, J = 20.7, P_γ); MS (ES^-): found *m/z*: 747.3 (M-1), 667.3 (M-PO_3H_2-1); HRMS (ES): *m/z* calcd for $C_{29}H_{30}O_{12}N_6P_3$: 747.11400; found: 747.11335.

7-[8''''-(2'',2''':6'',2'''-terpyridin-4''-yl)octa-1'''',7''''-diyn-l''''-yl]-7-deaza-2'-deoxyadenosine 5'-O-triphosphate ($dA^{Otpy}TP$, 26b).

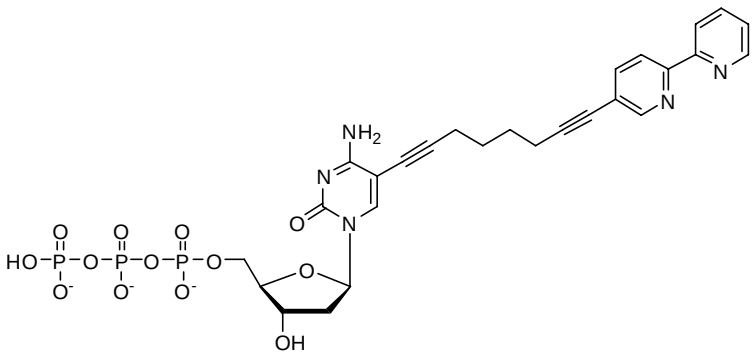
This compound was prepared according to the general procedure IX from **dA^{Otpy} (21b)** in the yield of 14% (13.2 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.29 (t, 27H, J_{vic} = 7.3, $C\mathbf{H_3}CH_2N$); 1.84 (m, 4H, C5-C≡C-$CH_2C\mathbf{H_2}C\mathbf{H_2}CH_2$-C≡C-C4''); 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, C5-C≡C-$C\mathbf{H_2}CH_2CH_2CH_2$-C≡C-C4''); 2.61 (t, 2H, J_{vic} = 6.8, C5-C≡C-$CH_2CH_2CH_2C\mathbf{H_2}$-C≡C-C4''); 3.18 (q, 18H, J_{vic} = 7.3, $CH_3C\mathbf{H_2}N$); 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 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C4"); 20.12 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C4"); 28.87, 28.93 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C4"); 41.85 (CH_2-2'); 47.44 (CH_3**C**H_2N); 66.87 (d, $J_{C,P}$ = 5.5, CH_2-5'); 72.56 (CH-3'); 72.74 (C5-**C**≡C); 80.10 (C4"-**C**≡C); 84.92 (CH-1'); 87.62 (d, $J_{C,P}$ = 8.5, CH-4'); 95.16 (C5-C≡**C**); 96.79 (C4"-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.

5-[8''''-(2'',2'''-bipyridin-5''-yl)-octa-1'''',7''''-diyn-1''''-yl)-2'-deoxycytidine 5'-O-triphosphate (dC^{Obpy}TP, 27a)

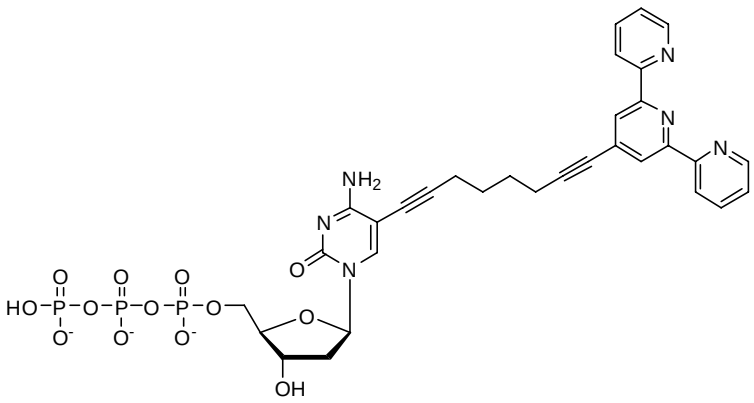


This compound was prepared according to the general procedure IX from **dC^{Obpy} (22a)** in the yield of 39% (41.2 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.30 (t, 27H, J_{vic} = 7.3, C**H$_3$**CH_2N); 1.80 (m, 4H, C5-C≡C-CH_2C**H$_2$**C**H$_2$**CH_2-C≡C-C5"); 2.18 (ddd, 1H, J_{gem} = 13.7, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.6, H-2'b); 2.34 (ddd, 1H, J_{gem} = 13.7, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 3.6, H-2'a); 2.55 (t, 2H, J_{vic} = 6.8, C5-C≡C-C**H$_2$**$CH_2CH_2CH_2$-C≡C-C5"); 2.57 (t, 2H, J_{vic} = 6.7, C5-C≡C-$CH_2CH_2CH_2$C**H$_2$**-C≡C-C5"); 3.19 (q, 18H, J_{vic} = 7.3, CH_3C**H$_2$**N); 4.08 (ddd, 1H, $J_{4',5'}$ = 4.8, 3.8, $J_{4',3'}$ = 3.6, H-4'); 4.20 (ddd, 1H, J_{gem} = 11.1, $J_{H,P}$ = 5.3,

$J_{5'b,4'}$ = 4.8, H-5'b); 4.28 (ddd, 1H, J_{gem} = 11.1, $J_{H,P}$ = 6.9, $J_{5'a,4'}$ = 3.8, H-5'a); 4.55 (dt, 1H, $J_{3',2'}$ = 6.6, 3.6, $J_{3',4'}$ = 3.6, H-3'); 6.23 (dd, 1H, $J_{1',2'}$ = 7.3, 6.0, H-1'); 7.44 (ddd, 1H, $J_{5''',4'''}$ = 7.4, $J_{5''',6'''}$ = 4.8, $J_{5''',3'''}$ = 1.0, H-5'''); 7.90 (dd, 1H, $J_{4'',3''}$ = 8.2, $J_{4'',6''}$ = 2.1, H-4''); 7.95 (ddd, 1H, $J_{4''',3'''}$ = 7.9, $J_{4''',5'''}$ = 7.4, $J_{4''',6'''}$ = 1.7, H-4'''); 8.04 (s, 1H, H-6); 8.29 (d, 1H, $J_{3'',4''}$ = 8.2, H-3''); 8.35 (d, 1H, $J_{3''',4'''}$ = 7.9, H-3'''); 8.63 (d, 1H, $J_{6'',4''}$ = 2.1, H-6''); 8.65 (d, 1H, $J_{6''',5'''}$ = 4.8, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 9.09 (**C**H_3CH_2N); 19.73 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C5''); 19.94 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C5''); 28.81, 28.98 (C5-C≡C-CH_2**C**H_2**C**H_2CH_2-C≡C-C5''); 41.23 (CH_2-2'); 47.33 (CH_3**C**H_2N); 66.60 (d, $J_{C,P}$ = 5.7, CH_2-5'); 71.71 (C5-**C**≡C); 71.93 (CH-3'); 78.49 (C5''-**C**≡C); 87.51 (CH-1'); 87.60 (d, $J_{C,P}$ = 8.8, CH-4'); 93.81 (C-5); 96.10 (C5''-C≡**C**); 97.95 (C5-C≡**C**); 121.79 (CH-3''); 122.72 (CH-3'''); 122.84 (C-5''); 125.42 (CH-5'''); 138.86 (CH-4'''); 140.87 (CH-4''); 144.82 (CH-6); 150.21 (CH-6''); 152.61 (CH-6'''); 155.11 (C-2''); 155.52 (C-2); 156.46 (C-2'''); 165.70 (C-4); ^{31}P{^{1}H} NMR (162.0 MHz, CD_3OD): -22.52 (t, J = 21, P_β); -9.97 (d, J = 21, P_α); -9.21 (d, J = 21.0, P_γ); MS (ES^-): found *m/z*: 724.1 (M-1), 644.1 (M-PO_3H_2-1); HRMS (ES): *m/z* calcd for $C_{27}H_{29}O_{13}N_5P_3$: 724.09802; found: 724.09741.

5-[8''''-(2'',2'''':6'',2'''-terpyridin-4''-yl)octa-1'''',7''''-diyn-1'''' yl]-2'-deoxycytidine 5'-O-triphosphate (dC^{Otpy}TP, 27b)



This compound was prepared according the general procedure IX from **dC^{Otpy} (22b)** in the yield of 31% (30.0 mg).

^{1}H NMR (600.1 MHz, CD_3OD): 1.30 (t, 27H, J_{vic} = 7.3, C**H**$_3$CH_2N); 1.85 (m, 4H, C5-C≡C-CH_2C**H**$_2$C**H**$_2$$CH_2$-C≡C-C4''); 2.16 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.6, H-2'b); 2.33 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 5.9, $J_{2'a,3'}$ = 3.5, H-2'a); 2.57 (t, 2H, J_{vic} = 6.8, C5-C≡C-C**H**$_2$$CH_2CH_2CH_2$-C≡C-C4''); 2.61 (t, 2H, J_{vic} = 6.8, C5-C≡C-$CH_2CH_2CH_2$C**H**$_2$-C≡C-C4''); 3.18 (q, 18H, J_{vic} = 7.3, CH_3C**H**$_2$N); 4.07 (ddd, 1H, $J_{4',5'}$ = 4.4, 4.0, $J_{4',3'}$ = 3.5, H-4'); 4.19 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 5.5, $J_{5'b,4'}$ = 4.4, H-5'b); 4.27 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 7.2, $J_{5'a,4'}$ = 4.0, H-5'a); 4.54 (dt, 1H, $J_{3',2'}$ = 6.6, 3.5, $J_{3',4'}$ =

3.5, H-3'); 6.20 (dd, 1H, $J_{1',2'}$ = 7.3, 5.9, H-1'); 7.47 (bdd, 2H, $J_{5''',4'''}$ = 7.0, $J_{5''',6'''}$ = 4.7, H-5'''); 7.98 (ddd, 2H, $J_{4''',3'''}$ = 8.0, $J_{4''',5'''}$ = 7.0, $J_{4''',6'''}$ = 1.6, H-4'''); 8.02 (s, 1H, H-6); 8.31 (s, 2H, H-3",5"); 8.59 (bd, 2H, $J_{3''',4'''}$ = 8.0, H-3'''); 8.68 (bd, 2H, $J_{6''',5'''}$ = 4.7, H-6'''); ^{13}C NMR (150.9 MHz, CD_3OD): 9.10 (**C**H_3CH_2N); 19.71 (C5-C≡C-$CH_2CH_2CH_2$**C**H_2-C≡C-C4"); 19.95 (C5-C≡C-**C**$H_2CH_2CH_2CH_2$-C≡C-C4"); 28.79, 28.85 (C5-C≡C-CH_2**C**H_2**C**$H_2CH_2CH_2$-C≡C-C4"); 41.23 (CH_2-2'); 47.36 (CH_3**C**H_2N); 66.63 (d, $J_{C,P}$ = 5.7, CH_2-5'); 71.89 (C5-**C**≡C); 71.94 (CH-3'); 80.01 (C4"-**C**≡C); 87.54 (CH-1'); 87.60 (d, $J_{C,P}$ = 8.9, CH-4'); 93.83 (C-5); 96.90 (C4"-C≡**C**); 97.89 (C5-C≡**C**); 122.78 (CH-3'''); 123.94 (CH-3",5"); 125.65 (CH-5'''); 135.62 (C-4"); 138.85 (CH-4'''); 144.78 (CH-6); 150.20 (CH-6'''); 155.76 (C-2); 156.60 (C-2",6"); 156.79 (C-2'''); 165.87 (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): -22.48 (bdd, J = 21.2, 20.6, P_β); -10.02 (d, J = 21.2, P_α); -9.21 (d, J = 20.6, P_γ); MS (ES^-): found *m/z*: 801.1 (M-1), 721.1 (M-PO_3H_2-1); HRMS (ES): *m/z* calcd for $C_{32}H_{32}O_{13}N_6P_3$: 801.12457; found: 801.12398.

6.2.2 Synthesis and analysis of PEX products

Synthetic ONs were purchased from Sigma Aldrich (USA). Primer: 5'-CAT GGG CGG CAT GGG-3' (prim^{rnd}); templates: 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3'(temp^{rnd16}), 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}) (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 polymerase from Finnzymes (Finland), KOD XL DNA polymerase from Novagen (EMD Chemicals, USA), Deep Vent DNA polymerase as well as T4 polynukleotide kinase and natural nucleoside triphosphates (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

The reaction mixture (20 µl) contained DNA polymerase: Pwo (0.1 U/µl, 2 µl), DyNAzyme II (0.2 U/µl, 1 µl), KOD XL (0.25 U/µl, 0.8 µl), Deep Vent (0.2 U/µl, 1 µl), dNTPs (either natural or modified, 4mM, 1 µl), ^{32}P-prelabelled primer at 5'-end (3µM, 1 µl) and template (3µM, 1.5 µl) in 2 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C.

For magnetoseparation unlabelled primers and biotinylated templates were used.

Primer extension for kinetics study

The reaction mixture (20 µl) contained DNA polymerase: Pwo (0.1 U/µl, 2 µl), dATP/**dA^{OR}TP 26a-b** or dCTP/**dC^{OR}TP 27a-b** (4mM, 1 µl), ^{32}P-prelabelled primer at 5'-end (3µM, 1 µl) and template temp^{A1} or temp^{C1} (3µM, 1.5 µl) in 2 µl of corresponding buffer suplied by manufacturer. 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, 20mM EDTA, 0.025% [w/v] bromphenole 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 1xTBE buffer (pH 8) and 7% urea at 60 W for ~ 60 min. Gel was dried and visualized by phosphoimager.

6.2.3 Complexation studies

Complexation of dN^{R}s

Complexes of modified nucleoside **dN^{OR}s** (**21a-b** or **22a-b**) with diverse transition metals were prepared by mixing of 100 µl of methanolic solution of corresponding nucleosides (100 µM) with 100 µl of methanolic solution of divalent metal ions M^{2+} (50µM, $Cu(BF_4)_2 \cdot 6H_2O$, $Ni(BF_4)_2 \cdot 6H_2O$, $Zn(BF_4)_2 \cdot H_2O$, $Fe(BF_4)_2 \cdot 6H_2O$) at room temperature for 10 minutes.

Complexation of ONs for recording UV-spectra

Double stranded DNAs were prepared by PEX-experiment on larger scale. The reaction mixture (100 µl) contained Deep Vent polymerase (2 U/µl, 7.5 µl) or DyNazyme II polymerase (2 U/µl, 7.5 µl), dNTPs (either natural or modified, 4mM, 15 µl), unlabeled primer (100µM, 6 µl), and temp^{rnd16} (100µM, 6 µl) in 10 µl of corresponding buffer supplied by manufacturer. 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, 1 equiv. of $Fe(BF_4)_2 \cdot 6H_2O$ to number of modification (0.24 µl, 10mM) 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, 2 µl) or DyNAzyme II (0.2 U/µl, 1 µl), dNTP (either natural or modified, 4mM, 1 µl), ^{32}P-prelabelled primer at 5'-end primer (3µM, 1 µl), and temp (3µM, 1.5 µl) in 2 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C. After addition of 1 µl of $Fe(BF_4)_2 \cdot 6H_2O$ (4mM), 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] bromphenol blue, 0.2% [w/v] xylene cyanol) subjected to gel electrophoresis in 8% non-denaturating polyacrylamide gel containing 1xSB buffer (pH 8) and at 600 V for ~ 3 h at room temperature. Gel was dried and visualized by phosphoimager.

MALDI-TOF experiment (ssDNA)

The reaction mixture (200 µl) contained DNA polymerase: Pwo (1 U/µl, 10 µl), Deep Vent (2 U/µl, 5µl) or DyNAzyme II (2 U/µl, 5 µl), dNTPs (either natural or modified, 4mM, 10 µl), unlabeled primer prim^{rnd} (10 µM, 40 µl, 5'-CAT GGG CGG CAT GGG-3') and biotinylated template temp^{A}-bio (10µM, 40 µl, 5'-**CCC T***CC CAT GCC GCC CAT G*-3'), temp^{C}-bio (10µM, 40 µl, 5'-**CCC G***CC CAT GCC GCC CAT G*-3') or

temp^{rnd16}-bio (10µM, 40 µl, 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3') in 20 µl of corresponding buffer suplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C. The separation on magnetic beads (50 µl, Novagen) was caried out according to standard technique (see 6.1.2, page 104). As matrix for MALDI-TOF measurement was used a mixture of HPA/PA/ammonium tartrate in ration 8/1/1 in 50% acetonitrile. Then 2 µl of the matrix and 1 µl of the sample were mixed on MTP 384 polished steel target by use of anchor-chip desk. The crystallized spots were washed once by 0.1% formic acid and once by water. The acceleration tension in reflectron mode was 19.5 kV and range of measurement 3 – 13 kDa. The found differences of 2-9 Da for 6 KDa DNA and 3-12 Da for 10 KDa DNA are still within the experimental error (ca 0.1%) of the low resolution machine also considering the very small amounts of DNA produced by PEX.

6.3 DNA complexes with acetylene linker vs. DNA complexes with octadiyne linker

6.3.1 Synthesis and analysis of PEX products

Synthetic ONs were purchased from Sigma Aldrich (USA). Primer: 5'-CAT GGG CGG CAT GGG-3' (prim^{rnd}); templates: 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3'(temp^{rnd16}) (segments forming duplex with the primer are in italics, the replicated segments are in bold). Deep Vent DNA polymerase and natural nucleoside triphosphates (dATP, dCTP, dGTP and dCTP) were obtained from New England Biolabs (Great Britain) and γ-^{32}P-ATP from Izotop, Institute of isotopes Co, Ltd. (Hungary).

Complexation of ON for gel electrophoresis

Double stranded ONs were prepared by PEX-experiment. The reaction mixture (20 µl) contained DNA polymerase: Deep Vent (0.2 U/µl, 1 µl), dNTP (either natural or modified, 4mM, 1 µl), ^{32}P-prelabelled primer at 5'-end primer (3µM, 1 µl), and temp^{rnd16} (3µM, 1.5 µl) in 2 µl of corresponding buffer supplied by manufacturer. Reaction mixture was incubated for 30 min at 60 °C. After addition of 1 µl of $Fe(BF_4)_2 \cdot 6H_2O$ (4mM), 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] bromphenol blue, 0.2% [w/v] xylene cyanol) subjected to gel electrophoresis in 8% non-denaturating polyacrylamide gel containing 1xSB buffer (pH 8) and at 600 V for ~ 3 h at room temperature. Gel was dried and visualized by phosphoimager.

MALDI-TOF experiment (dsDNA)

The reaction mixture (100 µl) contained DNA polymerase: Deep Vent (2 U/µl, 5µl), dNTPs (either natural or modified, 4mM, 10 µl), unlabeled primer prim^{rnd} (100µM, 4 µl, 5'-CAT GGG CGG CAT GGG-3') and temp^{rnd16} (100µM, 4 µl, 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3') in 10 µl of corresponding buffer suplied by manufacturer. 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, 1 equiv. of $Fe(BF_4)_2 \cdot 6H_2O$ to number of modification (0.8 µl, 1mM) was added to the corresponding sample and the solution was mixed overnight (25 °C, 550 rpm).

As matrix for MALDI-TOF measurement was used a mixture of HPA/PA/ammonium tartrate in ration 8/1/1 in 50% acetonitrile. Then 2 µl of the matrix and 1 µl of the sample were mixed on MTP 384 polished steel target by use of anchor-chip desk. The crystallized spots were washed once by 0.1% formic acid and once by water. The acceleration tension in reflectron mode was 19.5 kV and range of measurement 3 – 13 kDa. The found differences of 2-9 Da for 6 KDa DNA and 3-12 Da for 10 KDa DNA are still within the experimental error (ca 0.1%) of the low resolution machine also considering the very small amounts of DNA produced by PEX.

CD spectroscopy

CD spectra were determined for natural and functionalized DNA duplexes (either non-metalated or metalated) from PEX in large scale. The reaction mixture (400 µl) contained DNA polymerase: Deep Vent (2 U/µl, 30 µl), dNTPs (either natural or modified, 4mM, 60 µl), unlabeled primer prim^{rnd} (100µM, 24 µl, 5'-CAT GGG CGG CAT GGG-3') and temp^{rnd16} (100µM, 24 µl, 5'-**CTA GCA TGA GCT CAG T***CC CAT GCC GCC CAT G*-3') in 40 µl of corresponding buffer suplied by manufacturer.

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 modification (0.48 µl, 10mM) was added to the corresponding sample and the solution was mixed overnight (25 °C, 550 rpm).

CD spectra were recorded on Jasco 815 (Japan) at room temperature. The optical path length was 0.1 cm and the CD signal was monitored from 200 nm to 350 nm, when the scanning speed was 5 nm/min and response time was 32s. The CD spectra were expressed in optical density (OD).

7 References

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