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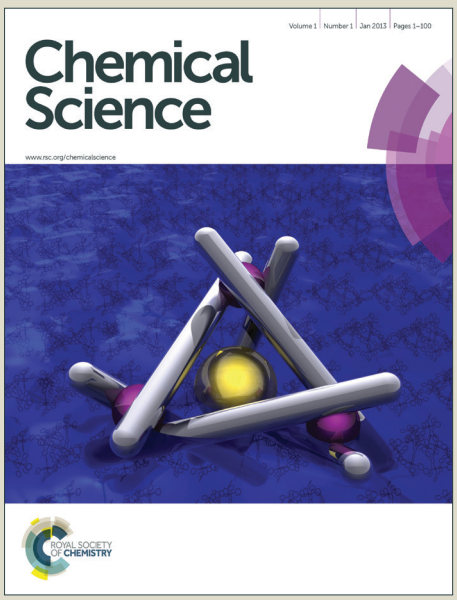

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EDGE ARTICLE

Azidophenyl as a click-transformable redox label of DNA suitable for electrochemical detection of DNA-protein interactions

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New redox labelling of DNA by azido group which can be chemically transformed to nitrophenyltriazole or silenced to phenyltriazole was developed and applied for electrochemical detection of DNA-protein interactions. 5-(4-Azidophenyl)-2'-deoxycytidine and 7-(4-azidophenyl)-7-deaza-2'-deoxyadenosine nucleosides were prepared by the aqueous-phase Suzuki cross-coupling and converted to nucleoside triphopshates (dNTPs) which served as substrates for incorporation to DNA by DNA polymerase. The azidophenyl-modifed nucleotides and DNA gave a strong signal in voltammetry at -0.9 V due to reduction of the azido function. Cu-catalyzed click reaction of azidophenyl-modified nucleosides or DNA with 4-nitrophenylacetylene gave nitrophenyl-substituted triazoles exerting a reduction at -0.4 V in voltammetry, whereas the click reaction with phenylacetylene gave electrochemically silent phenyltriazoles. The transformation of azidophenyl label to nitrophenyltriazole was used for electrochemical detection of DNA-protein interaction (p53 protein) since only those azidophenyl groups in the parts of the DNA not shielded by the bound p53 protein were transformed to nitrophenyltriazoles whereas those covered by the protein were not.

Introduction

Electrochemical detection of redox-labelled DNA[1] is an alternative to fluorescence techniques of DNA sequencing and diagnostics. However, despite of extensive research and number of available oxidizable or reducible labels,[2] the redox labelling of DNA often suffers from problems with sensitivity, stability and cross-reactivity of the labels. On the other hand, the use of several labels offers access to direct redox coding of DNA.[3] To the best of our knowledge, applications of redox labelling and electrochemistry for studying of DNA-protein interactions are still relatively scarce, limited to techniques based on changes in DNA-mediated charge transfer upon the protein binding (developed by J. K. Barton group[4]) and our recent studies utilizing immunoprecipitation at magnetic beads.[5] Most known methods for detection and footprinting of those interactions[6] are based on specific enzymatic or chemical cleavage of DNA.[7]

Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC or click reaction) is one of the most important bioorthogonal reactions[8] and has been widely used for modifications of oligonucleotides (ONs) and DNA.[9] Due to better compatibility with phosphoramidite synthesis, triphosphorylation and polymerase incorporations, base-modified nucleotides bearing an acetylene are typically incorporated to ON or DNA and are then clicked with an azido-derivative of the other component.[10] Only recently, 5-azidomethyl-dUTP has been synthesized and used for metabolic labelling through polymerase incorporation and click reaction with a fluorescent acetylene.[11] We have envisaged the azido group[12] as new redox label suitable for electrochemical detection but also transformable to another redox label through the click reaction.

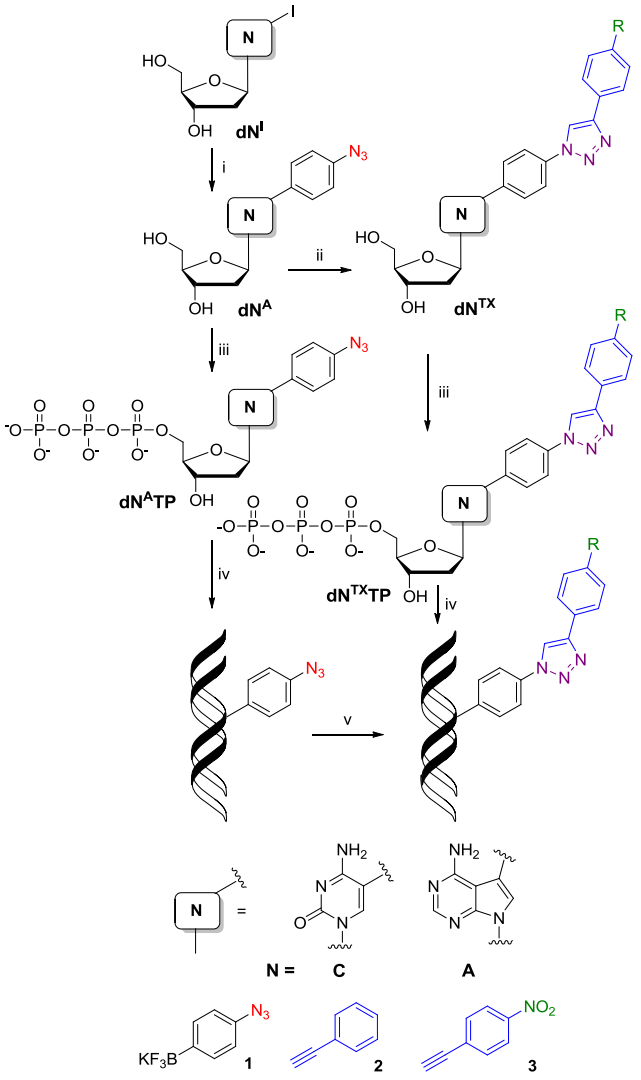
Results and Discussion

Synthesis of modified nucleosides and triphosphates

Our strategy for the synthesis of labelled ONs and DNA relied on polymerase incorporations[13] of base-modified nucleotides. The modified dNTPs necessary as substrates are available through triphosphorylation of modified nucleosides. The synthesis of the azidophenyl-modified nucleosides was based on the Suzuki-Miyaura cross-coupling reaction of unprotected halogenated nucleosides 5-iodocytidine (**dC^{I}**) and 7-deaza-7-iodoadenosine (**dA^{I}**) with 4-azidophenyltrifluoroborate (**1**).[14] The reactions were performed in the presence of $PdCl_2$(dppf) catalyst and Cs_2CO_3 in MeOH gave the desired modified nucleosides (**dC^{A}** and **dA^{A}**) in good yields of 58-63% (Scheme 1, Table 1, entries 1, 2). The Huisgen-Sharpless CuAAC reaction[15] between the azidophenyl-modified nucleosides (**dC^{A}** and **dA^{A}**) and an alkyne (phenylacetylene **2** or 1-ethynyl-4-nitrobenzene **3**) in the presence of copper (II) sulfate pentahydrate and sodium ascorbate as a reducing agent in *t*BuOH:H_2O (1:1) was used for the synthesis of 1,4-disubstituted 1,2,3-triazoles (**dN^{TP}** and **dN^{TNO2}**) in good yields of 40-94% (Scheme 1, Table 1, entries 5-8). The phenyltriazole (in **dN^{TP}**) was designed as an electrochemically silent group, whereas the nitrophenyltriazole (in **dN^{TNO2}**) should be reducible on electrode due to the nitro group.

For the preparation of **dN^{A}TPs** we have applied a

triphosphorylation[16] of the corresponding nucleosides **dN^{A}**s. The treatment of **dC^{A}** and **dA^{A}** with $POCl_3$ in $PO(OMe)_3$ followed by the addition of $(NHBu_3)_2H_2P_2O_7$, Bu_3N and treatment with TEAB (Scheme 1) gave the desired **dN^{A}TPs** (Table 1, entries 3, 4) in 21 and 34% yield after isolation by RP HPLC. Triazole-modified triphosphates **dN^{TP}TP**s and **dN^{TNO2}TP**s were prepared by analogous triphosphorylation of modified nucleosides **dN^{TP}**s and **dN^{TNO2}**s (Scheme 1, Table 1, entries 9-12) in 13-52% yield.



Scheme 1. Synthesis of modified nucleosides: i) Suzuki-Miyaura cross-coupling: **1**, $PdCl_2$(dppf), Cs_2CO_3, MeOH, 2h, 80ºC; ii) CuAAC: **2** (**3**), sodium ascorbate, $CuSO_4.5H_2O$, *t*BuOH:H_2O (1:1), 12 h, rt; iii) Triphosphorylation of modified nucleosides: 1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB; iv) PEX experiment; v) Azide-alkyne Huisgen cycloaddition: **2** (**3**), sodium ascorbate, CuBr, TBTA ligand, *t*BuOH:DMSO (1:3), 2 h, 37ºC.

Electrochemistry of modified dNTPs

All six modified dNTPs **dA^{A}TP**, **dC^{A}TP**, **dA^{TP}TP**, **dC^{TP}TP**, **dA^{TNO2}TP** and **dC^{TNO2}TP** were subjected to electrochemical study by cyclic voltammetry at the hanging mercury drop electrode (HMDE; Figure 1). The azidophenyl modified nucleotides **dA^{A}TP** and **dC^{A}TP** exerted a strong reduction peak at -0.9 V (peak N_3^{red}), whereas the phenyltriazole derivatives **dA^{TP}TP** and **dC^{TP}TP** did not give any redox signal of the label. On the other hand, the nitrophenyltriazole derivatives **dA^{TNO2}TP** and **dC^{TNO2}TP** gave a strong reduction peak at -0.4 V due to reduction of the nitro group (peak NO_2^{red}). Since the azidophenyl derivatives are easily transformed to both types of triazoles by CuAAC reactions with alkynes, the click reaction with phenylacetylene can be used for silencing of the redox signal of the azido group whereas the click reaction with nitrophenylacetylene can be used for transformation of one redox label (azido) into another one (nitro) exerting a different redox potential (vide infra for analytical applications of this finding).

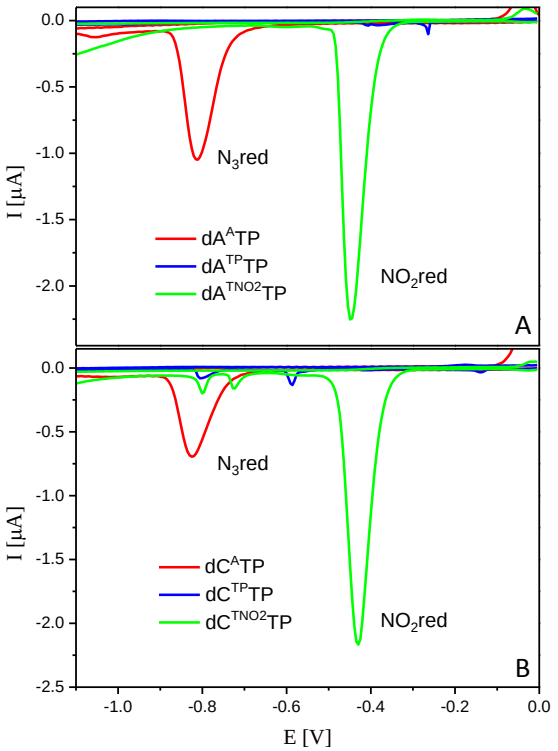


Figure 1. CV responses of **dN^{A}TPs**, **dN^{TP}TPs** and **dN^{TNO}TPs** at HMDE.

Enzymatic synthesis of modified DNA

The next goal was the polymerase-catalyzed synthesis of DNA bearing azidophenyl labels and the study of their conversion to (nitro)phenyltriazole groups by CuAAC of the azidophenyl modified DNA with acetylenes **2** or **3**. In comparison, a direct incorporation of triazole-modified nucleotides, using **dN^{TP}TP**s and **dN^{TNO2}TP**s as substrates, leading to the same triazole-modified DNA molecules were also tried.

The enzymatic incorporation of the azidophenyl modified nucleotide was studied in primer extension (PEX) using **dN^{A}TP**s as substrate together with a 19-nt template, radiolabeled 15-nt primer and a DNA polymerase, KOD XL (Figure 2) or Pwo (Figure S1 in ESI) and the products were analyzed by sequencing polyacrylamide gel electrophoresis (PAGE). In all cases we got fully extended products.

Then we performed a simple kinetics study to explore the efficiency of the PEX with the modified **dN^{A}TP**s in comparison to natural dNTPs. The rates of the PEX using Pwo DNA polymerase with temp^{C} (for C, without natural dGTP), temp^{Aterm} (for A) and prim^{rnd} were compared. The reaction mixtures were incubated for the time intervals indicated, followed by stopping

Table 1. Synthesis of modified nucleos(t)ides.

Entry	Starting compound	Reagent	Additives	Solvent	Product	Reaction time	Yield (%)
1	**dA^{I}**	**1**	$PdCl_2(dppf)$, Cs_2CO_3	MeOH	**dA^{A}**	2 h, 80ºC	58
2	**dC^{I}**	**1**	$PdCl_2(dppf)$, Cs_2CO_3	MeOH	**dC^{A}**	2 h, 80ºC	63
3	**dA^{A}**	1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB			**dA^{A}TP**	6 h, 0 ºC	34
4	**dC^{A}**	1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB			**dC^{A}TP**	6 h, 0 ºC	21
5	**dA^{A}**	**2**	sodium ascorbate, $CuSO_4.5H_2O$	*t*BuOH:H_2O (1:1)	**dA^{TP}**	12 h, rt	72
6	**dC^{A}**	**2**	sodium ascorbate, $CuSO_4.5H_2O$	*t*BuOH:H_2O (1:1)	**dC^{TP}**	12 h, rt	40
7	**dA^{A}**	**3**	sodium ascorbate, $CuSO_4.5H_2O$	*t*BuOH:H_2O (1:1)	**dA^{TNO2}**	12 h, rt	94
8	**dC^{A}**	**3**	sodium ascorbate, $CuSO_4.5H_2O$	*t*BuOH:H_2O (1:1)	**dC^{TNO2}**	12 h, rt	62
9	**dA^{TP}**	1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB			**dA^{TP}TP**	18 h, 0ºC	13
10	**dC^{TP}**	1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB			**dC^{TP}TP**	18 h, 0ºC	52
11	**dA^{TNO2}**	1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB			**dA^{TNO2}TP**	18 h, 0ºC	18
12	**dC^{TNO2}**	1. $PO(OMe)_3$, $POCl_3$, 0ºC; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0ºC; 3. TEAB			**dC^{TNO2}TP**	18 h, 0ºC	18

Table 2. Primers and templates used for PEX experimets[a]

Sequences	
Prim^{rnd}	5'-CATGGGCGGCATGGG-3'
Temp^{rnd16}	5'-CTAGCATGAGCTCAGT*CCCATGCCGCCCATG*-3'
Temp^{A}	5'-CCCTCCCATGCCGCCCATG-3'
Temp^{Aterm}	5'-TCCCATGCCGCCCATG-3'
Temp^{C}	5'-CCCGCCCATGCCGCCCATG-3'
Prim^{p53_15}	5'-GAATTCGATATCAAG-3'
Temp^{p53_2CON_4}	5'-TACCTTATCCATAATAGACATGTCTAGACAT GTCTCTTGATATCGAATTC-3'
Temp^{p53_1a2G}	5'-TAGGTTATGGATAATAAACATGTCTAGGCAT GTCTCTTGATATCGAATTC-3'
ON^{p53_2CON_4}	5'-GAATTCGATATCAAG***A*****G*****A*****C*****A***TGTCT***A*****G*****A*****C*****A***TGTCT **A**TT**A**TGG**A**T**AA**GGT**A**-3'
ON^{p53_1a2G}	5'-GAATTCGATATCAAG***A*****G*****A*****C*****A***TGCCT***A*****G*****A*****C*****A***TGTTT **A**TT**A**TCC**A**T**AA**CCT**A**-3'

[a] p53 recognition sequences are in italics and nucleotides bearing modification are in bold and underlined.

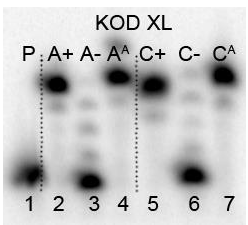


Figure 2. PEX single-incorporations into 19-nt DNA using **dN^{A}TP**, temp^{A} and temp^{C} template and KOD XL polymerase.

the reaction by addition of PAGE loading buffer and immediate heating. The incorporation of the natural nucleotides was finished in 1-2 minutes whereas the PEX with **dN^{A}TP**s took ca. 1-10 minutes to complete (Figures S3, S4 in ESI) but the synthesis was always safely finished within 10 minutes.

Multiple incorporations of **dN^{A}** nucleotides into random sequences were tested using 31-nt template in presence of KOD XL (Figure 3), Pwo (Figure S5 in ESI) or Vent (*exo*-) (Figure S6 in ESI). Both PEX reactions with modified **dN^{A}TP**s in presence of any of these polymerases were successful giving full-length products in PAGE analyses (Figure 3, lane 5, 8).

In order to study the CuAAC click modification, the azidophenyl-labelled dsDNA was synthesized in bigger scale (increased 10 times) and the PEX products were purified on QIAquick Nucleotide Removal Kit to remove dNTPs. Then the Cu(I)-catalyzed CuAAC reaction of the azidophenyl-labelled dsDNA with phenylacetylene or 1-ethynyl-4-nitrobenzene was performed under standard conditions in presence of CuBr, TBTA (tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine) ligand and sodium ascorbate in aqueous *t*BuOH:DMSO (1:3) at 37ºC for 2 hours. The products were purified once again and analyzed by PAGE (Figure 3, lane 6, 7, 9, 10) to show no apparent degradation of DNA and MALDI-TOF analysis (see SI, Figures S21-S24) to confirm the conversion.

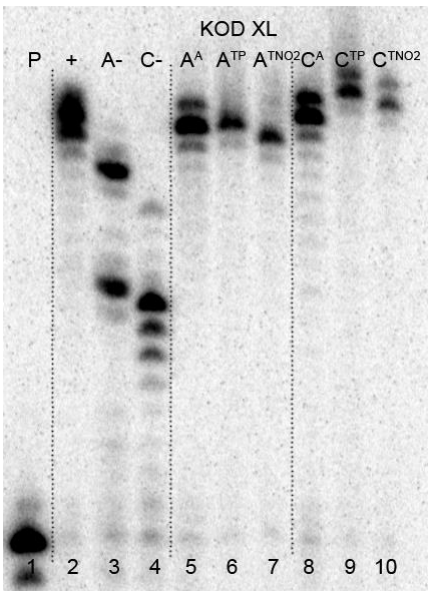


Figure 3. PEX incorporations into 31-nt DNA using **dN^{A}TP**, template temp^{rnd16} and KOD XL polymerase followed by click reaction with 1-ethynyl-4-nitrobenzene and phenylacetylene.

The direct PEX incorporation of **dN^{TP}** and **dN^{TNO2}** nucleotides into DNA was also studied with either 19-nt or 31-nt template and KOD XL (Figures 4,5) or Pwo polymerase and **dN^{TP}TP** or **dN^{TNO2}TP** as substrate (Figure S2 in ESI). In single incorporations all **dN^{TX}** nucleotides were successfully

incorporated into DNA (Figure 4, lane 4, 5, 8, 9). In multiple incorporations, **dC^{TX}TP**s gave fully extended products (Figure 5, lane 5, 7, 8), whereas the PEX using **dA^{TNO2}TP** (Figure 5, lane 6) stopped in the same line as negative control A- probably due to steric hindrance of the bulky nitrophenyltriazolylphenyl group.

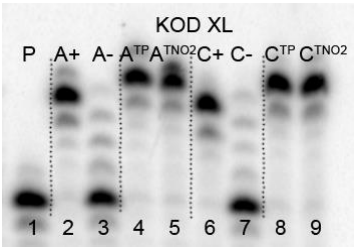


Figure 4. PEX single-incorporations into 19-nt DNA using **dN^{TNO2}TP** or **dN^{TP}TP**, temp^{A} or temp^{C} template and KOD XL polymerase.

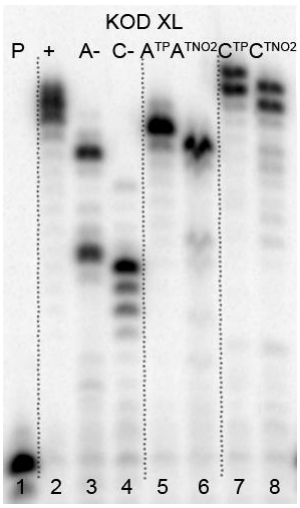


Figure 5. PEX reactions with **dN^{TP}TP** or **dN^{TNO2}TP** using template temp^{rnd16} and KOD XL polymerase leading to 31-nt DNA .

Electrochemistry of the modified DNA

The voltammetric properties of modified DNA (PEX products) containing **A^{A}** or **C^{A}** were studied by using cyclic voltammetry (CV) at the HMDE. Similarly to the electrochemistry of modifed **dN^{X}TP**s, cyclic voltammograms of PEX products prepared with temp^{rnd16} template containing azidophenyl-modified nucleobase (**A^{A}** or **C^{A}**) show an irreversible cathodic peak at around -0.9 V due to reduction of azido group (peak N_3^{red}, Figure 6). A detailed study of the electrode reaction mechanism will be published elsewhere. Negative control experiment of PEX reactions with no polymerase added to the mixture detected no signal of azido group what exclude the presence of unremoved **dN^{A}TPs** in the mixture. PEX products containing azido group **A^{A}** or **C^{A}** were transformed by click reaction to PEX products containing phenyltriazole group **A^{TP}** and **C^{TP}** with no redox signal of label (blue curves). On the other hand, the CuAAC click reaction of **N^{A}**-modified DNA with nitrophenylacetylene gives DNA products containing nitrophenyltriazole group **A^{TNO2}** and **C^{TNO2}** which exert the irreversible cathodic peak at around -0.4 V due to the reduction of nitro group (peak NO_2^{red}, Figure 6).

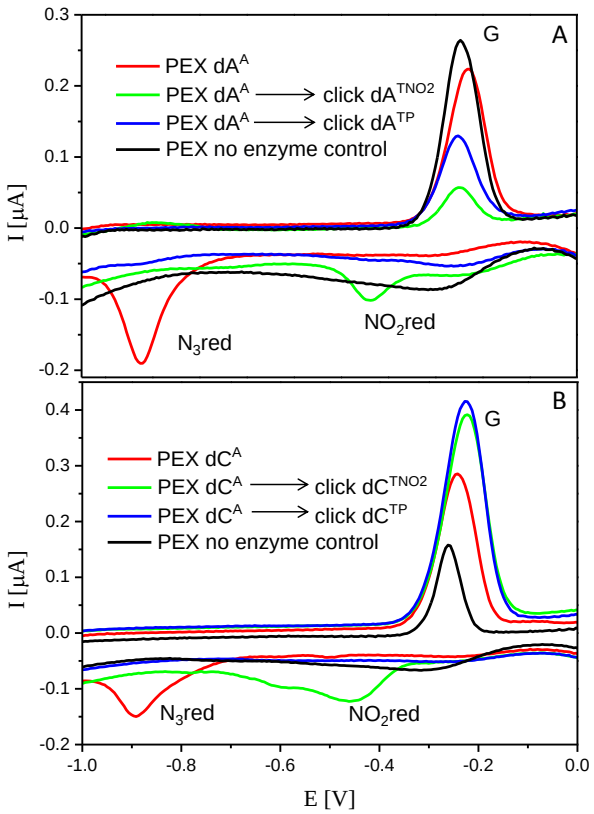


Figure 6. Detail of CV responses at HMDE of PEX products synthesized with temp^{rnd16} template and dNTP mixes containing **dN^{A}TP** conjugate (as specified in legend) complemented with three respective unmodified dNTPs and PEX products after click reaction with (nitro)phenyltriazole. Peak G corresponds to oxidation of a reduction product of guanine generated at the electrode.[1a] For full CV scans and other details see Figure S14-S15 and Experimental section.

For comparison, we also prepared the PEX products containing the **N^{TP}** and **N^{TNO2}** modifications by polymerase incorporation of the corresponding triazole-modified **dN^{TX}TP**s. Voltammetric responses of the PEX products were again measured at HMDE and Figure 7 confirms the irreversible cathodic peak NO_2^{red} at around -0.4 V corresponding to PEX containing nitrophenyltriazole label whereas PEX containing phenyltriazole label did not give any redox signal of the label.

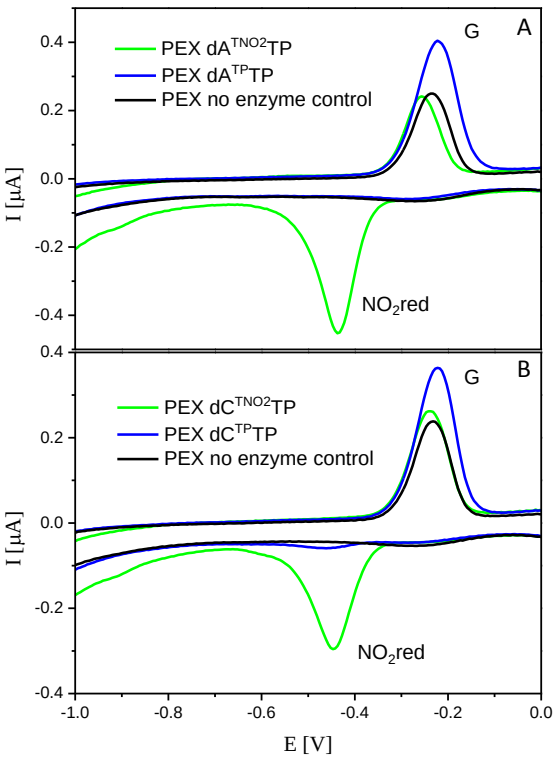
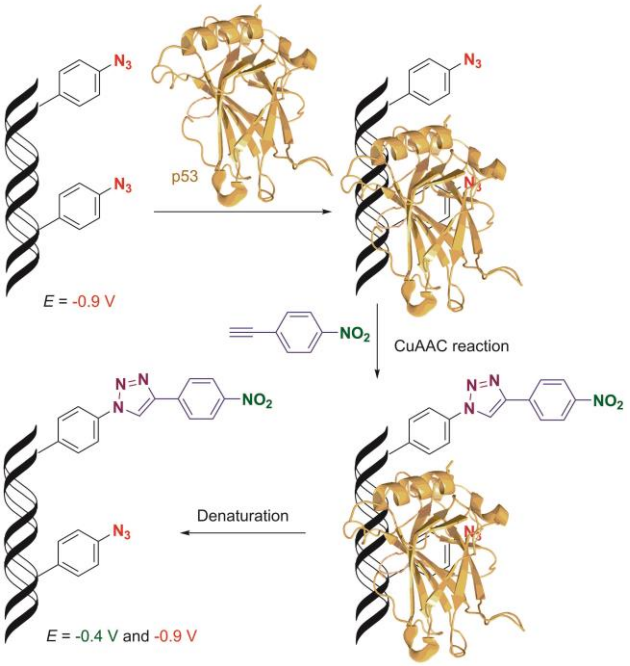


Figure 7. Detail of CV responses at HMDE of PEX products synthesized with temp^{rnd16} template and dNTP mixes containing **dN^{TX}TP** conjugate (as specified in legend) complemented with three respective unmodified dNTPs. For full CV scans and other details see Figure S16-S17 and Experimental section.

Application of the click transformations of the redox labels in detection of DNA-protein interactions

DNA-protein interactions are of paramount importance in DNA recombination, transcription, methylation and repair. The current techniques available for footprinting of these interactions are mostly based on specific DNA cleavage.[6,7] Based on our encouraging results in transformation of azido redox label to nitrophenyltriazole, we envisaged that this approach could be used as a new method for detection of protein-DNA interactions (Scheme 2). We assumed that if we incorporate several azidophenyl-modifications into DNA probe, incubate the probe with protein and perform the CuAAC click reaction with nitrophenylacetylene, only the freely accessible azido-groups (not shielded by the protein) should be transformed to nitrophenyltriazoles and the ratio of azido/nitro redox signals should indicate whether the protein was bound and how large was the sequence of contact.

To test this idea, we have chosen GST-tagged DNA binding (core) domain of tumor-suppressor protein p53[17,18] (p53CD_GST) as a biologically relevant example of a sequence-specific[19] binder to DNA. We have previously shown that p53 retained binding to a specific DNA sequence containing vinylsulfonamide modifications in the major groove which efficiently cross-linked with a cystein of p53 through Michael addition.[20] We prepared two different sequences of 50-bp DNA by PEX (using KOD XL polymerase and templates temp^{p53_1a2G} or temp^{p53_2CON_4}) in which 6 azido-groups are inside and 6 azido-groups are outside of sequence specifically recognized by p53. Both azido-modified **dN^{A}TP**s gave full lenth ON-products which were characterized by PAGE (Figure 8, lane 5, 6 and Figure S7 in ESI).



Scheme 2. The principle of electrochemical footprinting of protein-DNA interaction.

Figure 8. PEX reactions with **dN^{A}TP** using template temp$^{p53\text{-}1a2G}$ and KOD XL DNA polymerase giving 50-nt DNA products.

After successful synthesis of the azido-modified DNA, it was incubated with different ratios of p53CD_GST protein to test its binding activity. The recognition of the binding sequence by p53CD_GST was monitored by 6% native PAGE (Figure 9a, lane 1-10, and Figure S8 in ESI). For further experiments we decided to use 1.2 equiv. of protein p53CD_GST (under these conditions the modified DNA was quantitatively bound by the protein, forming predominantly 1:1 p53-DNA complex, Figure 9). Then it was necessary to test the thermal stability of complex DNA-protein (DNA_p53CD_GST complex), where created DNA_p53CD_GST complexes were incubated at mentioned temperatures for 1 h. DNA_p53CD_GST complex is stable at 20ºC for 1 hour (Figure S10, Figure S11, lane 3). At higher temperature binding of p53 to DNA is inefficient (Figure S10, Figure S11, lane 5).

Transition metals such as nickel, cobalt, zinc in high concentrations may also cause the inhibition or disruption of DNA-protein binding.[21] Therefore the stability of DNA_p53CD_GST complex was studied depending on Cu^{I} concentration. DNA_p53CD_GST complexes were mixed with different concentrations of CuBr solution in presence or absence of TBTA ligand at 20ºC for 1 h. Relatively low concentrations of CuBr (10 μM) prevent the inhibitory efect on binding of p53 to DNA (Figure S12, Figure S13, lane 6, 7). At higher concentrations of CuBr (20 μM), binding of p53 to DNA is completely inhibited (Figure S13, Figure S14, lane 8, 9). For next experiments we decided to use 5 μM CuBr. In the last control experiment, we tested the stability of DNA_p53CD_GST complex during the CuAAC click reaction. DNA_p53CD_GST complex was mixed with 0.5 mM 4-nitrophenylacetylene, 5 μM CuBr, 25 μM TBTA ligand, 65 μM sodium ascorbate at 20ºC for 1 h. Figure 9b, lane 13 (and Figure S9, lane 3 in ESI) shows that the DNA-protein complex is stable during the reaction under these conditions.

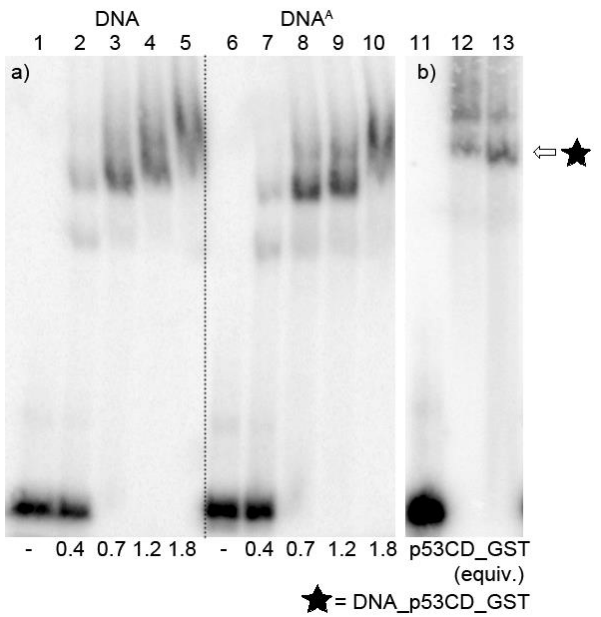


Figure 9. a) Native PAGE analysis of 50-mer DNA^{1a2G}_p53CD_GST complex. Lane 1: natural DNA; 2: 0.4 equiv.; 3: 0.7 equiv.; 4: 1.2 equiv.; 5: 1.8 equiv. of protein p53CD_GST to DNA; Lane 6: DNA^{A}; 7: 0.4 equiv.; 8: 0.7 equiv.; 9: 1.2 equiv.; 10: 1.8 equiv. of protein p53CD_GST to DNA. **b) Native PAGE analysis of stability of DNA_p53CD_GST complex after click reaction of DNA.** Template^{p53_1a2G}: Lane 11: DNA^{A}; lane 12: protein/DNA complex; lane 13: protein/DNA complex, 0.5 mM 4-nitrophenylacetylene, 5 μM CuBr; 25 μM TBTA ligand, 65 μM Na ascorbate, 20 ºC, 1h.

Then we proceeded to the experiments with electrochemical detection of DNA-protein interactions. The 50-bp dsDNA containing 12 azidophenyl groups was prepared by PEX with temp$^{p53\text{-}1a2G}$ template and the CV showed the characteristic peak of N_3 reduction at -0.9 V (Figure 10, red curve). This **A^{A}**-linked DNA was then reacted with nitrophenylacetylene (**3**) under the previously optimized conditions (suitable for survival of DNA-protein complexes) in presence CuBr, TBTA and sodium ascorbate and in the absence of p53CD_GST to show full conversion of all azido-groups to nitrophenyltriazoles which was confirmed by the disappearance of the signal at -0.9 V and appearance of the new signal at -0.4 V due to reduction of NO_2 group (Figure 10, green curve). In further experiment, the **A^{A}**-linked DNA was incubated with 1.2 equiv. of p53CD_GST (for 45 min on ice) to form the complex and then treated with nitrophenylacetylene (**3**) under the above mentioned conditions, followed by denaturation. The voltammetric analysis of the product (Figure 10, violet curve) revealed a ca. 50% decrease in intensity of the peak N_3^{red} of reduction of azido-group and an increase of the peak NO_2^{red} corresponding to the reduction of the nitro group (to ca. 50% intensity compared to the reaction in the absence of p53). This confirms that only those azido-groups which are not shielded by the protein binding can undergo the click tranformation to nitrophenyltriazole whereas the N_3 groups covered by the protein remain intact. As a control, we performed the CuAAC reaction of **A^{A}**-linked DNA in presence of bovine serum albumine (BSA), which does not bind DNA, to give the same results as the experiment in the absence of any protein (Figure 10, black curve, all azido groups were converted to nitrophenyltriazoles). Basically identical results were obtained with **A^{A}**-modified DNA synthesized on the temp$^{p53\text{-}2CON4}$ template (Figures S19, S20).

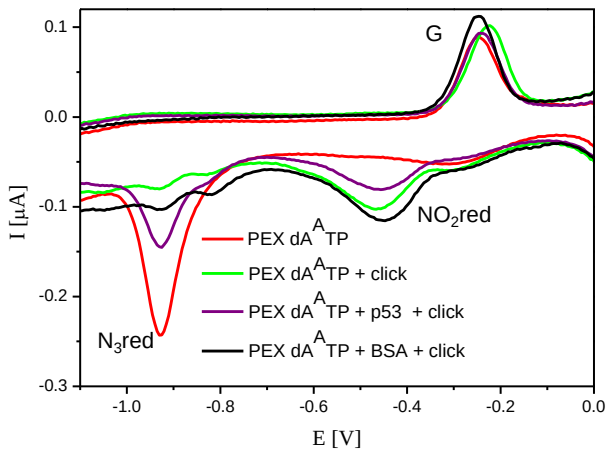


Figure 10. Detail of CV responses at HMDE of PEX products synthesized with template^{p53_1a2G} template and **dA^{A}TP** conjugate complemented with three respective unmodified dNTPs (red curve) and PEX products after click reaction with nitrophenylacetylene (green curve), DNA-p53 complex after click reaction followed by denaturation

(violet curve), the control with BSA (black curve). For full CV scans and other details see Figure S18 and Experimental section.

Conclusions

We have designed and prepared nucleosides and dNTPs bearing azidophenyl group and developed polymerase incorporation of azido-modified nucleotides to DNA by primer extension using these **dN^{A}TP**s as substrates. Both nucleotides and DNA bearing the azidophenyl modifications exert a strong reduction peak around -0.9 V in voltammetry. The azido-group readily undergoes the CuAAC click reaction with phenyl- or nitrophenylacetylene under the conditions compatible to working with dsDNA and DNA-protein complexes. The phenyltriazole-modified dNTPs do not give any reduction signal in voltammetry so the transformation of the azido-modification to TP gives silencing of the voltammetric signal. On the other hand, the nitrophenyltriazoles (resulting from CuAAC transformation of the azides with nitrophenylacetylene) give a strong reduction signal at -0.4 V. Thus the azidophenyl modified nucleotides are useful redox labels for DNA which can be easily either silenced or transformed to a different redox label with distinctly different redox potential. This transformation was utilized in detection and mapping of DNA-protein interactions. When an **A^{A}**-containing DNA is incubated with a protein, binding to a part of its sequence, and then undergoes the CuAAC reaction with nitrophenylacetylene, only the free azido-groups not covered by the protein would react whereas the azides in close contact with the protein remain intact. The electrochemical readout is used for the analysis of the outcome and one can not only distinguish whether or not the protein made a stable complex with the DNA but also directly deduce the length of the sequence in contact with the particular protein (from the ratio of transformed versus not transformed labels resulting in changes in intensity of the corresponding redox peaks in voltammetry). Apparently, by a proper design of a series of differently labelled probes, one could even determine the binding sequences for DNA-protein foot-printing. Moreover, the method has a potential for being parallelized and applied for high-throughput screening of ligands that interfere with protein-DNA binding. Since azido-group can be photolyzed to reactive nitrenes,[22] the azido-modified DNA could also be applied for cross-linking with proteins. Studies along these lines and toward practical applications of this approach will continue in our laboratories.

Experimental section:

NMR spectra were measured at 500 MHz for ^{1}H and 125.7 MHz for ^{13}C, or at 600 MHz for ^{1}H and 150.9 MHz for ^{13}C, in D_2O (reference to dioxane as internal standard, δ_H = 3.75 ppm, δ_C = 67.19 ppm) or in [D_6]DMSO (reference to the residual solvent signal). Chemical shifts are given in ppm (δ scale) and coupling constants (J) in Hz. Complete assignment of all NMR signals was achieved by using 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). Semipreparative separation of nucleoside triphosphates was performed by HPLC on a column packed with 10 μm C18 reversed phase (Phenomenex, Luna C18 (2)). IR spectra were measured by using the ATR technique or by using KBr tabletes. High-resolution mass spectra were measured by using ESI ionization technique. Mass spectra of functionalized DNA were measured by Maldi-TOF, Reflex IV (Bruker) with nitrogen laser. Melting points were determined on a Kofler block. Known starting compounds were prepared by literature procedures (compound potassium 4-azidophenyltrifluoroborate[14]).

Method A: Suzuki-Miyaura Cross-Coupling Reaction:

dC^{A}, dA^{A}: To a glass vial containing a stirring bar was added halogenated nucleosides **dN^{I}** (0.1 g, 0.2 mmol), potassium 4-azidophenyltrifluoroborate (95 mg, 0.4 mmol, 1.5 equiv.), Cs_2CO_3 (0.27 g, 0.8 mmol, 3 equiv.), $PdCl_2$(dppf) (21 mg, 0.02 mmol, 10 mol %). The vial was sealed with a septum and methanol (5 mL) was added via syringe. The reaction was heated in an oil bath at 80°C for 2 h until complete consumption of the starting material (the reaction was monitored by TLC), the reaction mixture was cooled to rt. The solvent was evaporated in vacuo. The products were purified by silica gel column chromatography using chloroform/methanol (0 to 10%) as eluent.

Method B: Synthesis of modified nucleosides triphosphates- Triphosphorylation:

dC^{A}TP, dA^{A}TP: Dry trimethyl phosphate (0.11 mL) was added to an argon-purged flask containing a nucleoside analogue **dN^{A}** (0.06 mmol, 1 equiv.) cooled to 0°C on ice followed by the addition of $POCl_3$ (7 μl, 0.07 mmol, 1.2 equiv.). After 4 h, a solution of $(NHBu_3)_2H_2P_2O_7$ (180 mg, 0.3 mmol, 5 equiv.), Bu_3N (0.06 mL, 0.3 mmol, 4.2 equiv.) in dry DMF (0.5 mL) was added to the reaction mixture and stirred for another 1.5 h and quenched by 2 M TEAB buffer (1 mL). Product was isolated from the crude reaction mixture by HPLC on a C18 column with the use of a linear gradient of 0.1 M TEAB (triethylammonium bicarbonate) in H_2O to 0.1 M TEAB in H_2O–MeOH (1 : 1) as eluent. Several co-distillations with water and conversion to sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave solid product.

Method C: General procedure for the CuAAC reactions[15]:

dC^{TP}, dA^{TP}, dC^{TNO2}, dA^{TNO2}: Azido-modified nucleoside **dN^{A}** (0.1 mmol), sodium ascorbate (12 mg, 0.06 mmol, 0.4 equiv.) and $CuSO_4.5H_2O$ (3 mg, 0.01 mmol, 0.08 equiv.) were suspended in 5 mL of H_2O/*t*BuOH (3:1). The appropriate alkyne (2 equiv.) was subsequently added, and the mixture was stirred overnight at room temperature. The 1,4-disubstituted 1,2,3-triazole derivatives (generally) precipitated from this reaction medium and were isolated by filtration with water.

Method D: Synthesis of modified nucleosides triphosphates- Triphosphorylation:

dC^{TP}TP, dA^{TP}TP, dC^{TNO2}TP, dA^{TNO2}TP: Dry trimethyl phosphate (0.11 mL) was added to an argon-purged flask containing a nucleoside analogue **dN^{TX}** (0.04 mmol, 1 equiv.) cooled to 0°C on ice followed by the addition of $POCl_3$ (4 μl, 0.04 mmol, 1.2 equiv.). After 16 h, a solution of $(NHBu_3)_2H_2P_2O_7$ (100 mg, 0.2 mmol, 5 equiv.), Bu_3N (0.04 mL, 0.15 mmol, 4.2 equiv.) in dry DMF (0.5 mL) was added to the reaction mixture and stirred for another 1.5 h and quenched by 2 M TEAB buffer (1 mL). Product was isolated from the crude reaction mixture by HPLC on a C18 column with the use of a linear gradient of 0.1 M TEAB (triethylammonium bicarbonate) in H_2O to 0.1 M TEAB in H_2O–MeOH (1 : 1) as eluent. Several co-distillations with water and conversion to sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave solid product.

5-(4-Azidophenyl)-2'-deoxycytidine (dC^{A}): Compound **dC^{A}** was prepared from **dC^{I}** according to general procedure (Method A). The product was isolated as a brown solid (61 mg, 63%); m.p. 145ºC. ^{1}H NMR (499.8 MHz, DMSO-d_6): 2.07 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 6.1, H-2'b); 2.15 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.6, H-2'a); 3.50, 3.56 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.0, $J_{5',4'}$ = 3.6, H-5'); 3.77 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.6, H-4'); 4.21 (m, 1H, $J_{3',2'}$ = 6.1, 3.6, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.6, H-3'); 4.95 (t, 1H, $J_{OH,5'}$ = 5.0, OH-5'); 5.19 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3'); 6.19 (dd, 1H, $J_{1',2'}$ = 6.7, 6.2, H-1'); 6.39 (bs, 1H, $NH_a\mathbf{H_b}$); 7.17 (m, 2H, H-*m*-phenylene); 7.39 (m, 2H, H-*o*-phenylene); 7.39 (bs, 1H, $\mathbf{N\!H_a}H_b$); 7.86 (s, 1H, H-6); ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.79 (CH_2-2'); 61.18 (CH_2-5'); 70.31 (CH-3'); 85.26 (CH-1'); 87.43 (CH-4'); 107.04 (C-5); 119.81 (CH-*m*-phenylene); 130.75 (CH-*o*-phenylene); 131.02 (C-*i*-phenylene); 138.74 (C-*p*-phenylene); 140.32 (CH-6); 154.58 (C-2); 163.53 (C-4); ν(KBr)/cm^{-1}: 3416, 3062, 2121, 2097, 1644, 1608, 1509, 1415, 1294, 1096, 1052, 787; MS (ESI+): *m/z* (%): 345.2 (75) [M+H], 367.2 (25) [M+Na]. HRMS (ESI+): calcd. for $C_{15}H_{17}N_6O_4$: 345.13058; found 345.13057.

7-(4-Azidophenyl)-7-deaza-2'-deoxyadenosine (dA^{A}): Compound **dA^{A}** was prepared from **dA^{I}** according to general procedure (Method A). The product was isolated as a yellow solid (56 mg, 58%); m.p. 96ºC. ^{1}H NMR (499.8 MHz, DMSO-d_6): 2.19 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 5.9, $J_{2'b,3'}$ = 2.7, H-2'b); 2.56 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.3, $J_{2'a,3'}$ = 5.9, H-2'a); 3.51 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 6.0, $J_{5'b,4'}$ = 4.3, H-5'b); 3.57 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 4.7, H-5'a); 3.83 (ddd, 1H, $J_{4',5'}$ = 4.7, 4.3, $J_{4',3'}$ = 2.4, H-4'); 4.36 (m, 1H, $J_{3',2'}$ = 5.9, 2.7, $J_{3',OH}$ = 4.0, $J_{3',4'}$ =

2.4, H-3'); 5.06 (dd, 1H, $J_{OH,5'}$ = 6.0, 5.2, OH-5'); 5.28 (d, 1H, $J_{OH,3'}$ = 4.0, OH-3'); 6.17 (bs 2H, NH_2); 6.58 (dd, 1H, $J_{1',2'}$ = 8.3, 5.9, H-1'); 7.23 (m, 2H, H-*m*-phenylene); 7.50 (m, 2H, H-*o*-phenylene); 7.54 (s, 1H, H-6); 8.14 (s, 1H, H-2); ^{13}C NMR (125.7 MHz, DMSO-d_6): 39.86 (CH_2-2'); 62.16 (CH_2-5'); 71.24 (CH-3'); 83.12 (CH-1'); 87.56 (CH-4'); 100.47 (C-4a); 115.77 (C-5); 119.87 (CH-*m*-phenylene); 120.93 (CH-6); 130.17 (CH-*o*-phenylene); 131.55 (C-*i*-phenylene); 138.09 (C-*p*-phenylene); 150.70 (C-7a); 151.93 (CH-2); 157.50 (C-4); ν(KBr)/cm^{-1}: 3418, 3394, 2126, 2092, 1583, 1501, 1621, 1128, 1094, 1053, 841; MS (ESI+): *m/z* (%): 368.1 (100) [M+H], 390.1 (10) [M+Na]. HRMS (ESI+): calcd. for $C_{17}H_{18}N_7O_3$: 368.14656; found 368.14648.

5-(4-Azidophenyl)-2'-deoxycytidine 5'-O-triphosphate (dC^{A}TP): Compound **dC^{A}TP** was prepared from **dC^{A}** according to general procedure (Method B). The product was isolated as a yellow solid (7 mg, 21%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.36 (ddd, 1H, J_{gem} = 14.2, $J_{2'b,1'}$ = 7.3, $J_{2'b,3'}$ = 6.4, H-2'b); 2.43 (ddd, 1H, J_{gem} = 14.2, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.6, H-2'a); 4.13 (m, 1H, H-5'b); 4.19 (m, 2H, H-4',5'a); 4.63 (dt, 1H, $J_{3',2'}$ = 6.4, 3.6, $J_{3',4'}$ = 3.6, H-3'); 6.35 (dd, 1H, $J_{1',2'}$ = 7.3, 6.3, H-1'); 7.21 (m, 2H, H-*m*-phenylene); 7.45 (m, 2H, H-*o*-phenylene); 7.77 (s, 1H, H-6); ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 41.66 (CH_2-2'); 67.86 (d, $J_{C,P}$ = 5.8, CH_2-5'); 73.15 (CH-3'); 88.26 (d, $J_{C,P}$ = 8.8, CH-4'); 88.61 (CH-1'); 112.85 (C-5); 122.47 (CH-*m*-phenylene); 131.41 (C-*i*-phenylene); 133.67 (CH-*o*-phenylene); 142.47 (CH-6); 142.99 (C-*p*-phenylene); 159.87 (C-2); 167.48 (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.47 (dd, *J* = 20.1, 16.3, P_β); -10.68 (d, *J* = 20.1, P_α); -5.50 (d, *J* = 16.3, P_γ); MS (ESI-): *m/z* (%): 503.3 (100) [M-H_2PO_3], 525.2 (75) [M-H-H_2PO_3+Na], 583.3 (10) [M-H]. HRMS (ESI-): calcd. for $C_{15}H_{18}N_6O_{13}P_3$: 583.01502; found 583.01516.

7-(4-Azidophenyl)-7-deaza-2'-deoxyadenosine 5'-O-triphosphate (dA^{A}TP): Compound **dA^{A}TP** was prepared from **dA^{A}** according to general procedure (Method B). The product was isolated as a yellow solid (13 mg, 34%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.48 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 3.3, H-2'b); 2.75 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.4, H-2'a); 4.12 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 5.5, $J_{5'b,4'}$ = 4.2, H-5'b); 4.19 (ddd, 1H, J_{gem} = 11.3, $J_{H,P}$ = 6.5, $J_{5'a,4'}$ = 4.2, H-5'a); 4.24 (td, 1H, $J_{4',5'}$ = 4.2, $J_{4',3'}$ = 3.3, H-4'); 4.79 (dt, 1H, $J_{3',2'}$ = 6.4, 3.3, $J_{3',4'}$ = 3.3, H-3'); 6.70 (dd, 1H, $J_{1',2'}$ = 7.9, 6.3, H-1'); 7.21 (m, 2H, H-*m*-phenylene); 7.54 (s, 1H, H-6); 7.55 (m, 2H, H-*o*-phenylene); 8.18 (s, 1H, H-2); ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 40.99 (CH_2-2'); 68.16 (d, $J_{C,P}$ = 5.5, CH_2-5'); 73.81 (CH-3'); 85.46 (CH-1'); 87.90 (d, $J_{C,P}$ = 9.0, CH-4'); 103.81 (C-4a); 120.34 (C-5); 122.26 (CH-*m*-phenylene); 122.92 (CH-6); 132.98 (C-*i*-phenylene); 133.00 (CH-*o*-phenylene); 141.81 (C-*p*-phenylene); 152.69 (C-7a); 154.23 (CH-2); 160.11 (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.44 (bdd, *J* = 19.6, 18.3, P_β); -10.39 (d, *J* = 19.6, P_α); -5.60 (bd, *J* = 18.3, P_γ); MS (ESI-): *m/z* (%): 526.3 (100) [M-H_2PO_3], 548.3 (100) [M-H-H_2PO_3+Na], 606.3 (5) [M-H], 628.3 (15) [M-2H+Na]. HRMS (ESI-): calcd. for $C_{17}H_{19}N_7O_{12}P_3$: 606.03100; found 606.03103.

5-[4-(4-Phenyl-1,2,3-triazol-1-yl)phenyl]-2'-deoxycytidine (dC^{TP}): Compound **dC^{TP}** was prepared from **dC^{A}** according to general procedure (Method C). The product was isolated as a green solid (26 mg, 40%); m.p. >300ºC. ^{1}H NMR (499.8 MHz, DMSO-d_6, t = 100°C): 2.15 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,3'}$ = 6.7, $J_{2'b,1'}$ = 6.4, H-2'b); 2.26 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.4, $J_{2'a,3'}$ = 4.0, H-2'a); 3.58, 3.64 (2 × bddd, 2 × 1H, J_{gem} = 12.0, $J_{5',OH}$ = 4.6, $J_{5',4'}$ = 3.8, H-5'); 3.84 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.8, H-4'); 4.28 (m, 1H, H-3'); 4.59 (bs, 1H, OH-5'); 4.85 (bs, 1H, OH-3'); 6.23 (t, 1H, $J_{1',2'}$ = 6.4, H-1'); 6.55 (bs, 2H, NH_2); 7.39 (m, 1H, H-*p*-Ph); 7.50 (m, 2H, H-*m*-Ph); 7.59 (m, 2H, H-*o*-phenylene); 7.92 (s, 1H, H-6); 7.96 (m, 2H, H-*o*-Ph); 8.00 (m, 2H, H-*m*-phenylene); 9.12 (s, 1H, H-5-triazole); ^{13}C NMR (125.7 MHz, DMSO-d_6, t = 100 °C): 40.52 (CH_2-2'); 61.01 (CH_2-5'); 70.00 (CH-3'); 85.34 (CH-1'); 87.25 (CH-4'); 106.60 (C-5); 119.06 (CH-5-triazole); 120.30 (CH-*m*-phenylene); 125.24 (CH-*o*-Ph); 127.86 (CH-*p*-Ph); 128.56 (CH-*m*-Ph); 130.06 (CH-*o*-phenylene); 130.13 (C-*i*-Ph); 134.33 (C-*i*-phenylene); 135.73 (C-*p*-phenylene); 140.29 (CH-6); 147.22 (C-4-triazole); 154.01 (C-2); 163.20 (C-4); ν(KBr)/cm^{-1}: 3464, 3363, 1647, 1482, 1457, 1411, 1353, 1254, 1187, 1096, 1042, 1026, 956; MS (ESI+): *m/z* (%): 447.3 (10) [M+H], 469.3 (100) [M+Na]. HRMS (ESI+): calcd. for $C_{23}H_{22}N_6O_4Na$: 469.15947; found 469.15920.

7-[4-(4-Phenyl-1,2,3-triazol-1-yl)phenyl]-7-deaza-2'-deoxyadenosine (dA^{TP}): Compound **dA^{TP}** was prepared from **dA^{A}** according to general procedure (Method C). The product was isolated as a yellow solid (34 mg, 72%); m.p. >300ºC. ^{1}H NMR (499.8 MHz, DMSO-d_6, t = 100°C): 2.30 (ddd, 1H, J_{gem} = 13.2, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 3.1, H-2'b); 2.60 (ddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 7.7, $J_{2'a,3'}$ = 6.1, H-2'a); 3.60, 3.66 (2 × bdt, 2 × 1H, J_{gem} = 11.7, $J_{5',OH}$ = $J_{5',4'}$ = 4.5, H-5'); 3.90 (td, 1H, $J_{4',5'}$ = 4.5, $J_{4',3'}$ = 3.0, H-4'); 4.43 (bm, 1H, H-3'); 4.64 (bs, 1H, OH-5'); 4.92 (bs, 1H, OH-3'); 5.91 (bs, 2H, NH_2); 6.52 (dd, 1H, $J_{1',2'}$ = 7.7, 6.1, H-1'); 7.39 (m, 1H, H-*p*-Ph); 7.51 (m, 2H, H-*m*-Ph); 7.58 (s, 1H, H-6); 7.72 (m, 2H, H-*o*-phenylene); 7.97 (m, 2H, H-*o*-Ph); 8.04 (m, 2H, H-*m*-phenylene); 8.20 (bs, 1H, H-2); 9.13 (bs, 1H, H-5-triazole); ^{13}C NMR (125.7 MHz, DMSO-d_6, t = 100 °C): 39.70 (CH_2-2'); 61.86 (CH_2-5'); 70.82 (CH-3'); 83.11 (CH-1'); 87.27 (CH-4'); 100.50 (C-4a); 114.99 (C-5); 119.13 (CH-5-triazole); 120.31 (CH-*m*-phenylene); 121.01 (CH-6); 125.23 (CH-*o*-Ph); 127.83 (CH-*p*-Ph); 128.56 (CH-*m*-Ph); 129.43 (CH-*o*-phenylene); 130.19 (C-*i*-Ph); 134.78 (C-*i*-phenylene); 135.21 (C-*p*-phenylene); 147.18 (C-4-triazole); 150.58 (C-7a); 151.50 (CH-2); 157.12 (C-4); ν(KBr)/cm^{-1}:3437, 1657, 1626, 1536, 1483, 1461, 1095, 1048, 1027, 960, 798; MS (ESI+): *m/z* (%): 470.3 (90) [M+H], 492.3 (100) [M+Na]. HRMS (ESI+): calcd. for $C_{25}H_{24}N_7O_3$: 470.19351; found 470.19342.

5-[4-(4-(4-Nitrophenyl)-1,2,3-triazol-1-yl)phenyl]-2'-deoxycytidine (dC^{TNO2}): Compound **dC^{TNO2}** was prepared from **dC^{PA}** according to general procedure (Method C). The product was isolated as a red solid (30 mg, 62%); m.p. 230ºC. ^{1}H NMR (600.1 MHz, DMSO-d_6): 2.12 (bdt, 1H, J_{gem} = 13.3, $J_{2'b,3'}$ = $J_{2'b,1'}$ = 6.3, H-2'b); 2.19 (bddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.5, H-2'a); 3.53, 3.60 (2 × bdt, 2 × 1H, J_{gem} = 11.9, $J_{5',OH}$ = $J_{5',4'}$ = 4.5, H-5'); 3.80 (btd, 1H, $J_{4',5'}$ = 4.5, $J_{4',3'}$ = 3.2, H-4'); 4.25 (m, 1H, H-3'); 5.00 (bt, 1H, $J_{OH,5'}$ = 4.5, OH-5'); 4.25 (bd, 1H, $J_{OH,5'}$ = 3.6, OH-3'); 6.22 (t, 1H, $J_{1',2'}$ = 6.3, H-1'); 6.68 (bs, 2H, NH_2); 7.61 (m, 2H, H-*o*-phenylene); 8.00 (s, 1H, H-6); 8.02 (m, 2H, H-*m*-phenylene); 8.23 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.40 (m, 2H, H-*m*-$C_6H_4NO_2$); 9.62 (s, 1H, H-5-triazole); ^{13}C NMR (150.9 MHz, DMSO-d_6): 40.86 (CH_2-2'); 61.13 (CH_2-5'); 70.23 (CH-3'); 85.49 (CH-1'); 87.25 (CH-4'); 106.50 (C-5); 120.65 (CH-*m*-phenylene); 121.80 (CH-5-triazole); 124.73 (CH-*m*-$C_6H_4NO_2$); 126.37 (CH-*o*-$C_6H_4NO_2$); 130.72 (CH-*o*-phenylene); 134.89 (C-*i*-phenylene); 135.74 (C-*p*-phenylene); 136.78 (C-*i*-$C_6H_4NO_2$); 140.82 (CH-6); 145.67 (C-4-triazole); 147.10 (C-*p*-$C_6H_4NO_2$); 154.44 (C-2); 163.40 (C-4); (KBr)/cm^{-1}:3454, 3320, 3206, 1657, 1643, 1606, 1519, 1481,1411, 1341, 1289, 1180, 1108, 1033, 855, 786, 636, 526; MS (ESI+): *m/z* (%): 514.3 (100) [M+H]. HRMS (ESI+): calcd. for $C_{23}H_{21}N_7O_6Na$: 514.14455; found 514.14447.

7-[4-(4-(4-Nitrophenyl)-1,2,3-triazol-1-yl)phenyl]-7-deaza-2'-deoxyadenosine (dA^{TNO2}): Compound **dA^{TNO2}** was prepared from **dA^{A}** according to general procedure (Method C). The product was isolated as a red solid (53 mg, 94%); m.p. 200ºC. ^{1}H NMR (600.1 MHz, DMSO-d_6): 2.24 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.7, H-2'b); 2.59 (bddd, 1H, J_{gem} = 13.2, $J_{2'a,1'}$ = 8.1, $J_{2'a,3'}$ = 5.9, H-2'a); 3.53, 3.60 (2 × bdt, 2 × 1H, J_{gem} = 11.6, $J_{5',OH}$ = $J_{5',4'}$ = 4.5, H-5'); 3.86 (td, 1H, $J_{4',5'}$ = 4.5, $J_{4',3'}$ = 2.6, H-4'); 4.39 (m, 1H, H-3'); 5.04 (bt, 1H, $J_{OH,5'}$ = 4.5, OH-5'); 5.30 (bd, 1H, $J_{OH,5'}$ = 4.1, OH-3'); 6.48 (bs, 2H, NH_2); 6.63 (dd, 1H, $J_{1',2'}$ = 8.1, 6.00, H-1'); 7.71 (s, 1H, H-6); 7.73 (m, 2H, H-*o*-phenylene); 8.07 (m, 2H, H-*m*-phenylene); 8.24 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.40 (m, 2H, H-*m*-$C_6H_4NO_2$); 9.63 (s, 1H, H-5-triazole); ^{13}C NMR (150.9 MHz, DMSO-d_6): 39.56 (CH_2-2'); 62.10 (CH_2-5'); 71.18 (CH-3'); 83.17 (CH-1'); 87.62 (CH-4'); 101.14 (C-4a); 115.80 (C-5); 120.71 (CH-*m*-phenylene); 121.80 (CH-5-triazole); 124.17 (CH-6); 124.72 (CH-*m*-$C_6H_4NO_2$); 126.32 (CH-*o*-$C_6H_4NO_2$); 129.93 (CH-*o*-phenylene); 135.17 (C-*i*,*p*-phenylene); 136.80 (C-*i*-$C_6H_4NO_2$); 145.61 (C-4-triazole); 147.06 (C-*p*-$C_6H_4NO_2$); 150.52 (C-7a); 151.97 (CH-2); 157.34 (C-4); ν(KBr)/cm^{-1}: 3440, 1657, 1625, 1607, 1589, 1536, 1517, 1481, 1466, 1408, 1342, 1289, 1107, 1067, 1038, 854, 796; MS (ESI+): *m/z* (%): 515.3 (100) [M+H]. HRMS (ESI+): calcd. for $C_{25}H_{23}N_8O_5$: 515.17859; found 515.17839.

5-[4-(4-Phenyl-1,2,3-triazol-1-yl)phenyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{TP}TP): Compound **dC^{TP}TP** was prepared from **dC^{TP}** according to general procedure (Method D). The product was isolated as a white solid (14 mg, 52%).^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.34 (ddd, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.5, H-2'b); 2.46 (ddd, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.6, H-2'a); 4.18-4.26 (bm, 3H, H-4',5'); 4.63 (dt, 1H, $J_{3',2'}$ = 6.5, 3.6, $J_{3',4'}$ = 3.6, H-3'); 6.24 (dd, 1H, $J_{1',2'}$ = 7.1, 6.3, H-1'); 7.42 (m, 1H, H-*p*-Ph); 7.49 (m, 2H, H-*m*-Ph); 7.55 (m, 2H, H-*o*-phenylene); 7.77 (s, 1H, H-6); 7.79 (m, 2H, H-*m*-phenylene); 7.80 (m, 2H, H-*o*-Ph); 8.75 (s, 1H, H-5-triazole); ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 42.09 (CH_2-2'); 67.93 (d, $J_{C,P}$ = 4.7, CH_2-5'); 73.21 (CH-3'); 88.37 (d, $J_{C,P}$ = 8.5, CH-4'); 89.03 (CH-1'); 111.70 (C-5); 122.96 (CH-5-triazole); 123.95 (CH-*m*-phenylene); 128.38 (CH-*o*-Ph); 131.60 (CH-*p*-Ph); 131.78 (C-*i*-Ph); 131.93 (CH-*m*-Ph); 133.18 (CH-*o*-phenylene); 135.91 (C-*i*-phenylene); 138.68 (C-*p*-phenylene); 142.82 (CH-6); 150.76 (C-4-triazole); 159.42 (C-2); 166.71 (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.56 (bm, P_β); -10.69 (bm, P_α); -6.88 (bm, P_γ); MS (ESI-): *m/z* (%): 525.3 (60) [M-$H_3P_2O_6$], 605.3 (100) [M-H_2PO_3], 627.2 (90) [M-H-H_2PO_3+Na], 685.3 (5) [M-H]. HRMS (ESI-): calcd. for $C_{23}H_{24}N_6O_{13}P_3$: 685.06197; found 685.06211.

7-[4-(4-Phenyl-1,2,3-triazol-1-yl)phenyl]-7-deaza-2'-deoxyadenosine 5'-O-triphosphate (dA^{TP}TP): Compound **dA^{TP}TP** was prepared from **dA^{TP}** according to general procedure (Method D). The product was isolated as a white solid (4 mg, 13%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.45 (ddd, 1H, J_{gem} = 13.8, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 3.0, H-2'b); 2.72 (bddd, 1H, J_{gem} = 13.8, $J_{2'a,1'}$ = 7.8, $J_{2'a,3'}$ =

6.4, H-2'a); 4.11, 4.17 (2 × bm, 2 × 1H, H-5'); 4.23 (bm, 1H, H-4'); 4.77 (bm, 1H, H-3'); 6.38 (bdd, 1H, $J_{1',2'}$ = 7.8, 6.1, H-1'); 7.32 (m, 2H, H-*m*-Ph); 7.35 (m, 1H, H-*p*-Ph); 7.47 (s, 1H, H-6); 7.48 (m, 2H, H-*o*-phenylene); 7.52 (m, 2H, H-*o*-Ph); 7.58 (m, 2H, H-*m*-phenylene); 8.02 (s, 1H, H-2); 8.51 (s, 1H, H-5-triazole); ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 40.77 (CH_2-2'); 68.21 (d, $J_{C,P}$ = 4.4, CH_2-5'); 73.74 (CH-3'); 85.34 (CH-1'); 87.76 (d, $J_{C,P}$ = 7.5, CH-4'); 103.15 (C-4a); 119.37 (C-5); 122.25 (CH-5-triazole); 123.32 (CH-6); 123.45 (CH-*m*-phenylene); 127.73 (CH-*o*-Ph); 131.21 (C-*i*-Ph); 131.43 (CH-*p*-Ph); 131.58 (CH-*m*-Ph); 132.00 (CH-*o*-phenylene); 137.05 (C-*i*-phenylene); 137.46 (C-*p*-phenylene); 150.47 (C-4-triazole); 152.63 (C-7a); 153.90 (CH-2); 159.59 (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.23 (bs, P_β); -10.32 (bs, P_α); -5.44 (bs, P_γ); MS (ESI-): *m/z* (%): 548.3 (100) [M-$H_3P_2O_6$], 628.3 (55) [M-H_2PO_3], 650.3 (50) [M-H-H_2PO_3+Na], 708.3 (10) [M-H]. HRMS (ESI-): calcd. for $C_{25}H_{25}N_7O_{12}P_3$: 708.07795; found 708.07822.

5-[4-(4-(4-Nitrophenyl)-1,2,3-triazol-1-yl)phenyl]-2'-deoxycytidine 5'-O-triphosphate (dC^{TNO2}TP): Compound **dC^{TNO2}TP** was prepared from **dC^{TNO2}** according to general procedure (Method D). The product was isolated as a brown solid (2.5 mg, 18%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.36 (ddd, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = 7.0, $J_{2'b,3'}$ = 6.4, H-2'b); 2.48 (ddd, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = 6.3, $J_{2'a,3'}$ = 3.9, H-2'a); 4.20-4.29 (bm, 3H, H-4',5'); 4.66 (m, 1H, H-3'); 6.27 (dd, 1H, $J_{1',2'}$ = 7.0, 6.3, H-1'); 7.55 (m, 2H, H-*o*-phenylene); 7.840 (m, 2H, H-*m*-phenylene); 7.843 (s, 1H, H-6); 7.99 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.28 (m, 2H, H-*m*-$C_6H_4NO_2$); 8.98 (s, 1H, H-5-triazole); ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 42.19 (CH_2-2'); 67.81 (d, $J_{C,P}$ = 4.6, CH_2-5'); 72.98 (CH-3'); 88.42 (d, $J_{C,P}$ = 8.8, CH-4'); 88.94 (CH-1'); 111.79 (C-5); 124.06 (CH-*m*-phenylene); 124.49 (CH-5-triazole); 127.21 (CH-*m*-$C_6H_4NO_2$); 129.05 (CH-*o*-$C_6H_4NO_2$); 133.38 (CH-*o*-phenylene); 136.19 (C-*i*-phenylene); 138.52, 138.55 (C-*i*-$C_6H_4NO_2$, C-*p*-phenylene); 142.94 (CH-6); 148.76 (C-4-triazole); 149.81 (C-*i*-$C_6H_4NO_2$); 159.54 (C-2); 166.82 (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): -21.23 (bm, P_β); -10.67 (bd, J = 16.8, P_α); -5.47 (bd, J = 19.1, P_γ); MS (ESI-): *m/z* (%): 570.3 (80) [M-$H_3P_2O_6$], 650.2 (95) [M-H_2PO_3], 672.2 (100) [M-H-H_2PO_3+Na], 731.2 (10) [M-H]. HRMS (ESI-): calcd. for $C_{23}H_{23}N_7O_{15}P_3$: 730.04705; found 730.04741.

7-[4-(4-(4-Nitrophenyl)-1,2,3-triazol-1-yl)phenyl]-7-deaza-2'-deoxyadenosine 5'-O-triphosphate (dA^{TNO2}TP): Compound **dA^{TNO2}TP** was prepared from **dA^{TNO2}** according to general procedure (Method D). The product was isolated as a brown solid (5.5 mg, 18%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.51 (ddd, 1H, J_{gem} = 13.8, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 3.5, H-2'b); 2.68 (bddd, 1H, J_{gem} = 13.8, $J_{2'a,1'}$ = 7.7, $J_{2'a,3'}$ = 6.4, H-2'a); 4.17, 4.22 (2 × bm, 2 × 1H, H-5'); 4.25 (bm, 1H, H-4'); 4.79 (m, 1H, H-3'); 6.34 (bdd, 1H, $J_{1',2'}$ = 7.7, 6.3, H-1'); 7.22 (m, 2H, H-*o*-phenylene); 7.37 (m, 2H, H-*m*-phenylene); 7.40 (s, 1H, H-6); 7.50 (m, 2H, H-*o*-$C_6H_4NO_2$); 7.79 (m, 2H, H-*m*-$C_6H_4NO_2$); 7.91 (s, 1H, H-2); 8.51 (s, 1H, H-5-triazole); ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 41.26 (CH_2-2'); 68.29 (d, $J_{C,P}$ = 5.7, CH_2-5'); 73.85 (CH-3'); 85.34 (CH-1'); 87.82 (d, $J_{C,P}$ = 8.8, CH-4'); 102.57 (C-4a); 119.07 (C-5); 122.64 (CH-*m*-phenylene); 123.26 (CH-5-triazole); 123.47 (CH-6); 126.24 (CH-*m*-$C_6H_4NO_2$); 128.20 (CH-*o*-$C_6H_4NO_2$); 131.54 (CH-*o*-phenylene); 136.61 (C-*i*-phenylene); 136.66 (C-*p*-phenylene); 137.50 (C-*i*-$C_6H_4NO_2$); 148.34 (C-4-triazole); 148.85 (C-*p*-$C_6H_4NO_2$); 152.27 (C-7a); 153.51 (CH-2); 159.14 (C-4); ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): -21.39 (bm, P_β); -10.87 (d, J = 18.2, P_α); -6.25 (bd, J = 15.5, P_γ); MS (ESI-): *m/z* (%):) 593.3 (50) [M-$H_3P_2O_6$], 673.3 (100) [M-H_2PO_3], 695.3 (90) [M-H-H_2PO_3+Na], 753.3 (5) [M-H]. HRMS (ESI-): calcd. for $C_{25}H_{24}N_8O_{14}P_3$: 753.06303; found 753.06312.

Primer extension experiment: The reaction mixture (20 µL) contained DNA polymerase [KOD XL, Pwo, Vent (exo-)], primer (0.15 µM), template (0.23 µM), natural and modified dNTPs (0.2 mM) in reaction buffer. Primer was labeled by use of [γ^{32}P]-ATP according to standard techniques. Reaction mixtures were incubated for 15-40 min at 60ºC and analysed by PAGE electrophoresis.

Kinetics of PEX (Figure S3-S4): The PEX reaction mixtures using Pwo DNA polymerase, temp^C and temp^{Aterm} templates were incubated for time intervals (0.1-10 min), followed by stopping the reaction by addition of PAGE loading buffer and immediate heating.

Click reaction of PEX product DNA[23] (Figure 2): dsDNA obtained by PEX experiments was purified using Qiagen Nucleotide Removal Kit purification columns. The solution of Cu(I) catalyst (10 mM) was freshly prepared just before the reaction by mixing CuBr (1 µL, 50 mM in DMSO/tBuOH 3:1), TBTA ligand (4 µL, 100 mM in DMSO/tBuOH 3:1) and DMSO/tBuOH 3:1 (3 µL). To the DNA solution (50 µL, 50 ng/ µL) a solution of acetylene (phenylacetylene or 1-ethynyl-4-nitrobenzene) (30 µL, 10 mM in DMSO), sodium ascorbate (2 µL, 5 mM in water), pre-complexed Cu(I) and 10 uL DMSO/tBuOH 3:1 were added. The sample was incubated for 2 h at 37°C and 500 rpm. After the reaction, the crude mixture was purified once again and then was desalted with dialysis membranes (Millipore).

Binding study using p53CD_GST protein: Native analysis of reaction mixtures with different p53CD_GST/DNA ratio: The reaction mixture (100 µl) contained primer (prim[15], 10 µl, 3 µM), template (template^{p53_1a2G} / temp^{p53_2CON_4}, 12 µl, 3 µM), KOD XL DNA polymerase (1 µl, 2.5 U/µl), dNTPs (either all natural or 3 natural and 1 modified, 5 µl, 4 mM) in KOD XL reaction buffer (10 µl) supplied by the manufacturer. Primers were labelled on their 5´-end by use of [γ^{32}P]-ATP according to standard techniques. The reaction mixture was incubated for 45 min at 60°C in a thermal cycler and purified using QIAquick Nucleotide Removal Kit (Qiagen). The PEX-product was eluted from the column by H_2O (pH 7.7, 50µl). The reaction mixtures for p53CD_GST protein binding (10 µl) were prepared from purified PEX-product (5 µl, 10 ng/µl), 50 mM KCl, 5 mM Tris pH 7.6 and p53CD_GST stock solution (750 ng/µl, 25 mM Hepes pH 7.6, 200 mM KCl, 10% glycerol, 0.1 mM PPh_3; 0.4, 0.7, 1.2, 1.7equiv.). Control sample was prepared analogously without p53CD_GST. All samples were incubated for 45 min on ice, glycerol was added (60%, 2 µl) and a part of the reaction mixture (3 µl) was separated by use of a 6% native PAGE (acrylamide/bisacrylamide 37.5:1; 4°C, 400 V/2.5 hours). Visualization was performed by phosphoimaging (Figure 9a, Figure S8).

Thermal stability of protein/DNA complexes (Figure S10-S11): The reaction mixtures for p53CD_GST protein binding (40 µl) were prepared from purified PEX-product (20 µl, 10 ng/µl), 50 mM KCl, 5 mM Tris pH 7.6 and p53CD_GST stock solution (750 ng/µl, 25 mM Hepes pH 7.6, 200 mM KCl, 10% glycerol, 0.1 mM PPh_3; 1.2 equiv.). Control sample was prepared analogously without p53CD_GST. Samples were incubated for 45 min on ice and then were divided into four vials and exposed to four different temperatures (0º, 20º, 37º, 50º) for 1 hour, glycerol was added (60%, 2 µl) and a part of the reaction mixture (3 µl) was separated by use of a 6% native PAGE (acrylamide/bisacrylamide 37.5:1; 4°C, 400 V/2.5 hours). Visualization was performed by phosphoimaging.

Cu^I concentration dependence of stability of protein/DNA complexes (Figure S12-S13): The reaction mixtures for p53CD_GST protein binding (80 µl) were prepared from purified PEX-product (40 µl, 10 ng/µl), 50 mM KCl, 5 mM Tris pH 7.6 and p53CD_GST stock solution (750 ng/µl, 25 mM Hepes pH 7.6, 200 mM KCl, 10% glycerol, 0.1 mM PPh_3; 1.2 equiv.). Control sample was prepared analogously without p53CD_GST. Samples were incubated for 45 min on ice and then were divided into eight vials and were incubated with various concentration of CuBr (5 µM, 10 µM, 20 µM) in/without presence of the ligand TBTA at 20ºC for 1h, glycerol was added (60%, 2 µl) and a part of the reaction mixture (3 µl) was separated by use of a 6% native PAGE (acrylamide/bisacrylamide 37.5:1; 4°C, 400 V/2.5 hours). Visualization was performed by phosphoimaging.

Stability of protein/DNA complex after click reaction of DNA (Figure 9b, Figure S9): The reaction mixtures for p53CD_GST protein binding (10 µl) were prepared from purified PEX-product (5 µl, 10 ng/µl), 50 mM KCl, 5 mM Tris pH 7.6 and p53CD_GST stock solution (750 ng/µl, 25 mM Hepes pH 7.6, 200 mM KCl, 10% glycerol, 0.1 mM PPh_3; 1.2 equiv.). Control sample was prepared analogously without p53CD_GST. Samples were incubated for 45 min on ice and then 4-nitrophenylacetylene (10 mM in MeOH, 0.75 µl), CuBr (100 µM in DMSO/tBuOH 3:1, 0.75 µl), TBTA (1 mM in DMSO/tBuOH 3:1, 0.4 µl), sodium ascorbate (5 mM in water, 0.2 µl), KCl (50mM, 1.5 µl), Tris (5mM, pH 7.6, 1.5 µl) were added to the reaction and sample was incubated at 20ºC for 1h, glycerol was added (60%, 2 µl) and a part of the reaction mixture (3 µl) was separated by use of a 6% native PAGE (acrylamide/bisacrylamide 37.5:1; 4°C, 400 V/2.5 hours). Visualization was performed by phosphoimaging. For electrochemical measurement we applied the same condition described above but in higher scope, three parallel samples for exact comparison (DNA mixed with binding protein, control sample − DNA mixed with BSA). BSA was used as control protein.

Electrochemical analysis: Nucleosides, dNTPs and other building blocks were analyzed by conventional in situ cyclic voltammetry (CV). PEX products were analyzed by ex situ (adsorptive transfer stripping, AdTS) CV or square-wave voltammetry (SWV). The PEX products (purified in their single-stranded form using streptavidin-coated magnetic beads or in their double-stranded forms using Qiagen Nucleotide Removal Kit) were accumulated at the surface of a working electrode (hanging mercury drop electrode, HMDE) for 60 s from 5-□L aliquots containing 0.2 M NaCl. The electrode was then rinsed with deionized water and placed into a electrochemical cell. CV settings: scan rate 1 V s^{-1}, initial potential 0.0 V, for switching potentials see Figure legends. SWV settings: initial potential 0 V, for final potentials see figure legends; frequency 200 Hz, amplitude 50 mV. Background

electrolyte: 0.5 M ammonium formate, 0.05 M sodium phosphate, pH 6.9. All measurements were performed at room temperature using an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-stand 663 (Metrohm, Herisau, Switzerland). The three-electrode system was used with Ag/AgCl/3M KCl electrode as a reference and platinum wire as an auxiliary electrode. Measurements of reduction signals were performed after deaeration of the solution by argon purging.

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1. a) E. Paleček, F. Jelen in *Electrochemistry of nucleic acids and proteins: Towards electrochemical sensors for genomics and proteomics* (Eds.: E. Paleček, F. Scheller, J. Wang), Elsevier, Amsterdam, 2005, 74–174; b) J. Wang, in *Electrochemistry of nucleic acids and proteins: Towards electrochemical sensors for genomics and proteomics* (Eds.: E. Paleček, F. Scheller, J. Wang), Elsevier, Amsterdam, 2005, 175–194; c) E. Palecek and M. Bartosik, *Chem. Rev*., 2012, **112**, 3427–3481.
2. a) M. Hocek, M. Fojta, *Chem. Soc. Rev.*, 2011, **40**, 5802–5814; b) P. Brázdilová, M. Vrábel, R. Pohl, H. Pivoňková, L. Havran, M. Hocek, M. Fojta, *Chem. Eur. J.*, 2007, **13**, 9527–9533; c) H. Cahová, L. Havran, P. Brázdilová, H. Pivoňková, R. Pohl, M. Fojta, M. Hocek, *Angew. Chem. Int. Ed.*, 2008, **47**, 2059–2062; d) J. Balintová, R. Pohl, P. Horáková, P. Vidláková, L. Havran, M. Fojta, M. Hocek, *Chem. Eur. J.*, 2011, **17**, 14063–14073; e) H. Macíčková-Cahová, R. Pohl, P. Horáková, L. Havran, J. Špaček, M. Fojta, M. Hocek, *Chem. Eur. J.*, 2011, **17**, 5833–5841; f) V. Raindlová, R. Pohl, B. Klepetářová, L. Havran, E. Šimková, P. Horáková, H. Pivoňková, M. Fojta, M. Hocek, *ChemPlusChem*, 2012, **77**, 652–662.
3. a) M. Vrábel, P. Horáková, H. Pivoňková, L. Kalachova, H. Černocká, H. Cahová, R. Pohl, P. Šebest, L. Havran, M. Hocek, M. Fojta, *Chem. Eur. J.*, 2009, **15**, 1144–1154; b) J. Balintová, M. Plucnara, P. Vidláková, R. Pohl, L. Havran, M. Fojta, M. Hocek, *Chem. Eur. J*., 2013, **19**, 12720–12731.
4. a) A. A. Gorodetsky, M. C. Buzzeo, J. K. Barton, *Bioconjugate Chemistry*, 2008, **19**, 2285–2296; b) H. F. Wang, N. B. Muren, D. Ordinario, A. A. Gorodetsky, J. K. Barton, C. Nuckolls, *Chem. Sci.*, 2012, **3**, 62–65.
5. a) P. Horakova, H. Macickova-Cahova, H. Pivonkova, J. Spacek, L. Havran, M. Hocek, M. Fojta, *Org. Biomol. Chem.*, 2011, **9**, 1366-1371; b) K. Nemcova, P. Sebest, L. Havran, P. Orsag, M. Fojta, H. Pivonkova, *Anal. Bioanal. Chem.*, 2014, in press: doi 10.1007/s00216-014-7996-0.
6. Review: B. Dey, S. Thukral, S. Krishnan, M. Chakrobarty, S. Gupta, C. Manghani, V. Rani, *Mol. Cell. Biochem*., 2012, **365**, 279–299.
7. a) D. J. Galas, A. Schmitz, *Nucleic Acids Res.*, 1978, **5**, 3157–3170; b) R. Sandaltzopoulos, P. B. Becker, *Nucleic Acids Res*., 1994, **22**, 1511–1512; c) M. J. Storek, A. Suciu, G. L. Verdine, *Org. Lett.*, 2002, **4**, 3867–3869; d) M. J. Storek, A. Ernst, G. L. Verdine, *Nature Biotechnology*, 2002, **20**, 183–186.
8. a) Review: H. C. Kolb, M. G. Finn, and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2001, **40**, 2004–2021; b) D. K. Scrafton, J. E. Taylor, M. F. Mahon, J. S. Fossey, T. D. James, *J. Org. Chem*, 2008, **73**, 2871–2874; c) F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc*., 2005, **127**, 210–216.
9. a) Review: A. H. El-Sagheer, T. Brown, *Chem. Soc. Rev.*, 2010, **39**, 1388–1405; b) J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.*, 2006, **8**, 3639–3642; c) A. H. El-Sagheer, T. Brown, *Chem. Commun.*, 2011, **47**, 12057–12058.
10. a) P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem., Int. Ed.*, 2008, **47**, 8350–8358; b) P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem., Int. Ed.*, 2008, **47**, 3442–3444; c) F. Seela, V. R. Sirivolu, P. Chittepu, *Bioconjugate Chem.*, 2008, **19**, 211–224; d) F. Seela, V. R. Sirivolu, *Org. Biomol. Chem.*, 2008, **6**, 1674–1687; e) K. Gutschmiedl, D. Fazio, T. Carell, *Chem. Eur. J.*, 2010, **16**, 6877-6883; f) S. A. Ingale, F. Seela, *J. Org. Chem.*, 2013, **78**, 3394−3399.
11. A. B. Neef, N. W. Luedtke, *ChemBioChem*, 2014, **15**, 789–793
12. Review on chemistry of azides: S. Bräse, C. Gil, K. Knepper, V. Zimmermann, *Angew. Chem. Int. Ed.*, 2005, **44**, 5188–5240.
13. Selected examples on polymerase incorporations of base-modified dNTPs: a) S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum, M. Famulok, *J. Am. Chem. Soc.*, 2005, **127**, 15071–15082; b) S. Obeid, M. Yulikow, G. Jeschke, A. Marx, *Angew. Chem. Int. Ed.*, 2008, **47**, 6782–6785; c) N. Ramsay, A.-S. Jemth, A. Brown, N. Crampton, P. Dear, P. Holliger, *J. Am. Chem. Soc.*, 2010, **132**, 5096–5104; d) A. Baccaro, A. Marx, *Chem. Eur. J.*, 2010, **16**, 218–226; e) P. Kielkowski, H. Macíčková-Cahová, R. Pohl, M. Hocek, *Angew. Chem. Int. Ed.*, 2011, **50**, 8727–8730; f) P. Ménová, M. Hocek, *Chem. Commun.*, 2012, **48**, 6921-6923; g) A. Baccaro, A.-L. Steck, A. Marx. *Angew. Chem. Int. Ed.*, 2012, **51**, 254-257; h) M. Hollenstein, *Chem. Eur. J.*, 2012, **18**, 13320–13330; i) J. Riedl, R. Pohl, N. P. Ernsting, P. Orsag, M. Fojta, M. Hocek, *Chem. Sci*., 2012, **3**, 2797-2806; j) S. Obeid, H. Busskamp, W. Welte, K. Diederichs, A. Marx, *J. Am. Chem. Soc.*, 2013, **135**, 15667–15669; k) Z. Vaníková, M. Hocek, *Angew. Chem. Int. Ed.*, 2014, **53**, 6734–6737; l) P. Kielkowski, J. Fanfrlík, M. Hocek, *Angew. Chem. Int. Ed.*, 2014, **53**, 7552–7555.
14. Y. A. Cho, D. Kim, H. R. Ahn, B. Canturk, G. A. Molander, J. Ham, *Org. Lett.*, 2009, **11**, 4330–4333.
15. For related CuAAC reactions of 2-azidopurines, see: L. Cosyn, K. K. Palaniappan, S.- K. Kim, H. T. Duong, Z.- G. Gao, K. A. Jacobson, S. van Calenbergh, *J. Med. Chem*., 2006, **49**, 7373–7383.
16. T. Kovacs, L. Ötvös, *Tetrahedron Lett.*, 1988, **29**, 4525–4528.
17. a) G. Matlashewski, P. Lamb, D. Pim, J. Peacock, L. Crawford, S. Benchimol, *EMBO J.*, 1984, **3**, 3257–3262; b) M. Isobe, B. S. Emanuel, D. Givol, M. Oren, C. M. Croce, *Nature*, 1986, **320**, 84–85; c) S. E. Kern, K. W. Kinzler, A. Bruskin, D. Jarosz, P. Friedman, C. Prives, B. Vogelstein, *Science*, 1991, **252**, 1708–1711; d) O. W. McBride, D. Merry, D. Givol, *Proc. Natl. Acad.Sci. USA*, 1986, **83**, 130–134.
18. For expression of p53, see: a) M. Brazdova, J. Palecek, D. I. Cherny, S. Billova, M. Fojta, P. Pecinka, B. Vojtesek, T. M. Jovin, E. Palecek, *Nucleic Acids Res.*, 2002, **30**, 4966–4974; b) M. Fojta, H. Pivonkova, M. Brazdova, K. Nemcova, J. Palecek, B. Vojtesek, *Eur. J. Biochem.*, 2004, **271**, 3865–3876; c) C. Klein, G. Georges, K.-P. Künkele, R. Huber, R. A. Engh, S. Hansen, *J. Biol. Chem.*, 2001, **276**, 37390–37401; d) J. Buzek, L. Latonen, S. Kurki, K. Peltonen,M. Laiho, *Nucleic Acids Res.*, 2002, **30**, 2340–2348.
19. a) W. S. El-Deiry, S. E. Kern, J. A. Pietenpol, K. W. Kinzler, B. Vogelstein, *Nat. Genet.*, 1992, **1**, 45–49; b) Y. Wang, J. F. Schwedes, D. Parks, K. Mann, P. Tegtmeyer, *Mol. Cell. Biol.*, 1995, **15**, 2157–2165; c) J. L. Kaar, N. Basse, A. C. Joerger, E. Stephens, T. J. Rutherford, A. R. Fersht, *Protein Sci.*, 2010, **19**, 2267–2278.

20. J. Dadová, P. Orság, R. Pohl, M. Brázdová, M. Fojta, M. Hocek, *Angew. Chem. Int. Ed.*, 2013, **52**, 10515–10518.
21. E. Paleček, M. Brázdová, H. Černocká, D. Vlk, V. Brázda, B. Vojtešek, *Oncogene*, 1999, **18**, 3617–3625.
22. a) J. Sumranjit and S. J. Chung, *Molecules*, 2013, **18**, 10425–10451; b) S.-W. Yang and H. A. Nash, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 12183–12187; c) R. K. Evans and B. E. Haley, *Biochemistry*, 1987, **26**, 269–276.
23. P. Ménová, V. Raindlová, M. Hocek, *Bioconjugate Chemistry*, 2013, **24**, 1081–1093.