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# Methoxyphenol and Dihydrobenzofuran as Oxidizable Labels for Electrochemical Detection of DNA

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4-Hydroxy-3-methoxyphenyl (MOP) and 2,3-dihydrobenzofuran-5-yl (DHB) groups were tested as potential new oxidizable labels for electrochemical detection of DNA. The corresponding 5-aryl-cytosine and 7-aryl-7-deazaadenine 2′-deoxyribonucleoside triphosphates (dNTPs) were prepared by the Suzuki coupling of halogenated dNTPs or by triphosphorylation of modified nucleosides, and were incorporated enzymatically into DNA by polymerase. In parallel, the electrochemical behavior of the labeled nucleosides, nucleotides, and DNA was studied to show that most of them give analytically useful signals of oxidation on the carbon electrode. Particularly, MOP appears to be a useful oxidizable DNA label since it offers independent detection of the modified DNA.

## Introduction

Electrochemical DNA biosensors[1] are increasingly applied in the life sciences and diagnostics and have become a viable alternative to the current fluorescence and microarray techniques.[2] Apart from inherent electrochemical detection of unlabeled nucleic acids, additional DNA labels have been developed extensively for diverse bioanalytical applications.[3] We have previously reported the labeling of nucleos(t)ides and DNA by several new redox labels, including ferrocene,[4] amino- and nitrophenyl,[5] $Os(bpy)_3$ (bpy = 2,2′-bipyridine),[6] tetrathiafulvalene,[7] anthraquinone,[8] alkylsulfanylphenyl,[9] hydrazones,[10] and benzofurazane,[11] and several more detailed electrochemical studies[12] of these labels. Some of these labels were combined in a new concept of multipotential redox coding of DNA nucleobases (each base was labeled by a different label with a different redox potential) but the first generation[6] suffered from weak and overlapping signals, limited stability, and difficult incorporation of some labels into DNA. Recently, we reported[11] the first orthogonal and ratiometric set of two reducible labels (nitrophenyl and benzofurazane) useful for electrochemical minisequencing of short DNA stretches.[5,6,11] However, for any practical bioanalytical applications, we need a fully orthogonal set of four labels that can be readily incorporated into DNA by polymerase and are then independently readable in the presence of all the other labels. This could then be used for simultaneous detection of multiple nucleobase mutations in short sequences (1–3 codons) in one experiment (as opposed to current sequencing techniques,[2] which always use detection of one nucleotide at a time).

Herein, we report the development of two new potential oxidizable labels, 4-hydroxy-3-methoxyphenyl (MOP) and 2,3-dihydrobenzofuran-5-yl (DHB) groups, and their electrochemical studies. 2-Methoxyphenol is known to undergo $2e^-$ electrooxidation to *o*-benzoquinone.[12] The electrochemistry of 2,3-dihydrobenzofurans has been less investigated[13] but they are known to be easily oxidized to aromatic benzofurans.[14]

## Results and Discussion

### Synthesis

The synthesis of model labeled nucleosides (2′-deoxycytidine and 2′-deoxy-7-deazaadenosine) and 2′-deoxyribonucleoside triphosphates (dNTPs) was based on aqueous-phase Suzuki–Miyaura cross-coupling reactions[15] of the corresponding halogenated nucleosides or dNTPs with commercially available MOP- or DHB-substituted boronic acids or pinacolatoboronates (MOP-B(pin) and DHB-$B(OH)_2$, Scheme 1). At first, the reactions were performed with nucleosides. Thus the reactions of either

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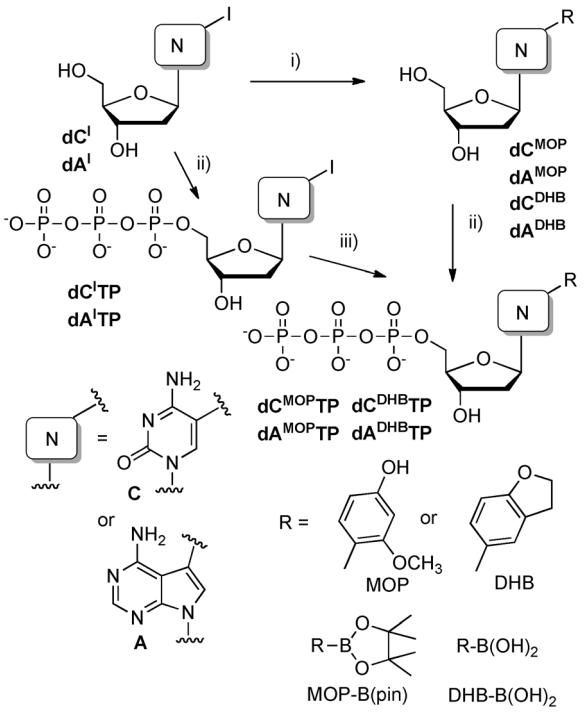


**Scheme 1.** Reagents and conditions: i) MOP-B(pin) or DHB-B(OH)$_2$, Pd(OAc)$_2$, TPPTS, $Cs_2CO_3$, MeCN/$H_2O$ (2:1), 1 h, 75 °C. ii) 1) $PO(OMe)_3$, $POCl_3$, 0 °C; 2) $(NHBu_3)_2H_2P_2O_7$, $Bu_3N$, DMF, 0 °C; 3) 2 M TEAB (phosphorylation of modified nucleosides to triphosphates was applied only in the case of **dC$^{MOP}$**). iii) MOP-B(pin) or DHB-B(OH)$_2$, Pd(OAc)$_2$, TPPTS, $Cs_2CO_3$, MeCN/$H_2O$ (2:1), 40 min, 50 °C.

**dA$^I$** or **dC$^I$** with MOP-B(pin) and DHB-B(OH)$_2$ were performed in the presence of Pd(OAc)$_2$, 3,3,′3"-tris(sulfonato)-triphenylphosphane (TPPTS), and $Cs_2CO_3$ in acetonitrile (MeCN)/$H_2O$ (2:1) at 75 °C for 1 hour. The desired aryl-substituted nucleosides **dA$^{MOP}$**, **dC$^{MOP}$**, **dA$^{DHB}$**, and **dC$^{DHB}$** were obtained in acceptable yields of 42–78% after chromatographic separation (Table 1). The same reactions were then performed with iodinated dNTPs (**dA$^I$TP** and **dC$^I$TP**) for 40 minutes at 50 °C (to avoid hydrolysis of the triphosphates). The desired labeled dNTPs (**dA$^{MOP}$TP**, **dC$^{MOP}$TP**, **dA$^{DHB}$TP**, and **dC$^{DHB}$TP**) were formed and isolated by semipreparative HPLC in approximately 40% yields (Table 1). Only in the case of **dC$^{MOP}$TP** was the product not isolated in pure form owing to a similar retention time to TPPTS. Therefore, this compound was prepared by an alternative approach using chemical triphosphorylation of the corresponding modified nucleoside **dC$^{MOP}$** with $POCl_3$ in $P(O)(OMe)_3$ followed by $(NHBu_3)_2H_2P_2O_7$, $Bu_3N$ in dimethylformamide (DMF), and triethylammonium bicarbonate (TEAB). The desired **dC$^{MOP}$TP** was isolated in pure form in 28% yield.

**Table 1.** Synthesis of MOP- and DHB-modified nucleosides and dNTPs

| Entry | Starting compound | Reagent | Product | Yield [%][a] |
|---|---|---|---|---|
| 1 | **dA$^I$** | MOP-B(pin) | **dA$^{MOP}$** | 77 |
| 2 | **dC$^I$** | MOP-B(pin) | **dC$^{MOP}$** | 42 |
| 3 | **dA$^I$** | DHB-B(OH)$_2$ | **dA$^{DHB}$** | 78 |
| 4 | **dC$^I$** | DHB-B(OH)$_2$ | **dC$^{DHB}$** | 69 |
| 5 | **dA$^I$TP** | MOP-B(pin) | **dA$^{MOP}$TP** | 42 |
| 6 | **dC$^I$TP** | MOP-B(pin) | **dC$^{MOP}$TP** | 35[b] |
| 7 | **dA$^I$TP** | DHB-B(OH)$_2$ | **dA$^{DHB}$TP** | 42 |
| 8 | **dC$^I$TP** | DHB-B(OH)$_2$ | **dC$^{DHB}$TP** | 41 |
| 9 | **dC$^{MOP}$** | 1) $PO(OMe)_3$,$POCl_3$; 2) $(NHBu_3)_2H_2P_2O_7$, $Bu_3N$, DMF; 3) 2 M TEAB | **dC$^{MOP}$TP** | 28 |

[a] Yield of isolated product. [b] Product was not separable from TPPTS.

## Polymerase incorporation of dN$^X$TPs

The four new dNTPs (**dA$^{MOP}$TP**, **dC$^{MOP}$TP**, **dA$^{DHB}$TP**, and **dC$^{DHB}$TP**) were then tested as substrates for DNA polymerases[16] in primer extension experiments (PEX) using KOD XL and Pwo polymerases (for sequences of primers and templates, see Table 2). At first, single nucleotide incorporation of each of the **dN$^X$TP**s into a 15-nucleotide (nt) primer was followed by three natural dG using temp$^A$ or temp$^C$ templates (Figure 1). In all cases, fully extended product was observed on polyacrylamide gel electrophoresis (PAGE). All the products were also characterized by MALDI-TOF mass spectrometry (see Table 3). Kinetic experiments (see Figures S1–S12 in the Supporting Information) showed that the modified **dN$^X$TP**s were incorporated with only slightly slower rate than dATP or dCTP.

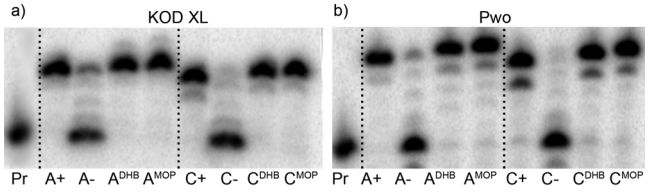


**Figure 1.** Primer extension with a) KOD XL and b) Pwo polymerases using prim$^{rnd}$, temp$^A$, and temp$^C$: (**Pr**) primer (5′-$^{32}$P-end labeled); (**A+**) dATP, dGTP; (**A−**) dGTP; (**A$^{DHB}$**) **dA$^{DHB}$TP**, dGTP; (**A$^{MOP}$**) **dA$^{MOP}$TP**, dGTP; (**C+**) dCTP, dGTP; (**C−**) dGTP; (**C$^{DHB}$**) **dC$^{DHB}$TP**, dGTP; (**C$^{MOP}$**) **dC$^{MOP}$TP**, dGTP.

Then, the **dN$^X$TP**s were incorporated individually into a longer oligonucleotide (ON) by using temp$^{rnd16}$ designed to encode for four modifications at each nucleobase. Also here, the PEX gave full-length products in the presence of KOD XL polymerase (Figure 2). Also, a combination of a modified A and modified C (**A$^{DHB}$** + **C$^{MOP}$** or **A$^{MOP}$** + **C$^{DHB}$**) was successfully incorporated to give full-length ONs bearing two different modifications at A and C bases. These ONs were characterized by MALDI-TOF mass spectrometry (Table 3).

Then we tried to challenge the polymerases by incorporation of four modifications in a row by using temp$^{4C}$ or temp$^{4A}$. Figure 3 shows that KOD XL gave full-length products in all cases (also confirmed by MALDI-TOF analysis), whereas Pwo gave mixtures of full-length and $n+1$ products in the case of incorporation of **A$^{DHB}$** and **A$^{MOP}$** (Figure 3a) and $n-1$ products with **C$^{MOP}$** (Figure 3b).

To study the compatibility of the MOP label with previously reported aminophenyl[5] and benzofurazane[11] groups, we prepared PEX products containing combinations of **C$^{MOP}$** with 7-

**Table 2.** List of sequences of templates, primer, and products.[a]

| Oligonucleotide | Sequence |
|---|---|
| primer$^{rnd}$ | 5′-CATGGGCGGCATGGG-3′ |
| temp$^{rnd16}$ | 5′-CTAGCATGAGCTCAGT*CCCATGCCGCCCATG*-3′ |
| temp$^{4A}$ | 5′-TTTT*CCCATGCCGCCCATG*-3′ |
| temp$^{4C}$ | 5′-GGGG*CCCATGCCGCCCATG*-3′ |
| temp$^{A}$ | 5′-CCCT*CCCATGCCGCCCATG*-3′ |
| temp$^{C}$ | 5′-CCCG*CCCATGCCGCCCATG*-3′ |
| temp$^{tempA}$ | 5′-*TCCCATGCCGCCCATG*-3′ |
| temp$^{tempC}$ | 5′-*GCCCATGCCGCCCATG*-3′ |
| ON$^{rnd16}$ **A$^{DHB}$** | 5′-CATGGGCGGCATGGG**A$^{DHB}$**CTG**A$^{DHB}$**GCTC**A$^{DHB}$**TGCT**A$^{DHB}$**G-3′ |
| ON$^{rnd16}$ **A$^{MOP}$** | 5′-CATGGGCGGCATGGG**A$^{MOP}$**CTG**A$^{MOP}$**GCTC**A$^{MOP}$**TGCT**A$^{MOP}$**G-3′ |
| ON$^{rnd16}$ **C$^{DHB}$** | 5′-CATGGGCGGCATGGG**AC$^{DHB}$**TGAG**C$^{DHB}$C$^{DHB}$**ATG**C$^{DHB}$**TAG-3′ |
| ON$^{rnd16}$ **C$^{MOP}$** | 5′-CATGGGCGGCATGGG**AC$^{MOP}$**TGAG**C$^{MOP}$T$^{MOP}$**ATG**C$^{MOP}$**TAG-3′ |
| ON$^{rnd16}$ **A$^{DHB}$ C$^{MOP}$** | 5′-CATGGGCGGCATGGG**A$^{DHB}$C$^{MOP}$**TG**A$^{DHB}$**G**C$^{MOP}$**T**C$^{MOP}$**A$^{DHB}$**TG**C$^{MOP}$**TA$^{DHB}$**G-3′ |
| ON$^{rnd16}$ **A$^{MOP}$ C$^{DHB}$** | 5′-CATGGGCGGCATGGG**A$^{MOP}$C$^{DHB}$**TG**A$^{MOP}$**G**C$^{DHB}$**T**C$^{DHB}$**A$^{MOP}$**TG**C$^{DHB}$**TA$^{MOP}$**G-3′ |
| ON$^{rnd16}$ **A$^{NH2}$ C$^{MOP}$** | 5′-CATGGGCGGCATGGG**A$^{NH2}$C$^{MOP}$**TG**A$^{NH2}$**G**C$^{MOP}$**T**C$^{MOP}$**A$^{NH2}$**TG**C$^{MOP}$**TA$^{NH2}$**G-3′ |
| ON$^{rnd16}$ **A$^{BF}$ C$^{MOP}$** | 5′-CATGGGCGGCATGGG**A$^{BF}$C$^{MOP}$**TG**A$^{BF}$**G**C$^{MOP}$**T**C$^{MOP}$**A$^{BF}$**TG**C$^{MOP}$**TA$^{BF}$**-3′ |
| ON$^{4A}$ **A$^{DHB}$** | 5′-CATGGGCGGCATGGG **A$^{DHB}$ A$^{DHB}$ A$^{DHB}$ A$^{DHB}$**-3′ |
| ON$^{4A}$ **A$^{MOP}$** | 5′-CATGGGCGGCATGGG **A$^{MOP}$ A$^{MOP}$ A$^{MOP}$ A$^{MOP}$**-3′ |
| ON$^{4A1C}$ **A$^{DHB}$** | 5′-CATGGGCGGCATGGG **A$^{DHB}$ A$^{DHB}$ A$^{DHB}$ A$^{DHB}$**-3′ |
| ON$^{4A1C}$ **A$^{MOP}$** | 5′-CATGGGCGGCATGGG **A$^{MOP}$ A $^{MOP}$ A$^{MOP}$ A$^{MOP}$**-3′ |
| ON$^{4C}$ **C$^{DHB}$** | 5′-CATGGGCGGCATGGG **C$^{DHB}$ C$^{DHB}$ C$^{DHB}$ C$^{DHB}$**-3′ |
| ON$^{4C}$ **C$^{MOP}$** | 5′-CATGGGCGGCATGGG **C$^{MOP}$ C$^{MOP}$ C$^{MOP}$ C$^{MOP}$**-3′ |
| ON$^{A}$ **A$^{DHB}$** | 5′-CATGGGCGGCATGGG **A$^{DHB}$** GGG-3′ |
| ON$^{A}$ **A$^{MOP}$** | 5′-CATGGGCGGCA GGG **A$^{MOP}$** GGG-3′ |
| ON$^{C}$ **C$^{DHB}$** | 5′-CATGGGCGGCATGGG **C$^{DHB}$** GGG-3′ |
| ON$^{C}$ **C$^{MOP}$** | 5′-CATGGGCGGCATGGG **C$^{MOP}$** GGG-3′ |
| ON$^{tempA}$ **A$^{DHB}$** | 5′-CATGGGCGGCATGGG **A$^{DHB}$**-3′ |
| ON$^{tempA}$ **A$^{MOP}$** | 5′-CATGGGCGGCATGGG **A$^{MOP}$**-3′ |
| ON$^{tempC}$ **C$^{DHB}$** | 5′-CATGGGCGGCATGGG **C$^{DHB}$**-3′ |
| ON$^{tempC}$ **C$^{MOP}$** | 5′-CATGGGCGGCATGGG **C$^{MOP}$**-3′ |

[a] For magnetic separation of the extended primer strands, the templates were 5′-end biotinylated; acronyms used in the text for primer extension products correspond to those used for the templates.

(3-aminophenyl)-7-deazaadenine (**A$^{NH2}$**) or with 7-(benzofurazan-5-yl)-7-deazaadenine (**A$^{BF}$**). The PEX experiments were performed using KOD XL or Pwo polymerases and template temp$^{rnd16}$. Figure 4 shows the incorporation of either **dC$^{MOP}$TP** or **dA$^{NH2}$TP** separately and in combination. In all cases, full-length ONs bearing either one or two different labels at A and/or C bases were obtained.

Similarly, PEX incorporations of **dC$^{MOP}$TP** in combination with **dA$^{BF}$TP** were performed (Figure 5). KOD XL polymerase gave clean full-length products for each modified DNA, whereas when using Pwo, some shorter ON side-products were also observed on PAGE. Therefore, labeled ONs prepared by KOD XL polymerase incorporations were further used in electrochemical studies. These products were also characterized by MALDI-TOF mass spectrometry (Table 3).

Next, we tried incorporation of a large number of modifications into double-stranded DNA by the polymerase chain reaction (PCR). The experiments using 98-mer template and each of the modified **dN$^{X}$TP**s in the presence of KOD XL polymerase proceeded well to give good amplification of the modified DNA. Also, both combinations of the modified A and C (**A$^{DHB}$** + **C$^{MOP}$** or **A$^{MOP}$** + **C$^{DHB}$**) gave the correct product (Figure 6). This result confirms that all four modified **dN$^{X}$TP**s are very good substrates, at least for KOD XL polymerase.

**Table 3.** List of MALDI-TOF mass spectrometry data of ONs bearing MOP and DHB derivatives.

| Oligonucleotide | *M* calcd [Da] | *M* found [Da] |
|---|---|---|
| ON$^{rnd16}$ **A$^{DHB}$** | 10085.90 | 10085.9 |
| ON$^{rnd16}$ **A$^{MOP}$** | 10101.86 | 10101.3 |
| ON$^{rnd16}$ **C$^{DHB}$** | 10089.90 | 10090.6 |
| ON$^{rnd16}$ **C$^{MOP}$** | 10105.86 | 10107.0 |
| ON$^{rnd16}$ **A$^{DHB}$ C$^{MOP}$** | 10574.46 | 10575.2 |
| ON$^{rnd16}$ **A$^{MOP}$ C$^{DHB}$** | 10574.46 | 10575.3 |
| ON$^{rnd16}$ **A$^{NH2}$ C$^{MOP}$** | 10466.34 | 10467.8 |
| ON$^{rnd16}$ **A$^{BF}$ C$^{MOP}$** | 10574.26 | 10575.8 |
| ON$^{4A}$ **A$^{DHB}$** | 6395.50 | 6396.7 |
| ON$^{4A}$ **A$^{MOP}$** | 6411.50 | 6433.3[a] |
| ON$^{4C}$ **C$^{DHB}$** | 6303.40 | 6304.9 |
| ON$^{4C}$ **C$^{MOP}$** | 6319.36 | 6320.7 |
| ON$^{A}$ **A$^{DHB}$** | 6092.05 | 6093.4 |
| ON$^{A}$ **A$^{MOP}$** | 6096.04 | 6097.2 |
| ON$^{C}$ **C$^{DHB}$** | 6069.05 | 6070.1 |
| ON$^{C}$ **C$^{MOP}$** | 6073.04 | 6074.0 |

[a] Found mass corresponds to $[M+Na]^+$.

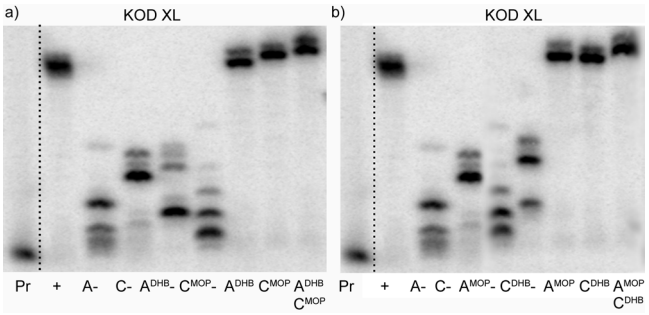


**Figure 2.** Denaturing PAGE analysis of a) **A$^{DHB}$TP** and **C$^{MOP}$TP**, and b) **A$^{MOP}$TP** and **C$^{DHB}$TP** by using KOD XL polymerase, prim$^{rnd}$, and temp$^{rnd16}$. Compositions of the dNTP mixes and nucleotide labeling are as follows: (**Pr**) primer (5′-$^{32}$P-end labeled); (**+**) natural dNTPs; (**A**−) dCTP, dTTP, dGTP; (**C**−) dATP, dTTP, dGTP; (**A$^{DHB}$**−) **dC$^{MOP}$TP**, dTTP, dGTP; (**A$^{MOP}$**−) **dC$^{DHB}$TP**, dTTP, dGTP; (**C$^{MOP}$**−) **dA$^{DHB}$TP**, dTTP, dGTP; (**C$^{DHB}$**−) **dA$^{MOP}$TP**, dTTP, dGTP; (**A$^{DHB}$**) **dA$^{DHB}$TP**, dCTP, dTTP, dGTP; (**A$^{MOP}$**) **dA$^{MOP}$TP**, dCTP, dTTP, dGTP; (**C$^{MOP}$**) **dC$^{MOP}$TP**, dATP, dTTP, dGTP; (**C$^{DHB}$**) **dC$^{DHB}$TP**, dATP, dTTP, dGTP; (**A$^{DHB}$C$^{MOP}$**) **dA$^{DHB}$TP**, **dC$^{MOP}$TP**, dTTP, dGTP; (**A$^{MOP}$C$^{DHB}$**) **dA$^{MOP}$TP**, **dC$^{DHB}$TP**, dTTP, dGTP.

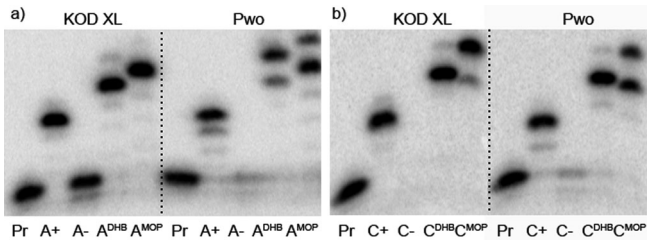


**Figure 3.** Primer extension with a) temp$^{4A}$ and b) temp$^{4C}$ templates using prim$^{rnd}$, KOD XL, and Pwo polymerases: (**Pr**) primer (5′-$^{32}$P-end labeled); (**A+**) dATP; (**A**−) no dNTPs; (**A$^{DHB}$**) **dA$^{DHB}$TP**; (**A$^{MOP}$**) **dA$^{MOP}$TP**; (**C+**) dCTP; (**C**−) no dNTPs; (**C$^{DHB}$**) **dC$^{DHB}$TP**; (**C$^{MOP}$**) **dC$^{MOP}$TP**.

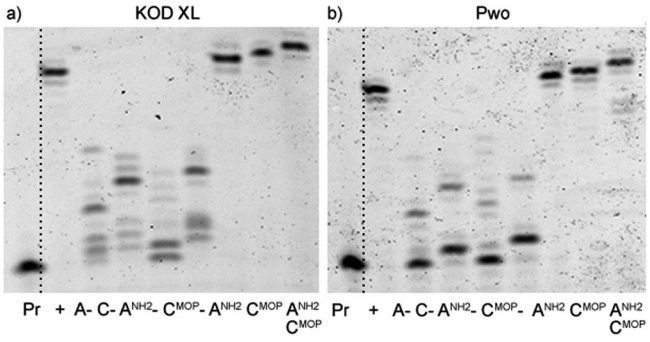


**Figure 4.** Denaturing PAGE analysis of **A$^{NH2}$TP** and **C$^{MOP}$TP** by using a) KOD XL or b) Pwo polymerases, prim$^{rnd}$, and temp$^{rnd16}$. Compositions of the dNTP mixes and nucleotide labeling are as follows: (**Pr**) primer (5′-fluorescein amidite (FAM)■■OK?■■ labeled); (**+**) natural dNTPs; (**A**−) dCTP, dTTP, dGTP; (**C**−) dATP, dTTP, dGTP; (**A$^{NH2}$**−) **dC$^{MOP}$**TP, dTTP, dGTP; (**C$^{MOP}$**−) **dA$^{NH2}$TP**, dTTP, dGTP; (**A$^{NH2}$**) **dA$^{NH2}$TP**, dCTP, dTTP, dGTP; (**C$^{MOP}$**) **dC$^{MOP}$TP**, dATP, dTTP, dGTP; (**A$^{NH2}$C$^{MOP}$**) **dA$^{NH2}$TP**, **dC$^{MOP}$TP**, dTTP, dGTP.

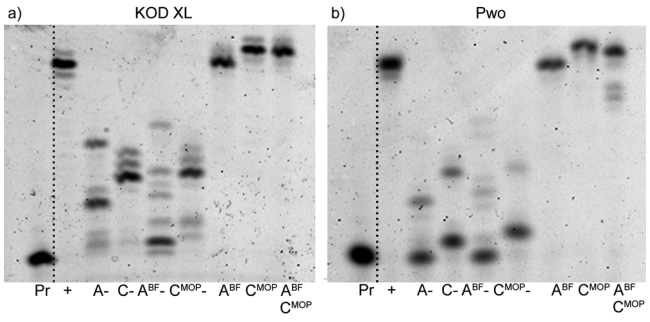


**Figure 5.** Denaturing PAGE analysis of **A$^{BF}$TP** and **C$^{MOP}$TP** by using a) KOD XL or b) Pwo polymerases, prim$^{rnd}$, and temp$^{rnd16}$. Compositions of the dNTP mixes and nucleotide labeling are as follows: (**Pr**) primer (5′-FAM labeled); (**+**) natural dNTPs; (**A**−) dCTP, dTTP, dGTP; (**C**−) dATP, dTTP, dGTP; (**A$^{BF}$**−) **dC$^{MOP}$**TP, dTTP, dGTP; (**C$^{MOP}$**−) **dA$^{BF}$TP**, dTTP, dGTP; (**A$^{BF}$**) **dA$^{BF}$TP**, dCTP, dTTP, dGTP; (**C$^{MOP}$**) **dC$^{MOP}$TP**, dATP, dTTP, dGTP; (**A$^{BF}$C$^{MOP}$**) **dA$^{BF}$TP**, **dC$^{MOP}$TP**, dTTP, dGTP.

## Electrochemistry

The electrochemical behavior was first studied on model nucleosides (**dA$^{MOP}$**, **dC$^{MOP}$**, **dA$^{DHB}$**, and **dC$^{DHB}$**) and **dN$^{X}$TP**s (**dA$^{MOP}$TP**, **dC$^{MOP}$TP**, **dA$^{DHB}$TP**, and **dC$^{DHB}$TP**) and compared to that of the starting boronic acids/boronates MOP-B(pin) and DHB-B(OH)$_2$ by using square-wave voltammetry (SWV) at the basal-plane pyrolytic graphite electrode (Figure 7). The MOP-B(pin) yielded under these conditions a single anodic (oxidation) peak around 600 mV (peak MOP$^{ox}$, Figure 7a, Table 4), whereas DHB-B(OH)$_2$ produced two distinct signals at 785 and 950 mV (peaks marked DHB$^{ox}$, Figure 7a). All electrode process-

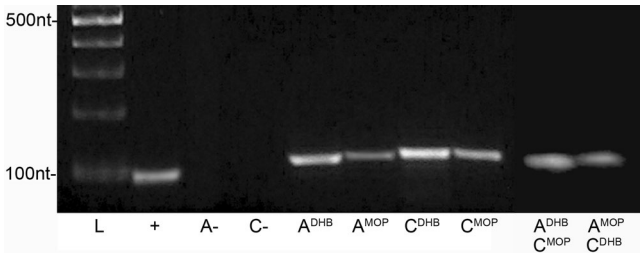


**Figure 6.** PCR synthesis of 98-mer by KOD XL polymerase: (**L**) DNA ladder; (**+**) dATP, dCTP, dTTP, dGTP; (**A**−) dCTP, dTTP, dGTP; (**C**−) dATP, dTTP, dGTP; (**A$^{DHB}$**) **dA$^{DHB}$TP**, dCTP, dTTP, dGTP; (**A$^{MOP}$**) **A$^{MOP}$TP**, dCTP, dTTP, dGTP; (**C$^{DHB}$**) **dC$^{DHB}$TP**, dATP, dTTP, dGTP; (**C$^{MOP}$**) **C$^{MOP}$TP**, dATP, dTTP, dGTP; (**A$^{DHB}$C$^{MOP}$**) **dA$^{DHB}$TP**, **dC$^{MOP}$TP**, dTTP, dGTP, (**A$^{MOP}$C$^{DHB}$**) **dA$^{MOP}$TP**, **dC$^{DHB}$TP**, dTTP, dGTP.

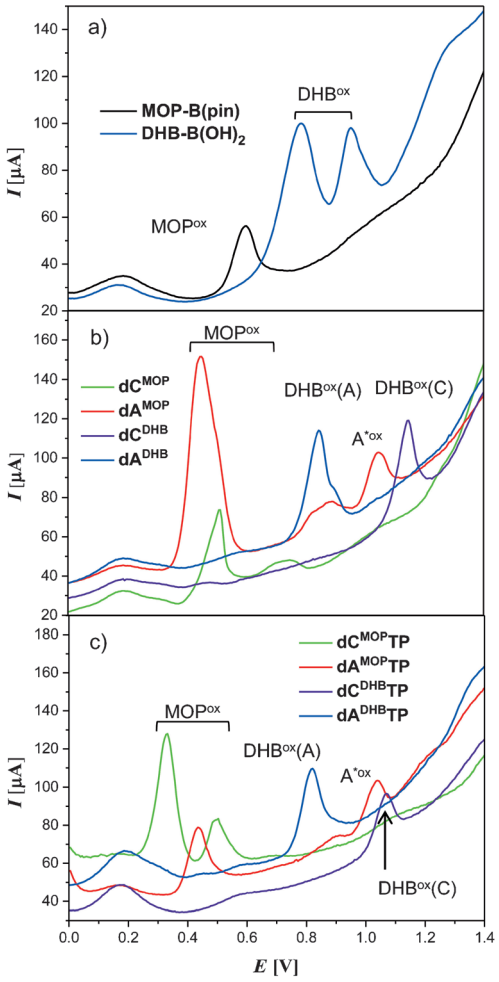


**Figure 7.** Square-wave voltammograms of boronic acids (a), modified nucleosides (b), and **dN$^{X}$TPs** (c). All experiments were done at a pyrolytic graphite electrode in 0.2 M acetate buffer (pH 5.0) with 40 μM concentration of samples. The peak around 200 mV is related to oxygen-containing groups at the graphite electrode surface.

**Table 4.** Potential peaks produced by boronic acids, nucleosides, **dN*TP**s, and PEX products measured at a pyrolytic graphite electrode using SWV (values in mV against Ag/AgCl/3 M KCl reference electrode).

| Sample | Peaks | | | | |
|---|---|---|---|---|---|
| | $MOP^{ox}$ | $DHB^{ox}$ | $A^{*ox}$ | $G^{ox}$ | $A^{ox}$ |
| MOP-B(pin) | 600 | – | – | – | – |
| DHB-$B(OH)_2$ | – | 785, 950 | – | – | – |
| **$dC^{MOP}$** | 510 | – | – | – | – |
| **$dA^{MOP}$** | 450 | – | 1045 | – | – |
| **$dC^{DHB}$** | – | 1150 | – | – | – |
| **$dA^{DHB}$** | – | 845 | – | – | – |
| **$dC^{MOP}TP$** | 330, 500 | – | – | – | – |
| **$dA^{MOP}TP$** | 435 | – | 1040 | – | – |
| **$dC^{DHB}TP$** | – | 1070 | – | – | – |
| **$dA^{DHB}TP$** | – | 820 | – | – | – |
| PEX **$C^{MOP}$** | 590 | – | – | 1100 | 1360 |
| PEX **$A^{MOP}$** | 510 | – | – | 1095 | 1370 |
| PEX **$A^{Ph}$** | – | – | – | 1100 | 1380 |
| PEX **$C^{DHB}$** | – | 1100?[a] | – | 1100[a] | 1360 |
| PEX **$A^{DHB}$** | – | 870 | – | 1090 | 1360 |
| unlabeled PEX | – | – | – | 1100 | 1380 |

[a] Overlapping signals.

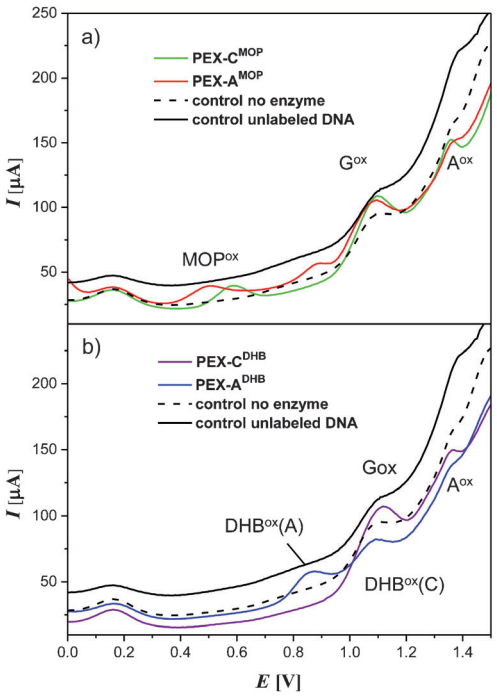


**Figure 8.** AdTS square-wave voltammograms of single-stranded PEX products bearing four MOP (a) or four DHB (b) labels in sequences synthesized on the $temp^{rnd16}$ template.

es giving rise to these signals took place in the adsorbed state and were irreversible (data not shown).

Oxidation signals of the MOP moiety were retained in both nucleoside conjugates (Figure 7b) and appeared at less positive potentials than observed for the corresponding boronic acid (around 450 mV for **$dA^{MOP}$** and around 510 mV for **$dC^{MOP}$**, Figure 7b). In addition to these well-developed peaks, both MOP-labeled nucleosides produced poorly developed double peaks around 750 and 880 mV for **$dA^{MOP}$** and **$dC^{MOP}$**, respectively. Another distinct anodic signal was produced by **$dA^{MOP}$** at 1045 mV (peak $A^{*ox}$), which can be attributed to oxidation of the 7-deazaadenine moiety[17] in the nucleobase conjugate.[6] The DHB-labeled nucleosides gave single peaks around 1070 mV (peak $DHB^{ox}$(C) of **$dC^{DHB}$**, Figure 7b) or 820 mV (peak $DHB^{ox}$(A) of **$dA^{DHB}$**; interestingly, no separate signal corresponding to the 7-deazaadenine oxidation was detected in the DHB conjugate). The nucleoside triphosphates (Figure 7c) displayed qualitatively the same electrochemical behavior as the corresponding nucleosides (Figure 7b) with the exception of **$dC^{MOP}TP$**, which yielded two well-developed anodic signals in the potential region around 300 and 500 mV. Differences in the peak heights produced by corresponding species (boronic acid versus nucleosides versus triphosphates) may be a result of effects of the nucleobase type and/or of the negatively charged triphosphate group on the adsorbability of the given substance at the positively charged electrode surface and accessibility of the oxidizable moieties for the electrode reaction.

Then, the single-stranded DNA PEX products prepared by the magnetoseparation procedure, each bearing four modifications, were studied by adsorptive transfer stripping (AdTS) SWV (Figure 8). Regardless of the presence or absence of any of the two labels, all PEX products gave peaks $G^{ox}$ (around 1100 mV) and $A^{ox}$ (around 1370 mV) owing to oxidation of natural guanine and adenine nucleobases, respectively. For ON labeled with **$C^{MOP}$**, an additional anodic signal appeared around 590 mV (Figure 8a) whereas ON bearing the **$A^{MOP}$** conjugate gave two signals at potentials of 510 and 900 mV. For the **$A^{DHB}$**-labeled ON (Figure 8b), an extra peak was detected at 870 mV. For ON bearing the **$C^{DHB}$** conjugates, no signal outside the region of natural purine oxidation was detected but a remarkable increase of the peak occurring at 1100 V was observed, which suggests that in the ON, the peak of DHB oxidation in **$C^{DHB}$** overlapped with the peak $G^{ox}$ (Figure 8b).

Taken together, the MOP moiety appears to be a potentially useful label for both cytosine and adenine nucleobases, as the corresponding conjugates produce signals detectable in a potential region in which natural DNA components (as well as unlabeled 7-deazapurines[17]) are electrochemically silent. Difference in peak potentials makes it possible to distinguish between DNA bearing **$C^{MOP}$** and that containing **$A^{MOP}$**. Moreover, **$C^{MOP}$** producing a single oxidation signal can easily be used for multipotential DNA coding in combination with other oxidizable labels differing in the potentials of their electrooxidation. For example, a PEX product labeled simultaneously with **$C^{MOP}$** and **$A^{NH2}$** produced two independently readable peaks $MOP^{ox}$ and $NH_2{}^{ox}$ (the latter a result of oxidation of the aminophenyl moiety[5] around 830 mV, Figure 9a). An example of combination of MOP with an irreversibly reducible label, benzofurazane (BF),[11] is demonstrated in Figure 9b and c. In this case the oxidizable label MOP produces a specific signal only in the anodic potential scan and BF only in the cathodic one (peak $BF^{red}$ around −900 mV, Figure 9c), thereby making their differentiation even more obvious.

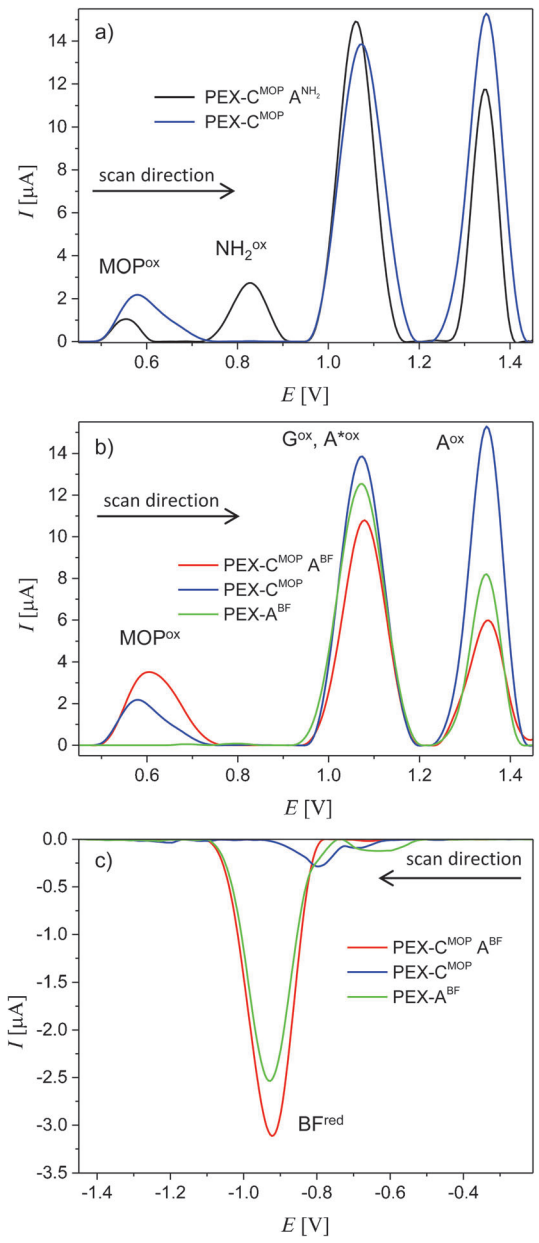


**Figure 9.** Sections of baseline-corrected AdTS square-wave voltammograms of PEX products labeled with $\mathbf{C}^{MOP}$ in combination with $\mathbf{A}^{BF}$ (a, b) or with $\mathbf{A}^{NH2}$ (c); in (a, c) electrochemical oxidation and in (b) electrochemical reduction are shown. The $temp^{rnd16}$ template was used for the PEX.

On the other hand, utilization of DHB as a DNA label is limited to $\mathbf{A}^{DHB}$ owing to coincidence of $\mathbf{C}^{DHB}$ oxidation with guanine oxidation. Moreover, DHB cannot be used for independent DNA labeling in combination with $\mathbf{A}^{MOP}$ because of coincidence of their oxidation peaks around 900 mV. Compared with $\mathbf{C}^{MOP}$, the applicability of both $\mathbf{A}^{DHB}$ and $\mathbf{A}^{MOP}$ in the multipotential labeling scheme in combination with earlier introduced oxidizable labels is also limited as a result of interference of oxidation signals close to 900 mV, at which the oxidation signals of aminophenyl (Figure 9a) and 7-deazaguanine[17] also appear.

## Conclusion

4-Hydroxy-3-methoxyphenyl (MOP) and 2,3-dihydrobenzofuran-5-yl (DHB) groups were attached to nucleosides and 2′-deoxyribonucleoside triphosphates (dNTPs) and tested as new oxidizable redox labels for DNA. The synthesis of the modified nucleosides and dNTPs by the aqueous-phase Suzuki cross-coupling reactions is straightforward and efficient. The corresponding modified $\mathbf{dN^XTP}$s were good substrates for DNA polymerases and were easily incorporated into DNA by primer extension experiments or polymerase chain reaction. The labeled nucleosides, dNTPs, and DNA were studied systematically by several electrochemical techniques. In nucleosides and dNTPs, both MOP and DHB labels show distinct signals resulting from oxidation of the label. However, after incorporation into DNA, only the MOP label appears to be useful since its oxidation signals occur at about 500–600 mV, at which they do not interfere either with any signals of inherent oxidation of DNA or with those of some other earlier designed oxidizable labels. Particularly, the $\mathbf{C}^{MOP}$ conjugate appears to be a candidate for application in multipotential DNA labeling in combination with other oxidizable or reducible labels. The use of DHB label would be limited only for modified adenine, which gives an oxidation signal at 870 mV, whereas the oxidation of the corresponding cytosine conjugate ($\mathbf{C}^{DHB}$) interferes with oxidation of guanine.

## Experimental Section

### General

NMR spectra were measured on a Bruker Avance 600 spectrometer (600 MHz for $^1$H and 151 MHz for $^{13}$C nuclei) and a Bruker 500 spectrometer (500 MHz for $^1$H, 125.7 MHz for $^{13}$C, and 202.3 for $^{31}$P) in $D_2O$ (referenced to dioxane as internal standard, $\delta H = 3.75$ ppm, $\delta C = 67.19$ ppm, standard for $^{31}$P NMR was external $H_3PO_4$). Chemical shifts are given in ppm ($\delta$ scale), and coupling constants ($J$) in Hertz. Complete assignment of all NMR signals was performed using a combination of H,H-COSY, H,C-HSQC, and H,C-HMBC experiments. Mass spectra were measured on an LCQ classic (Thermo-Finnigan) spectrometer using electrospray ionization (ESI) or Q-Tof Micro (Waters, ESI source, internal calibration with lockspray). Preparative HPLC separations were performed on a column packed with 10 µm C18 reversed phase (Phenomenex, Luna C18(2)). IR spectra were measured by using the attenuated total reflectance technique or by using KBr tablets. High-resolution mass spectra were measured by using the ESI technique. Mass spectra of functionalized DNA were measured by MALDI-TOF, Reflex IV (Bruker) with a nitrogen laser. UV/Vis spectra were measured on a Varian CARY 100 Bio spectrophotometer at room temperature. Melting points were determined on a Kofler block. Known starting compounds $\mathbf{dA^ITP}$,[5] $\mathbf{dC^ITP}$,[15b] $\mathbf{dA^{Ph}TP}$,[18] and $\mathbf{dA^{BF}TP}$[11] were prepared by literature procedures.

### General procedure for the synthesis of modified nucleosides: Suzuki–Miyaura cross-coupling

Method A: A 2:1 mixture of $H_2O/CH_3CN$ (2 mL) was added through a septum to an argon-purged flask containing halogenated nucleosides $\mathbf{dN^I}$ (1 equiv), boronic acid/boronate (2 equiv), and $Cs_2CO_3$

(3 equiv). In a separate flask, $Pd(OAc)_2$ (10 mol%) and TPPTS (2.5 equiv with respect to Pd) were combined, the flask was evacuated and purged with argon, and then a 2:1 mixture of $H_2O/CH_3CN$ (1 mL) was added. This catalyst solution was injected into the reaction mixture, which was then stirred at 75 °C for 1 h until complete consumption of the starting material and then evaporated in vacuo. The products were purified by silica gel column chromatography using chloroform/methanol (0 to 10%) as eluent.

### General procedure for the synthesis of modified nucleotide triphosphates: Suzuki–Miyaura cross-coupling

Method B: A 2:1 mixture of $H_2O/CH_3CN$ (1 mL) was added through a septum to an argon-purged flask containing halogenated nucleotides **dN$^{I}$TP** (1 equiv), boronic acid/boronate (2 equiv), and $Cs_2CO_3$ (3 equiv). In a separate flask, $Pd(OAc)_2$ (10 mol%) and TPPTS (2.5 equiv with respect to Pd) were combined, the flask was evacuated and purged with argon, and then a 2:1 mixture of $H_2O/CH_3CN$ (0.5 mL) was added. This catalyst solution was injected into the reaction mixture, which was then stirred at 50 °C for 40 min until complete consumption of the starting material and then evaporated in vacuo. The 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 triethylammonium bicarbonate (TEAB) 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 a solid product.

### Synthesis of modified nucleoside triphosphates: triphosphorylation

Method C: $POCl_3$ (1.2 equiv) in $PO(OMe)_3$ was added through a septum to an argon-purged flask containing modified nucleosides **dN$^{X}$** (1 equiv). The reaction mixture was then stirred at 0 °C for 3 h until complete consumption of the starting material. Then an ice-cooled solution of $(NHBu_3)_2H_2P_2O_7$ (5 equiv) and $Bu_3N$ (4.2 equiv) in dry DMF (2 mL) was added and the mixture was stirred at 0 °C for another 1.5 h. The reaction was quenched by addition of 2 M aqueous TEAB (2 mL), the solvents were evaporated in vacuo, and the residue was co-distilled with water three times. The product was isolated by HPLC on a C18 column with the use of a linear gradient of 0.1 M TEAB 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 a solid product.

**dA$^{MOP}$**: Compound **dA$^{MOP}$** was prepared from **dA$^{I}$** according to the general procedure (Method A). The product was isolated as a yellow solid (38 mg, 77%). M.p. 90 °C; $^1$H NMR (500.0 MHz, [D$_6$]DMSO): $\delta$ = 2.18 (ddd, $J_{gem}$ = 13.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6 Hz, 1H; H-2′b), 2.56 (ddd, $J_{gem}$ = 13.1, $J_{2'a,1'}$ = 8.3, $J_{2'a,3'}$ = 5.7 Hz, 1H; H-2′a), 3.50, 3.57 (2×ddd, $J_{gem}$ = 11.7, $J_{5',OH}$ = 5.3, $J_{5',4'}$ = 4.4 Hz, 2×1H; H-5′), 3.81 (s, 3H; $CH_3O$), 3.82 (td, $J_{4',5'}$ = 4.4, $J_{4',3'}$ = 2.5 Hz, 1H; H-4′), 4.35 (bm, 1H; H-3′), 5.07 (bt, $J_{OH,5'}$ = 5.3 Hz, 1H; OH-5′), 5.27 (bd, $J_{OH,3'}$ = 2.9 Hz, 1H; OH-3′), 6.12 (bs, 2H; $NH_2$), 6.57 (dd, $J_{1',2'}$ = 8.3, 6.0 Hz, 1H; H-1′), 6.84 (dd, $J_{6,5}$ = 8.0, $J_{6,2}$ = 1.9 Hz, 1H; H-6-$C_6H_3$OHOMe), 6.87 (d, $J_{5,6}$ = 8.0 Hz, 1H; H-5-$C_6H_3$OHOMe), 6.98 (d, $J_{2,6}$ = 1.9 Hz, 1H; H-2-$C_6H_3$OHOMe), 7.41 (s, 1H; H-6), 8.12 (s, 1H; H-2), 9.14 ppm (bs, 1H; OH-4-$C_6H_3$OHOMe); $^{13}$C NMR (125.7 MHz, [D$_6$]DMSO): $\delta$ = 39.53 ($CH_2$-2′), 55.76 ($CH_3O$), 62.20 ($CH_2$-5′), 71.25 (CH-3′), 83.06 (CH-1′), 87.49 (CH-4′), 100.79 (C-4a), 113.02 (CH-2-$C_6H_3$OHOMe), 116.04 (CH-5-$C_6H_3$OHOMe), 116.86 (C-5), 120.03 (CH-6), 121.08 (CH-6-$C_6H_3$OHOMe), 125.57 (C-1-$C_6H_3$OHOMe), 146.03 (C-4-$C_6H_3$OHOMe), 147.96 (C-3-$C_6H_3$OHOMe), 150.34 (C-7a), 151.77 (CH-2), 157.48 ppm (C-4); IR (KBr): $\tilde{\nu}$ = 3497, 3385, 2968, 2865, 1735, 1630, 1470, 1291, 1097, 1052 cm$^{-1}$; MS (ESI+): *m/z* (%): 373.2 (100) [$M^+$+H], 395.2 (54) [$M^+$+Na]; HRMS (ESI+): *m/z* calcd for $C_{18}H_{21}N_4O_5$: 373.15060; found: 373.15065; calcd for $C_{18}H_{20}N_4O_5Na$: 395.13254; found: 395.3259.

**dC$^{MOP}$**: Compound **dC$^{MOP}$** was prepared from **dC$^{I}$** according to the general procedure (Method A). The product was isolated as a yellow solid (48 mg, 83%). M.p. 204 °C; $^1$H NMR (499.8 MHz, [D$_6$]DMSO): $\delta$ = 2.12 (m, 2H; H-2′), 3.50, 3.57 (2×ddd, $J_{gem}$ = 11.7, $J_{5',OH}$ = 5.1, $J_{5',4'}$ = 3.4 Hz, 2×1H; H-5′), 3.77 (q, $J_{4',3'}$ = $J_{4',5'}$ = 3.4 Hz, 1H; H-4′), 3.78 (s, 3H; $CH_3O$), 4.22 (m, 1H; H-3′), 4.96 (t, $J_{OH,5'}$ = 5.1 Hz, 1H; OH-5′), 5.19 (d, $J_{OH,3'}$ = 4.2 Hz, 1H; OH-3′), 6.20 (dd, $J_{1',2'}$ = 7.1, 6.1 Hz, 1H; H-1′), 6.35 (bs, 1H; $NH_aH_b$), 6.70 (dd, $J_{6,5}$ = 8.0, $J_{6,2}$ = 2.0 Hz, 1H; H-6-$C_6H_3$OHOMe), 6.81 (d, $J_{5,6}$ = 8.0 Hz, 1H; H-5-$C_6H_3$OHOMe), 6.83 (d, $J_{2,6}$ = 2.0 Hz, 1H; H-2-$C_6H_3$OHOMe), 7.35 (bs, 1H; $NH_aH_b$), 7.79 (s, 1H; H-6), 9.10 ppm (bs, 1H; OH-4-$C_6H_3$OHOMe); $^{13}$C NMR (125.7 MHz, [D$_6$]DMSO): $\delta$ = 40.75 ($CH_2$-2′), 55.60 ($CH_3O$), 61.25 ($CH_2$-5′), 70.37 (CH-3′), 85.20 (CH-1′), 87.41 (CH-4′), 108.11 (C-5), 113.00 (CH-2-$C_6H_3$OHOMe), 115.98 (CH-5-$C_6H_3$OHOMe), 121.40 (CH-6-$C_6H_3$OHOMe), 124.75 (C-1-$C_6H_3$OHOMe), 139.64 (CH-6), 146.41 (C-4-$C_6H_3$OHOMe), 147.86 (C-3-$C_6H_3$OHOMe), 154.52 (C-2), 163.73 ppm (C-4); IR (KBr): $\tilde{\nu}$ = 3305, 2928, 2859, 1744, 1642,.1601, 1473, 1280, 1092, 1059 cm$^{-1}$; MS (ESI+): *m/z* (%): 350.2 (20) [$M^+$+H]; HRMS (ESI+): *m/z* calcd for $C_{16}H_{20}N_3O_6$: 350.13466; found: 350.13466; calcd for $C_{18}H_{20}N_4O_5Na$: 395.13254; found: 395.3259.

**dC$^{DHB}$**: Compound **dC$^{DHB}$** was prepared from **dC$^{I}$** according to the general procedure (Method A). The product was isolated as a dark purple solid (20 mg, 69%). M.p. 178 °C; $^1$H NMR (500.0 MHz, [D$_6$]DMSO): $\delta$ = 2.05 (ddd, $J_{gem}$ = 13.1, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.1 Hz 1H;, H-2′b), 2.13 (ddd, $J_{gem}$ = 13.1, $J_{2'a,1'}$ = 6.1, $J_{2'a,3'}$ = 3.5 Hz, 1H; H-2′a), 3.19 (t, $J_{3,2}$ = 8.7 Hz, 2H; H-3-dihydrobenzofuryl), 3.50, 3.55 (2×ddd, $J_{gem}$ = 11.8, $J_{5',OH}$ = 5.0, $J_{5',4'}$ = 3.5 Hz, 2×1H; H-5′), 3.76 (q, $J_{4',3'}$ = $J_{4',5'}$ = 3.5 Hz, 1H; H-4′), 4.21 (m, 1H; H-3′), 4.54 (t, $J_{2,3}$ = 8.7 Hz, 2H; H-2-dihydrobenzofuryl), 4.94 (t, $J_{OH,5'}$ = 5.0 Hz, 1H; OH-5′), 5.19 (d, $J_{OH,3'}$ = 4.2 Hz, 1H; OH-3′), 6.20 (dd, $J_{1',2'}$ = 7.1, 6.1 Hz, 1H; H-1′), 6.24 (bs, 1H; $NH_aH_b$), 6.79 (d, $J_{7,6}$ = 8.2 Hz, 1H; H-7-dihydrobenzofuryl), 7.01 (dd, $J_{6,7}$ = 8.2, $J_{6,4}$ = 2.0 Hz, 1H; H-6-dihydrobenzofuryl), 7.16 (d, $J_{4,6}$ = 2.0 Hz, 1H; H-4-dihydrobenzofuryl), 7.34 (bs, 1H; $NH_aH_b$), 7.75 ppm (s, 1H; H-6); $^{13}$C NMR (125.7 MHz, [D$_6$]DMSO): $\delta$ = 29.31 ($CH_2$-3-dihydrobenzofuryl), 40.70 ($CH_2$-2′), 61.27 ($CH_2$-5′), 70.41 (CH-3′), 71.30 ($CH_2$-2-dihydrobenzofuryl), 85.12 (CH-1′), 87.36 (CH-4′), 108.04 (C-5), 109.43 (CH-7-dihydrobenzofuryl), 125.95 (C-5-dihydrobenzofuryl), 126.08 (CH-4-dihydrobenzofuryl), 128.24 (C-3a-dihydrobenzofuryl), 128.86 (CH-6-dihydrobenzofuryl), 139.58 (CH-6), 154.72 (C-2), 159.53 (C-7a-dihydrobenzofuryl), 163.94 ppm (C-4); IR (KBr): $\tilde{\nu}$ = 3410, 2929, 2860, 1649, 1601, 1475, 1233,1192, 1094, 1053 cm$^{-1}$; MS (ESI+): *m/z* (%): 346.1 (35) [*M*++H]; 368.1 (100) [*M*++Na]; HRMS (ESI+): *m/z* calcd for $C_{17}H_{20}O_5N_3$: 346.13976; found: 346.13975; calcd for $C_{17}H_{19}O_5N_3Na$: 368.12157; found: 368.12169.

**dA$^{DHB}$**: Compound **dA$^{DHB}$** was prepared from **dA$^{I}$** according to the general procedure (Method A). The product was isolated as a weak yellow solid (38 mg, 78%). M.p. 107 °C; $^1$H NMR (499.8 MHz, [D$_6$]DMSO): $\delta$ = 2.18 (ddd, $J_{gem}$ = 13.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6 Hz, 1H; H-2′b), 2.55 (ddd, $J_{gem}$ = 13.1, $J_{2'a,1'}$ = 8.4, $J_{2'a,3'}$ = 5.8 Hz, 1H; H-2′a), 3.23 (t, $J_{3,2}$ = 8.7 Hz, 2H; H-3-dihydrobenzofuryl), 3.50, 3.57 (2×ddd, $J_{gem}$ = 11.7, $J_{5',OH}$ = 5.5, $J_{5',4'}$ = 4.5 Hz, 2×1H; H-5′), 3.82 (td, $J_{4',5'}$ = 4.5, $J_{4',3'}$ = 2.4 Hz, 1H; H-4′), 4.35 (bm, 1H; H-3′), 4.57 (t, $J_{2,3}$ = 8.7 Hz, 2H; H-2-dihydrobenzofuryl), 5.05 (t, $J_{OH,5'}$ = 5.5 Hz, 1H; OH-5′), 5.25 (d, $J_{OH,3'}$ = 4.1 Hz, 1H; OH-3′), 6.10 (bs, 2H; $NH_2$), 6.57 (dd, $J_{1',2'}$ = 8.4 Hz, 1H; 6.0, H-1′), 6.86 (dd, $J_{7,6}$ = 8.1, $J_{7,4}$ = 0.5 Hz, 1H; H-7-dihydroben-

zofuryl), 7.16 (ddt, $J_{6,7}=8.1$, $J_{6,4}=2.0$, $J_{6,3}=0.7$ Hz, 1H; H-6-dihydrobenzofuryl), 7.31 (dtd, $J_{4,6}=2.0$, $J_{4,3}=1.1$, $J_{4,7}=0.5$ Hz, 1H; H-4-dihydrobenzofuryl), 7.40 (s, 1H; H-6), 8.12 ppm (s, 1H; H-2); $^{13}$C NMR (125.7 MHz, [D$_6$]DMSO): $\delta=29.33$ (CH$_2$-3-dihydrobenzofuryl), 39.82 (CH$_2$-2′), 62.21 (CH$_2$-5′), 71.27 (CH$_2$-2-dihydrobenzofuryl), 71.28 (CH-3′), 83.06 (CH-1′), 87.50 (CH-4′), 100.77 (C-4a), 109.35 (CH-7-dihydrobenzofuryl), 116.70 (C-5), 120.11 (CH-6), 125.64 (CH-4-dihydrobenzofuryl), 126.65 (C-5-dihydrobenzofuryl), 128.34 (C-3a-dihydrobenzofuryl), 128.39 (CH-6-dihydrobenzofuryl), 150.35 (C-7a), 151.73 (CH-2), 157.44 (C-4), 159.17 ppm (C-7a-dihydrobenzofuryl); IR (KBr): $\tilde{\nu}=3332$, 3191, 2929, 2860,1493, 1300, 1223, 1093, 1050 cm$^{-1}$; MS (ESI+): *m/z* (%): 369.2 (100) [$M^+$+H]; HRMS (ESI+): *m/z* calcd for $C_{19}H_{21}N_4O_4$: 369.15573; found: 369.15575.

**dA$^{DHB}$TP**: Compound **dA$^{DHB}$TP** was prepared from **dA$^{I}$TP** according to the general procedure (Method B). The product was isolated as a purple solid (14 mg, 42%). $^1$H NMR (499.8 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=3.75 ppm): $\delta=2.46$ (ddd, $J_{gem}=14.0$, $J_{2'b,1'}=6.2$, $J_{2'b,3'}=3.2$ Hz, 1H; H-2′b), 2.72 (ddd, $J_{gem}=14.0$, $J_{2'a,1'}=7.8$, $J_{2'a,3'}=6.6$ Hz, 1H; H-2′a), 3.76 (t, $J_{3,2}=8.7$ Hz, 2H; H-3-dihydrobenzofuryl), 4.11 (ddd, $J_{gem}=11.0$, $J_{H,P}=5.0$, $J_{5'b,4'}=4.4$ Hz, 1H; H-5′b), 4.17 (ddd, $J_{gem}=11.0$, $J_{H,P}=6.4$, $J_{5'a,4'}=4.4$ Hz, 1H; H-5′a), 4.24 (bq, $J_{4',5'}=J_{4',3'}=4.4$ Hz, 1H; H-4′), 4.63 (t, $J_{2,3}=8.7$ Hz, 2H; H-2-dihydrobenzofuryl), 4.80 (m, 1H; H-3′ overlapped with HDO signal), 6.66 (dd, $J_{1',2'}=7.8$, 6.2 Hz, 1H; H-1′), 6.90 (d, $J_{7,6}=8.2$ Hz, 1H; H-7-dihydrobenzofuryl), 7.24 (dd, $J_{6,7}=8.2$, $J_{6,4}=1.7$ Hz, 1H; H-6-dihydrobenzofuryl), 7.35 (d, $J_{4,6}=2.0$ Hz, 1H; H-4-dihydrobenzofuryl), 7.42 (s, 1H; H-6), 8.15 ppm (s, 1H; H-2); $^{13}$C NMR (125.7 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=69.3 ppm): $\delta=31.67$ (CH$_2$-3-dihydrobenzofuryl), 40.92 (CH$_2$-2′), 68.27 (d, $J_{C,P}=5.5$ Hz; CH$_2$-5′), 73.88 (CH-3′), 74.69 (CH$_2$-2-dihydrobenzofuryl), 85.42 (CH-1′), 87.76 (d, $J_{C,P}=8.7$ Hz; CH-4′), 103.88 (C-4a), 112.16 (CH-7-dihydrobenzofuryl), 121.05 (C-5), 122.29 (CH-6), 128.57 (CH-4-dihydrobenzofuryl), 128.96 (C-5-dihydrobenzofuryl), 131.27 (CH-6-dihydrobenzofuryl), 131.45 (C-3a-dihydrobenzofuryl), 152.37 (C-7a), 153.90 (CH-2), 159.88 (C-4), 161.24 ppm (CH-7a-dihydrobenzofuryl); $^{31}$P NMR (202.3 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(phosphate buffer)=2.35 ppm): $\delta=-21.53$ (t, $J=19.6$ Hz; P$_\beta$), −10.55 (d, $J=19.6$ Hz; P$_\alpha$), −6.48 ppm (d, $J=19.6$ Hz; P$_\gamma$); MS (ESI+): *m/z* (%): 629.0 (8) [$M^+$+Na]; 651.0 (20) [$M^+$+2Na]; HRMS (ESI+): *m/z* calcd for $C_{19}H_{22}O_{13}N_4P_3$: 607.04017; found: 607.04022; calcd for $C_{19}H_{21}O_{13}N_4NaP_3$: 629.02211; found: 629.02208.

**dA$^{MOP}$TP**: Compound **dA$^{MOP}$TP** was prepared from **dA$^{I}$TP** according to the general procedure (Method B). The product was isolated as a purple solid (21 mg, 42%). $^1$H NMR (500.0 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=3.75 ppm): $\delta=2.46$ (ddd, $J_{gem}=14.0$, $J_{2'b,1'}=6.2$, $J_{2'b,3'}=3.0$ Hz, 1H; H-2′b), 2.73 (ddd, $J_{gem}=14.0$, $J_{2'a,1'}=8.1$, $J_{2'a,3'}=6.4$ Hz, 1H; H-2′a), 3.88 (s, 3H; CH$_3$O), 4.11 (ddd, $J_{gem}=10.7$, $J_{H,P}=5.6$, $J_{5'b,4'}=4.4$ Hz, 1H; H-5′b), 4.17 (ddd, $J_{gem}=10.7$, $J_{H,P}=6.3$, $J_{5'a,4'}=4.4$ Hz, 1H; H-5′a), 4.24 (td, $J_{4',5'}=4.4$, $J_{4',3'}=3.0$ Hz, 1H; H-4′), 4.76 (dt, $J_{3',2'}=6.4$, 3.0, $J_{3',4'}=3.0$ Hz, 1H; H-3′), 6.67 (dd, $J_{1',2'}=8.1$, 6.2 Hz, 1H; H-1′), 6.98 (dd, $J_{6,5}=8.0$, $J_{6,2}=1.7$ Hz, 1H; H-6-C$_6$H$_3$OHOMe), 7.01 (d, $J_{5,6}=8.0$ Hz, 1H; H-5-C$_6$H$_3$OHOMe), 7.10 (d, $J_{2,6}=1.7$ Hz, 1H; H-2-C$_6$H$_3$OHOMe), 7.45 (s, 1H; H-6), 8.16 ppm (s, 1H; H-2); $^{13}$C NMR (125.7 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=69.3 ppm): $\delta=40.93$ (CH$_2$-2′), 58.73 (CH$_3$O), 68.31 (d, $J_{C,P}=5.5$ Hz; CH$_2$-5′), 73.95 (CH-3′), 85.46 (CH-1′), 87.79 (d, $J_{C,P}=8.9$ Hz; CH-4′), 103.82 (C-4a), 115.81 (CH-2-C$_6$H$_3$OHOMe), 118.68 (CH-5-C$_6$H$_3$OHOMe), 120.89 (C-5), 122.36 (CH-6), 124.55 (CH-6-C$_6$H$_3$OHOMe), 128.93 (C-1-C$_6$H$_3$OHOMe), 147.14 (C-4-C$_6$H$_3$OHOMe), 150.38 (C-3-C$_6$H$_3$OHOMe), 152.40 (C-7a), 153.70 (CH-2), 159.86 ppm (C-4); $^{31}$P NMR (202.3 MHz, $D_2O$, pD=7.1, ref(phosphate buffer)=2.35 ppm): $\delta=-21.49$ (dd, $J=19.8$, 19.2 Hz; P$_\beta$), −10.44 (d, $J=19.2$ Hz; P$_\alpha$), −6.65 ppm (d, $J=19.8$ Hz; P$_\gamma$); MS (ESI+): *m/z* (%): 611.1 (8) [$M^+$+H]; 633.0 (20) [$M^+$+Na]; HRMS (ESI+): *m/z* calcd for $C_{18}H_{22}O_{14}N_4P_3$: 611.03508; found: 611.03483; calcd for $C_{18}H_{21}O_{14}N_4NaP_3$: 633.01703; found: 633.01664.

**dC$^{DHB}$TP**: Compound **dC$^{DHB}$TP** was prepared from **dC$^{I}$TP** according to the general procedure (Method B). The product was isolated as a white solid (17 mg, 41%). $^1$H NMR (500.0 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=3.75 ppm): $\delta=2.35$ (ddd, $J_{gem}=14.1$, $J_{2'b,1'}=7.5$, $J_{2'b,3'}=6.4$ Hz, 1H; H-2′b), 2.42 (ddd, $J_{gem}=14.1$, $J_{2'a,1'}=6.3$, $J_{2'a,3'}=3.6$ Hz, 1H; H-2′a), 3.28 (t, $J_{3,2}=8.7$ Hz, 2H; H-3-dihydrobenzofuryl), 4.15 (m, 2H; H-5′), 4.21 (m, 1H; H-4′), 4.61 (dt, $J_{3',2'}=6.4$,. 3.6, $J_{3',4'}=3.6$ Hz, 1H; H-3′), 4.64 (t, $J_{2,3}=8.7$ Hz, 2H; H-2-dihydrobenzofuryl), 6.35 (dd, $J_{1',2'}=7.5$, 6.3 Hz, 1H; H-1′), 6.91 (d, $J_{7,6}=8.2$ Hz, 1H; H-7-dihydrobenzofuryl), 7.18 (dd, $J_{6,7}=8.2$, $J_{6,4}=2.0$ Hz, 1H; H-6-dihydrobenzofuryl), 7.31 (d, $J_{4,6}=2.0$ Hz, 1H; H-4-dihydrobenzofuryl), 7.70 ppm (s, 1H; H-6); $^{13}$C NMR (125.7 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=69.3 ppm): $\delta=31.62$ (CH$_2$-3-dihydrobenzofuryl), 41.59 (CH$_2$-2′), 68.03 (d, $J_{C,P}=5.4$ Hz; CH$_2$-5′), 73.37 (CH-3′), 74.76 (CH$_2$-2-dihydrobenzofuryl), 88.16 (d, $J_{C,P}=8.8$ Hz; CH-4′), 88.62 (CH-1′), 112.38 (CH-7-dihydrobenzofuryl), 113.56 (C-5), 127.38 (C-5-dihydrobenzofuryl), 129.13 (CH-4-dihydrobenzofuryl), 131.68 (C-3a-dihydrobenzofuryl), 131.96 (CH-6-dihydrobenzofuryl), 142.03 (CH-6), 159.88 (C-2), 161.99 (CH-7a-dihydrobenzofuryl), 167.79 ppm (C-4); $^{31}$P NMR (202.3 MHz, $D_2O$, pD=7.1, ref(phosphate buffer)=2.35 ppm): $\delta=-21.60$ (t, $J=19.7$ Hz; P$_\beta$), −10.74 (d, $J=19.7$ Hz; P$_\alpha$), −6.63 ppm (d, $J=19.7$ Hz; P$_\gamma$); MS (ESI+): *m/z* (%): 628.0 (15) [$M^+$+2Na]; HRMS (ESI+): *m/z* calcd for $C_{17}H_{21}O_{14}N_3P_3$: 584.02418; found: 584.02419; calcd for $C_{17}H_{20}O_{14}N_3NaP_3$: 606.00613; found: 606.00623.

**dC$^{MOP}$TP**: Compound **dC$^{MOP}$TP** was prepared from **dC$^{I}$** according to the general procedure (Method C). The product was isolated as a white solid (11 mg, 28%). $^1$H NMR (500.0 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=3.75 ppm): $\delta=2.36$ (ddd, $J_{gem}=14.0$, $J_{2'b,1'}=7.4$, $J_{2'b,3'}=6.4$ Hz, 1H; H-2′b), 2.42 (ddd, $J_{gem}=14.0$, $J_{2'a,1'}=6.4$, $J_{2'a,3'}=3.5$ Hz, 1H; H-2′a), 3.89 (s, 3H; CH$_3$O), 4.15 (m, 2H; H-5′), 4.22 (td, $J_{4',5'}=4.4$, $J_{4',3'}=3.5$ Hz, 1H; H-4′), 4.60 (dt, $J_{3',2'}=6.4$,. 3.5, $J_{3',4'}=3.5$ Hz, 1H; H-3′), 6.35 (dd, $J_{1',2'}=7.4$, 6.4 Hz, 1H; H-1′), 6.93 (dd, $J_{6,5}=8.1$, $J_{6,2}=2.0$ Hz, 1H; H-6-C$_6$H$_3$OHOMe), 7.02 (d, $J_{5,6}=8.1$ Hz, 1H; H-5-C$_6$H$_3$OHOMe), 7.03 (d, $J_{2,6}=2.0$ Hz, 1H; H-2-C$_6$H$_3$OHOMe), 7.71 ppm (s, 1H; H-6); $^{13}$C NMR (125.7 MHz, $D_2O$, pD=7.1, phosphate buffer, ref(dioxane)=69.3 ppm): $\delta=41.59$ (CH$_2$-2′), 58.72 (CH$_3$O), 68.05 (d, $J_{C,P}=5.5$ Hz; CH$_2$-5′), 73.41 (CH-3′), 88.18 (d, $J_{C,P}=8.7$ Hz; CH-4′), 88.70 (CH-1′), 113.36 (C-5), 116.11 (CH-2-C$_6$H$_3$OHOMe), 118.90 (CH-5-C$_6$H$_3$OHOMe), 125.22 (CH-6-C$_6$H$_3$OHOMe), 127.33 (C-1-C$_6$H$_3$OHOMe), 142.12 (CH-6), 147.97 (C-4-C$_6$H$_3$OHOMe), 150.42 (C-3-C$_6$H$_3$OHOMe), 159.88 (C-2), 167.73 ppm (C-4); $^{31}$P NMR (202.3 MHz, $D_2O$, pD=7.1, ref(phosphate buffer)=2.35 ppm): $\delta=-21.57$ (t, $J=19.6$ Hz; P$_\beta$), −10.72 (d, $J=19.6$ Hz; P$_\alpha$), −6.60 ppm (d, $J=19.6$ Hz; P$_\gamma$); MS (ESI+): *m/z* (%): 610.0 (15) [$M^+$+H]; 653.9 (10) [$M^+$+Na]; HRMS (ESI+): *m/z* calcd for $C_{16}H_{20}O_{15}N_3P_3$: 610.00104; found: 610.00062; calcd for $C_{16}H_{18}O_{15}N_3Na_3P_3$: 653.96493; found: 653.96465.

## Materials for biochemistry

Synthetic oligonucleotides (ONs) and unmodified nucleoside triphosphates (dATP, dTTP, dCTP, and dGTP) were purchased from Sigma, Dynabeads M-270 Streptavidin (DBStv) were obtained from Dynal A.S. (Norway), Pwo polymerase was from New England Biolabs (Great Britain), KOD XL DNA from Novagen, and γ-$^{32}$P-ATP from MP Empowered Discovery (USA). Other chemicals were of analytical grade.

### PEX experiment

The reaction mixture (20 μL) contained template (for sequences, see Table 2, 3 μM), primer (5′-$^{32}$P- or 5′-FAM-labeled, for sequences see Table 2, 3 μM), dNTPs (all natural, three natural and one modified, or two natural and two modified; 1, 2, or 4 mM depending on the experiment), DNA polymerase (0.125–0.5 U KOD XL or 0.25–0.5 U Pwo), and buffer. Primer was labeled at its 5′-end by use of [γ-$^{32}$P]-ATP according to standard techniques. The reaction mixture was incubated for 10 min (20 min for temp$^{rnd16}$) at 60 °C. The PEX reaction was stopped by the addition of PAGE stop solution [40 μL, formamide (80%, v/v), ethylenediaminetetraacetic acid (EDTA, 20 mM), bromophenol blue (0.025%, w/v), xylene cyanol (0.025%,w/v)] and heating for 2 min at 95 °C. Samples were analyzed by use of a 12.5% denaturing polyacrylamide gel electrophoresis (PAGE; 1 h, 50 °C) and visualized by phosphorimager (Typhoon 9410, Amersham Biosciences).

Kinetics of PEX: The PEX reaction mixtures using KOD XL and Pwo DNA polymerases were incubated for time intervals followed by stopping the reaction by addition of PAGE loading buffer and immediate heating (see Figures S1–S12).

### MALDI-TOF experiment

The MALDI-TOF spectra were measured on an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) with 1 kHz smartbeam II laser technology. The measurements were done in reflectron mode by the droplet technique, with the mass range up to 30 kDa. The matrix consisted of 3-hydroxypicolinic acid/picolinic acid/ammonium tartrate in the ratio 9:1:1. The matrix (1 μL) was applied on the target (ground steel) and dried down at room temperature. The sample (1 μL) and matrix (1 μL) were mixed and added on top of the dried matrix preparation spot and dried at room temperature.

Preparation of ONs for MALDI-TOF analysis: Streptavidin magnetic particles stock solution (Roche, 50 μL) was washed with binding buffer (3×200 μL, 10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.5). The PEX solution (prepared as described above) and binding buffer (50 μL) were added. The suspension was shaken (1200 rpm) for 30 min at 15 °C. The magnetic beads were collected on a magnet (DynaMag-2, Invitrogen) and washed with wash buffer (3×200 μL, 10 mM Tris, 1 mM EDTA, 500 mM NaCl, pH 7.5) and water (4×200 μL). Then water (50 μL) was added and the sample was denatured for 2 min at 60 °C and 900 rpm. The beads were collected on a magnet and the solution was transferred into a clean vial. The product was analyzed by MALDI-TOF mass spectrometry (the results are summarized in Table 3; for copies of mass spectra, see Figures S18–S33).

### Electrochemical analysis

Nucleosides, **dN$^{X}$TP**s, and boronic acids were analyzed by conventional in situ square-wave voltammetry (SWV). Purified PEX products were analyzed by ex situ (adsorptive transfer stripping) SWV. The PEX products were accumulated for 60 s from aliquots (5 μL) containing 0.2 M NaCl at the surface of the working electrode (basal-plane pyrolytic graphite). The electrode was then rinsed with deionized water and placed in the electrochemical cell. SWV settings: initial potential 0.0 V, end potential +1.5 V (for oxidation) or −1.5 V (for reduction, Figure 9c), frequency 200 Hz, amplitude 50 mV. Background electrolyte: 0.2 M sodium acetate pH 5.0. All measurements were performed at room temperature using an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-stand 663 apparatus (Metrohm, Herisau, Switzerland). The three-electrode system was used with an Ag/AgCl/3 M KCl electrode as a reference and platinum wire as an auxiliary electrode.

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**Keywords:** DNA · electrochemistry · nucleotides · polymerase chain reactions · redox labeling

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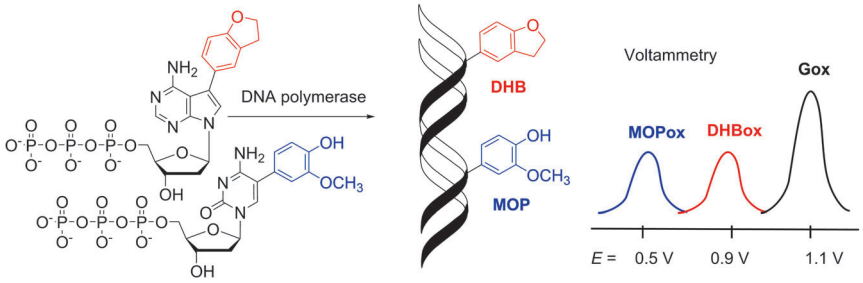


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**Methoxyphenol and Dihydrobenzofuran as Oxidizable Labels for Electrochemical Detection of DNA**

**Clearly labeled**: 4-Hydroxy-3-methoxyphenyl (MOP) and 2,3-dihydrobenzofuran-5-yl (DHB) groups have been developed as new oxidizable labels for the electrochemical detection of DNA and for redox coding of nucleotides (see figure). MOP offers independent detection of the modified DNA.