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Alkylsulfanylphenyl Derivatives of Cytosine and 7-Deazaadenine Nucleosides, Nucleotides and Nucleoside Triphosphates: Synthesis, Polymerase Incorporation to DNA and Electrochemical Study

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Abstract: Aqueous Suzuki–Miyaura cross-coupling reactions of halogenated nucleosides, nucleotides and nucleoside triphosphates derived from 5-iodocytosine and 7-iodo-7-deazaadenine with methyl-, benzyl- and tritylsulfanylphenylboronic acids gave the corresponding alkylsulfanylphenyl derivatives of nucleosides and nucleotides. The modified nucleoside triphosphates were incorporated into DNA by primer extension by using Vent(*exo*-) polymerase. The electrochemical behaviour of the alkylsulfanylphenyl nucleosides indicated formation of compact layers on the electrode. Modified nucleotides and DNA with incorporated benzyl- or tritylsulfanylphenyl moieties produced signals in $[Co(NH_3)_6]^{3+}$ ammonium buffer, attributed to the Brdička catalytic response, depending on the negative potential applied. Repeated constant current chronopotentiometric scans in this medium showed increased Brdička catalytic response, which suggests the deprotection of the alkylsulfanyl derivatives to free thiols under the conditions.

Keywords: DNA polymerases · electrochemistry · nucleosides · nucleotides · organosulfur compounds

Introduction

Attachment of thiol-end-labelled oligonucleotides (HS-ONs) at gold surfaces to facilitate the formation of a DNA self-assembled monolayer (SAM)[1] is one of the most frequently used methods of DNA immobilisation on solid surfaces. This approach is widely applied in the construction of diverse sensors of DNA hybridisation, DNA chips and other important technologies.[2] In addition, formation of a SAM of HS-ONs at mercury electrodes[3] was used to generate electrochemical sensors of DNA hybridisation. In all of these applications, the HS-ONs used are end-labelled with a thiol group tethered by a longer alkyl linker. The conformational behaviour of such HS-ON probes is quite complex and not yet fully understood,[4] and the separation of the ON probe from the thiol group by the saturated tether inherently decreases the sensitivity of the electrochemical sensors. In many applications, the free thiols can be replaced by dialkylsulfides (in particular methylsulfanyl derivatives),[5] which also bind efficiently to the gold surface. We envisaged that preparation of ON probes with an alkylsulfanyl group attached to a nucleobase through a conjugated system may lead to the construction of more sensitive and accurate electrochemical DNA sensors and may also potentially be used for multiple attachment(s) of an ON to a metal surface to form stable loops, hairpins and other structures.

Polymerase incorporations of base-modified 2′-deoxynucleoside triphosphates (dNTPs) is now a very popular approach for the construction of functionalised oligonucleotides and DNA.[6] It has been used for attachment of diverse fluorescent,[7] redox[8] or spin[9] labels, and reactive functional groups.[10] Within our project of "multi-colour" redox labelling of DNA,[11] we are interested in the development of other alternative electroreducible or -oxidisable labels (both reversible and irreversible) with different redox potentials. Alkylsulfanyl groups, apart from being anchors for binding to metal surfaces,[5] are potentially interesting both as redox labels and as precursors of free thiols by chemical deprotection[12] or electroreduction.[13]

The goals of our present study were the synthesis of nucleosides, nucleotides and dNTPs bearing either a free sulfanyl- or alkylsulfanylphenyl group attached directly to a nucleobase and the development of polymerase incorporations of the sulfur-containing dNTPs into DNA. Additionally, we studied the electrochemical behaviour of the modified nucleotides and DNA and their assembly onto the metal electrode surfaces.

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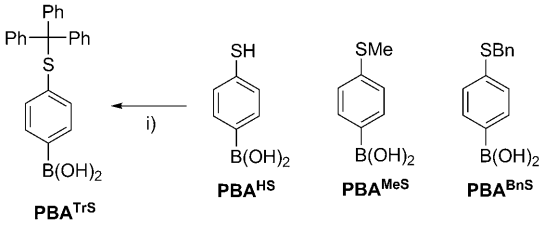
Results and Discussion

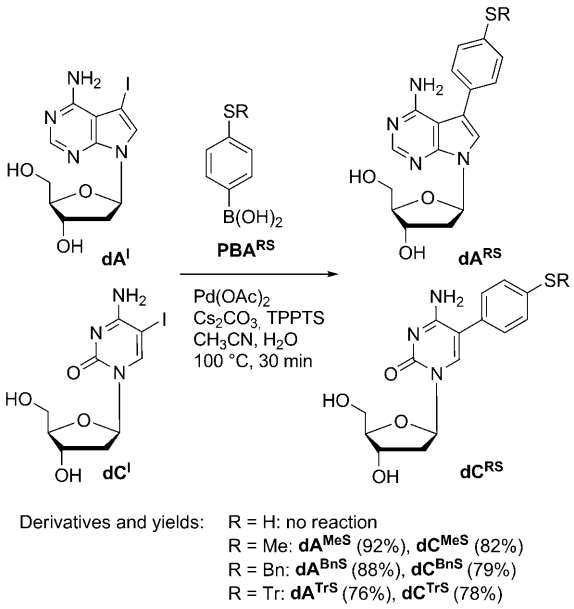
Chemistry: Previously, we discovered that the best position for attachment of a modification at pyrimidine nucleobases is the 5-position, whereas purines should be replaced by 7-deazapurines modified at the 7-position.[14] Such modifications are accommodated into the major groove of DNA in which they do not destabilise duplexes (in some cases they even stabilise them). Moreover, dNTPs modified at these positions are good substrates for polymerases and can be enzymatically incorporated into DNA. Therefore, our target compounds were nucleotides functionalised with alkylsulfanylphenyl groups at the 5- (pyrimidine) or 7-position (deazapurine), respectively.

Suzuki–Miyaura cross-coupling reactions are considered to be the superior method for introduction of functionalised aryl groups to unprotected nucleosides or nucleotides in aqueous solutions.[15] Therefore, our project started with a model study of cross-couplings of halogenated nucleosides with alkylsulfanylphenylboronic acids (**PBA^{RS}**). Because the free-sulfanyl phenylboronic acid **PBA^{HS}** was presumed to be potentially unreactive because of Pd-catalyst poisoning, we selected stable methylsulfanyl derivatives, as well as more labile benzyl- and tritylsulfanyl derivatives with potentially cleavable protecting groups at sulfur. Sulfanyl-, methylsulfanyl- and benzylsulfanylphenylboronic acids were commercially available, whereas novel *S*-trityl-protected sulfanylphenylboronic acid was prepared in excellent yield by the reaction of triphenylmethanol with **PBA^{HS}** in the presence of trifluoroacetic acid (TFA) in dichloromethane (Scheme 1).[16]



Scheme 1. Phenylboronic acids used in this study. i) TrOH, TFA, CH_2Cl_2, RT, 1 h (95%).

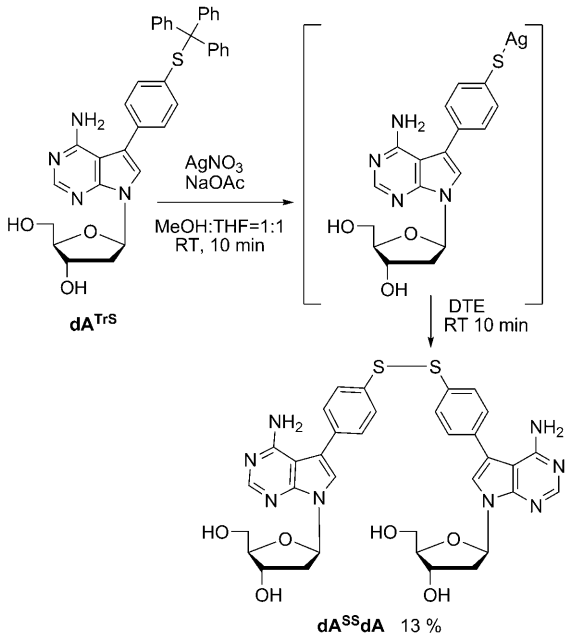
Iodinated nucleosides, 7-iodo-7-deaza-2′-deoxyadenosine (**dA^{I}**) and 5-iodo-2′-deoxycytidine (**dC^{I}**) were selected as model starting compounds for the development of the aqueous Suzuki–Miyaura cross-coupling methodology. Previously reported conditions ($Pd(OAc)_2$, $P(m\text{-}C_6H_4SO_3Na)_3$ (TPPTS) ligand, Cs_2CO_3, 2:1 water/acetonitrile)[4] were applied in reactions with **PBA^{HS}** and **PBA^{RS}**. The reactions were performed at 100 °C for 30 min, for compatibility with the synthesis of labile dNTPs. The attempted reactions of **dA^{I}** or **dC^{I}** with **PBA^{HS}** did not proceed, which confirmed the expected outcome of catalyst poisoning. On the other hand, all of the 4-(alkylsulfanyl)phenylboronic acids (**PBA^{MeS}**, **PBA^{BnS}** and **PBA^{TrS}**) reacted with **dA^{I}** to give the desired 7-[(alkylsulfanyl)phenyl]-7-deaza-2′-deoxyadenosines **dA^{MeS}**, **dA^{BnS}** and **dA^{TrS}** in excellent yields (76–92%, Scheme 2). Similarly, the same boronic acids reacted with **dC^{I}** to give the corresponding 5-substituted 2′-deoxycytidines **dC^{MeS}**, **dC^{BnS}** and **dC^{TrS}** in good yields (78–82%, Scheme 2).



Scheme 2. Synthesis of sulfur-containing nucleosides.

Apparently, the free HS-containing nucleosides could not be prepared by direct cross-couplings, therefore, possible deprotection methods were considered for the cleavage of the benzyl or trityl protecting groups. Unfortunately, the portfolio of R–S cleavage methods operable under mild conditions, and thus compatible with fragile nucleosides or nucleotides, is very limited. Therefore, we focused on the S-detritylation.

Several methodologies for cleavage of this group are known, but most of them were excluded due to the requirement for harsh reaction conditions or extremely toxic reagents (e.g. HCl in AcOH (aq);[17] $Hg(OAc)_2$, reflux, then H_2S;[17] $(SCN)_2$;[18] electrolysis;[19] PhHgOAc, then H_2S[20]). Two methods were selected as potentially useful for our purposes. Treatment of **dA^{TrS}** with triethylsilane in dichloromethane and TFA[21] gave an inseparable mixture of products. On the other hand, the reaction of **dA^{TrS}** with $AgNO_3$[22] proceeded smoothly (TLC analysis showed complete conversion of the starting compound to an insoluble polar product) to give a silver salt of the desired thiolate **dA^{HS}**. The procedure was followed by trapping of Ag^+ by dithioerythritol (DTE) under mild conditions[22] (Scheme 3). Unfortunately, the crude product obtained after the treatment with DTE was unstable and decomposed during workup and chromatographic purification. The only product isolated after a combination of silica gel and reversed-phase flash chromatography was the disulfide dinucleoside **dA^{SS}dA**, in a low yield of 13%. Apparently, even if an efficient method for the synthesis of free sulfanylphenyl nucleosides was developed,

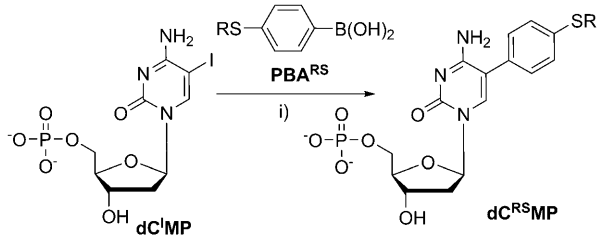


Scheme 3. Attempted trityl deprotection of **dA^{TrS}**.

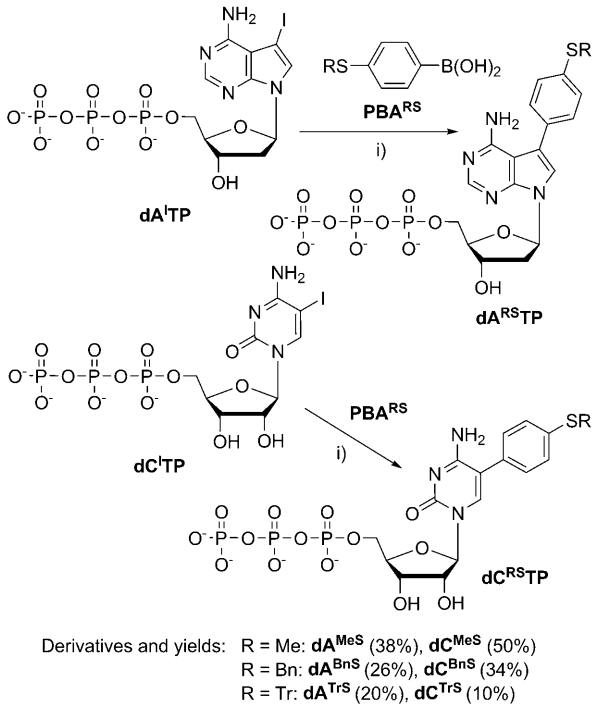
these compounds would be very unstable and prone to oxidation to disulfides. Therefore, no further attempts on the thiol nucleosides were pursued and we focused on the synthesis and use of the more stable alkylsulfanyl nucleotides.

To act as simplified models of DNA for electrochemical studies, a small series of 5-(alkylsulfanylphenyl)-2′-deoxycytidine monophosphates (**dC^{RS}MP**) was prepared. Thus, the reactions of 5-iodo-2′-deoxycytidine monophosphate (**dC^{I}MP**) with boronic acids **PBA^{RS}** under the conditions described above proceeded well to give the methylsulfanyl and benzylsulfanyl derivatives **dC^{MeS}MP** and **dC^{BnS}MP** in good yields (Scheme 4). The reaction with **PBA^{TrS}** gave a much lower conversion and the desired nucleotide **dC^{TrS}MP** was isolated in only 14% yield.

Finally, we applied the cross-coupling reactions for the synthesis of modified dNTPs, substrates for polymerase incorporation to DNA. The reactions of iodinated dNTPs



Scheme 4. Synthesis of sulfur-containing 2′-deoxycytidines. i) 1. $Pd(OAc)_2$, Cs_2CO_3, TPPTS, CH_3CN, H_2O, 100 °C, 30 min; 2. Dowex 50WX8 (Na^+ cycle) conversion to sodium salts.



Scheme 5. Synthesis of sulfur-containing dNTPs. i) 1. $Pd(OAc)_2$, Cs_2CO_3, TPPTS, CH_3CN, H_2O, 100 °C, 30 min; 2. Dowex 50WX8 (Na^+ cycle) conversion to sodium salts.

dA^{I}TP and **dC^{I}TP** with the series of alkylsulfanylphenylboronic acids **PBA^{RS}** proceeded under the same conditions within 30 min. (Scheme 5), with similar efficiency as for the monophosphates (Scheme 4). However, the cross-coupling reactions were accompanied by unwanted partial hydrolysis of the dNTPs (both starting compounds and products) to diphosphates. The hydrolytic reactions dramatically decreased the isolated yields of the dNTPs. In all cases, the desired triphosphates **dN^{RS}TP** were isolated by semi-preparative HPLC and were accompanied by substantial amounts (15–25%) of the corresponding diphosphates. The isolated yields of the methyl- and benzylsulfanylphenyl derivatives (**dA^{MeS}TP**, **dA^{BnS}TP**, **dC^{MeS}TP** and **dC^{BnS}TP**) were acceptable (26–50%), whereas the tritylsulfanylphenyl derivatives (**dA^{TrS}TP** and **dC^{TrS}TP**) were only obtained in moderate yields of 20 and 10%, respectively. An alternative synthesis of **dC^{TrS}TP** by triphosphorylation of 5-tritylsulfanyl-2′-deoxycytidine under standard conditions[8c] was attempted but the yield was also low (approximately 15%).

Enzymatic incorporations: The sulfur-containing dNTPs were then tested as substrates of DNA polymerases, to serve as building blocks for enzymatic DNA synthesis. Primer extension (PEX) experiments with **dA^{MeS}TP**, **dA^{BnS}TP**, **dA^{TrS}TP**, **dC^{MeS}TP**, **dC^{BnS}TP** and **dC^{TrS}TP** were performed with Vent(*exo*-) DNA polymerase and temp^{rnd16} template (see the Experimental Section). The PEX reaction extended the primer by 16 nucleotides, which included four A and four C nucleotides. The Vent(*exo*-) polymerase showed

good tolerance towards the presence of the sulfur modifications on the dNTPs. Namely, the enzymatic incorporation of **dA^{MeS}TP**, **dA^{BnS}TP**, **dC^{MeS}TP** and **dC^{BnS}TP** gave the desired full-length products with four modifications within one DNA molecule in every case (Figure 1, lines 4, 5, 9 and 10).

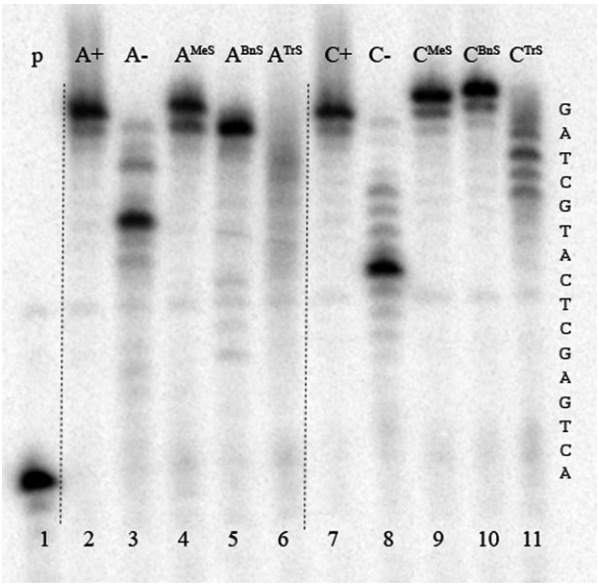


Figure 1. PEX with temp^{rnd16} in the presence of: dCTP, dGTP, dTTP and dATP (lines 2, 7); dCTP, dGTP and dTTP (line 3); dATP, dGTP and dTTP (line 8); dCTP, dGTP, dTTP and **dA^{RS}TP** (lines 4, 5, 6); dATP, dGTP, dTTP and **dC^{RS}TP** (lines 9, 10, 11). Line 1: ^{32}P radiolabelled primer.

The incorporation of **dA^{TrS}TP** and **dC^{TrS}TP** was more complicated (Figure 1, lines 6 and 11); mixtures of products of various lengths were observed in both cases (Figure 1). Other DNA polymerases (DyNAzyme and Klenow fragment) were tested with similar results (not shown). The methyl- and benzylsulfanyl dNTPs (**dA^{MeS}TP**, **dA^{BnS}TP**, **dC^{MeS}TP** and **dC^{BnS}TP**) are excellent substrates, with good potential for the construction of sulfur-containing DNA, whereas the tritylsulfanylphenyl group is probably too bulky to be incorporated by the polymerase at multiple positions in random sequences. Nevertheless, even the bulky **dA^{TrS}TP** and **dC^{TrS}TP** are substrates for polymerases and can be incorporated into DNA.

Electrochemistry: Electrochemical and interfacial properties of the title compounds, their respective building blocks and modified ONs were studied by means of cyclic voltammetry (CV), AC voltammetry (ACV) or constant current chronopotentiometric stripping (CPS) at basal plane pyrolytic graphite (PGE) or hanging mercury drop (HMDE) electrodes. At the PGE we only observed the oxidation signal of the 7-deazaadenine residue[11,23] in the **dA^{RS}** compounds and their respective monophosphates and triphosphates (not shown). The behaviour of the alkylsulfanylphenyl nucleosides at the HMDE was more complex, which reflected the presence of the sulfur groups and/or strongly adsorbing aromatic moieties coupled to the nucleobases. The 2′-deoxynucleosides and 2′-deoxynucleoside monophosphates showed cathodic peaks due to the nucleobase reduction in acetate buffer, pH 5.0. This is illustrated in Figure 2 for cytosine derivatives, which produced peak C^{red}. This peak was shifted towards less negative potentials by 50 mV in **dC^{BnS}** relative to unmodified 2′-deoxycytidine (**dC**) and by a further 40 mV in **dC^{BnS}MP**. The **dC^{RS}** compounds produced two sharp

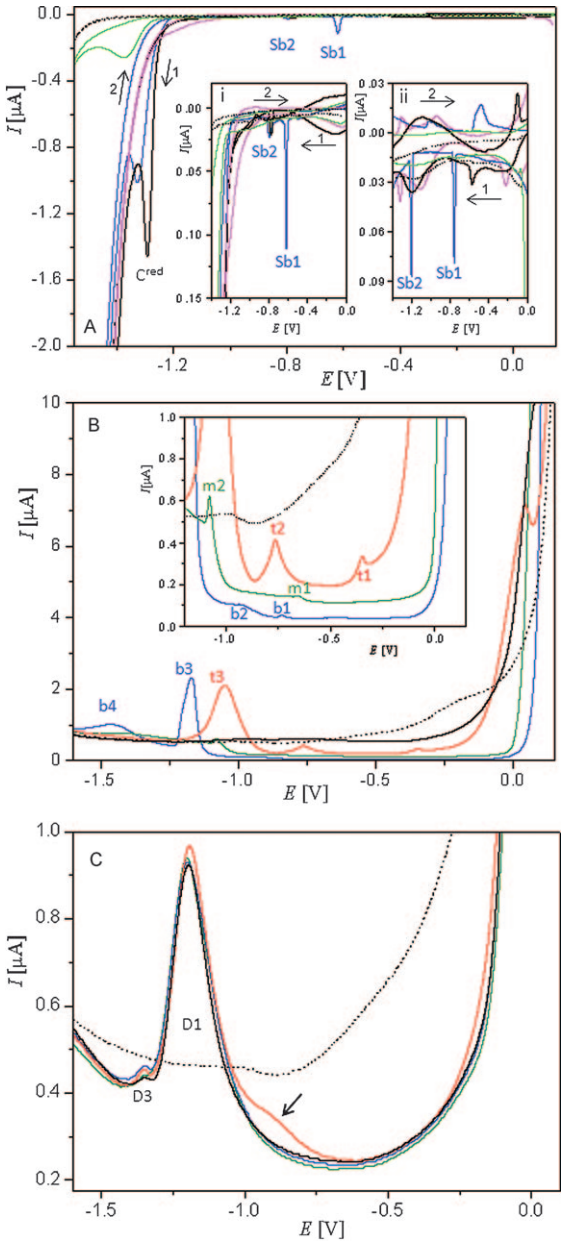


Figure 2. A) CV in sodium acetate buffer, pH 5.0. **dC^{BnS}** (——); **dC^{BnS}MP** (——); **dC** (——); **PBA^{BnS}** (——); background electrolyte (•••••). Insets: details of CV measured in i) sodium acetate buffer; ii) borax buffer. B) ACV of **dC^{RS}**: R = methyl (——), benzyl (——), trityl (——); **dC^{BnS}MP** (——); background electrolyte (•••••). Inset: detail. C) ACV of PEX^{rnd16} products. Unmodified (——) and modified with 5-(RS)cytidine: R = methyl (——), benzyl (——), trityl (——); background electrolyte (•••••).

cathodic peaks (spikes) around −0.6 and −0.8 V in the acetate buffer. These spikes were best developed in **dC^{BnS}** (Figure 2A; peaks Sb1 and Sb2), followed by **dC^{MeS}**, then **dC^{TrS}** (not shown). In borax buffer (pH 9.3), the spikes were shifted to more negative potentials and the more negative Sb2 was much better developed than at the weakly acidic pH (Figure 2A, inset i). The spikes were only observed with the alkylsulfanylphenyl-modified nucleosides; neither of the building blocks (unmodified nucleosides, **PBA^{RS}**) nor the **dN^{RS}MP** (N = A or C) compounds produced such effects, which suggests the involvement of both the nucleobase and the RS moiety. Occurrence of sharp spikes in voltammograms has been shown to be connected with phase transitions in compact, two-dimensionally condensed layers of various surface-active substances (including nucleic acid components) at electrically charged surfaces.[24] The 2D condensation processes are usually connected with a strong decrease of differential capacity of the electrode bilayer in AC voltammetric modes, reflected in the decrease of capacitive currents well below values that correspond to a clean electrode surface in the background electrolyte. Formation of capacitance pits with flat bottoms and distinct edges that correspond to the above mentioned spikes are also observed, at potentials where the phase transitions (condensation/reorientation/disruption of the compact layers) occur. ACV of the modified nucleosides revealed such behaviour in the borax buffer for all three **dC^{RS}** compounds (Figure 2B), but not for unmodified **dC**, **dC^{RS}MP** or **dC^{RS}TP**. The tendency towards compact-layer formation and its stability at the negatively charged HMDE surface decreased in the order **dC^{BnS}** > **dC^{MeS}** > **dC^{TrS}**. Hence, attachment of the alkylsulfanylphenyl groups to **dC** contributed significantly to adsorption of the nucleoside at the HMDE surface to form the compact layers. In **dC^{BnS}**, the third sterically unconstrained planar aromatic moiety further enhanced these effects, whereas the presence of the bulky, non-planar trityl group in **dC^{TrS}** made formation of a regular surface structure less feasible. In the **dN^{RS}MP** and **dN^{RS}TP** series, electrostatic repulsion between the molecules and between a molecule and the negatively charged electrode surface prevented the condensation processes. More detailed analysis of the properties of adsorption and interactions at the electrode surfaces is out of the scope of this paper and will be published at a later date.

ACV traces of the oligonucleotide PEX products with incorporated sulfanyl groups did not significantly differ from those of unmodified ONs. For **dC^{RS}** (Figure 2C), two capacitive peaks were produced at potentials −1.20 and −1.34 V due to reorientation/desorption of the polyanionic ON chains at the negatively charged electrode surface.[25] Such behaviour was in agreement with the strong adsorption of single-stranded nucleic acids at mercury and amalgam electrodes through hydrophobic nucleobase residues. The excess of unmodified nucleotides in flat-lying ON chains at the HMDE[3] dictated the overall behaviour of the ONs that contained the alkylsulfanylphenyl conjugates, the contributions of which were insignificant under the given conditions (with the exception of **dC^{TrS}**, which produced an extra signal close to −0.9 V, Figure 2C). Similarly, CV analysis in neutral ammonium formate background electrolyte[25] showed redox processes of C, A and G nucleobases but no significant contribution from the conjugate groups (not shown).

Further, we studied the behaviour of the modified dNMPs and ONs in ammonium buffer that contained $[Co(NH_3)_6]^{3+}$ ions by CPS. In solutions that contain cobalt ions, thiol and disulfide compounds are known to give characteristic catalytic currents (namely, the Brdička catalytic response, BCR) at mercury[26] and silver amalgam electrodes,[27] whereas thioethers have been reported to be inactive. Notably, our results revealed the BCR appearance with dNMPs and ONs that possessed trityl- or benzylsulfanylphenyl groups (Figure 3), suggestive of deprotection of the thiol directly at

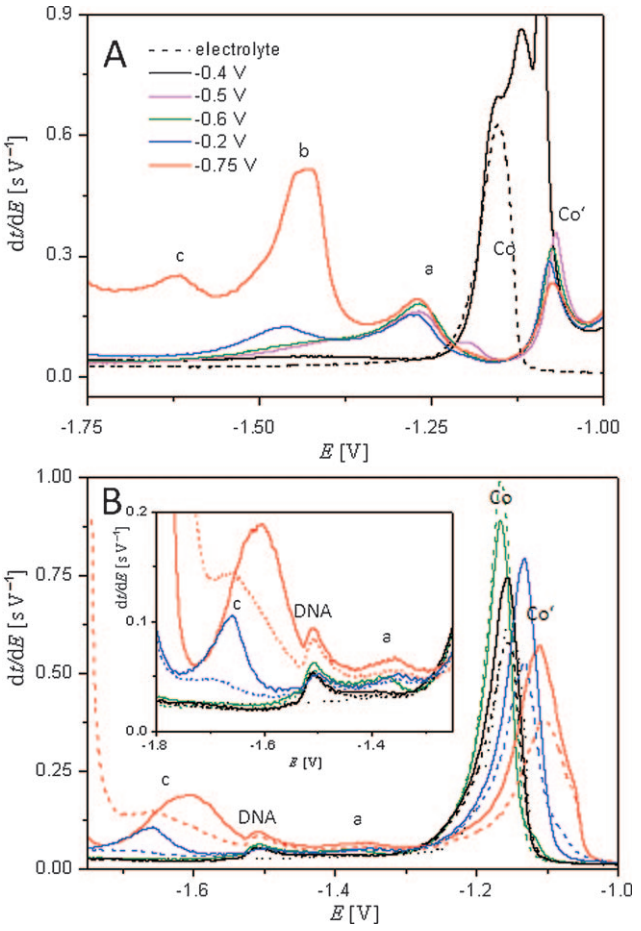
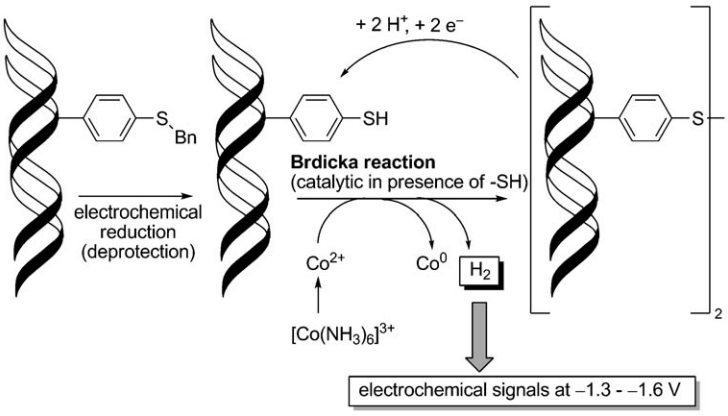


Figure 3. A) BCR of **dC^{TrS}MP** after pre-polarisation to negative potentials. −0.25 V (——), −0.4 V (——), −0.5 V (——), −0.6 V (——), −0.75 V (——), background electrolyte (-----). Peaks Co and Co′ correspond to reduction of Co^{II} and its complexes; peaks a, b and c have been attributed to catalytic hydrogen evolution in the presence of Co and thiol species. B) BCR of **PEX^{RS}** products modified with: **C^{MeS}** (——), **C^{BnS}** (——), **C^{TrS}** (——); negative PEX control (no modified base incorporated, ——). Dashed and solid curves correspond to first and second scans, respectively, of the same DNA adsorbed at the HMDE. The peak denoted as "DNA" is due to adsorption/desorption processes of ON chains. Inset: detailed section. Peaks Co and Co′ correspond to reduction of Co^{II} and its complexes; peaks a, b and c have been attributed to catalytic hydrogen evolution in the presence of Co and thiol species.

the electrode in the cobalt-containing medium, depending on the negative potential applied. Compound **dC^{TrS}MP** was exposed to various negative potentials (by applying potential cycles between 0.0 V and the given more negative potential) prior to measurement of the BCR. As shown in Figure 3A, there was practically no signal in the region of the BCR (i.e. more negative than the cobalt reduction peak) after pre-polarisation to −0.25 V. Application of potentials of −0.4 or −0.5 V resulted in the appearance of a small peak a close to −1.3 V (Figure 3A, peak a). After pre-polarisation to −0.6 V, a peak around −0.5 V appeared (Figure 3A, peak b). Pre-polarisation to −0.75 V resulted in a significant increase of the intensity of peak b and appearance of another signal at −1.62 V (Figure 3A, peak c). In the absence of cobalt, there were no such effects, which strongly suggests that the appearance of peaks b and c was due to reductive deprotection of the thioether groups to free thiols.

Figure 3B shows the results from CPS of PEX^{rnd16} products generated from **dC^{RS}TP** conjugates. It has been shown previously that unmodified DNA did not give the BCR, whereas end-alkylthiolated ONs produced specific catalytic currents in the presence of cobalt ions.[28] In accordance with the former observation, unmodified PEX^{rnd16} did not produce the BCR. The only distinct signal (around −1.5 V), produced by the unmodified PEX^{rnd16} at potentials more negative than the cobalt reduction peak, was dependent on the presence of neither cobalt ions (not shown), nor the modified nucleotides (compare curves in Figure 3B). Most likely the signal corresponded to the tensammetric DNA signal (see ACV traces in Figure 2C). The PEX product obtained from **dC^{MeS}TP** also did not show any significant effects. On the other hand, ONs that contained 5-benzylsulfanyl- or 5-tritylsulfanylphenyl cytidine gave distinct signals in the presence, but not absence, of cobalt ions. These signals were attributed to the BCR and suggest the presence of unprotected thiol groups. PEX^{rnd16} derived from **dC^{BnS}TP** produced only a weak BCR peak at −1.7 V (Figure 3B, peak c) in the first CPS scan. The signal increased significantly in the second CPS scan, which suggested deprotection of more thiol groups due to repeated negative polarisation of the HMDE with adsorbed modified DNA and/or reorientation of the DNA layer to facilitate communication of the sulfur groups with the electrode surface. For the PEX product containing **C^{TrS}**, stronger BCR responses were observed and the effect of repeated potential scanning on the BCR intensity was observed as well. Notably, the ON containing **C^{MeS}** did not give the BCR signal, even in the second CPS scan. Such behaviour accorded well with a more difficult reductive cleavage of the Me–S bond relative to Bn–S and, especially, Tr–S bonds.

It has been reported that benzyl or trityl protective groups were cleaved electrochemically from aryl thiols, cysteine and peptides at platinum or mercury electrodes in ammonia, methanol or DMF solutions only at highly negative potentials (approximately −2.5 V).[29] Such cleavages are not useful for analytical or preparative applications with regards to DNA (for example, nucleobases would be reduced under these conditions). Our results indicate that in the aqueous ammonium buffer, the complex redox electrochemistry of $[Co(NH_3)_6]^{3+}$ coupled with catalytic hydrogen gas evolution might facilitate the C–S cleavage under less drastic conditions (Scheme 6).



Scheme 6. Debenzylation of benzylsulfanylphenyl-modified DNA by electroreduction followed by the Brdička reaction.

Conclusion

Methyl-, benzyl- and tritylsulfanylphenyl-substituted 7-deazaadenosine and cytosine nucleosides, nucleotides and nucleoside triphosphates were prepared by Suzuki–Miyaura cross-coupling reactions of halogenated nucleosides and nucleotides with the appropriate alkylsulfanylphenylboronic acids. The attempted deprotection of the tritylsulfanylphenyl derivatives was unsuccessful. The methyl- and benzylsulfanyl dNTPs (**dA^{MeS}TP**, **dA^{BnS}TP**, **dC^{MeS}TP** and **dC^{BnS}TP**) were good substrates for DNA polymerases and were successfully incorporated into DNA by primer extension. The trityl derivatives (**dA^{TrS}TP** and **dC^{TrS}TP**) were less suitable substrates; they were incorporated but did not lead to fully extended products.

Electrochemical deprotection of the alkylsulfanylphenyl nucleosides and DNA by using literature conditions[29] was not considered due to the incompatibility of highly negative potentials with the preparation of functional DNA probes. However, a remarkable tendency to condensation of the alkylsulfanyl nucleosides into layers at the electrode was observed, which suggests that they might bind to the metal surface. Moreover, behaviour of the benzyl- or trityl-protected DNA in $[Co(NH_3)_6]^{3+}$-containing ammonium buffer suggests that electrochemical deprotection may be achieved at potentials less negative than the potentials of nucleobase reduction.[25] Repeated potential scans in this medium showed increased formation of characteristic peaks of the BCR, which indicates the presence of free (deprotected) SH groups. The most promising building block for the applications is **dC^{BnS}TP**, which is perfectly incorporated by DNA polymerases to give full-length DNA products and appears

to be efficiently deprotected by electrochemical reduction at moderate potentials in the presence of cobalt ions. Exploitation of these outcomes would enable immobilisation of ON probes at the electrode surfaces by using protected building blocks and enzymatically constructed probes to avoid oxidative dimerisation or coupling to other thiol species (such as proteins), followed by deprotection directly at the electrode to which the probe is to be attached. Recently, it has been shown that not only traditional gold electrodes, but also mercury electrodes, are suitable for the preparation of thiol-terminated ON monolayers.[3] Moreover, formation of such monolayers at mercury-based electrodes (including silver amalgam) has been reported to be more facile. Above and beyond the prospective utilisation of the sulfur moieties as anchors, we have shown that the BCR in the presence of cobalt ions can be exploited analytically to monitor the modified ON synthesis.

Experimental Section

General procedure for Suzuki–Miyaura cross-coupling reactions of halogenated deoxynucleosides with alkylsulfanylphenylboronic acids: Water/acetonitrile mixture (2:1, 1.5 mL) was added through septum to an argon-purged vial that contained halogenated dNTP (0.14 mmol), boronic acid (0.28 mmol) and Cs_2CO_3 (228 mg, 0.7 mmol). A solution of Pd(OAc)$_2$ (3.1 mg, 0.014 mmol) and TPPTS (40 mg, 0.07 mmol) in water/acetonitrile (2:1, 1.2 mL) was added and the mixture was stirred and heated to 100 °C for 30 min. Products were isolated from crude reaction mixture by silica gel column chromatography with gradient elution ($CHCl_3$/0–10 % MeOH) and evaporation of the solvents, then dried under vacuum.

7-[(4-Benzylsulfanyl)phenyl]-7-deaza-2′-deoxyadenosine (dA^{BnS}): Yellowish foam, 88%; m.p. 76–81 °C; ^{1}H NMR (499.8 MHz, CD_3OD): δ = 2.32 (ddd, J = 13.4, 6.0, 2.7 Hz, 1H; H-2′b), 2.68 (ddd, J = 13.4, 8.3, 6.0 Hz, 1H; H-2′a), 3.72 (dd, J = 12.1, 3.6 Hz, 1H; H-5′b), 3.80 (dd, J = 12.1, 3.2 Hz, 1H; H-5′b), 4.01 (ddd, J = 3.6, 3.2, 2.7 Hz, 1H; H-4′), 4.13 (s, 2H; CH_2S), 4.53 (dt, J = 6.0, 2.7 Hz, 1H; H-3′), 6.55 (dd, J = 8.3, 6.0 Hz, 1H; H-1′), 7.18 (m, 1H; H-*p*-Ph), 7.23 (m, 2H; H-*m*-Ph), 7.28 (m, 2H; H-*o*-Ph), 7.30 (m, 2H; H-*o*-phenylene), 7.33 (m, 2H; H-*m*-phenylene), 7.36 (s, 1H; H-8), 8.12 ppm (brs, 1H; H-2); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 39.30 (CH_2S), 41.42 (CH_2-2′), 63.65 (CH_2-5′), 73.03 (CH-3′), 86.48 (CH-1′), 89.05 (CH-4′), 102.73 (C-5), 118.02 (C-7), 122.65 (CH-8), 128.15 (CH-*p*-Ph), 129.41 (CH-*m*-Ph), 129.96 (CH-*o*-Ph), 130.21 (CH-*o*-phenylene), 131.28 (CH-*m*-phenylene), 133.50 (C-*i*-phenylene), 136.96 (C-*p*-phenylene), 138.92 (C-*i*-Ph), 151.07 (C-4), 152.22 (CH-2), 158.79 ppm (C-6); IR (KBr): $\tilde{\nu}$ = 3474, 3388, 3351, 3063, 1658, 1620, 1558, 1548, 1536, 1493, 1466, 1454, 1300, 1216, 1093, 1050, 700 cm^{-1}; MS (ESI$^+$): m/z (%): 449 (100) $[M+H]^+$, 471 (80) $[M+Na+H]^+$; HRMS: m/z calcd for $C_{24}H_{24}O_3N_4S$: 449.1642; found: 449.1642.

General procedure for Suzuki–Miyaura cross-coupling reactions of 5-iodo-dCMP or halogenated dNTPs with alkylsulfanylphenylboronic acids: The reactions were performed as described above for sulfur-containing deoxynucleosides. Products were isolated from the crude reaction mixture by HPLC (C18 column, linear gradient elution: 0.1 M triethylammonium bicarbonate (TEAB) in H_2O to 0.1 M TEAB in 1:1 H_2O/MeOH). Several co-distillations with water and conversion to the sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave white solid products.

5-[(4-Methylsulfanyl)phenyl]-2′-deoxycytidine 5′-*O*-monophosphate (dC^{MeS}MP): Yield: 73 %; ^{1}H NMR (499.8 MHz, D_2O, ref(dioxane) = 3.75 ppm): δ = 1.27 (t, J = 7.3 Hz, 9H; CH_3CH_2N), 2.33 (ddd, J = 14.2, 7.5, 6.3 Hz, 1H; H-2′b), 2.45 (ddd, J = 14.2, 6.2, 3.2 Hz, 1H; H-2′a), 2.53 (s, 3H; CH_3S), 3.19 (q, J = 7.3 Hz, 6H; CH_3CH_2N), 4.00 (dd, J = 5.5, 4.0 Hz, 2H; H-5′), 4.19 (td, J = 4.0, 3.2 Hz, 1H; H-4′), 4.51 (dt, J = 6.3, 3.2 Hz, 1H; H-3′), 6.33 (dd, J = 7.5, 6.2 Hz, 1H; H-1′), 7.37 (m, 2H; H-*o*-phenylene), 7.41 (m, 2H; H-*m*-phenylene), 7.75 ppm (s, 1H; H-6); ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): δ = 10.95 (**C**H_3CH_2N), 17.05 (CH_3S), 42.06 (CH_2-2′), 49.39 (CH_3**C**H_2N), 67.34 (d, J(C,P) = 4.9 Hz; CH_2-5′), 73.91 (CH-3′), 88.40 (d, J(C,P) = 8.5 Hz; CH-4′), 89.03 (CH-1′), 112.84 (C-5), 129.37 (CH-*m*-phenylene), 131.55 (C-*i*-phenylene), 132.62 (CH-*o*-phenylene), 141.34 (C-*p*-phenylene), 142.41 (CH-6), 159.49 (C-2), 167.19 ppm (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): δ = 1.26 ppm; MS (ESI$^-$): m/z (%): 428 (100) $[M]^+$; HRMS: m/z calcd for $C_{16}H_{19}O_7N_3PS$: 428.0687; found: 428.0687.

7-[(4-Methylsulfanyl)phenyl]-7-deaza-2′-deoxyadenosine 5′-*O*-triphosphate (dA^{MeS}TP): Yield: 38 %; ^{1}H NMR (500.0 MHz, D_2O, pD = 7.1, phosphate buffer, ref(dioxane) = 3.75 ppm): δ = 2.44 (ddd, J = 14.0, 6.0, 3.0 Hz, 1H; H-2′b), 2.52 (s, 3H; CH_3S), 2.69 (ddd, J = 14.0, 7.5, 6.0 Hz, 1H; H-2′a), 4.11 (brm, 1H; H-5′), 4.15 (brm, 1H; H-5′), 4.23 (brm, 1H; H-4′), 4.74 (dt, J = 6.0, 3.0 Hz, 1H; H-3′), 6.62 (dd, J = 7.3, 6.4 Hz, 1H; H-1′), 7.33 (m, 2H; H-*m*-phenylene), 7.36 (m, 2H; H-*o*-phenylene), 7.45 (s, 1H; H-8), 8.13 ppm (s, 1H; H-2); ^{13}C NMR (125.7 MHz, D_2O, pD = 7.1, phosphate buffer, ref(dioxane) = 69.3 ppm): δ = 17.31 (CH_3S), 40.98 (CH_2-2′), 68.30 (d, J(C,P) = 5.6 Hz; CH_2-5′), 73.90 (CH-3′), 85.48 (CH-1′), 87.85 (d, J(C,P) = 8.5 Hz; CH-4′), 103.58 (C-5), 120.50 (C-7), 122.75 (CH-8), 129.73 (CH-*m*-phenylene), 131.92 (CH-*o*-phenylene), 133.01 (C-*i*-phenylene), 139.65 (C-*p*-phenylene), 152.48 (C-4), 153.69 (CH-2), 159.61 ppm (C-6); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, pD = 7.1, phosphate buffer, ref(phosphate buffer) = 2.35 ppm): −21.09 (brt, J = 19.0 Hz; P_β), −10.24 (d, J = 19.0 Hz; P_α), −6.37 ppm (d, J = 19.0 Hz; P_γ); MS (ESI$^-$): m/z (%): 611 (100) $[M-H]^+$, 633 (40) $[M+Na-H]^+$; HRMS: m/z calcd for $C_{11}H_{22}O_{12}N_4P_3S$: 611.0173; found: 611.0160.

7-[(4-Benzylsulfanyl)phenyl]-7-deaza-2′-deoxyadenosine 5′-*O*-triphosphate (dA^{BnS}TP): Yield: 26 %; ^{1}H NMR (499.8 MHz, D_2O, pD = 7.1, ref(dioxane) = 3.75 ppm): δ = 2.41 (ddd, J = 13.9, 6.0, 3.2 Hz, 1H; H-2′b), 2.65 (ddd, J = 13.9, 7.1, 6.2 Hz, 1H; H-2′a), 4.10 (dt, J = 10.6, 4.5 Hz, 1H; H-5′b), 4.12 (s, 2H; CH_2S), 4.14 (dt, J = 10.6, 4.5 Hz, 1H; H-5′a), 4.23 (td, J = 4.5, 3.2 Hz, 1H; H-4′), 4.71 (dt, J = 6.2, 3.2 Hz, 1H; H-3′), 6.58 (dd, J = 7.1, 6.0 Hz, 1H; H-1′), 7.20–7.28 (m, 7H; H-*o,m,p*-Ph, H-*o*-phenylene), 7.30 (m, 2H; H-*m*-phenylene), 7.41 (s, 1H; H-8), 8.13 ppm (brs, 1H; H-2); ^{13}C NMR (125.7 MHz, D_2O, pD = 7.1, ref(dioxane) = 69.3 ppm): δ = 40.45 (CH_2S), 40.99 (CH_2-2′), 68.35 (d, J(C,P) = 5.6 Hz; CH_2-5′), 73.93 (CH-3′), 85.54 (CH-1′), 87.74 (d, J(C,P) = 8.6 Hz; CH-4′), 103.44 (C-5), 120.41 (C-7), 123.03 (CH-8), 130.08 (CH-*p*-Ph), 131.35 (CH-*m*-Ph), 131.56 (CH-*o*-Ph), 131.80 (CH-*o*-phenylene), 133.12 (CH-*m*-phenylene), 134.43 (C-*i*-phenylene), 136.78 (C-*p*-phenylene), 140.30 (C-*i*-Ph), 152.34 (C-4), 153.13 (CH-2), 159.12 ppm (C-6); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, pD = 7.1, ref(phosphate buffer) = 2.35 ppm): δ = −21.04 (brdd, J = 19.4, 18.4 Hz; P_β), −9.76 (d, J = 19.4 Hz; P_α), −7.32 ppm (d, J = 18.4 Hz; P_γ); MS (ESI$^-$): m/z (%): 687 (100) $[M-H]^+$, 709 (20) $[M+Na-H]^+$; HRMS: m/z calcd for $C_{24}H_{25}O_{12}N_4P_3S/2$: 343.0207; found: 343.0196.

7-[(4-Triphenylmethylsulfanyl)phenyl]-7-deaza-2′-deoxyadenosine 5′-*O*-triphosphate (dA^{TrS}TP): Yield: 20 %; ^{1}H NMR (499.8 MHz, CD_3OD): δ = 1.29 (brt, J = 6.9 Hz, 18H; CH_3CH_2N), 2.25 (ddd, J = 13.1, 5.6, 2.9 Hz, 1H; H-2′b), 2.43 (ddd, J = 13.1, 8.1, 5.6 Hz, 1H; H-2′a), 3.18 (brq, J = 6.9 Hz, 12H; CH_3CH_2N), 4.14 (m, 1H; H-4′), 4.22 (brt, J = 5.0 Hz, 2H; H-5′), 4.57 (brdt, J = 5.6, 2.9 Hz, 1H; H-3′), 6.57 (dd, J = 8.1, 5.6 Hz, 1H; H-1′), 6.99 (m, 4H; H-*o,m*-phenylene), 7.22 (m, 9H; H-*m,p*-trityl), 7.41 (m, 6H; H-*o*-trityl), 7.54 (s, 1H; H-8), 8.23 ppm (brs, 1H; H-2); ^{13}C NMR (150.9 MHz, CD_3OD): δ = 9.12 (CH_3CH_2N), 41.16 (CH_2-2′), 47.47 (CH_3CH_2N), 66.91 (d, J(C,P) = 4.3 Hz; CH_2-5′), 72.40 (CH-3′), 72.42 (C-trityl), 84.44 (CH-1′), 87.28 (d, J(C,P) = 8.7 Hz; CH-4′), 100.50 (C-5), 119.40 (C-7), 123.04 (CH-8), 127.99 (CH-*p*-trityl), 128.83 (CH-*m*-trityl), 129.32 (CH-*o*-phenylene), 131.18 (CH-*o*-trityl), 133.90 (C-*i*-phenylene), 135.25 (C-*p*-phenylene), 136.45 (CH-*m*-phenylene), 145.90 (C-*i*-trityl), 147.30 (CH-2), 150.21 ppm (C-4), C-6 not detected; ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): δ = −22.46 (br; P_β), −10.14 (d, J = 19.8 Hz; P_α), −9.44 ppm (d, J = 18.6 Hz; P_γ); MS (ESI$^-$): m/z (%): 839 (20) $[M-H]^+$, 759 (50) $[M-PO_3H-H]^+$, 516 (100) $[M-PO_3H-Tr-H]^+$; HRMS: m/z calcd for $C_{36}H_{33}O_{12}N_4P_3S/2$: 419.0520; found: 419.0516.

5-[(4-Methylsulfanyl)phenyl]-2′-deoxycytidine 5′-*O*-triphosphate (dC^{MeS}TP): Yield: 50%; ^{1}H NMR (600.1 MHz, D_2O, pD = 7.1, phosphate buffer, ref(dioxane) = 3.75 ppm): δ = 2.36 (ddd, J = 14.3, 7.3, 6.6 Hz, 1H; H-2′b), 2.42 (ddd, J = 14.3, 6.4, 3.3 Hz, 1H; H-2′a), 2.53 (s, 3H; CH_3S), 4.15 (br m, 2H; H-5′), 4.22 (br m, 1H; H-4′), 4.60 (dt, J = 6.6, 3.3 Hz, 1H; H-3′), 6.34 (dd, J = 7.3, 6.4 Hz, 1H; H-1′), 7.38 (m, 2H; H-*o*-phenylene), 7.42 (m, 2H; H-*m*-phenylene), 7.73 ppm (s, 1H; H-6); ^{13}C NMR (150.9 MHz, D_2O, pD = 7.1, phosphate buffer, ref(dioxane) = 69.3 ppm): δ = 17.07 (CH_3S), 41.63 (CH_2-2′), 68.04 (d, J(C,P) = 5.4 Hz; CH_2-5′), 73.36 (CH-3′), 88.16 (d, J(C,P) = 8.7 Hz; CH-4′), 88.75 (CH-1′), 112.97 (C-5), 129.42 (CH-*m*-phenylene), 131.62 (C-*i*-phenylene), 132.63 (CH-*o*-phenylene), 141.27 (C-*p*-phenylene), 142.35 (CH-6), 159.83 (C-2), 167.42 ppm (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, pD = 7.1, phosphate buffer, ref(phosphate buffer) = 2.35 ppm): δ = −21.16 (br t, J = 19.3 Hz; P_β), −10.52 (d, J = 19.3 Hz; P_α), −6.31 ppm (d, J = 19.3 Hz; P_γ); MS (ESI$^-$): m/z (%): 588 (100) $[M-H]^+$, 610 (40) $[M+Na-H]^+$; HRMS: m/z calcd for $C_{16}H_{21}O_{13}N_3P_3S$: 588.0013; found: 587.9992.

5-[(4-Benzylsulfanyl)phenyl]-2′-deoxycytidine-5′-*O*-triphosphate (dC^{BnS}TP): Yield: 34%; ^{1}H NMR (499.8 MHz, D_2O, pD = 7.1, ref(dioxane) = 3.75 ppm): δ = 2.35 (ddd, J = 14.1, 7.5, 6.3 Hz, 1H; H-2′b), 2.43 (ddd, J = 14.1, 6.3, 3.4 Hz, 1H; H-2′a), 4.13 (ddd, J = 11.3, 5.8, 4.3 Hz, 1H; H-5′), 4.16 (ddd, J = 11.3, 5.8, 4.3 Hz, 1H; H-5′), 4.22 (td, J = 4.3, 3.4 Hz, 1H; H-4′), 4.28 (s, 2H; CH_2S), 4.59 (dt, J = 6.3, 3.4 Hz, 1H; H-3′), 6.32 (dd, J = 7.5, 6.3 Hz, 1H; H-1′), 7.30 (m, 1H; H-*p*-Ph), 7.34 (m, 2H; H-*o*-phenylene), 7.35 (m, 2H; H-*m*-Ph), 7.42 (m, 2H; H-*o*-Ph), 7.47 (m, 2H; H-*m*-phenylene), 7.72 ppm (s, 1H; H-6); ^{13}C NMR (125.7 MHz, D_2O, pD = 7.1, ref(dioxane) = 69.3 ppm): δ = 39.91 (CH_2S), 41.60 (CH_2-2′), 68.08 (d, J(C,P) = 5.7 Hz; CH_2-5′), 73.44 (CH-3′), 88.17 (d, J(C,P) = 8.7 Hz; CH-4′), 88.81 (CH-1′), 112.79 (C-5), 130.16 (CH-*p*-Ph), 131.49 (CH-*m*-Ph), 131.59 (CH-*o*-Ph), 132.63, 132.66 (CH-*o,m*-phenylene), 133.08 (C-*i*-phenylene), 138.45 (C-*p*-phenylene), 140.43 (C-*i*-Ph), 142.49 (CH-6), 159.77 (C-2), 167.28 ppm (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, pD = 7.1, ref(phosphate buffer) = 2.35 ppm): δ = −21.38 (t, J = 19.4 Hz; P_β), −10.35 (d, J = 19.4 Hz; P_α), −7.07 ppm (d, J = 19.4 Hz; P_γ); MS (ESI$^-$): m/z (%): 664 (50) $[M-H]^+$, 584 (100) $[M-PO_3H-H]^+$; HRMS: m/z calcd for $C_{22}H_{24}O_{13}N_3P_3S/2$: 331.5127; found: 331.5125.

5-[(4-Triphenylmethylsulfanyl)phenyl]-2′-deoxycytidine 5′-*O*-triphosphate (dC^{TrS}TP): Yield: 10%; ^{1}H NMR (499.8 MHz, CD_3OD): δ = 1.29 (t, J = 7.0 Hz, 36H; CH_3CH_2N), 2.19 (ddd, J = 13.7, 7.6, 6.7 Hz, 1H; H-2′b), 2.36 (ddd, J = 13.7, 5.9, 3.1 Hz, 1H; H-2′a), 3.18 (q, J = 7.0 Hz, 24H; CH_3CH_2N), 4.11 (m, 2H; H-5′), 4.23 (m, 1H; H-4′), 4.55 (dt, J = 6.7, 3.1 Hz, 1H; H-3′), 6.26 (dd, J = 7.6, 5.9 Hz, 1H; H-1′), 7.08 (m, 4H; H-*o,m*-phenylene), 7.21 (m, 3H; H-*p*-trityl), 7.26 (m, 6H; H-*m*-trityl), 7.41 (m, 6H; H-*o*-trityl), 7.69 ppm (s, 1H; H-6); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 9.11 (CH_3CH_2N), 41.14 (CH_2-2′), 47.39 (CH_3CH_2N), 66.72 (d, J(C,P) = 7.4 Hz; CH_2-5′), 72.16 (C-trityl), 72.31 (CH-3′), 87.56 (CH-1′), 87.71 (d, J(C,P) = 8.2 Hz; CH-4′), 109.93 (C-5), 127.99 (CH-*p*-trityl), 128.83 (CH-*m*-trityl), 130.01 (CH-*o*-phenylene), 131.18 (CH-*o*-trityl), 133.65 (C-*i*-phenylene), 135.89 (CH-*m*-phenylene), 136.51 (C-*p*-phenylene), 140.93 (CH-6), 145.84 (C-*i*-trityl), 157.54 (C-2), 165.33 ppm (C-4); ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): δ = −22.54 (br; P_β), −10.51 (d, J = 20.6 Hz; P_α), −9.43 (d, J = 18.8 Hz; P_γ); MS (ESI$^-$): m/z (%): 816 (40) $[M-H]^+$, 736 (30) $[M-PO_3H-H]^+$, 493 ppm (100) $[M-PO_3H-Tr-H]^+$; HRMS: m/z calcd for $C_{34}H_{33}O_{13}N_3P_3S$: 816.0952; found: 816.0936.

Primer extension experiment: The reaction mixture (20 μL) contained Vent(*exo*-) DNA polymerase (New England Biolabs, 0.1 unit), natural dNTPs (Fermentas, 0.2 mM), modified surrogates **dA^{RS}TP** or **dC^{RS}TP** (0.2 mM), 3′-GGGTACGGCGGGTAC-5′ primer (Sigma–Aldrich oligoes, 0.15 μM), temp^{rnd16} template 5′-CTAGCATGAGCTCAGTCC-CATGCCGCCCATG-3′ (Sigma–Aldrich oligoes, 0.225 μM) in 1×ThermoPol reaction buffer. The primer was labelled with of [γ^{32}P]-ATP by using standard techniques. Reaction mixtures were incubated for 30 min at 60 °C in a thermal cycler and were stopped by addition of the stop solution (40 μL, 80% *v/v*, formamide, 20 mM ethylenediaminetetraacetic acid, 0.025% *w/v*, bromophenol blue, 0.025% *w/v*, xylene cyanol). Reaction mixtures were separated by use of 12.5% denaturing polyacrylamide gel electrophoresis. Visualisation was performed by phosphoimaging.

Electrochemical analysis: Nucleosides, dNMPs and other building blocks were analysed in situ by conventional CV, ACV and CPS. The PEX products were analysed by ex situ (adsorptive transfer stripping) CV, ACV and CPS. The PEX products were accumulated over 60 s from 5 μL aliquots that contained 0.2 M NaCl at the surface of the working electrode (HMDE). The electrode was then rinsed with deionised water and placed in the electrochemical cell. CV settings: scan rate 0.5 V s^{-1}, initial potential 0.15 V, switching potential −1.6 V (electrolyte: 0.2 M acetate buffer, pH 5.0); −1.9 V (electrolyte: 0.05 M sodium tetraborate, pH 9.3), end potential 0.15 V. ACV settings: frequency 230 Hz, amplitude 10 mV, initial potential 0.15 V, end potential −1.9 V (electrolyte: 0.05 M sodium tetraborate, pH 9.3). CPS settings: stripping current −10 μA, initial potential 0 V (electrolyte: 0.1 M ammonium buffer + 1 mM $[Co(NH_3)_6]^{3+}$, pH 9.5). All electrochemical measurements were performed with an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-stand 663 (Metrohm, Herisau, Switzerland). The three-electrode system was used with a Ag/AgCl/3 M KCl electrode as a reference and platinum wire as an auxiliary electrode. As a working electrode, HMDE with an area of 0.4 mm^2 was used. CV measurements were performed after the solution was purged with argon. All electrochemical measurements were performed at room temperature.

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