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Aqueous Heck Cross-Coupling Preparation of Acrylate-Modified Nucleotides and Nucleoside Triphosphates for Polymerase Synthesis of Acrylate-Labeled DNA

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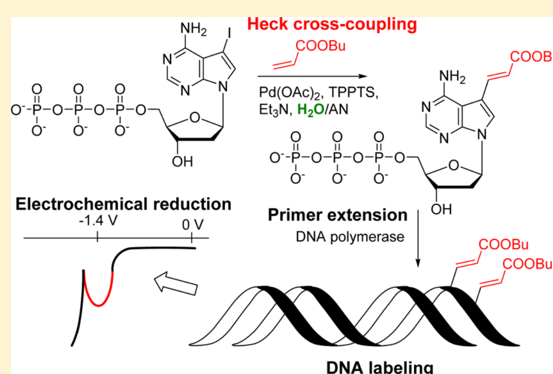
S *Supporting Information*

ABSTRACT: Aqueous-phase Heck coupling methodology was developed for direct attachment of butyl acrylate to 5-iodoracil, 5-iodocytosine, 7-iodo-7-deazaadenine, and 7-iodo-7-deazaguanine 2′-deoxyribonucleoside 5′-*O*-monophosphates (dNMPs) and 5′-*O*-triphosphates (dNTPs) and compared with the classical approach of phosphorylation of the corresponding modified nucleosides. The 7-substituted 7-deazapurine nucleotides (**dA^{BA}MP**, **dA^{BA}TP**, **dG^{BA}MP**, and **dG^{BA}TP**) were prepared by the direct Heck coupling of nucleotides in good yields (35−55%), whereas the pyrimidine nucleotides reacted poorly and the corresponding BA-modified dNTPs were prepared by triphosphorylation of the modified nucleosides. The acrylate-modified **dN^{BA}TPs** (N = A, C, and U) were good substrates for DNA polymerases and were used for enzymatic synthesis of acrylate-modified DNA by primer extension, whereas **dG^{BA}TP** was an inhibitor of polymerases. The butyl acrylate group was found to be a useful redox label giving a strong reduction peak at −1.3 to −1.4 V in cyclic voltammetry.



■ INTRODUCTION

Base-modified 2′-deoxyribonucleoside triphosphates (dNTPs) bearing chemical modifications at position 5 of pyrimidines or at position 7 of 7-deazapurines are generally good substrates for DNA polymerases. Diverse protocols for enzymatic synthesis of base-modified DNA have been developed and have been extensively used in the past decade.[1] The methods include primer extension (PEX) or PCR,[2] site-specific single-nucleotide incorporation,[3] or nicking-enzyme amplification reaction (NEAR).[4] The applications cover fluorescent,[5,6] redox,[7] spin,[8] barcode,[9] and reactive[10] labeling, protection,[11] and incorporation of protein-like groups for catalysis.[12] The modified dNTPs are usually synthesized by triphosphorylation of modified nucleosides which is laborious and sometimes incompatible with the introduced functionality.

Aqueous-phase cross-coupling reactions are an increasingly popular and useful tool in modifications of polar molecules[13] and are of particular importance for biomolecules. The very first report was on the Sonogashira coupling of 5-iodo-2′-deoxyuridine and dUMP with propargylamine by Casalnuovo.[14] Later on, the Shaughnessy group has developed the first aqueous Suzuki−Miyaura coupling of unprotected halogenated purine nucleosides,[15] whereas the Burgess group reported the first direct Sonogashira coupling of 5-iodoracil dNTP.[5] In our laboratory, the aqueous Suzuki coupling of halogenated nucleotides and dNTPs with diverse boronic acids was developed.[16] More recently, the Suzuki reactions of oligonucleotides have also been reported.[17] We extensively use both Suzuki and Sonogashira couplings for modification of pyrimidine and 7-deazapurine dNTPs linked through aryl groups (by the Suzuki coupling) or through the alkynyl moiety (by the Sonogashira coupling).[6,7,10,11] Both these reactions are performed in water/acetonitrile mixtures using triphenylphosphine-3,3′,3″-trisulfonate (TPPTS) as water-soluble ligand for palladium catalyst. Both these reactions are very tolerant to the presence of large variety of functional groups and allow a single-step preparation of modified dNTPs without the need of protection group manipulation. Development of other aqueous coupling reactions applicable for other types of carbon substituents is still highly desirable to extend the portfolio of bioorthogonal modifications of nucleic acids.

The Heck reaction is another very useful type of cross-coupling extensively applied in attachment of alkenyl groups.[18]

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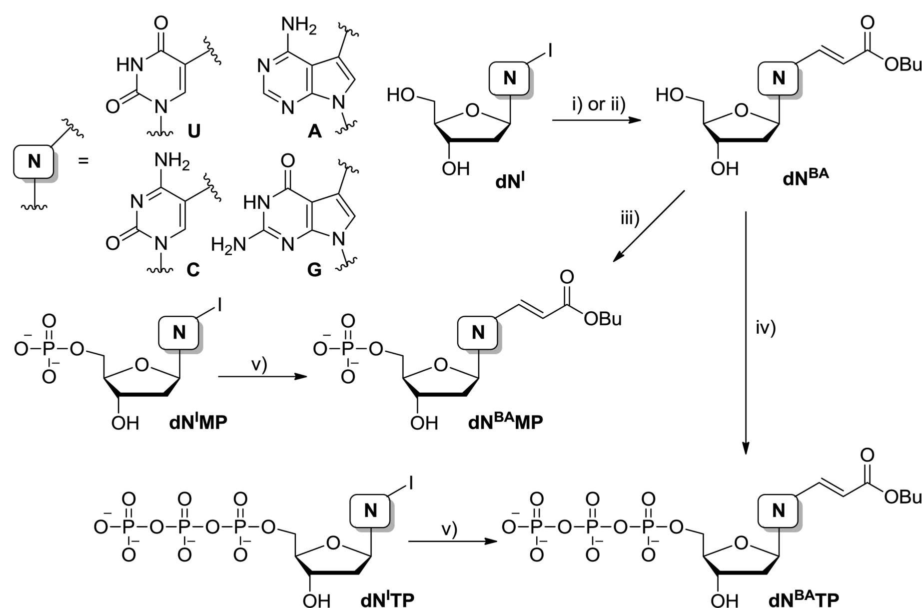
Scheme 1. Synthesis of Butyl Acrylate Modified Nucleosides (dN^{BA}s), Nucleoside Mono- (dN^{BA}MPs), and Triphosphates (dN^{BA}TPs)[a]



[a]Reagents and conditions: (i) butyl acrylate, $Pd(OAc)_2$, PPh_3, Et_3N, DMF; (ii) butyl acrylate, $Pd(OAc)_2$, TPPTS, Et_3N, CH_3CN/H_2O (1:1); (iii) $PO(OMe)_3$, $POCl_3$, 0 °C; (iv) (1) $PO(OMe)_3$, $POCl_3$, 0 °C, (2) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0 °C, (3) 2 M TEAB; (v) butyl acrylate, $Pd(OAc)_2$, TPPTS, Et_3N, CH_3CN/H_2O (1:1).

Table 1. Synthesis of Butyl Acrylate Modified Nucleosides and Nucleotides

entry	starting compd	product	catalyst	additives	solvent	yield (%)
1	**dC^I**	**dC^{BA}**	$Pd(OAc)_2$, PPh_3	Et_3N	DMF	14
2	**dU^I**	**dU^{BA}**	$Pd(OAc)_2$, PPh_3	Et_3N	DMF	93
3	**dA^I**	**dA^{BA}**	$Pd(OAc)_2$, PPh_3	Et_3N	DMF	97
4	**dG^I**	**dG^{BA}**	$Pd(OAc)_2$, PPh_3	Et_3N	DMF	83
5	**dC^I**	**dC^{BA}**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	16
6	**dU^I**	**dU^{BA}**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	98
7	**dA^I**	**dA^{BA}**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	81
8	**dG^I**	**dG^{BA}**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	84
9	**dC^IMP**	**dC^{BA}MP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	-
10	**dU^IMP**	**dU^{BA}MP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	35
11	**dA^IMP**	**dA^{BA}MP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	55
12	**dG^IMP**	**dG^{BA}MP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	38
13	**dC^ITP**	**dC^{BA}TP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	-
14	**dU^ITP**	**dU^{BA}TP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	4
15	**dU^ITP**	**dU^{BA}TP**	$Pd(OAc)_2$, PPh_3	Et_3N	DMF	14
16	**dA^ITP**	**dA^{BA}TP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	43
17	**dG^ITP**	**dG^{BA}TP**	$Pd(OAc)_2$, TPPTS	Et_3N	CH_3CN/H_2O (1:1)	44
18	**dU^{BA}**	**dU^{BA}MP**	$PO(OMe)_3$, $POCl_3$, 0 °C			42
19	**dA^{BA}**	**dA^{BA}MP**	$PO(OMe)_3$, $POCl_3$, 0 °C			54
20	**dC^{BA}**	**dC^{BA}TP**	(1) $PO(OMe)_3$, $POCl_3$, 0 °C; (2) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0 °C; (3) 2 M TEAB			19
21	**dU^{BA}**	**dU^{BA}TP**	(1) $PO(OMe)_3$, $POCl_3$, 0 °C; (2) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0 °C; (3) 2 M TEAB			40
22	**dA^{BA}**	**dA^{BA}TP**	(1) $PO(OMe)_3$, $POCl_3$, 0 °C; (2) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0 °C; (3) 2 M TEAB			28
23	**dG^{BA}**	**dG^{BA}TP**	(1) $PO(OMe)_3$, $POCl_3$, 0 °C; (2) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF, 0 °C; (3) 2 M TEAB			25

In nucleoside chemistry it has been often used for modifications of pyrimidines[19] but is difficult for modifications of purines.[20] Only very recently, Shaughnessy reported[21] the first aqueous-phase Heck reactions for modification of 5-iodo-2′-deoxyuridine. Here we report on the development of the aqueus Heck coupling for modifications of pyridimine and 7-deazapurine nucleoside mono- and triphosphates and the use of acrylate-modified dNTPs for enzymatic synthesis of modified DNA.

RESULTS AND DISCUSSION

Synthesis of Acrylate-Modified Nucleosides and Nucleotides by Aqueous Heck Coupling. The acrylate

Table 2. List of Oligodeoxyribonucleotides Used or Synthesized[a]

oligonucleotide	sequence
prim	5′-CATGGGCGGCATGGG-3′
temp^{1C}	5′-**CCCG**CCCATGCCGCCCATG-3′
temp^{1T}	5′-**CCCA**CCCATGCCGCCCATG-3′
temp^{1A}	5′-**CCCT**CCCATGCCGCCCATG-3′
temp^{1G}	5′-**AAAC**CCCATGCCGCCCATG-3′
temp^{4}	5′-**CTAGCATGAGCTCAGT**CCCATGCCGCCCATG-3′
ON^{1C}	5′-CATGGGCGGCATGGG**C^{BA}**GGG-3′
ON^{1T}	5′-CATGGGCGGCATGGG**U^{BA}**GGG-3′
ON^{1A}	5′-CATGGGCGGCATGGG**A^{BA}**GGG-3′
ON^{1G}	5′-CATGGGCGGCATGGG**G^{BA}**TTT-3′
ON^{4C}	5′-CATGGGCGGCATGGGA**C^{BA}**TGAG**C^{BA}**T**C^{BA}**ATG**C^{BA}**TAG-3′
ON^{4T}	5′-CATGGGCGGCATGGGAC**U^{BA}**GAGC**U^{BA}**CA**U^{BA}**GC**U^{BA}**AG-3′
ON^{4A}	5′-CATGGGCGGCATGGG**A^{BA}**CTG**A^{BA}**GCTC**A^{BA}**TGCT**A^{BA}**G-3′
ON^{4G}	5′-CATGGGCGGCATGGGACU**G^{BA}**A**G^{BA}**CUCAU**G^{BA}**CUA**G^{BA}**-3′

[a] In the template (temp) ONs the segment-forming duplex with the primer are underlined and the replicated segments are in bold. For magnetic separation of the extended primer strands, the templates were 5′-end biotinylated. Acronyms used in the text for primer extension products are analogous to those introduced for the templates (e.g., ssDNA PEX product **ON^{1A}** was synthesized on temp^{1A} etc.).

ester moiety is an attractive label for DNA since it can be in principle further derivatized by amide formation, conjugate additions, reductions, etc. and also could be reduced on electrode to serve as redox label for electrochemistry. Jäger et al.[2] prepared methyl acrylate-modified dUTP by triphosphorylation of nucleoside and showed its successful incorporation by Vent(*exo*-) polymerase but never reported any further use. We wanted to study the aqueous Heck reaction of iodinated pyrimidine and deazapurine nucleotides and dNTPs. However, at first we tested the Heck reactions of 2′-deoxy-5-iodocytidine (**dC^{I}**), 2′-deoxy-5-iodoridine (**dU^{I}**), 2′-deoxy-7-iodo-7-deazaadenosine (**dA^{I}**) and 2′-deoxy-7-iodo-7-deazaguanosine (**dG^{I}**) nucleosides with *n*-butyl acrylate (6 equiv) in the presence of $Pd(OAc)_2$ (10 mol %), triphenylphosphine, and triethylamine in DMF (Scheme 1). While the reactions with **dU^{I}**, **dA^{I}**, and **dG^{I}** proceeded almost quantitatively to give the desired acrylate-modified nucleosides in excellent yields (93% **dU^{BA}**, 97% **dA^{BA}**, and 83% **dG^{BA}**; Table 1, entries 2−4), **dC^{I}** showed much lower reactivity and the desired **dC^{BA}** was isolated in poor yield (14%; Table 1, entry 1). Then, for comparison, aqueous Heck reactions of iodinated nucleosides with *n*-butyl acrylate were carried out in the mixture water/acetonitrile (1:1) in the presence of water-soluble phosphine ligand tris(3-sulfonatophenyl)phosphine (TPPTS, Scheme 1). The results were comparable to the DMF protocol in all cases. The couplings on **dU^{I}**, **dA^{I}** ,and **dG^{I}** proceeded smoothly with good isolated yields of modified nucleosides (98% **dU^{BA}**, 81% **dA^{BA}**, and 84% **dG^{BA}**; Table 1, entries 6−8), whereas **dC^{I}** gave **dC^{BA}** in an isolated yield of 16% (Table 1, entry 5).

Then the aqueous conditions were tested with halogenated nucleoside monophosphates (dNMPs) which are relatively stable and water-soluble compounds suitable as models for nucleic acids (Scheme 1). Thus, the aqueous Heck coupling of **dC^{I}MP**, **dU^{I}MP**, **dA^{I}MP**, and **dG^{I}MP** with *n*-butyl acrylate (the excess was increased to 10 equiv) were performed in the presence of $Pd(OAc)_2$ (10 mol % were necessary) and TPPTS (25 mol %). The reactions proceeded less efficiently (compared to nucleosides) with ca. 60−70% conversion to give modified nucleotides **dU^{BA}MP**, **dA^{BA}MP**, and **dG^{BA}MP** in isolated yields of 35%, 55%, and 38%, respectively (Table 1, entries 10−12). The formation of **dC^{BA}MP** was not observed (Table 1, entry 9). Finally, the aqueous phase Heck coupling was performed on nucleoside triphosphates (**dC^{I}TP**, **dU^{I}TP**, **dA^{I}TP**, and **dG^{I}TP**, Scheme 1). To minimize hydrolysis of triphosphate group, the reaction time was shortened to 1 h under the same conditions as above to reach ca. 50% conversion. The deazapurine dNTPs reacted well to obtain **dA^{BA}TP** and **dG^{BA}TP** in acceptable yields of 43% and 44%, respectively (Table 1, entries 16 and 17). On the other hand, pyrimidine dNTPs reacted poorly. **dC^{BA}TP** was not detected in the reaction mixture, whereas **dU^{BA}TP** was isolated in 4% yield only due to substantial hydrolysis of both starting **dU^{I}TP** and the product (Table 1, entries 13 and 14). To prevent the hydrolysis, the Heck coupling of **dU^{I}TP** was also performed in DMF under the conditions described above for the synthesis of modified nucleosides. The conversion increased to ca. 80%, but the isolated yield of **dU^{BA}TP** was 14% (Table 1, entry 15) and substantive amounts of the corresponding mono- and diphosphate were observed. It indicates that **dU^{BA}TP** was largely hydrolyzed during the isolation and purification process.

For comparison of the efficiency of the aqueous-phase direct modification of nucleotides with the classical approach, the acrylate modified nucleosides (**dC^{BA}**, **dU^{BA}**, **dA^{BA}**, and **dG^{BA}**) were phosphorylated to obtain **dN^{BA}MPs** and **dN^{BA}TPs** (Scheme 1, Table 1). The treatment of **dU^{BA}** or **dA^{BA}** with $POCl_3$ in $PO(OMe)_3$ at 0 °C followed by quenching the reaction with triethylammonium bicarbonate (TEAB, 2 M) gave the desired modified **dN^{BA}MPs** in acceptable isolated yields (42% for **dU^{BA}MP** and 54% for **dA^{BA}MP**). Triphosphorylation of nucleosides **dC^{BA}**, **dU^{BA}**, **dA^{BA}**, and **dG^{BA}** was performed under standard conditions[22] by treatment with $POCl_3$ in $PO(OMe)_3$ at 0 °C followed by addition of $(NHBu_3)_2H_2P_2O_7$ in DMF in the presence of tributylamine. The reaction was quenched with TEAB and after purification, **dN^{BA}TPs** were obtained in acceptable yields (19% for **dC^{BA}TP**, 40% for **dU^{BA}TP**, 28% for **dA^{BA}TP**, and 25% for **dG^{BA}TP**).

The yields of both approaches were then compared. The efficiency of direct aqueous Heck reaction of halogenated nucleotides was comparable with phosphorylation in the case of both tested monophosphates (**dU^{BA}MP** and **dA^{BA}MP**) giving yields of 35−55%. It was even more efficient for the synthesis of deazapurine dNTPs: **dA^{BA}TP** (43% compared to 28% yield of triphosphorylation) and **dG^{BA}TP** (44% compared to 25% yield of triphosphorylation). However, in the preparation of

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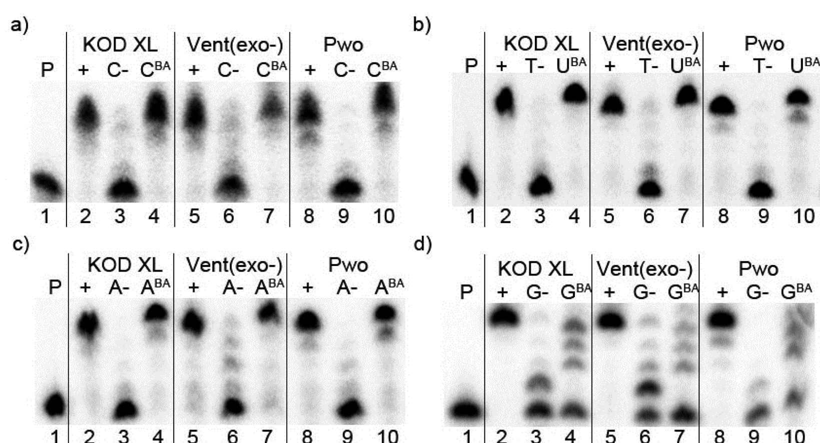


Figure 1. Primer extension with (a) temp^{1C}; (b) temp^{1T}; (c) temp^{1A}; (d) temp^{1G}. Key: P, primer; C$^+$, dCTP, dGTP; C$^-$, dGTP; C^{BA}, **dC^{BA}TP**, dGTP; T$^+$, dTTP, dGTP; T$^-$, dGTP; U^{BA}, **dU^{BA}TP**, dGTP; A$^+$, dATP, dGTP; A$^-$, dGTP; A^{BA}, **dA^{BA}TP**, dGTP; G$^+$, dGTP, dTTP; G$^-$, dTTP; G^{BA}, **dG^{BA}TP**, dTTP.

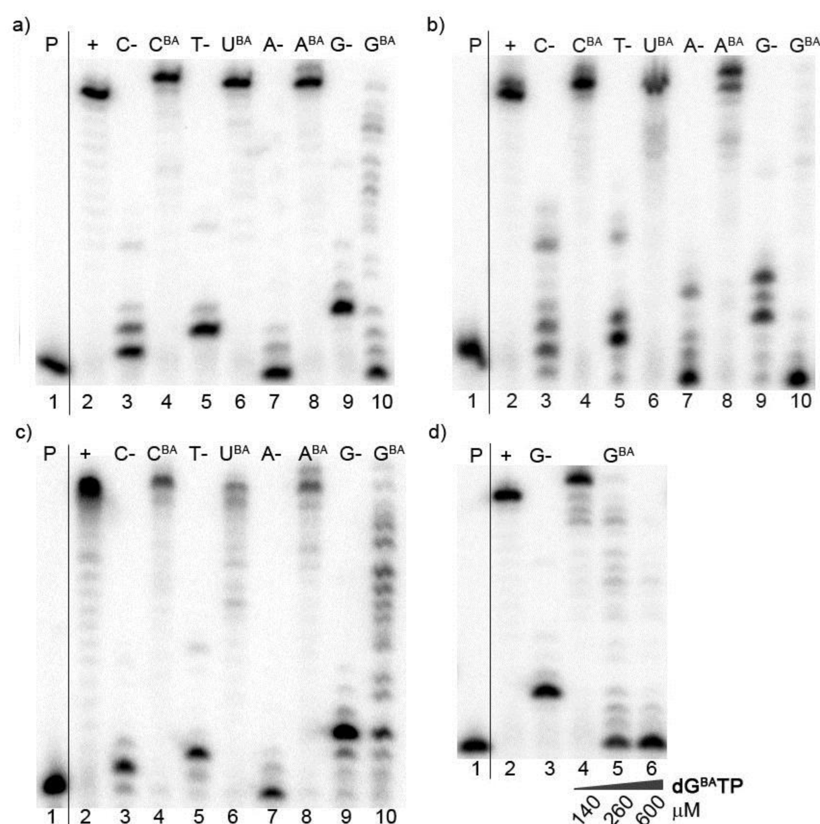


Figure 2. Primer extension with temp^4 using (a) KOD XL; (b) Vent(*exo*-); (c) Pwo DNA polymerase; (d) inhibition of KOD XL DNA polymerase by **dG^{BA}TP**. Key: P, primer; +, all natural dNTPs; C$^-$, dATP, dTTP, dGTP; C^{BA}, **dC^{BA}TP**, dATP, dTTP, dGTP; T$^-$, dATP, dCTP, dGTP; U^{BA}, **dU^{BA}TP**, dATP, dCTP, dGTP; A$^-$,dTTP, dCTP, dGTP; A^{BA}, **dA^{BA}TP**, dTTP, dCTP, dGTP; G$^-$, dTTP, dCTP, dATP; G^{BA}, **dG^{BA}TP**, dTTP, dCTP, dATP. In (d): lane 4, 140 μM; lane 5, 260 μM; lane 6, 600 μM **dG^{BA}TP**.

dU^{BA}TP and **dC^{BA}TP**, the Heck reaction of **dU^{I}TP** proceeded only in low yield accompanied by large hydrolysis and the reaction of **dC^{I}TP** did not occur at all. Significantly higher yields were observed by triphosphorylation of **dU^{BA}** (40%) or **dC^{BA}** (19%).

Incorporation of dN^{BA}TPs into DNA by PEX. The enzymatic synthesis of *n*-butyl acrylate modified oligonucleotides (**ON^{BA}**s) was studied by primer extension experiment (PEX) using KOD XL, Vent(*exo*-), or Pwo polymerases. The templates and primer (for sequences see Table 2) were chosen in order to introduce one (**ON^{1X}**) or four modifications (**ON^{4X}**) to the extended primer strand.

dC^{BA}TP, **dU^{BA}TP**, and **dA^{BA}TP** were found to be good substrates for all of the tested enzymes and were successfully incorporated into DNA bearing one modification (**ON^{1X}**). Figure 1 shows the denaturing PAGE where only full length products were observed in all cases (except of weak additional bands of $n - 1$ products with Pwo polymerase). **dG^{BA}TP** was not incorporated into DNA (**ON^{1G}**, Figure 1d) by the tested enzymes under the same conditions.

All $dN^{BA}TP$s were also tested for multiple incorporations into DNA of a mixed sequence bearing four modifications (Figure 2). All tested DNA polymerases gave full length products with $dC^{BA}TP$ and $dA^{BA}TP$. KOD XL and Vent(*exo-*) (but not Pwo) were shown to be suitable enzymes for multiple incorporation of $dU^{BA}TP$ (Figure 2a,b; lane 6). On the other hand, $dG^{BA}TP$ was not only a poor substrate for these polymerases but also apparently inhibited the enzymatic synthesis of DNA by KOD XL and Vent(*exo-*) (Figure 2a,b; lane 10) where only unextended primers were detected. Therefore, the PEX using KOD XL was performed with decreasing concentrations of $dG^{BA}TP$. Figure 2d clearly confirms that higher concentrations of $dG^{BA}TP$ inhibited polymerase activity, whereas at lower concentration of $dG^{BA}TP$, the desired full-length PEX product has been obtained.

All ON^{BA}s prepared by PEX with biotinylated template using KOD XL DNA polymerase were isolated by magnetoseparation[7] and analyzed by MALDI (data are summarized in Table 3; for copies of spectra see Figures S1−S8 in the Supporting Information). KOD XL DNA polymerase was then also used for preparation of ON^{BA}s for the electrochemical studies.

Table 3. MALDI Data of ONs Bearing Butyl Acrylate

oligonucleotide	*M* (calcd) (Da)	*M* (found) [M + H]$^+$ (Da)
ON^{1C}	6077.1	6078.6
ON^{1T}	6078.0	6079.1
ON^{1A}	6100.1	6101.2
ON^{1G}	6041.1	6042.2
ON^{4C}	10122.1	10123.2
ON^{4T}	10065.6	10066.1
ON^{4A}	10118.1	10122.3
ON^{4G}	10118.1	10119.2

Electrochemical Study. Electrochemistry has proved to be potent, widely applicable tool for analysis of nucleic acids modified with diverse oxidizable or reducible extrinsic moieties.[7] In our previous work, we demonstrated that such species can be applied for redox coding of nucleotide sequences and/or individual nucleobases with potential applications in DNA diagnostics. To complete the palette of redox tags for independent coding of all four nucleobases, new potential labels are sought among all newly synthesized dNTP conjugates. Moreover, not only species applied purposely as redox DNA labels but also functional groups serving for further derivatization (DNA postsynthetic modification) introduced into DNA can be determined and their chemical conversion monitored by means of simple voltammetric techniques.[23] Thus, we were interested in electrochemical properties of the **BA** nucleos(t)ide conjugates. Since the dC^{BA} nucleotides were difficult to synthesize (due to low reactivity) and $dG^{BA}TP$ was an inhibitor of DNA polymerases, we have chosen dU^{BA}- and dA^{BA}-modified ONs for the electrochemical studies. As evident from cyclic voltammograms (CVs) measured on a hanging mercury electrode (HMDE) shown in Figure 3A, dA^{BA} as well $dA^{BA}MP$ produced two separated irreversible cathodic peaks which can be assigned to electrochemical reduction of adenine (peak A^{red} at −1.430 V) and of the BA moiety at a less negative potential (peak BA^{red} at −1.295 V; compare with the behavior of modified DNA, vide infra). For dU^{BA} and $dU^{BA}MP$ only one irreversible signal was observed in agreement with the fact that

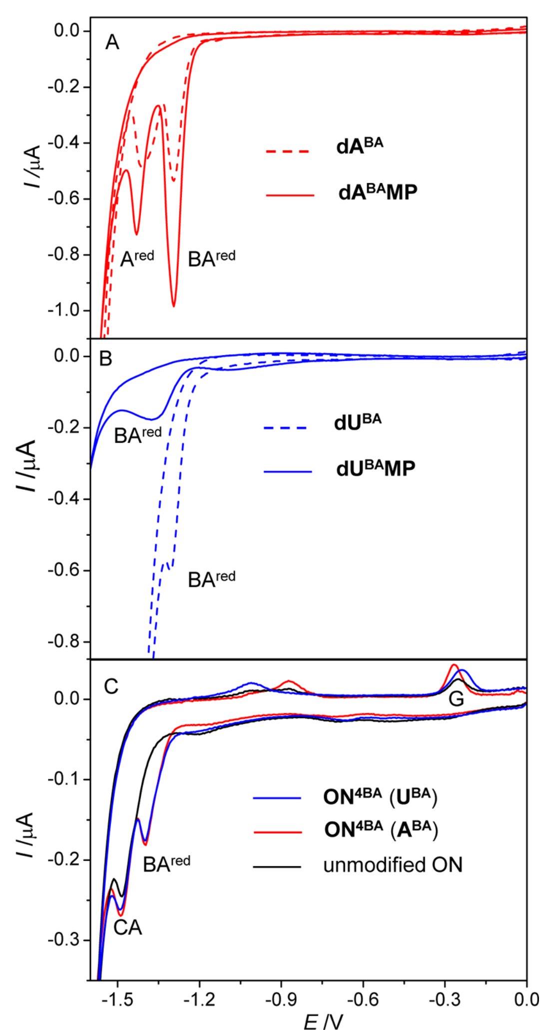


Figure 3. (A, B) Cyclic voltammograms of dA^{BA}, $dA^{BA}MP$ (A), dU^{BA} and $dU^{BA}MP$ (B). Concentrations of all substances 40 μM, background electrolyte sodium acetate buffer pH 5.0. (C) Ex situ CVs of unmodified ON and ON^{4BA}s with A^{BA} or U^{BA} incorporated; measured in ammonium formate/sodium phosphate background electrolyte. See the Experimental Section for details.

reduction of uracil cannot be measured at mercury electrodes in aqueous media. In analogy with previously studied *α,β*-unsaturated carbonyl compounds,[24] including ketones,[24a] esters, amides,[24b] and acrylate anion,[24c] the primary electroreduction of **BA** moiety can be expected at the *α,β* C=C double bond probably via two successive one-electron steps, in aqueous medium resulting in the double bond hydrogenation. Different shapes and potential shifts of the BA^{red} peak among the **BA**-modified nucleos(t)ides can be ascribed to different adsorbability of these species, effect of negative charge of the phosphate group and influence of these phenomena on the reduction mechanism of the given compound.

Figure 3C shows ex situ CVs obtained for unmodified ON (product of PEX with all four unmodified dNTPs on temp[4] template) and ON^{4BA}s with incorporated either A^{BA} or U^{BA} conjugates. All ONs yielded a cathodic peak CA at −1.490 V due to irreversible reduction of adenines and cytosines and an anodic peak G at −0.255 V due to reoxidation of guanine reduction product generated at potentials more negative than −1.6 V.[25] Both ON^{4BA}s produced and additional reduction signal, peak BA^{red}, at −1.400 V, i.e., at a potential less negative

than potential of peak CA (compare behavior of **dA^{BA}(MP)** above). In contrast to nucleo(s)tides, the modified ON produce practically identical shape of the voltammograms regardless of which modified nucleotide was incorporated. Such behavior could be expected considering the major contribution of the polyanionic ON molecule containing excess of unmodified nucleotides, dictating the overall interaction of the ON with the negatively charged electrode surface. Some differences were observed in the anodic branch of CVs where broad peaks appeared with the modified ONs in the region between −1.160 and −0.750 V and intensities and potential of peak G were also influenced by the modifications (Figure 3C) which may be ascribed to effects of the BA reduction products on electrode processes at negatively charged HMDE surface. Explanation of these phenomena will require more detailed study which is beyond the scope of this report. Nevertheless, data presented here demonstrate applicability of the **BA** electrochemical reduction for monitoring of DNA modification with this functional group.

■ CONCLUSIONS

The first aqueous Heck cross-coupling of halogenated nucleotides and dNTPs has been developed and tested on the synthesis of acrylate-modified **dN^{BA}MP**s and **dN^{BA}TP**s. For modification of 7-deazapurine nucleotides (synthesis of **dA^{BA}MP**, **dA^{BA}TP**, **dG^{BA}MP**, and **dG^{BA}TP**), the direct aqueous coupling procedure is comparable or more efficient than phosphorylation of modified nucleosides. However, the Heck coupling of pyrimidine dNTPs gave only traces of **dU^{BA}TP** or no reaction at all (for **dC^{I}MP** and **dC^{I}TP**), and therefore, the phosphorylation approach is necessary for the synthesis of these compounds. It can be concluded that the aqueous Heck cross-coupling is a possible reaction for modification of deazapurine dNTPs; however, apparently it is far less general and efficient than the Suzuki and Sonogashira reactions developed previously.[5−7,10,11]

dC^{BA}TP, **dU^{BA}TP**, and **dA^{BA}TP** were found to be good substrates for DNA polymerases and were efficiently incorporated to ssONs and dsDNAs by PEX, whereas **dG^{BA}TP** was found to be inhibitor at higher concentrations. Electrochemical properties of the butyl acrylate group were also studied on **dU^{BA}**- and **dA^{BA}**-modified ONs to show that it gives an analytically useful signal of reduction at −1.4 V in cyclic voltammetry suitable for monitoring DNA modification with **BA**. Considering the position of the **BA** reduction signal at a potential less negative than potential of nucleobase reduction but more negative than potential of reduction of previously introduced redox tags,[7] it is promising also for possible redox coding of nucleobases in combination with other redox labels[7] as an extension of the available palette of reducible tags toward more negative potential region. Another field of potential applications are further postsynthetic chemical transformations of the butyl acrylate group in DNA. Further studies along these lines are under way.

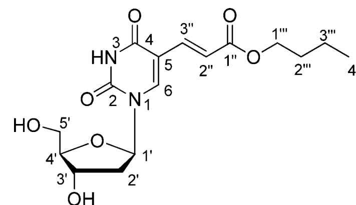
■ EXPERIMENTAL SECTION

NMR spectra were recorded on a 600 MHz (600.1 MHz for ^{1}H, 150.9 MHz for ^{13}C) or a 500 MHz (499.8 or 500.0 MHz for ^{1}H, 202.3 or 202.4 MHz for ^{31}P, 125.7 MHz for ^{13}C) spectrometer from sample solutions in D_2O or CD_3OD. Chemical shifts (in ppm, δ scale) were referenced as follows: D_2O (referenced to dioxane as internal standard: 3.75 ppm for ^{1}H NMR and 69.30 ppm ^{13}C NMR); CD_3OD (referenced to solvent signal: 3.31 ppm for ^{1}H NMR and 49.00 ppm for ^{13}C NMR). ^{31}P chemical shifts were referenced to H_3PO_4 as external reference or to phosphate buffer signal 2.35 ppm in the case of measurement in phosphate buffer. Coupling constants (J) are given in hertz. NMR spectra of dNTPs were measured in phosphate buffer at pH 7.1. Complete assignment of all NMR signals was achieved by using a combination of H,H-COSY, H,C-HSQC, and H,C-HMBC experiments. Mass spectra and high-resolution mass spectra were measured using ESI ionization technique. Mass spectra of functionalized ONs were measured by MALDI-TOF with 1 kHz smartbeam II laser. Water used in synthetic part was of HPLC quality. Ultrapure water (18 MΩ.cm) was used for all biochemical experiments. All chemicals, oligonucleotides, enzymes, streptavidine magnetic particles, and isolation kits were purchased from commercial suppliers. Synthesis and characterization data for 2′-deoxy-5-iodocytidine 5′-*O*-triphosphate,[7b] 2′-deoxy-5-iodouridine 5′-*O*-triphosphate,[26a] 2′-deoxy-7-iodo-7-deazaadenosine 5′-*O*-triphosphate,[16b] 2′-deoxy-7-iodo-7-deazaguanosine 5′-*O*-triphosphate[26b] were reported previously.

General Procedure I: Preparation of Butylacrylate-Modified Nucleosides (dN^{BA}s). *Method Ia.* Nucleoside (**dN^{I}**), butyl acrylate, $Pd(OAc)_2$, and TPPTS were dissolved in a mixture of water/acetonitrile (1:1, 3 mL) under argon atmosphere followed by addition of trielthylamine. The reaction mixture was stirred at 80 °C for 1.5 h and then evaporated in vacuo. The products were purified by column chromatography.

Method Ib. Nucleoside (**dN^{I}**), butyl acrylate (6 equiv), $Pd(OAc)_2$ (10 mol %), and PPh_3 (20 mol %) were dissolved in DMF (3 mL) under argon atmosphere followed by addition of trielthylamine (2 equiv). The reaction mixture was stirred at 100 °C and then evaporated in vacuo. The products were purified by column chromatography.

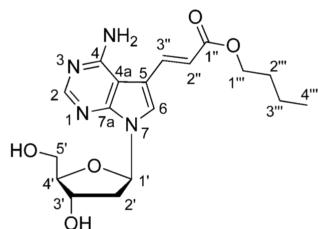
(*E*)-5-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxyuridine (dU^{BA}).



According to general method Ia, 2′-deoxy-5-iodouridine (71 mg, 0.201 mmol), butyl acrylate (172 μL, 1.202 mmol), $Pd(OAc)_2$ (3.4 mg, 0.010 mmol), TPPTS (17.1 mg, 0.020 mmol), and Et_3N (172 μL, 0.402 mmol) were heated. The crude product was purified by column chromatography using chloroform/methanol (10:1) as a mobile phase. **dU^{BA}** was isolated as white powder (70 mg, 98%).

According to general method Ib, 2′-deoxy-5-iodouridine (100 mg, 0.283 mmol), butyl acrylate (243 μL, 1.698 mmol), $Pd(OAc)_2$ (6.3 mg, 0.028 mmol), PPh_3 (15.0 mg, 0.057 mmol), and Et_3N (79 μL, 0.566 mmol) were heated for 45 min. The crude product was purified by column chromatography using chloroform/methanol (7:1) as a mobile phase. **dU^{BA}** was isolated as white powder (93 mg, 93%). ^{1}H NMR (600.1 MHz, CD_3OD): 0.97 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.43 (m, 2H, H-3‴); 1.66 (m, 2H, H-2‴); 2.27 (dt, 1H, J_{gem} = 13.6, $J_{2'b,1'}$ = $J_{2'b,3'}$ = 6.5, H-2′b); 2.34 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 4.1, H-2′a); 3.76 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.4, H-5′b); 3.86 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.0, H-5′a); 3.95 (ddd, 1H, $J_{4',3'}$ = 3.6, $J_{4',5'}$ = 3.4, 3.0, H-4′); 4.16 (t, 2H, $J_{1''',2'''}$ = 6.7, H-1‴); 4.42 (dddd, 1H, $J_{3',2'}$ = 6.5, 4.1, $J_{3',4'}$ = 3.6, $J_{3',1'}$ = 0.5, H-3′); 6.26 (ddd, 1H, $J_{1',2'}$ = 6.5, 6.2, $J_{1',3'}$ = 0.5, H-1′); 6.89 (dd, 1H, $J_{2'',3''}$ = 15.8, $J_{2'',6}$ = 0.3, H-2″); 7.39 (dd, 1H, $J_{3'',2''}$ = 15.8, $J_{3'',6}$ = 0.6, H-3″); 8.49 (dd, 1H, $J_{6,3''}$ = 0.6, $J_{6,2''}$ = 0.3, H-6). ^{13}C NMR (150.9 MHz, CD_3OD): 14.1 (CH_3-4‴); 20.2 (CH_2-3‴); 31.9 (CH_2-2‴); 41.9 (CH_2-2′); 62.4 (CH_2-5′); 65.3 (CH_2-1‴); 71.7 (CH-3′); 87.1 (CH-1′); 89.2 (CH-4′); 110.4 (C-5); 118.8 (CH-2″); 138.6 (CH-3″); 144.8 (CH-6); 151.1 (C-2); 163.7 (C-4); 169.4 (C-1″). MS (ESI$^+$): m/z 377.2 (100) $[M + Na]^+$; 731.5 (50) $[2M + Na]^+$. HR/MS (ESI$^+$) for $C_{16}H_{22}O_7N_2Na$: $[M + Na]^+$ calcd 377.1319, found 377.1318.

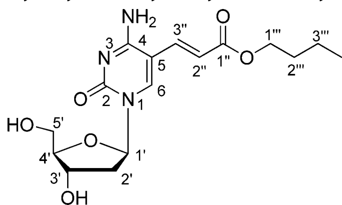
(*E*)-7-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxy-7-deazaadenosine (dA^{BA}).



According to general method Ia, 2′-deoxy-7-iodo-7-deazaadenosine (100 mg, 0.266 mmol), butyl acrylate (381 μL, 2.660 mmol), $Pd(OAc)_2$ (6.1 mg, 0.027 mmol), TPPTS (37.8 mg, 0.067 mmol), and Et_3N (111 μL, 0.798 mmol) were reacted. The crude product was purified by column chromatography using chloroform/methanol (10:1). **dA^{BA}** was isolated as white powder (81 mg, 81%).

According to general method Ib, 2′-deoxy-7-iodo-7-deazaadenosine (100 mg, 0.266 mmol), butyl acrylate (229 μL, 1.596 mmol), $Pd(OAc)_2$ (6.1 mg, 0.027 mmol), PPh_3 (13.9 mg, 0.053 mmol), and Et_3N (74 μL, 0.532 mmol) were heated for 1.5 h. The crude product was purified by column chromatography using chloroform/methanol (10:1) as a mobile phase. **dA^{BA}** was isolated as white powder (97 mg, 97%). ^{1}H NMR (600.1 MHz, CD_3OD): 0.98 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.46 (m, 2H, H-3‴); 1.70 (m, 2H, H-2‴); 2.35 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.8, H-2′b); 2.64 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 8.0, $J_{2'a,3'}$ = 6.0, H-2′a); 3.75 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 3.6, H-5′b); 3.82 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 3.2, H-5′a); 4.02 (ddd, 1H, $J_{4',5'}$ = 3.6, 3.2, $J_{4',3'}$ = 2.8, H-4′); 4.21 (t, 2H, $J_{1''',2'''}$ = 6.6, H-1‴); 4.53 (dtd, 1H, $J_{3',2'}$ = 6.0, 2.8, $J_{3',4'}$ = 2.8, $J_{3',1'}$ = 0.6, H-3′); 6.41 (d, 1H, $J_{2'',3''}$ = 15.8, H-2″); 6.54 (dd, 1H, $J_{1',2'}$ = 8.0, 6.0, H-1′); 7.95 (dd, 1H, $J_{3'',2''}$ = 15.8, $J_{3'',6}$ = 0.8, H-3″); 7.98 (bd, 1H, $J_{6,3''}$ = 0.8, H-6); 8.12 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 14.1 (CH_3-4‴); 20.2 (CH_2-3‴); 32.0 (CH_2-2‴); 41.7 (CH_2-2′); 63.5 (CH_2-5′); 65.5 (CH_2-1‴); 72.9 (CH-3′); 86.6 (CH-1′); 89.2 (CH-4′); 103.0 (C-4a); 113.2 (C-5); 117.6 (CH-2″); 125.2 (CH-6); 137.9 (CH-3″); 152.0 (C-7a); 152.8 (CH-2); 159.4 (C-4); 169.4 (C-1″). MS (ESI$^+$): m/z 377.1 (100) [M + H]$^+$; 399.1 (20) [M + Na]$^+$. HR/MS (ESI$^+$) for $C_{18}H_{25}O_5N_4$: [M + H]$^+$ calcd 377.1820, found 377.1819.

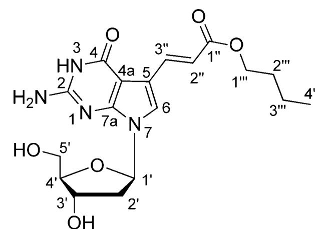
(*E*)-5-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxycytidine (dC^{BA}).



2′-Deoxy-5-iodocytidine (100 mg, 0.284 mmol), butyl acrylate (404 μL, 2.840 mmol), $Pd(OAc)_2$ (6.4 mg, 0.028 mmol), and TPPTS (32.0 mg, 0.056 mmol) were dissolved in mixture water/acetonitrile (1:1, 6 mL) under argon atmosphere followed by addition of triethylamine (80 μL, 0.568 mmol). The reaction mixture was stirred at 80 °C for 2 h and then evaporated in vacuo. The product was purified by column chromatography using chloroform/methanol (7:1) as a mobile phase. **dC^{BA}** was isolated as pale yellow powder (16 mg, 16%) after final purification using reversed-phase HPLC (water/methanol, 5−100%).

According to general method Ib, 2′-deoxy-5-iodocytidine (100 mg, 0.284 mmol), butyl acrylate (243 μL, 1.704 mmol), $Pd(OAc)_2$ (6.4 mg, 0.028 mmol), PPh_3 (14.7 mg, 0.056 mmol), and Et_3N (79 μL, 0.566 mmol) were heated for 2 h. The crude product was purified by column chromatography using chloroform/methanol (7:1) as a mobile phase. **dC^{BA}** was isolated as pale yellow powder (14 mg, 14%). ^{1}H NMR (499.8 MHz, CD_3OD): 0.97 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.44 (m, 2H, H-3‴); 1.68 (m, 2H, H-2‴); 2.22 (ddd, 1H, J_{gem} = 13.6, $J_{2'b,3'}$ = 6.3, $J_{2'b,1'}$ = 5.8, H-2′b); 2.42 (ddd, 1H, J_{gem} = 13.6, $J_{2'a,1'}$ = 6.4, $J_{2'a,3'}$ = 4.9, H-2′a); 3.77 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.2, H-5′b); 3.89 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 2.9, H-5′a); 3.96 (ddd, 1H, $J_{4',3'}$ = 4.3, $J_{4',5'}$ = 3.2, 2.9, H-4′); 4.18 (t, 2H, $J_{1''',2'''}$ = 6.7, H-1‴); 4.40 (ddd, 1H, $J_{3',2'}$ = 6.53, 4.9, $J_{3',4'}$ = 4.3, H-3′); 6.21 (dd, 1H, $J_{1',2'}$ = 6.4, 5.8, H-1′); 6.33 (d, 1H, $J_{2'',3''}$ = 15.7, H-2″); 7.59 (dd, 1H, $J_{3'',2''}$ = 15.7, $J_{3'',6}$ = 0.7, H-3″); 8.72 (d, 1H, $J_{6,3''}$ = 0.6, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.07 (CH_3-4‴); 20.19 (CH_2-3‴); 31.93 (CH_2-2‴); 42.63 (CH_2-2′); 62.00 (CH_2-5′); 65.53 (CH_2-1‴); 71.08 (CH-3′); 88.04 (CH-1′); 89.06 (CH-4′); 103.98 (C-5); 118.25 (CH-2″); 136.88 (CH-3″); 142.85 (CH-6); 157.16 (C-2); 165.26 (C-4); 168.48 (C-1″). MS (ESI$^+$): m/z 354.3 (50) [M + H]$^+$; 376.2 (100) [M + Na]$^+$. HR/MS (ESI$^+$) for $C_{16}H_{24}O_6N_3$: [M + H]$^+$ calcd 354.16596, found 354.16598.

(*E*)-7-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxy-7-deazaguanosine (dG^{BA}).



According to general method Ia, 2′-deoxy-7-iodo-7-deazaguanosine (50 mg, 0.128 mmol), butyl acrylate (182 μL, 1.28 mmol), $Pd(OAc)_2$ (2.9 mg, 0.013 mmol), TPPTS (18.2 mg, 0.032 mmol), and Et_3N (54 μL, 0.384 mmol) were heated. The crude product was purified by column chromatography using chloroform/methanol (10:1) as a mobile phase. **dG^{BA}** was isolated as white powder (42 mg, 84%) after final purification using reversed-phase HPLC (water/methanol, 5−100%).

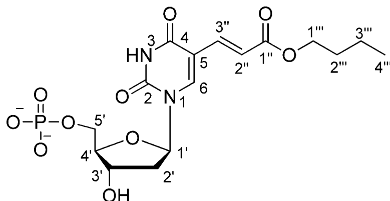
According to general method Ib, 2′-deoxy-7-iodo-7-deazaguanosine (100 mg, 0.256 mmol), butyl acrylate (366 μL, 2.560 mmol), $Pd(OAc)_2$ (5.7 mg, 0.026 mmol), PPh_3 (13.4 mg, 0.051 mmol), and Et_3N (72 μL, 0.512 mmol) were heated for 1 h. The crude product was purified by column chromatography using chloroform/methanol (7:1) as a mobile phase. **dG^{BA}** was isolated as white powder (83 mg, 83%). ^{1}H NMR (500.0 MHz, CD_3OD): 0.97 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.44 (m, 2H, H-3‴); 1.67 (m, 2H, H-2‴); 2.28 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 3.0, H-2′b); 2.50 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.1, H-2′a); 3.71 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 4.2, H-5′b); 3.77 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.8, H-5′a); 3.94 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.0, $J_{4',3'}$ = 2.9, H-4′); 4.15 (t, 2H, $J_{1''',2'''}$ = 6.6, H-1‴); 4.47 (dtd, 1H, $J_{3',2'a}$ = 6.0, $J_{3',2'b}$ = $J_{3',4'}$ = 3.0, $J_{3',1'}$ = 0.6, H-3′); 6.40 (bdd, 1H, $J_{1',2'a}$ = 7.9, $J_{1',2'b}$ = 6.0, H-1′); 7.19 (dd, 1H, $J_{2'',3''}$ = 15.7, $J_{2'',6}$ = 0.6, H-2″); 7.43 (q, 1H, $J_{6,2''}$ = $J_{6,3''}$ = $J_{6,1'}$ = 0.5, H-6); 7.66 (dd, 1H, $J_{3'',2''}$ = 15.7, $J_{3'',6}$ = 0.6, H-3″). ^{13}C NMR (125.7 MHz, CD_3OD): 14.08 (CH_3-4‴); 20.27 (CH_2-3‴); 32.02 (CH_2-2‴); 41.40 (CH_2-2′); 63.51 (CH_2-5′); 65.04 (CH_2-1‴); 72.79 (CH-3′); 85.29 (CH-1′); 88.76 (CH-4′); 99.44 (C-4a); 118.05 (C-5); 118.14 (CH-2″); 124.42 (CH-6); 138.60 (CH-3″); 154.51 (C-7a); 154.67 (C-2); 161.52 (C-4); 170.38 (C-1″). MS (ESI$^+$): m/z 393.2 (100) [M + H]$^+$; 415.2 (35) [M + Na]$^+$; 785.5 (42) [2M + H]$^+$; 807.5 (26) [2M + Na]$^+$. HR/MS (ESI$^+$) for $C_{18}H_{25}O_6N_4$: [M + H]$^+$ calcd 393.1769, found 393.1768.

General Procedure II: Preparation of Butyl Acrylate Modified Nucleoside Monophosphates (dN^{BA}MPs). ***Method IIa: Heck Coupling of Butyl Acrylate to dN^{I}MPs.*** Nucleoside monophosphate (**dN^{I}MP**), butyl acrylate (10 equiv), $Pd(OAc)_2$ (10 mol %), and TPPTS (25 mol %) were dissolved in a mixture water/acetonitrile (1:1, 2 mL) under argon atmosphere followed by addition of triethylamine (3 equiv). The reaction mixture was stirred at 80 °C for 2 h and then evaporated in vacuo. The products were purified by C18 reversed-phase HPLC using water/methanol (5 to 100%) containing 0.1 M TEAB buffer as eluent. Several codistillations with water and conversion to sodium salt (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the desired **dN^{BA}MPs** as white solids.

*Method IIb: Phosphorylation of **dN^{BA}**s.* Acrylate-modified nucleoside (**dN^{BA}**) was dried at 80 °C for 2 h in vacuo. After cooling, $PO(OMe)_3$ and $POCl_3$ were added on ice under argon atmosphere. The resulting mixture was stirred at 0 °C. The phosphorylation was stopped by addition of TEAB (2 M, 2 mL) and water (2 mL). The products were purified by C18 reversed-phase HPLC using water/methanol (5 to 100%) containing 0.1 M TEAB buffer as eluent.

Several codistillations with water and conversion to sodium salt (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the desired **dN^{BA}MP**s as white solids.

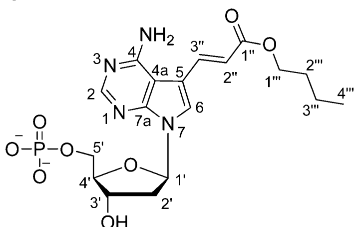
***(E)*-5-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxyuridine 5′-*O*-Phosphate (dU^{BA}MP).**



According to general method IIa, **dU^{I}MP** (50.0 mg, 0.110 mmol), butyl acrylate (150 μL, 1.100 mmol), $Pd(OAc)_2$ (2.3 mg, 11 μmol), TPPTS (14.9 mg, 27 μmol), and Et_3N (44 μL, 0.330 mmol) were reacted to yield **dU^{BA}MP** (17.5 mg, 35%).

According to general method IIb, **dU^{BA}** (60 mg, 0.169 mmol), $PO(OMe)_3$ (0.6 mL), and $POCl_3$ (60 μL) were stirred at 0 °C for 4 h and then kept in the refrigerator overnight. **dU^{BA}MP** was isolated as a white powder (25 mg, 42%). ^{1}H NMR (600.1 MHz, D_2O): 0.92 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.39 (m, 2H, H-3‴); 1.68 (m, 2H, H-2‴); 2.42 (m, 2H, H-2′); 4.03 (m, 2H, H-5′); 4.20 (m, 1H, H-4′); 4.21 (t, 2H, $J_{1''',2'''}$ = 6.7, H-1‴); 4.57 (dt, 1H, $J_{3',2'}$ = 5.6, 3.7, $J_{3',4'}$ = 3.7, H-3′); 6.30 (t, 1H, $J_{1',2'}$ = 6.9, H-1′); 6.90 (d, 1H, $J_{2'',3''}$ = 15.9, H-2″); 7.46 (d, 1H, $J_{3'',2''}$ = 15.9, H-3″); 8.19 (s, 1H, H-6). ^{13}C NMR (150.9 MHz, D_2O): 15.7 (CH_3-4‴); 21.3 (CH_2-3‴); 32.7 (CH_2-2‴); 41.8 (CH_2-2′); 67.0 (d, $J_{C,P}$ = 4.7, CH_2-5′); 68.2 (CH_2-1‴); 73.9 (CH-3′); 88.8 (CH-1′); 88.9 (d, $J_{C,P}$ = 8.4, CH-4′); 112.6 (C-5); 120.9 (CH-2″); 140.7 (CH-3″); 146.9 (CH-6); 153.3 (C-2); 166.4 (C-4); 172.7 (C-1″). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): 2.35. MS (ESI$^-$): m/z 433.1 (100) $[M + H]^-$. HR/MS (ESI$^-$) for $C_{16}H_{22}O_{10}N_2P$: $[M + H]^-$ calcd 433.1018, found 433.1019.

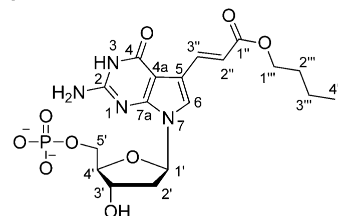
***(E)*-7-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxy-7-deazaadenosine 5′-*O*-Phosphate (dA^{BA}MP).**



According to general method IIa, **dA^{I}MP** (20.0 mg, 41.9 μmol), butyl acrylate (57 μL, 0.419 mmol), $Pd(OAc)_2$ (0.9 mg, 4.2 μmol), TPPTS (5.7 mg, 10.5 μmol), and Et_3N (17 μL, 0.125 mmol) were heated to yield **dA^{BA}MP** (10.5 mg, 55%).

According to general method IIb, **dA^{BA}** (50.0 mg, 0.133 mmol), $PO(OMe)_3$ (0.5 mL), and $POCl_3$ (25 μL) were stirred at 0 °C for 45 min. **dA^{BA}MP** was isolated as white powder (27.1 mg, 54%). ^{1}H NMR (600.1 MHz, D_2O): 0.95 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.42 (m, 2H, H-3‴); 1.71 (m, 2H, H-2‴); 2.49 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.1, H-2′b); 2.73 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 8.0, $J_{2'a,3'}$ = 6.2, H-2′a); 3.85 (dt, 1H, J_{gem} = 11.1, $J_{H,P}$ = $J_{5'b,4'}$ = 5.0, H-5′b); 3.89 (ddd, 1H, J_{gem} = 11.1, $J_{H,P}$ = 5.4, $J_{5'a,4'}$ = 5.0, H-5′a); 4.18 (td, 1H, $J_{4',5'}$ = 5.0, $J_{4',3'}$ = 3.1, H-4′); 4.23 (t, 2H, $J_{1''',2'''}$ = 6.8, H-1‴); 4.68 (dt, 1H, $J_{3',2'}$ = 6.2, 3.1, $J_{3',4'}$ = 3.1, H-3′); 6.33 (d, 1H, $J_{2'',3''}$ = 15.8, H-2″); 6.56 (dd, 1H, $J_{1',2'}$ = 8.0, 6.2, H-1′); 7.75 (dd, 1H, $J_{3'',2''}$ = 15.8, $J_{3'',6}$ = 0.6, H-3″); 7.91 (s, 1H, H-6); 8.10 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, D_2O): 15.8 (CH_3-4‴); 21.3 (CH_2-3‴); 32.8 (CH_2-2‴); 40.9 (CH_2-2′); 66.6 (d, $J_{C,P}$ = 4.5, CH_2-5′); 68.2 (CH_2-1‴); 74.4 (CH-3′); 85.6 (CH-1′); 88.6 (d, $J_{C,P}$ = 8.4, CH-4′); 104.0 (C-4a); 115.2 (C-5); 119.4 (CH-2″); 126.0 (CH-6); 139.8 (CH-3″); 153.3 (C-7a); 154.5 (CH-2); 160.2 (C-4); 172.3 (C-1″). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): 4.45. MS (ESI$^-$): m/z 455.1 (100) $[M + H]^-$. HR/MS (ESI$^-$) for $C_{18}H_{24}O_8N_4P$: $[M + H]^-$ calcd 455.1337, found 455.1341.

***(E)*-7-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxy-7-deazaguanosine 5′-*O*-Phosphate (dG^{BA}MP).**

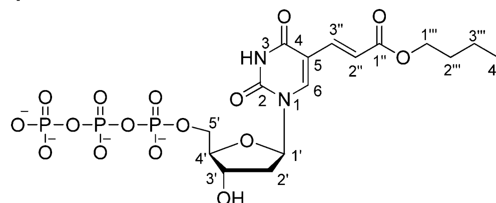


According to general method IIa, **dG^{I}MP** (30.0 mg, 63.8 μmol), butyl acrylate (91 μL, 0.638 mmol), $Pd(OAc)_2$ (1.4 mg, 6.4 μmol), TPPTS (9.1 mg, 16.0 μmol), and Et_3N (27 μL, 0.191 mmol) were heated to yield **dG^{BA}MP** (11.5 mg, 38%). ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 0.94 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.42 (m, 2H, H-3‴); 1.69 (m, 2H, H-2‴); 2.41 (ddd, 1H, J_{gem} = 13.9, $J_{2'b,1'}$ = 6.3, $J_{2'b,3'}$ = 3.3, H-2′b); 2.65 (ddd, 1H, J_{gem} = 13.9, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.3, H-2′a); 3.87 (t, 2H, $J_{H,P}$ = $J_{5',4'}$ = 5.3, H-5′); 4.14 (td, 1H, $J_{4',5'}$ = 5.3, $J_{4',3'}$ = 3.3, H-4′); 4.20 (t, 2H, $J_{1''',2'''}$ = 6.7, H-1‴); 4.64 (dt, 1H, $J_{3',2'}$ = 6.3, 3.3, $J_{3',4'}$ = 3.3, H-3′); 6.34 (dd, 1H, $J_{1',2'}$ = 7.9, 6.3, H-1′); 6.90 (d, 1H, $J_{2'',3''}$ = 15.7, H-2″); 7.47 (s, 1H, H-6); 7.63 (d, 1H, $J_{3'',2''}$ = 15.7 H-3″). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 15.76 (CH_3-4‴); 21.35 (CH_2-3‴); 32.82 (CH_2-2‴); 40.82 (CH_2-2′); 66.70 (d, $J_{C,P}$ = 4.5, CH_2-5′); 67.96 (CH_2-1‴); 74.33 (CH-3′); 85.56 (CH-1′); 88.38 (d, $J_{C,P}$ = 8.3, CH-4′); 100.79 (C-4a); 119.06 (C-5); 119.14 (CH-2″); 126.10 (CH-6); 140.64 (CH-3″); 155.69 (C-7a); 155.99 (C-2); 163.52 (C-4); 173.31 (C-1″). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): 3.83. MS (ESI$^-$): m/z 471.1 (100) $[M + H]^-$. HR/MS (ESI$^-$) for $C_{18}H_{24}O_9N_4P$: $[M + H]^-$ calcd 471.1286, found 471.1287.

General Procedure III: Preparation of Butyl Acrylate Modified Nucleoside Triphosphates (dN^{BA}TPs). *Method IIIa: Heck Coupling of Butyl Acrylate to **dN^{I}TP**s.* Nucleoside monophosphate (**dN^{I}TP**), butyl acrylate (10 equiv), $Pd(OAc)_2$ (10 mol %), and TPPTS (25 mol %) were dissolved in a mixture water/acetonitrile (1:1, 2 mL) under argon atmosphere followed by addition of triethylamine (3 equiv). The reaction mixture was stirred at 80 °C for 1 h and then evaporated in vacuo. The products were purified by C18 reversed-phase HPLC using water/methanol (5 to 100%) containing 0.1 M TEAB buffer as eluent. Several codistillations with water and conversion to sodium salt (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the desired **dN^{BA}TP**s as white solids.

*Method IIIb: Triphosphorylation of **dN^{BA}s**.* Acrylate-modified nucleoside (**dN^{BA}**) was dried at 80 °C for 2 h in vacuo. After cooling, $PO(OMe)_3$ and $POCl_3$ were added on ice under argon atmosphere. The resulting mixture was stirred at 0 °C. In a separate flask, the mixture of $(NHBu_3)_2H_2P_2O_7$ and tributylamine in dry DMF was prepared under argon atmosphere, cooled to 0 °C, and then added by syringe to the reaction mixture. The mixture was stirred at 0 °C. The phosphorylation was stopped by addition of TEAB (2 M, 2 mL) and water (2 mL). The products were purified by C18 reversed-phase HPLC using water/methanol (5 to 100%) containing 0.1 M TEAB buffer as eluent. Several codistillations with water and conversion to sodium salt (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the desired **dN^{BA}TP**s as white solids.

***(E)*-5-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxyuridine 5′-*O*-Triphosphate (dU^{BA}TP).**

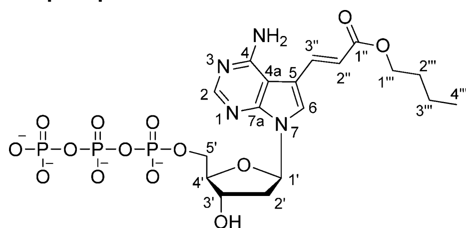


According to general method IIIa, **dU^{I}TP** (40 mg, 58.7 μmol), butyl acrylate (84 μL, 0.587 mmol), $Pd(OAc)_2$ (1.3 mg, 5.9 μmol), TPPTS (8.3 mg, 14.7 μmol), and Et_3N (25 μL, 0.176 mmol) were reacted to yield **dU^{BA}TP** (1.5 mg, 4%).

According to general method IIIb, **dU^BA** (30.0 mg, 84.7 μmol), $PO(OMe)_3$ (0.3 mL), and $POCl_3$ (30 μL) were stirred at 0 °C for 4 h and then kept in the refrigerator overnight. A cool solution of $(NHBu_3)_2H_2P_2O_7$ (0.3 g) and tributylamine (120 μL) in dry DMF (1.2 mL) was added, and the resulting mixture was stirred at 0 °C for 2 h. **dU^BATP** was isolated as a white powder (22.0 mg, 40%).

Method IIIc. Triethylammonium salt of **dU^ITP** (50 mg, 55.8 μmol), butyl acrylate (48 μL, 0.335 mmol), $Pd(OAc)_2$ (1.3 mg, 5.6 μmol), and PPh_3 (2.9 mg, 11.2 μmol) were dissolved in DMF (3 mL) under argon atmosphere followed by addition of trielthylamine (16 μL, 0.112 mmol). The reaction mixture was stirred at 100 °C for 1 h and then evaporated in vacuo. The product was purified by C18 reversed-phase HPLC using water/methanol (5 to 100%) containing 0.1 M TEAB buffer as eluent. Several codistillations with water and conversion to sodium salt (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave **dU^BATP** (5.0 mg, 14%). ^{1}H NMR (600.1 MHz, D_2O, pD = 7.1, phosphate buffer): 0.92 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.39 (m, 2H, H-3‴); 1.68 (m, 2H, H-2‴); 2.43 (m, 2H, H-2′); 4.20−4.27 (m, 5H, H-1‴,4′,5′); 4.67 (m, 1H, H-3′); 6.29 (t, 1H, $J_{1',2'}$ = 6.6, H-1′); 6.91 (d, 1H, $J_{2'',3''}$ = 15.9, H-2″); 7.48 (d, 1H, $J_{3'',2''}$ = 15.9, H-3″); 8.17 (s, 1H, H-6). ^{13}C NMR (150.9 MHz, D_2O, pD = 7.1, phosphate buffer): 15.7 (CH_3-4‴); 21.3 (CH_2-3‴); 32.7 (CH_2-2‴); 41.5 (CH_2-2′); 67.9 (d, $J_{C,P}$ = 5.7, CH_2-5′); 68.2 (CH_2-1‴); 73.1 (CH-3′); 88.5 (d, $J_{C,P}$ = 8.8, CH-4′); 88.7 (CH-1′); 112.6 (C-5); 120.9 (CH-2″); 140.7 (CH-3″); 146.9 (CH-6); 153.3 (C-2); 166.4 (C-4); 172.7 (C-1″). ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, pD = 7.1, phosphate buffer): −21.31 (t, J = 19.8, P_β); −10.34 (d, J = 19.8, P_α); −6.77 (bd, J = 19.8, P_γ). MS (ESI$^-$): m/z 297.0 (60) [M − $3PO_3$ − C_4H_9]$^-$; 433.1 (50) [M − $2PO_3$ + H]$^-$; 513.1 (100) [M + H − PO_3]$^-$; 535.1 (90) [M + Na − PO_3]$^-$; 593.1 (10) [M + 3H]$^-$; 615.1 (30) [M + 2H + Na]$^-$; 637.1 (20) [M + H + 2Na]$^-$. HR/MS (ESI$^-$) for $C_{16}H_{24}O_{16}N_2P_3$: [M + 3H]$^-$ calcd 593.0344, found 593.0341.

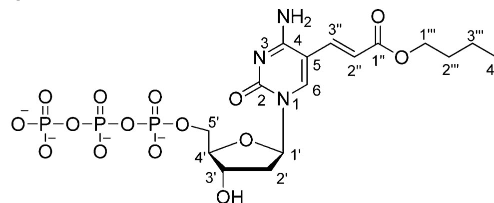
(*E*)-7-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxy-7-deazaadenosine 5′-*O*-Triphosphate (dA^BATP).



According to general method IIIa, **dA^ITP** (40 mg, 56.8 μmol), butyl acrylate (81 μL, 0.568 mmol), $Pd(OAc)_2$ (1.3 mg, 5.7 μmol), TPPTS (8.1 mg, 14.2 μmol), and Et_3N (24 μL, 0.170 mmol) were reacted to yield **dA^BATP** (15.5 mg, 43%).

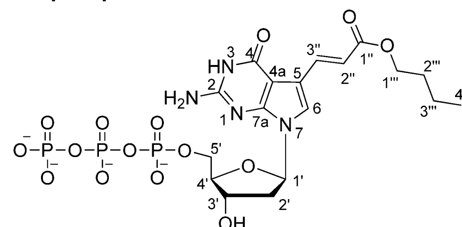
According to general method IIIb, **dA^BA** (30 mg, 79.7 μmol), $PO(OMe)_3$ (0.3 mL), and $POCl_3$ (15 μL) were stirred at 0 °C for 45 min. A cool solution of $(NHBu_3)_2H_2P_2O_7$ (0.3 g) and tributylamine (120 μL) in dry DMF (1.2 mL) was added, and the resulting mixture was stirred at 0 °C for 2 h. **dA^BATP** was isolated as a white powder (15.2 mg, 28%). ^{1}H NMR (600.1 MHz, D_2O, pD = 7.1, phosphate buffer): 0.95 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.42 (m, 2H, H-3‴); 1.71 (m, 2H, H-2‴); 2.51 (ddd, 1H, J_{gem} = 13.8, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.2, H-2′b); 2.71 (ddd, 1H, J_{gem} = 13.8, $J_{2'a,1'}$ = 8.0, $J_{2'a,3'}$ = 6.6, H-2′a); 4.12 (dt, 1H, J_{gem} = 11.2, $J_{H,P}$ = $J_{5'b,4'}$ = 5.3, H-5′b); 4.18 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.2, $J_{5'a,4'}$ = 4.4, H-5′a); 4.23 (t, 2H, $J_{1''',2'''}$ = 6.7, H-1‴); 4.24 (m, 1H, H-4′); 4.76 (m, 1H, H-3′ − overlapped with HDO signal); 6.31 (d, 1H, $J_{2'',3''}$ = 15.7, H-2″); 6.55 (dd, 1H, $J_{1',2'}$ = 8.0, 6.2, H-1′); 7.70 (d, 1H, $J_{3'',2''}$ = 15.7, H-3″); 7.87 (s, 1H, H-6); 8.08 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, D_2O, pD = 7.1, phosphate buffer): 15.8 (CH_3-4‴); 21.3 (CH_2-3‴); 32.8 (CH_2-2‴); 41.1 (CH_2-2′); 68.2 (d, $J_{C,P}$ = 5.7, CH_2-5′); 68.2 (CH_2-1‴); 73.8 (CH-3′); 85.7 (CH-1′); 88.0 (d, $J_{C,P}$ = 8.8, CH-4′); 103.9 (C-4a); 115.2 (C-5); 119.4 (CH-2″); 125.9 (CH-6); 139.6 (CH-3″); 153.2 (C-7a); 154.3 (CH-2); 160.0 (C-4); 172.3 (C-1″). ^{31}P{^{1}H} NMR (202.3 MHz, D_2O, pD = 7.1, phosphate buffer): −21.22 (t, J = 19.4, P_β); −10.27 (d, J = 19.4, P_α); −6.53 (bd, J = 19.4, P_γ). MS (ESI$^-$): m/z 516.1 (100) [M + 3H − PO_3H]$^-$; 538.1 (90) [M + 2H + Na − PO_3H]$^-$; 618.0 (15) [M + 2H + Na]$^-$. HR/MS (ESI$^-$) for $C_{17}H_{20}O_{13}N_5P_3Na$: [M + 2H + Na]$^-$ calcd 618.0174, found 618.0169.

(*E*)-5-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxycytidine 5′-*O*-Triphosphate (dC^BATP).



According to general method IIIb, **dC^BA** (30.0 mg, 84.9 μmol), $PO(OMe)_3$ (0.3 mL), and $POCl_3$ (16 μL) were stirred at 0 °C for 1 h. Cool solution of $(NHBu_3)_2H_2P_2O_7$ (0.3 g) and tributylamine (120 μL) in dry DMF (1.3 mL) was added and the resulting mixture was stirred at 0 °C for 1.5 h. **dC^BATP** was isolated as a white powder (9.2 mg, 19%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm, pD = 7.1, phosphate buffer): 0.92 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.40 (m, 2H, H-3‴); 1.69 (m, 2H, H-2‴); 2.37 (ddd, 1H, J_{gem} = 14.2, $J_{2'b,1'}$ = 7.1, $J_{2'b,3'}$ = 6.4, H-2′b); 2.46 (ddd, 1H, J_{gem} = 14.2, $J_{2'a,1'}$ = 6.2, $J_{2'a,3'}$ = 3.7, H-2′a); 4.19−4.27 (m, 5H, H-1‴,4′,5′); 4.63 (dt, 1H, $J_{3',2'}$ = 6.2, 3.7, $J_{3',4'}$ = 3.7, H-3′); 6.25 (dd, 1H, $J_{1',2'}$ = 7.1, 6.2, H-1′); 6.39 (d, 1H, $J_{2'',3''}$ = 15.9, H-2″); 7.55 (d, 1H, $J_{3'',2''}$ = 15.9, H-3″); 8.17 (s, 1H, H-6). ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm, pD = 7.1, phosphate buffer): 15.71 (CH_3-4‴); 21.24 (CH_2-3‴); 32.71 (CH_2-2‴); 42.05 (CH_2-2′); 67.95 (d, $J_{C,P}$ = 5.7, CH_2-5′); 68.33 (CH_2-1‴); 73.23 (CH-3′); 88.50 (d, $J_{C,P}$ = 8.6, CH-4′); 89.43 (CH-1′); 106.90 (C-5); 121.53 (CH-2″); 138.81 (CH-3″); 144.33 (CH-6); 159.24 (C-2); 166.62 (C-4); 171.66 (C-1″). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O, ref(phosphate buffer) = 2.35 ppm, pD = 7.1, phosphate buffer): −21.38 (t, J = 19.8, P_β); −10.44 (d, J = 19.8, P_α); −6.84 (bd, J = 19.8, P_γ). MS (ESI$^-$): m/z 512.2 (100) [M + 3H − PO_3H]$^-$; 534.2 (98) [M + 2H + Na − PO_3H]$^-$; 432.2 (45) [M + H − $2PO_3$H]$^-$; 614.2 (30) [M + 2H + Na]$^-$; 636.2 (26) [M + H + 2Na]$^-$. HR/MS (ESI$^-$) for $C_{16}H_{24}O_{15}N_3P_3Na$: [M + 2H + Na]$^-$ calcd 614.0323, found 614.0328.

(*E*)-7-[2-(*n*-Butyloxycarbonyl)vinyl]-2′-deoxy-7-deazaguanosine 5′-*O*-Triphosphate (dG^BATP).



According to general method IIIa, **dG^ITP** (30 mg, 47.8 μmol), butyl acrylate (68 μL, 0.478 mmol), $Pd(OAc)_2$ (1.1 mg, 4.8 μmol), TPPTS (6.8 mg, 12.0 μmol), and Et_3N (20 μL, 0.143 mmol) were reacted to yield **dG^BATP** (13.0 mg, 44%).

According to general Method IIIb, **dG^BA** (38.0 mg, 96.9 μmol), $PO(OMe)_3$ (0.3 mL), and $POCl_3$ (18 μL) were stirred at 0 °C for 1 h. A cool solution of $(NHBu_3)_2H_2P_2O_7$ (360 mg) and tributylamine (146 μL) in dry DMF (1.5 mL) was added, and the resulting mixture was stirred at 0 °C for 1.5 h. **dG^BATP** was isolated as a white powder (15.4 mg, 25%). ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 0.94 (t, 3H, $J_{4''',3'''}$ = 7.4, H-4‴); 1.42 (m, 2H, H-3‴); 1.69 (m, 2H, H-2‴); 2.42 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.3, H-2′b); 2.65 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.3, H-2′a); 4.10 − 4.23 (m, 3H, H-4′,5′); 4.20 (t, 2H, $J_{1''',2'''}$ = 6.7, H-1‴); 4.72 (bdt, 1H, $J_{3',2'a}$ = 6.2, $J_{3',2'b}$ = $J_{3',4'}$ = 3.2, H-3′); 6.35 (dd, 1H, $J_{1',2'a}$ = 7.9, $J_{1',2'b}$ = 6.2, H-1′); 7.91 (d, 1H, $J_{2'',3''}$ = 15.8, H-2″); 7.46 (s, 1H, H-6); 7.63 (d, 1H, $J_{3'',2''}$ = 15.8, H-3″). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 15.77 (CH_3-4‴); 21.35 (CH_2-3‴); 32.82 (CH_2-2‴); 40.95 (CH_2-2′); 67.96 (CH_2-1‴); 68.32 (d, $J_{C,P}$ = 5.9, CH_2-5′); 73.87 (CH-3′); 85.80 (CH-1′); 87.80 (d, $J_{C,P}$ = 8.8, CH-4′); 100.82 (C-4a); 119.09 (C-5); 119.17 (CH-2″); 126.10 (CH-6); 140.62 (CH-3″); 155.68 (C-7a); 155.94 (C-2); 163.49 (C-4); 173.31 (C-1″). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): −21.84 (t, J = 19.7, P_β); −10.47 (d, J =

19.7, P_α); −7.95 (bd, J = 19.6, P_γ). MS (ESI$^-$): m/z 551.2 (100) [M + 3H-PO_3H]$^-$; 573.2 (90) [M + 2H + Na − PO_3H]$^-$; 471.3 (43) [M + 3H + 2PO_3H]$^-$; 653.2 (30) [M + 2H + Na]$^-$; 631.2 (15) [M + 3H]$^-$. HR/MS (ESI$^-$) for $C_{18}H_{26}O_{15}N_4P_3$: [M + 3H]$^-$ calcd 631.0613, found 631.0614.

Incorporation of Butyl Acrylate Modified Triphosphates into DNA by PEX. The reaction mixture (20 μL) contained primer (4 μM), template (4 μM), DNA polymerase (0.075 U KOD XL, 0.1 U Vent(exo-) or 0.5 U Pwo), and **dNTPs** (either all natural or 3 natural and 1 modified, 260 μM; for the inhibition studies shown in Figure 2d, 140; 260 and 600 μM **dG^{BA}TP** was used) in enzyme reaction buffer supplied by the manufacturer. Primer was labeled on its 5′-end by use of [γ^{32}P]-ATP according to standard techniques. The reaction mixture was incubated for 40 min at 60 °C in a thermal cycler. Primer extension was stopped by addition of stop solution (40 μL, 80% (v/v) formamide, 20 mM EDTA, 0.025% (w/v) bromophenol blue, 0.025% (w/v) xylene cyanol) and heated for 5 min at 95 °C. Samples were separated by 12.5% PAGE under denaturing conditions (42 mA, 1 h). Visualization was performed by phosphoimaging (Figures 1 and 2).

MALDI-TOF Experiments. The MALDI-TOF spectra were measured on a MALDI-TOF/TOF mass spectrometer with 1 kHz smartbeam II laser. The measurements were done in reflectron mode by droplet technique, with the mass range up to 30 kDa. The matrix consisted of 3-hydroxypicolinic acid (HPA)/picolinic acid (PA)/ ammonium tartrate in 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 the top of the dried matrix preparation spot and dried at room temperature.

Preparation of ON^{BA}s 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. 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 55 °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 S1−S8, Supporting Information).

Electrochemistry. Nucleosides and dNMPs were analyzed by conventional in situ cyclic voltammetry (CV) while ONs (PEX products) by ex situ (adsorptive transfer stripping, AdTS) CV. The PEX products (purified in their single-stranded form using streptavidin-coated magnetic beads as above) were accumulated at the surface of a hanging mercury drop electrode (HMDE) for 60 s from 5-μL aliquots containing 0.2 M NaCl. The electrode was then rinsed with deionized water and placed into an electrochemical cell. CV settings: scan rate 1 V s^{-1}, initial potential 0.0 V, switching potential −1.85 V. Background electrolyte: 0.3 M ammonium formate, 0.05 M sodium phosphate, pH 6.9 (for ON measurements) or 0.2 M sodium acetate pH 5.0 (for measurements of nucleos(t)ides). All measurements were performed at room temperature using an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-stand 663 (Metrohm, Herisau, Switzerland) using a three-electrode system with a Ag/AgCl/3 M KCl electrode as a reference and platinum wire as an auxiliary electrode in solution deareated by argon purging.

■ ASSOCIATED CONTENT

Supporting Information

Copies of NMR and MALDI spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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