JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Additions of Thiols to 7-Vinyl-7-deazaadenine Nucleosides and Nucleotides. Synthesis of Hydrophobic Derivatives of 2′-Deoxyadenosine, dATP and DNA

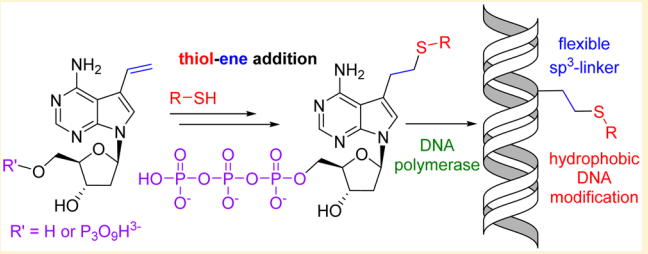
Michaela Slavíčková,[†] Radek Pohl,[†] and Michal Hocek*[,†,‡]

[†]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Gilead & IOCB Research Center, Flemingovo namesti 2, CZ-16610 Prague 6, Czech Republic

[‡]Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, Prague-2 12843, Czech Republic

Supporting Information

ABSTRACT: Additions of alkyl- or arylthiols to 7-vinyl-7-deaza-2′-deoxyadenosine gave a series of 7-[2-(alkyl- or arylsulfanyl)-ethyl]-7-deaza-2′-deoxyadenosines in 45−85% yields. The nucleosides were converted to 5′-*O*-mono-(**dA^{SR}MP**) or triphosphates (**dA^{SR}TP**) by phosphorylation. The modified triphosphates were also prepared by thiol addition to 7-vinyl-7-deaza-dATP. The triphosphates **dA^{SR}TP** were good substrates for DNA polymerases useful in the enzymatic synthesis of base-modified oligonucleotides (ONs) or DNA containing flexibly linked hydrophobic substituents in the major groove. Primer extension was used for the synthesis of ONs with one or several modifications, PCR was used for the synthesis of heavily modified DNA, whereas terminal deoxynucleotidyl transferase was used for a single-nucleotide labeling of the 3′-end.



INTRODUCTION

Lipophilic or amphiphilic derivatives of DNA attract considerable attention due to diverse applications in medicinal chemistry, chemical biology, and material sciences.[1,2] For example, the lipophilized oligonucleotides (ON) or DNA were used for surface modification,[3] liposome[4] or cell-membrane[5] labeling, gene or therapeutic ON delivery,[6] and for stabilization of ONs against nuclease degradation.[7] Other potential fields of application are modified aptamers[8] targeting hydrophobic proteins or DNAzymes soluble in organic solvents.[9] Apart from classical chemical 3′- or 5′-end labeling[10] or phosphoramidite synthesis on solid support,[11] modified DNA can be synthesized by polymerase reactions using functionalized 2′-deoxyribonucleoside triphosphates (dNTPs) as substrates.[12,13] Several examples of polymerase incorporation of lipophilic nucleotides, including PEG-ylated,[7,14−16] grafted-polyamide-,[16] diamondoid-,[17] or steroid-linked[18] dNTPs have been reported, and also an engineered DNA polymerase capable of efficient multiple incorporations of some of these nucleotides has been developed.[14]

5-Substituted pyrimidine or 7-substituted 7-deazapurine dNTPs are typically good substrates for DNA polymerases and therefore are the most frequently used building blocks for polymerase synthesis of base-modified DNA.[13] They can be synthesized either by triphosphorylation of modified nucleosides[13] or by direct cross-coupling reactions of halogenated dNTPs in aqueous solutions.[13,19] From the cross-coupling reactions, the aqueous Suzuki-Miyaura reaction with arylboronic acids[20] and the Sonogashira reactions with terminal acetylenes[21] are the most general, versatile, and useful reactions, whereas some examples of the Stille coupling[22] with stannanes and Heck reaction[23] with acrylates were also reported but have limited scope. However, all of these reactions can be only efficiently used for the introduction of rigid sp^1- (alkynes) or sp^2-hybridized (alkenyl, aryl) substituents, while the introduction of flexible sp^3-hybridized substituents (i.e., alkyls) remains a challenge.

Thiol−ene additions are one of the most useful and frequently reported click-reactions in bioconjugations or surface-modifications.[24] When using electron-poor conjugate alkenes, the reaction proceeds without radical initiation via Michael-type nucleophilic addition.[25] Vinyl-substituted purines and pyrimidines are sufficiently reactive for nucleophilic additions of amines or thiols and have been used for conjugations[26] or cross-linking in DNA[27] or for the synthesis of sulfide-modified derivatives.[28] We envisaged that easily available 7-vinyl-7-deazaadenine nucleosides and nucleotides[29,30] might have the potential for thiol additions in order to synthesize novel lipophilic sp^3-linked 7-(alkyl- or aryl-sulfanylethyl)-7-deazapurine derivatives, and we report here on the results of this study.

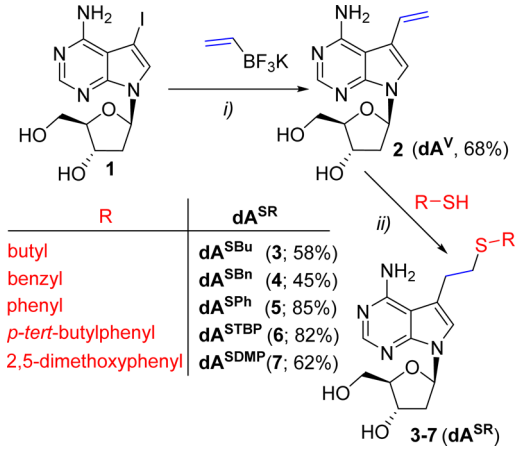
RESULTS AND DISCUSSION

The starting 7-vinyl-7-deaza-2′-deoxyadenosine (**2**)[30] was prepared by the aqueous Suzuki-Miyaura cross-coupling reaction of unprotected 7-iodo-7-deazadeoxyadenine nucleoside **1** with

Received: August 25, 2016
Published: October 6, 2016

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

Scheme 1[a]



R	dA^{SR}
butyl	dA^{SBu} (**3**; 58%)
benzyl	dA^{SBn} (**4**; 45%)
phenyl	dA^{SPh} (**5**; 85%)
p-tert-butylphenyl	dA^{STBP} (**6**; 82%)
2,5-dimethoxyphenyl	dA^{SDMP} (**7**; 62%)

[a]Reagents and conditions: (i) potassium vinyltrifluoroborate, Cs_2CO_3, $Pd(OAc)_2$, TPPTS, CH_3CN/H_2O (1:2), 80°C, Ar, 2 h; (ii) thiol, CH_2Cl_2/MeOH (1:1), Ar, r.t., 3–5 days.

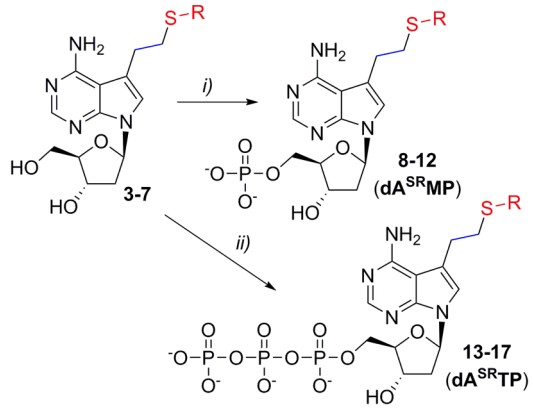
potassium vinyltrifluoroborate (Scheme 1). The reaction proceeded, similarly to our previously published work,[29] in the presence of $Pd(OAc)_2$, tris(3-sulfonylphenyl)phosphine trisodium salt (TPPTS), and Cs_2CO_3 in a water/acetonitrile mixture (2:1) at 80 °C and gave the desired vinyl derivative **2** in 68% yield, which is comparable to the previously reported synthesis[30] by the Stille coupling.

In order to develop mild procedures further applicable for fragile nucleoside triphosphates, the additions of thiols to 7-vinyl-7-deaza-deoxyadenosine **2** were performed at room temperature without any base or radical initiator. Because of the solubility of both nucleoside **2** and thiols, we used a mixture of CH_2Cl_2/MeOH as solvent. We tested two aliphatic (butyl and benzyl) and three aromatic thiols in large excess (10 equiv). The reactions were quite sluggish and took 3 days to reach acceptable conversions (5 days in case of BuSH). Only the dimethoxythiophenol reacted faster to give 62% yield in 24 h when using lower 5-fold excess of the thiol. Since further prolongation of the reaction time or addition of either catalytic or stoichiometric amounts of a base (NaH) did not improve the conversions, the desired 7-(2-alkylsulfanyl)ethyl- (**3** or **4**) or 7-(2-arylsulfanyl)ethyl-7-deazaadenine (**5**–**7**) nucleosides dA^{SR} were isolated by column chromatography in moderate but still acceptable (45–58% for alkylthiols) to good (62–85% for arylthiols) yields (Scheme 1).

The sulfide-linked nucleosides dA^{SR} (**3**–**7**) were then converted to nucleotides, model nucleoside monophosphates, and nucleoside triphosphates, which were needed as substrates for polymerase synthesis of modified DNA. First, the nucleosides **3**–**7** were phosphorylated with phosphoryl chloride in trimethyl phosphate at 0 °C to give the corresponding 5′-*O*-monophosphates $dA^{SR}MP$ (**8**–**12**) in 47–84% yields (Scheme 2). The triphosphorylation of dA^{SR} (**3**–**7**) with phosphoryl chloride in trimethyl phosphate followed by treatment with tributylammonium pyrophosphate gave the corresponding nucleoside triphosphates $dA^{SR}TP$ (**13**-**17**) in 52–81% yield (Scheme 2).

Then, we decided to explore the possibility of direct thiol additions vinyl-substituted nucleoside triphosphates. For this purpose, 7-vinyl-7-deazaadenosine 5′-*O*-triphosphate ($dA^{V}TP$, **18**) was prepared in 33% yield (19% overall yield from **1**) by triphosphorylation of 7-vinyl-2′-deoxy-7-deazaadenosine

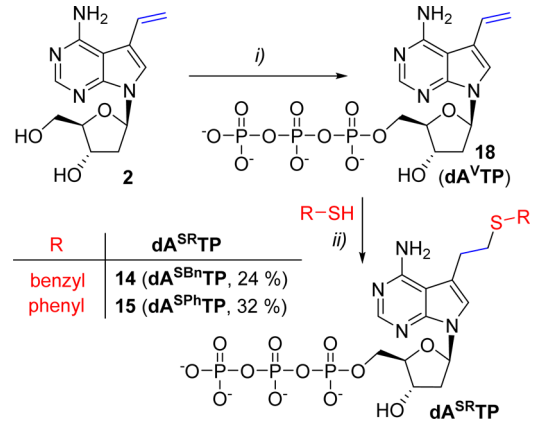
Scheme 2[a]



R	$dA^{SR}MP$	$dA^{SR}TP$
butyl	$dA^{SBu}MP$ (**8**; 47%)	$dA^{SBu}TP$ (**13**; 52%)
benzyl	$dA^{SBn}MP$ (**9**; 72%)	$dA^{SBn}TP$ (**14**; 64%)
phenyl	$dA^{SPh}MP$ (**10**; 75%)	$dA^{SPh}TP$ (**15**; 78%)
p-tert-butyl-phenyl	$dA^{STBP}MP$ (**11**; 48%)	$dA^{STBP}TP$ (**16**; 81%)
2,5-dimethoxyphenyl	$dA^{SDMP}MP$ (**12**; 84%)	$dA^{SDMP}TP$ (**17**; 54%)

[a]Reagents and conditions: (i) $POCl_3$, $PO(OMe)_3$, 0°C; (ii) 1. $POCl_3$, $PO(OMe)_3$, 0 °C; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF.

Scheme 3[a]



R	$dA^{SR}TP$
benzyl	**14** ($dA^{SBn}TP$, 24 %)
phenyl	**15** ($dA^{SPh}TP$, 32 %)

[a]Reagents and conditions: (i) 1. $POCl_3$, $PO(OMe)_3$, 0 °C; 2. $(NHBu_3)_2H_2P_2O_7$, Bu_3N, DMF; (ii) dry methanol, thiol, r.t., 20 h.

(dA^{V}, **2**, Scheme 3). The same compound ($dA^{V}TP$) was previously prepared[29] by the Suzuki cross-coupling of 7-iodo-2′-deoxy-7-deazaadenosine 5′-*O*-triphosphate with potassium vinyl-trifluoroborate in slightly higher 22% overall yield. We have chosen one aliphatic (BnSH) thiol and one aromatic (thiophenol) thiol for the additions to $dA^{V}TP$, and the reactions were carried out in dry methanol under argon atmosphere. We used triethylammonium salt of $dA^{V}TP$ (**18**) because of its better solubility in methanol. The reactions with 10 equiv of thiols were performed for 20 h and gave the desired hydrophobic sulfide-linked dNTPs after isolation by reversed-phase HPLC in acceptable yields of 24% for $dA^{SBn}TP$ (**14**, Scheme 3) or 32% for $dA^{SPh}TP$ (**15**). Thus, we proved that the thiol–ene additions are suitable for direct modification of vinyl-substituted dNTPs. However, the above-mentioned alternative approach consisting of thiol additions to vinyl-linked nucleoside **2** followed by triphopshorylation gave significantly higher overall yields (compounds **14** and **15** were prepared in

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115–11125

20 or 45% overall yields from **1** by the first approach, whereas the overall yields by the thiol additions to **18** were 5 and 8% only).

Hydrophobic dA^{SR}TP as Substrates for Polymerase Synthesis of DNA. The novel hydrophobic sulfide-linked dNTP analogues (**dA^{SR}TPs**, **13**−**17**) were then studied as substrates for DNA polymerases in primer extension reactions (PEX). We tested three different DNA polymerases: KOD XL, Vent(*exo*$^-$), and Pwo. The templates and primer (for sequences see Table 1) were chosen in order to introduce one (temp^{oligo1A}, 19-mer) or four (temp^{Prb4baseII}, 31-mer) modifications to the extended primer strand. We found that all sulfide-linked nucleoside triphosphates (**dA^{SR}TP**) were good substrates for KOD XL and Vent(exo-) DNA polymerases and were successfully incorporated into DNA bearing one or four modifications (Figure 1, Figures S1a an S2a in Supporting Information). However, when using the Pwo polymerase, it was very difficult to optimize the reaction conditions for PEX, and we observed the formation of some truncated products (shorter by one or two nucleotides) in some cases (Figures S1b and S2b in Supporting Information). In all cases, the corresponding alkyl- or arylsulfanylethyl-modified single-stranded oligonucleotides (ONs) were prepared by PEX using a biotinylated template in the presence of KOD XL DNA polymerase and were isolated by magnetoseparation and analyzed by MALDI-TOF to confirm the identity of the modified ONs (Table 2; for copies of spectra, see Figures S4−S13 in Supporting Information).

To further quantify the substrate activities of the hydrophobic sulfide-linked **dA^{SR}TPs**, we performed a simple kinetic analysis of single nucleotide extension using KOD XL DNA polymerase and temp^{1A_term} (Figure 2). The rates of incorporation of all modified nucleotides were compared with natural dATP. The reaction mixtures were incubated for the time intervals from 6 s to 5 min, then the reactions were stopped by the addition of PAGE loading buffer and immediately heated. The single nucleotide extension using **dA^{SBu}TP**, **dA^{SBn}TP**, or **dA^{SPh}TP** were completed within 60 s which was comparable to the rate of incorporation of natural dATP, whereas the incorporation of the more functionalized **dA^{STBP}TP** and **dA^{SDMP}TP** nucleotides took ca. 2 min to reach completion (Figure 2).

Next, incorporation of a large number of modifications into double-stranded DNA by the polymerase chain reaction (PCR) was tested. The experiments were performed using a 98-mer (temp^{FVL-A}, Table 1), a modified nucleotide (**dA^{SR}TP**), and three natural nucleotide triphosphates in the presence of KOD XL DNA polymerase. PCR experiments gave good amplification of the modified DNA only in the case of dNTPs bearing smaller unfunctionalized substituents (**dA^{SBu}TP**, **dA^{SPh}TP**, and **dA^{SBn}TP**, Figure 3). The bulkier *t*-Bu- or 2,5-dimethoxyphenylsulfanyl-linked triphosphates **dA^{STBP}TP** and **dA^{SDMP}TP** did not give the amplified PCR product.

Finally, we explored the possibility of using these modified **dA^{SR}TPs** as substrates for nontemplated 3′-end elongation of ONs catalyzed by terminal deoxynucleotidyl transferase (TdT),[31] which has been previously used[32,33] for 3′-end labeling of DNA or ONs with modified nucleotides. This enzyme is known[32,33] to extend the ONs in the absence of template, and usually, the major problem is to optimize the concentrations and reaction time to obtain mixtures of elongated products with lower dispersity (typically several or even many nucleotides are incorporated[32,33] depending on the concentration and time). We have tested two modified dNTPs (**dA^{SBu}TP** and **dA^{SPh}TP**)

Table 1. List of ON Sequences Used in This Study

oligo	sequence
primer$^{248\text{-}sh}$	5′-CATGGGCGGCATGGG-3′
temp^{oligo1A}	5′-CCCTCCCATGCCGCCCATG-3′
temp^{1A_term}	5′-TCCCATGCCGCCCATG-3′
temp^{Prb4baseII}	5′-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′
primer^{LT25TH}	5′-CAAGGACAAAATACCTGTATTCCTT-3′
primer^{L20}	5′-GACATCATGAGACATCGC-3′
temp^{FVL-A}	5′-GACATCATGAGACATCGCCTCTGGCTAATAGGACTACTTCTAATCTGTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGGTATTTTGTCCTTG-3′

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

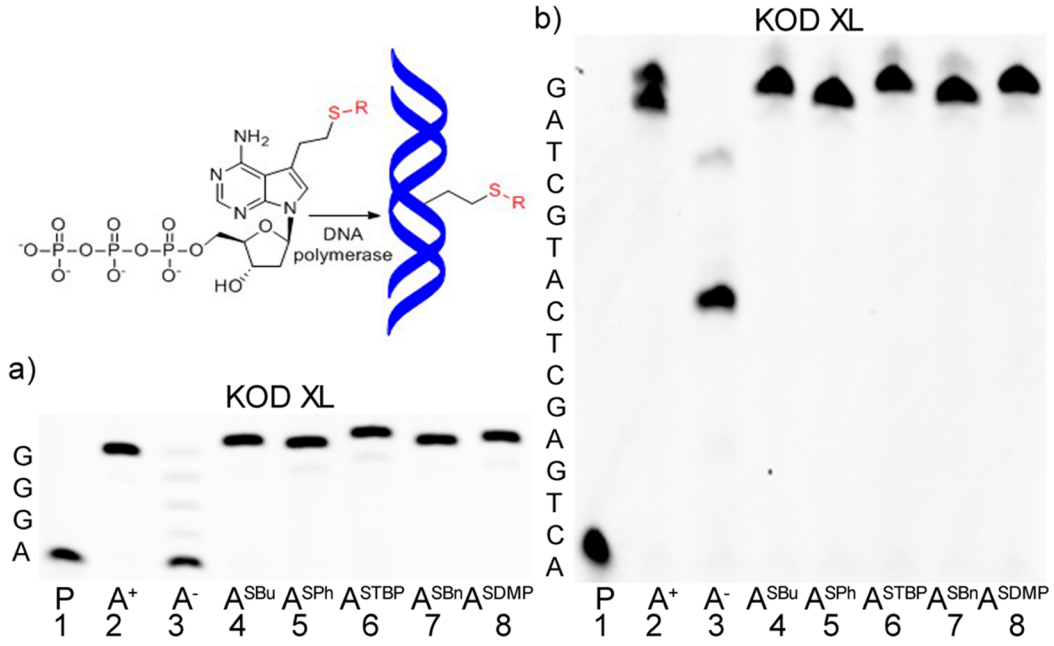


Figure 1. PEX reactions with temp^{oligo1A} (a) and temp^{Prb4baseII} (b) using KOD XL DNA polymerase. Lane 1, P, primer; lane 2, A$^+$, products of PEX with natural dNTPs; lane 3, A$^-$, products of PEX with dTTP, dCTP, and dGTP; lanes 4–8, **A^{SR}**, products of PEX with dTTP, dCTP, dGTP, and functionalized **dA^{SR}TP**.

Table 2. MALDI-TOF Data of Modified Oligodeoxyribonucleotides

ssDNA	M (calc.) (Da)	M (found) [M or M+H]$^+$ (Da)
ON^{1Bu}	6089.9	6090.9
ON^{1Ph}	6109.9	6110.2
ON^{1TBP}	6165.9	6167.4
ON^{1Bn}	6123.9	6124.2
ON^{1DMP}	6169.9	6171.2
ON^{4Bu}	10077.3	10078.3
ON^{4Ph}	10157.3	10158.6
ON^{4TBP}	10381.3	10382.4
ON^{4Bn}	10213.3	10213.4
ON^{4DMP}	10397.3	10398.1

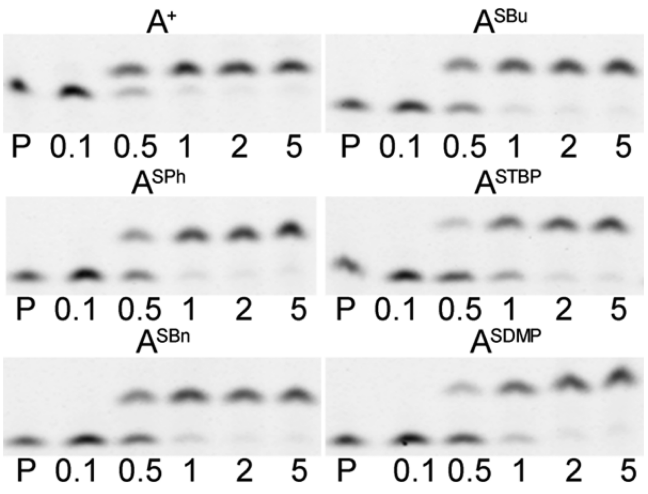


Figure 2. PAGE analyses of kinetic single nucleotide extension experiments with temp^{1A_term} using KOD XL DNA polymerases and **dA^{SBu}TP**, **dA^{SPh}TP**, **dA^{STBP}TP**, **dA^{SBn}TP**, and **dA^{SDMP}TP** in comparison with natural dATP (+). Time intervals are given in minutes.

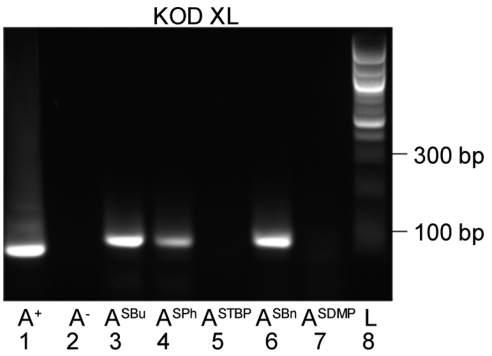


Figure 3. PCR experiments using KOD XL DNA polymerases. Lane 1, A$^+$, products of PEX with natural dNTPs; lane 2, A$^-$, products of PEX with dTTP, dCTP, and dGTP; lanes 3–7, **A^{SR}**, products of PCR with dTTP, dCTP, dGTP, and functionalized **dA^{SR}TP**; and lane 8, L, ladder.

in different reaction times and compared with natural dATP. Figure 4 (and Figure S3 in SI) shows that the TdT elongation using dATP gave long polyadenylate tails (depending on the reaction time), whereas the reaction with **dA^{SBu}TP** or **dA^{SPh}TP** gave exclusively one-nucleotide elongation which was not further extended (presumably due to the hydrophobic character of the substituent). Therefore, the TdT catalyzed 3′-elongation with hydrophobic **dA^{SR}TP**s has a good potential in attachment of a single lipophilic group at the 3′- end of ONs which can be useful for DNA lipidation,[1,2] etc.

CONCLUSIONS

Additions of thiols to 7-vinyl-7-deazaadenine 2′-deoxyribonucleoside gave the desired 7-substituted nucleosides bearing hydrophobic substituents linked via flexible sp^3-hybridized linkage to the nucleobase. Such alkyl-type modifications are difficult to synthesize by cross-coupling reactions. The 7-[2-(arylsylfanyl)ethyl]- or 7-[2-(alkylsylfanyl)ethyl]-7-deazaadenine

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115–11125

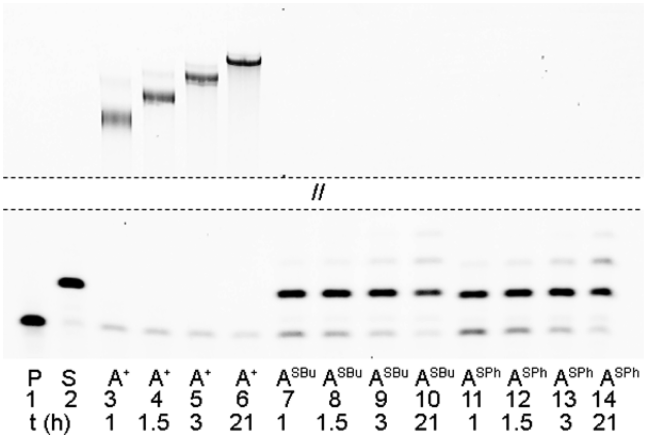


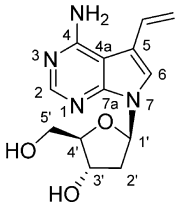
Figure 4. TdT-catalyzed DNA chain elongation. Lane 1, P, 15-mer primer$^{248\text{-sh}}$; lane 2, S, 16-mer ON standard (PEX extended primer$^{248\text{-sh}}$ containing an additional dA^{SBu}); lanes 3−14, A^{+}, A^{SBu}, and A^{SPh}, products of primer$^{248\text{-sh}}$ elongation using terminal transferase and either dATP, **dA^{SBu}TP**, or **dA^{SPh}TP** as substrate (for the full gel image, see Figure S3 in SI).

2′-deoxyribonucleoside monophosphates (**dA^{SR}MPs**) or triphosphates (**dA^{SR}TPs**) can be prepared either by (tri)phosphorylation of the modified nucleosides or by direct thiol−ene additions to 7-vinyl-7-deaza-dATP. The latter reaction extends the portfolio of transformations for direct modifications of fragile dNTPs.[34] The base-modified **dA^{SR}TPs** were good substrates for DNA polymerases and could be used for enzymatic synthesis of modified ONs (by PEX) or double-stranded DNA (by PCR) bearing hydrophobic modifications in the major groove. Finally, we found that TdT can only incorporate one modified nucleotide, and therefore, it can be advantageously used for specific 3′-end labeling of ONs or DNA with a single nucleotide bearing a hydrophobic group. This has potential in DNA lipidation and other applications.

EXPERIMENTAL SECTION

General. NMR spectra were recorded on 600 MHz (600.1 MHz for ^{1}H, 150.9 MHz for ^{13}C), 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), or 400 MHz (400.0 MHz for ^{1}H, 162 MHz for ^{31}P, 100 MHz for ^{13}C) spectrometers from sample solutions in D_2O, DMSO-d_6 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); and DMSO-d_6 (referenced to solvent signal: 2.50 ppm for ^{1}H NMR and 39.70 ppm for ^{13}C NMR). Coupling constants (J) are given in Hz. Complete assignment of all NMR signals was achieved by using a combination of H,H−COSY, H,C-HSQC, and H,C-HMBC experiments. Mass spectra and high resolution mass spectra were measured using an ESI ionization technique and Orbitrap separator. The water used in the synthetic part was of HPLC quality and in the biochemical part of ultrapure quality (18 MΩ.cm). The MALDI-TOF spectra were measured on a MALDI-TOF/TOF mass spectrometer with a 1 kHz smartbeam II laser. The measurements were done in reflectron mode by a 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 top of the dried matrix preparation spot and dried down at room temperature. Chemicals, synthetic oligodeoxyribonucleotides, and enzymes were purchased from commercial suppliers and were used without further purification.

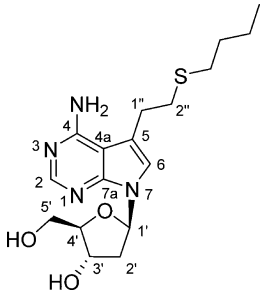
*7-Vinyl-2′-deoxy-7-deazaadenosine (**2; dA^{V}**).*[30]



A mixture of acetonitrile/water 1:2 (7 mL) was added through a septum to an argon-purged vial containing **dA^{I}** (**1**) (100.0 mg, 266.0 μmol), $C_2H_3BF_3K$ (42.7 mg, 319.0 μmol), Cs_2CO_3 (433 mg, 1.33 mmol), $Pd(OAc)_2$ (6.0 mg, 27 μmol), and TPPTS (15.1 mg, 27 μmol). After the solids were dissolved, the reaction mixture was stirred at 80 °C for 2 h. The product was isolated from a crude reaction mixture by column chromatography in CH_2Cl_2/MeOH 10:1 (v:v) to give **2** as a yellow solid (50 mg, 68%). ^{1}H NMR (600.1 MHz, CD_3OD): 2.31 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6, H-2′b); 2.66 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 8.3, $J_{2'a,3'}$ = 6.0, H-2′a); 3.73 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.80 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.4, H-5′a); 4.00 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.6, H-4′); 4.52 (dtd, 1H, $J_{3',2'}$ = 6.0, 2.6, $J_{3',4'}$ = 2.6, $J_{3',1'}$ = 0.6, H-3′); 5.26 (dd, 1H, J_{cis} = 10.9, J_{gem} = 1.5, CH_aH_b=CH−); 5.58 (dd, 1H, J_{trans} = 17.3, J_{gem} = 1.5, CH_aH_b=CH−); 6.51 (dd, 1H, $J_{1'2'}$ = 8.3, 6.0, H-1′); 6.99 (ddd, 1H, J_{trans} = 17.3, J_{cis} = 10.9, ^{4}J = 1.0, CH_aH_b=CH−); 7.51 (d, 1H, ^{4}J = 1.0, H-6); 8.06 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 41.4 (CH_2−2′); 63.7 (CH_2−5′); 73.1 (CH-3′); 86.5 (CH-1′); 89.1 (CH-4′); 103.0 (C-4a); 115.5 (CH_2=CH−); 116.6 (C-5); 121.3 (CH-6); 129.7 (CH_2=CH−); 151.1 (C-7a); 152.1 (CH-2); 159.3 (C-4). MS: m/z (%): 277.1 $[M + H]^+$ (100), 299.1 $[M + Na]^+$ (50). HRMS: calculated for $[C_{13}H_{16}N_4O_3+H]^+$, 277.12952; found, 277.12956.

Additions of Thiols to Vinyl-2′-deoxy-7-deazaadenosine (2; dA^{V}): General Procedure A. A mixture of CH_2Cl_2/MeOH (1:1) was added through a septum to an argon-purged vial containing **dA^{V}** (**2**), followed by the addition of thiol, and the reaction mixture was stirred at room temperature for 24−120 h. The solvents were evaporated, and the product was isolated by column chromatography in CH_2Cl_2/MeOH (10:1).

*7-[2-(Butylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine (**3; dA^{SBu}**).*

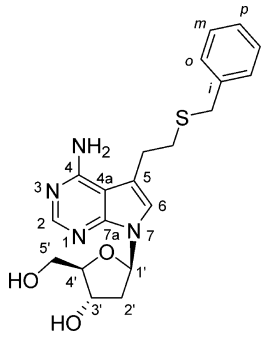


Compound **3** was prepared according to general procedure A from **2** (30 mg, 109 μmol) and 1-butanethiol (120 μL, 1.09 mmol), reaction time 120 h, and isolated as an amorphous white solid (22.9 mg, 58%). ^{1}H NMR (500.0 MHz, CD_3OD): 0.88 (t, 3H, J_{vic} = 7.3, $CH_3CH_2CH_2CH_2S$); 1.37 (m, 2H, $CH_3CH_2CH_2CH_2S$); 1.52 (m, 2H, $CH_3CH_2CH_2CH_2S$); 2.28 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6, H-2′b); 2.50 (m, 2H, $CH_3CH_2CH_2CH_2S$); 2.66 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 8.4, $J_{2'a,3'}$ = 6.0, H-2′a); 2.80 (m, 2H, H-2″); 3.04 (m, 2H, H-1″); 3.72 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.79 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.4, H-5′a); 4.00 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.6, H-4′); 4.51 (dt, 1H, $J_{3',2'}$ = 6.0, 2.6, $J_{3',4'}$ = 2.6, H-3′); 6.48 (dd, 1H, $J_{1'2'}$ = 8.4, 6.0, H-1′); 7.17 (s, 1H, H-6); 8.04 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.0 ($CH_3CH_2CH_2CH_2S$); 22.9 ($CH_3CH_2CH_2CH_2S$); 28.1 (CH_2−1″); 32.82, 32.86 ($CH_3CH_2CH_2CH_2S$); 33.7 (CH_2−2″); 41.2 (CH_2−2′); 63.8 (CH_2−5′); 73.1 (CH-3′); 86.4 (CH-1′); 88.9 (CH-4′); 104.4 (C-4a); 115.6 (C-5); 122.0 (CH-6); 151.0 (C-7a); 151.8 (CH-2); 159.1 (C-4). MS ESI^{+}: m/z (%): 251.2 $[M\text{-}C_6H_9O_3+2H]^+$ (25), 367.3

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

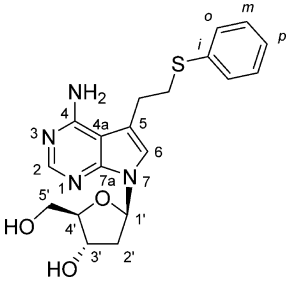
$[M + H]^+$(100), 389.3 $[M + Na]^+$(25). HRMS: calculated for $[C_{17}H_{26}N_4O_3S+H]^+$, 367.17984; found, 367.17980.

*7-[2-(Benzylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine (**4; dA^{SBn}**).*



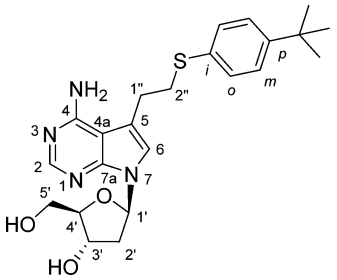
Compound **4** was prepared according to general procedure A from **2** (25 mg, 91 μmol) and benzylthiol (110 μL, 905 μmol), reaction time 72 h, and isolated as an amorphous white solid (16.4 mg, 45%). ^{1}H NMR (499.8 MHz, CD_3OD): 2.28 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.5, H-2′b); 2.66 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 8.4, $J_{2'a,3'}$ = 5.9, H-2′a); 2.71 (m, 2H, CH_2CH_2S); 3.01 (m, 2H, CH_2CH_2S); 3.71 (s, 2H, CH_2Ph); 3.72 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.80 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.4, H-5′a); 4.01 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.5, H-4′); 4.52 (dtd, 1H, $J_{3',2'}$ = 6.0, 2.5, $J_{3',4'}$ = 2.5, $J_{3',1'}$ = 0.5, H-3′); 6.47 (dd, 1H, $J_{1'2'}$ = 8.4, 6.0, H-1′); 7.13 (t, 1H, ^4J = 0.9, H-6); 7.22 (m, 1H, H-*p*-Ph); 7.28 (m, 4H, H-*o*,*m*-Ph); 8.04 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 27.8 (CH_2CH_2S); 32.9 (CH_2CH_2S); 37.1 (CH_2Ph); 41.2 (CH_2−2′); 63.8 (CH_2−5′); 73.2 (CH-3′); 86.4 (CH-1′); 88.9 (CH-4′); 104.3 (C-4a); 115.5 (C-5); 122.1 (CH-6); 127.9 (CH-*p*-Ph); 129.4 (CH-*m*-Ph); 130.0 (CH-*o*-Ph); 140.0 (C-*i*-Ph); 151.0 (C-7a); 151.8 (CH-2); 159.1 (C-4). MS ESI$^+$: *m*/*z* (%): 285.0 $[M-C_6H_9O_3+2H]^+$ (50), 401.1 $[M + H]^+$ (100), 423.1$[M + Na]^+$ (10). HRMS: calculated for $[C_{20}H_{24}N_4O_3S+H]^+$, 401.16419; found, 401.16415.

*7-[2-(Phenylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine (**5; dA^{SPh}**).*



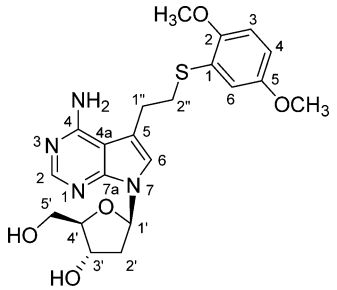
Compound **5** was prepared according to general procedure A from **2** (25 mg, 91 μmol) and thiophenol (93 μL, 905 μmol), reaction time 72 h, and was isolated as an amorphous white solid (30 mg, 85%). ^{1}H NMR (600.1 MHz, CD_3OD): 2.26 (ddd, 1H, J_{gem} = 13.4, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6, H-2′b); 2.61 (ddd, 1H, J_{gem} = 13.4, $J_{2'a,1'}$ = 8.3, $J_{2'a,3'}$ = 6.0, H-2′a); 3.08 (m, 2H, CH_2CH_2S); 3.22 (m, 2H, CH_2CH_2S); 3.71 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.78 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.4, H-5′a); 3.99 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.6, H-4′); 4.50 (dtd, 1H, $J_{3',2'}$ = 6.0, 2.6, $J_{3',4'}$ = 2.6, $J_{3',1'}$ = 0.4, H-3′); 6.44 (dd, 1H, $J_{1'2'}$ = 8.3, 6.0, H-1′); 7.12 (m, 1H, H-*p*-Ph); 7.14 (t, 1H, ^4J = 0.9, H-6); 7.22 (m, 2H, H-*m*-Ph); 7.31 (m, 2H, H-*o*-Ph); 8.02 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 27.7 (CH_2CH_2S); 35.5 (CH_2CH_2S); 41.3 (CH_2−2′); 63.8 (CH_2−5′); 73.1 (CH-3′); 86.3 (CH-1′); 88.9 (CH-4′); 104.4 (C-4a); 115.0 (C-5); 122.3 (CH-6); 127.0 (CH-*p*-Ph); 129.9 (CH-*m*-Ph); 130.5 (CH-*o*-Ph); 137.7 (C-*i*-Ph); 151.0 (C-7a); 151.7 (CH-2); 159.0 (C-4). MS ESI$^+$: *m*/*z* (%): 271.1$[M-C_6H_9O_3+2H]^+$ (100), 387.02 $[M + H]^+$ (50), 409.2 $[M + Na]^+$ (40). HRMS: calculated for $[C_{19}H_{22}N_4O_3S+H]^+$, 387.14854; found, 387.14847.

*7-{2-[(4-tert-Butylphenyl)sulfanyl]ethyl}-2′-deoxy-7-deazaadenosine (**6; dA^{STBP}**).*



Compound **6** was prepared according to general procedure A from **2** (30 mg, 109 μmol) and 4-*tert*-butylbenzenethiol (190 μL, 1090 μmol), reaction time 72 h, and isolated as an amorphous white solid (39.2 mg, 82%). ^{1}H NMR (499.8 MHz, CD_3OD): 1.27 (s, 9H, $(CH_3)_3C$); 2.26 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 2.6, H-2′b); 2.62 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 8.3, $J_{2'a,3'}$ = 6.0, H-2′a); 3.06 (m, 2H, H-1″); 3.19 (m, 2H, H-2″); 3.71 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.79 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.4, H-5′a); 3.99 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.6, H-4′); 4.50 (dtd, 1H, $J_{3',2'}$ = 6.0, 2.6, $J_{3',4'}$ = 2.6, $J_{3',1'}$ = 0.4, H-3′); 6.44 (dd, 1H, $J_{1'2'}$ = 8.3, 6.0, H-1′); 7.14 (t, 1H, ^4J = 0.9, H-6); 7.22 (m, 2H, H-*o*-phenylene); 7.25 (m, 2H, H-*m*-phenylene); 8.00 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 27.9 (CH_2−1″); 31.7 ($(CH_3)_3C$); 35.3 ($(CH_3)_3C$); 35.9 (CH_2−2″); 41.3 (CH_2−2′); 63.8 (CH_2−5′); 73.1 (CH-3′); 86.4 (CH-1′); 88.9 (CH-4′); 104.5 (C-4a); 115.0 (C-5); 122.4 (CH-6); 126.9 (CH-*m*-phenylene); 130.8 (CH-*o*-phenylene); 134.0 (C-*i*-phenylene); 150.5 (C-p-phenylene); 151.0 (C-7a); 151.7 (CH-2); 159.0 (C-4). MS ESI$^+$: *m*/*z* (%): 327.2 $[M-C_6H_9O_3+2H]^+$ (50), 443.3 $[M + H]^+$ (100), 465.2 $[M + Na]^+$ (15). calculated for $[C_{23}H_{30}N_4O_3S+H]^+$, 443.21114; found, 443.21122.

*7-{2-[(2,5-Dimethoxyphenyl)sulfanyl]ethyl}-2′-deoxy-7-deazaadenosine (**7; dA^{SDMP}**).*

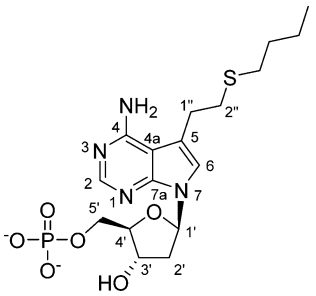


Compound **7** was prepared according to general procedure A from **2** (30 mg, 109 μmol) and 2,5-dimethoxythiophenol (81 μL, 540 μmol), reaction time 24 h, and isolated as a white solid (30.3 mg, 62%), mp 168−171 °C. ^{1}H NMR (500.0 MHz, DMSO-d_6): 2.11 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 5.9, $J_{2'b,3'}$ = 2.6, H-2′b); 2.47 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.4, $J_{2'a,3'}$ = 5.7, H-2′a); 3.08 (m, 2H, H-1″); 3.14 (m, 2H, H-2″); 3.48 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 6.0, $J_{5'b,4'}$ = 4.3, H-5′b); 3.55 (dt, 1H, J_{gem} = 11.7, $J_{5'a,4'}$ = $J_{5'a,OH}$ = 5.0, H-5′a); 3.68 (s, 3H, CH_3O-5-$C_6H_3(OMe)_2S$); 3.73 (s, 3H, CH_3O-2-$C_6H_3(OMe)_2S$); 3.79 (ddd, 1H, $J_{4',5'}$ = 5.0, 4.3, $J_{4',3'}$ = 2.6, H-4′); 4.32 (m, 1H, H-3′); 5.10 (dd, 1H, $J_{OH,5'}$ = 6.0, 5.0, OH-5′); 5.25 (d, 1H, $J_{OH,3'}$ = 4.0, OH-3′); 6.46 (dd, 1H, $J_{1'2'}$ = 8.4, 5.9, H-1′); 6.59 (bs, 2H, NH_2); 6.70 (dd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 2.9, H-4-$C_6H_3(OMe)_2S$); 6.76 (d, 1H, $J_{6,4}$ = 2.9, H-6-$C_6H_3(OMe)_2S$); 6.88 (d, 1H, $J_{3,4}$ = 8.8, H-3-$C_6H_3(OMe)_2S$); 7.21 (s, 1H, H-6); 8.02 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 25.7 (CH_2−1″); 31.6 (CH_2−2″); 39.6 (CH_2−2′); 55.6 (CH_3O-5-$C_6H_3(OMe)_2S$); 56.4 (CH_3O-2-$C_6H_3(OMe)_2S$); 62.4 (CH_2−5′); 71.3 (CH-3′); 83.1 (CH-1′); 87.4 (CH-4′); 102.3 (C-4a); 110.4 (CH-4-$C_6H_3(OMe)_2S$); 111.9 (CH-3-$C_6H_3(OMe)_2S$); 113.4 (C-5); 113.6 (CH-6-$C_6H_3(OMe)_2S$); 119.8 (CH-6); 123.4 (C-1-$C_6H_3(OMe)_2S$); 150.56 (C-2-$C_6H_3(OMe)_2S$); 150.60 (C-7a); 151.6 (CH-2); 153.8 (C-5-$C_6H_3(OMe)_2S$); 157.8 (C-4). MS ESI$^+$: *m*/*z* (%): 331.2 $[M-C_6H_9O_3+2H]^+$ (90), 447.2 $[M + H]^+$ (100), 469.2 $[M + Na]^+$(20). HRMS: calculated for $[C_{21}H_{26}N_4O_5S+H]^+$, 447.16967; found, 447.16971.

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

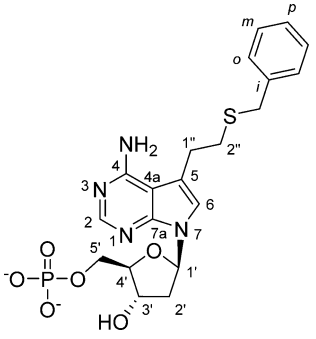
Preparation of Sulfide-Linked Nucleoside Monophosphates: General Procedure B. Sulfide-linked nucleoside **dA^{SR}** (**3**−**7**) was dried at 80 °C for 2 h in vacuo, and after cooling, it was suspended in $PO(OMe)_3$ at 0 °C, and $POCl_3$ (1.2 equiv) was added. The reaction mixture was stirred at 0 °C for 2 h, then quenched by the addition of 2 M aqueous TEAB (triethylammonium bicarbonate, 2 mL), and the solvents were evaporated under vacuum. Products were isolated from a crude reaction mixture by HPLC on a C18 column with the use of a linear gradient of 0.1 M TEAB in H_2O to 0.1 M TEAB in H_2O/MeOH (1:1) as eluent. Several codistillations with water and conversion to the sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the desired nucleoside monophosphates sodium salts as white solid products.

*7-[2-(Butylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine 5′-O-Phosphate (**8; dA^{SBu}MP**).*



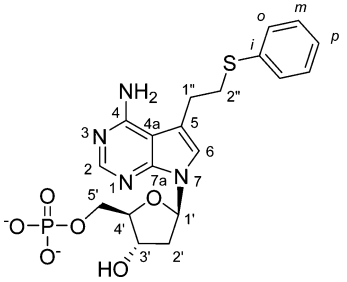
Compound **8** was prepared according to general procedure B from **3** (22.4 mg, 61.1 μmol) and $POCl_3$ (6.8 μL, 73 μmol) in $PO(OMe)_3$ (1 mL) and isolated as a white solid (13.3 mg, 47%). ^{1}H NMR (600.1 MHz, D_2O): 0.77 (t, 3H, J_{vic} = 7.4, C**H**$_3$CH$_2$CH$_2$CH$_2$S); 1.23 (m, 2H, CH$_3$C**H**$_2$CH$_2$CH$_2$S); 1.42 (m, 2H, CH$_3$CH$_2$C**H**$_2$CH$_2$S); 2.38−2.46 (m, 3H, H-2′b, CH$_3$CH$_2$CH$_2$C**H**$_2$S); 2.69 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 8.2, $J_{2'a,3'}$ = 6.3, H-2′a); 2.79−2.88 (m, 2H, H-2″); 2.96−3.05 (m, 2H, H-1″); 3.85, 3.88 (2 × dt, 1H, J_{gem} = 11.0, $J_{5',4'}$ = $J_{H,P}$ = 5.6, H-5′); 4.15 (td, 1H, $J_{4',5'}$ = 5.6, $J_{4',3'}$ = 3.0, H-4′); 4.66 (dt, 1H, $J_{3',2'}$ = 6.3, 3.0, $J_{3',4'}$ = 3.0, H-3′); 6.56 (dd, 1H, $J_{1'2'}$ = 8.2, 6.2, H-1′); 7.25 (s, 1H, H-6); 8.06 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, D_2O): 15.3 (**C**H$_3$CH$_2$CH$_2$CH$_2$S); 24.0 (CH$_3$**C**H$_2$CH$_2$CH$_2$S); 29.1 (CH$_2$−1″); 33.6, 34.0 (CH$_3$CH$_2$**C**H$_2$**C**H$_2$S); 34.6 (CH$_2$−2″); 40.7 (CH$_2$−2′); 66.7 (d, $J_{C,P}$ = 4.6, CH$_2$−5′); 74.5 (CH-3′); 85.1 (CH-1′); 88.1 (d, $J_{C,P}$ = 8.3, CH-4′); 105.3 (C-4a); 117.9 (C-5); 122.4 (CH-6); 152.6 (C-7a); 153.8 (CH-2); 160.1 (C-4). ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): 4.61. MS ESI$^-$: *m*/*z* (%): 445.3 [M + H]$^-$ (100) calculated for [$C_{17}H_{25}N_4O_6PS$+H]$^-$, 445.13161; found, 445.13049.

*7-[2-(Benzylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine 5′-O-Phosphate (**9; dA^{SBn}MP**).*



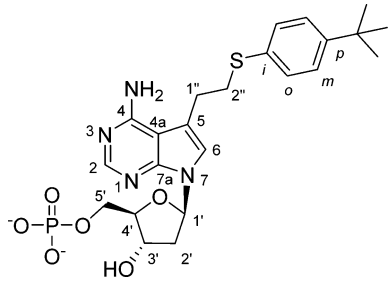
Compound **9** was prepared according to general procedure B from **4** (36.0 mg, 90 μmol) and $POCl_3$ (10.1 μL, 108 μmol) in $PO(OMe)_3$ (1.5 mL) and isolated as a white solid (32.7 mg, 72%). ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.33 (ddd, 1H, J_{gem} = 13.8, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.3, H-2′b); 2.49−2.59 (m, 2H, H-2′a,2″b); 2.63 (dt, 1H, J_{gem} = 13.2, $J_{2''a,1''}$ = 6.6, H-2″a); 2.66−2.77 (m, 2H, H-1″); 3.46, 3.50 (2 × d, 2 × 1H, J_{gem} = 13.5, CH$_2$Ph); 3.94 (dd, 2H, $J_{H,P}$ = 5.9, $J_{5',4'}$ = 5.1, H-5′); 4.17 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.3, H-4′); 4.62 (dt, 1H, $J_{3',2'}$ = 6.3, 3.3, $J_{3',4'}$ = 3.3, H-3′); 6.42 (dd, 1H, $J_{1'2'}$ = 7.9, 6.2, H-1′); 7.01 (s, 1H, H-6); 7.03 (m, 2H, H-*o*-Ph); 7.08 (m, 1H, H-*p*-Ph); 7.12 (m, 2H, H-*m*-Ph); 7.96 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 28.6 (CH$_2$−1″); 33.8 (CH$_2$−2″); 38.3 (CH$_2$Ph); 40.9 (CH$_2$−2′); 67.2 (d, $J_{C,P}$ = 4.7, CH$_2$−5′); 74.3 (CH-3′); 85.1 (CH-1′); 87.9 (d, $J_{C,P}$ = 8.2, CH-4′); 104.9 (C-4a); 117.6 (C-5); 122.6 (CH-6); 129.6 (CH-*p*-Ph); 131.1 (CH-*m*-Ph); 131.3 (CH-*o*-Ph); 140.9 (C-*i*-Ph); 151.9 (C-7a); 152.1 (CH-2); 158.6 (C-4). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): 3.38. MS ESI$^-$: *m*/*z* (%): 479.2 [M + H]$^-$ (100), 501.2 [M + Na]$^-$ (10). HRMS: calculated for [$C_{20}H_{23}N_4O_6PS$+H]$^-$, 479.11596; found, 479.11502.

*7-[2-(Phenylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine 5′-O-Phosphate (**10; dA^{SPh}MP**).*



Compound **10** was prepared according to general procedure B from **5** (45.0 mg, 116 μmol) and $POCl_3$ (13 μL, 140 μmol) in $PO(OMe)_3$ (1.5 mL) and isolated as a white solid (42.7 mg, 75%). ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.33 (ddd, 1H, J_{gem} = 13.9, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.2, H-2′b); 2.49 (ddd, 1H, J_{gem} = 13.9, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.3, H-2′a); 2.76−2.89 (m, 2H, H-1″); 3.01−3.13 (m, 2H, 2″); 3.95 (dd, 2H, $J_{H,P}$ = 5.8, $J_{5',4'}$ = 5.0, H-5′); 4.15 (td, 1H, $J_{4',5'}$ = 5.0, $J_{4',3'}$ = 3.2, H-4′); 4.60 (dt, 1H, $J_{3',2'}$ = 6.3, 3.2, $J_{3',4'}$ = 3.2, H-3′); 6.31 (dd, 1H, $J_{1'2'}$ = 7.9, 6.2, H-1′); 6.90−7.06 (m, 6H, H-6, H-*o*,*m*,*p*-Ph); 7.87 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 29.3 (CH$_2$−1″); 36.6 (CH$_2$−2″); 41.1 (CH$_2$−2′); 67.2 (d, $J_{C,P}$ = 4.7, CH$_2$−5′); 74.2 (CH-3′); 85.1 (CH-1′); 87.9 (d, $J_{C,P}$ = 8.2, CH-4′); 105.1 (C-4a); 117.5 (C-5); 123.1 (CH-6); 128.7 (CH-*p*-Ph); 131.2 (CH-*m*-Ph); 131.8 (CH-*o*-Ph); 137.4 (C-*i*-Ph); 151.3 (CH-2); 151.5 (C-7a); 158.2 (C-4). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): 3.21. MS ESI$^-$: *m*/*z* (%): 465.2 [M + H]$^-$ (100). HRMS: calculated for [$C_{19}H_{21}N_4O_6PS$+H]$^-$, 465.10031; found, 465.09967.

*7-{2-[(4-tert-Butylphenyl)sulfanyl]ethyl}-2′-deoxy-7-deazaadenosine 5′-O-Phosphate (**11; dA^{STBP}MP**).*

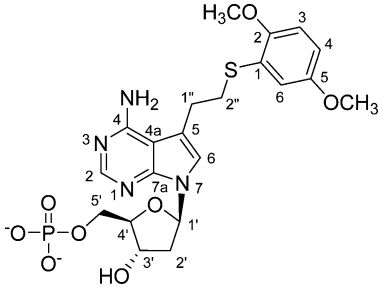


Compound **11** was prepared according to general procedure B from **6** (32.0 mg, 72 μmol) and $POCl_3$ (8.1 μL, 87 μmol) in $PO(OMe)_3$ (1.5 mL) and isolated as a white solid (19.0 mg, 48%). ^{1}H NMR (600.1 MHz, D_2O, ref(dioxane) = 3.75 ppm): 0.97 (s, 9H, (CH$_3$)$_3$C); 2.31, 2.50 (2 × bm, 2 × 1H, H-2′); 2.86, 2.93 (2 × bm, 2 × 1H, H-1″); 3.09 (bm, 2H, 2″); 3.09−4.01 (bm, 2H, H-5′); 4.15 (td, 1H, $J_{4',5'}$ = 5.0, $J_{4',3'}$ = 3.4, H-4′); 4.66 (bm, 1H, H-3′); 6.28 (t, 1H, $J_{1'2'}$ = 6.9, H-1′); 6.87 (m, 2H, H-*m*-phenylene); 6.94 (m, 2H, H-*o*-phenylene); 6.99 (s, 1H, H-6); 7.94 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, D_2O, ref(dioxane) = 69.3 ppm): 29.7 (CH$_2$−1″); 33.5 ((**C**H$_3$)$_3$C); 36.5 ((CH$_3$)$_3$**C**); 36.7 (CH$_2$−2″); 41.0 (CH$_2$−2′); 67.3 (d, $J_{C,P}$ = 4.3, CH$_2$−5′); 74.1 (CH-3′); 85.2 (CH-1′); 87.9 (d, $J_{C,P}$ = 7.9, CH-4′); 105.2 (C-4a); 117.4 (C-5); 123.1 (CH-6); 128.1 (CH-*m*-phenylene); 131.9 (CH-*o*-phenylene); 134.4 (C-*i*-phenylene);

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

151.6 (CH-2); 151.7 (C-7a, C-*p*-phenylene); 158.4 (C-4). $^{31}P\{^1H\}$ NMR (202.4 MHz, D_2O): 2.99. MS ESI$^-$: *m*/*z* (%): 521.2 $[M + H]^-$ (100), 543.2 $[M + Na]^-$ (10). HRMS: calculated for $[C_{23}H_{29}N_4O_6PS + H]^-$, 521.16292; found, 521.16223.

*7-{2-[(2,5-Dimethoxyphenyl)sulfanyl]ethyl}-2′-deoxy-7-deazaadenosine 5′-O-Phosphate (**12; dA^{SDMP}MP**).*

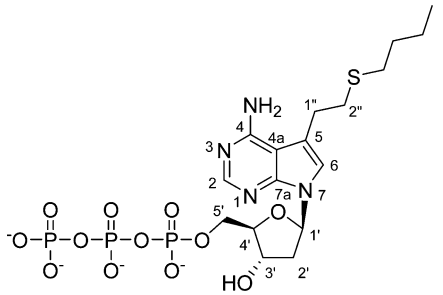


Compound **12** was prepared according to general procedure B from **7** (26 mg, 58 μmol) and $POCl_3$ (6.5 μL, 70 μmol) in $PO(OMe)_3$ (1 mL) and isolated as a white solid of triethylammonium salt (30.8 mg, 84%). ^1H NMR (500.0 MHz, DMSO-d_6): 1.13 (t, 9H, J_{vic} = 7.3, C**H**$_3$CH$_2$N); 2.12 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 3.0, H-2′b); 2.46 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 8.2, $J_{2'a,3'}$ = 5.9, H-2′a); 2.96 (q, 6H, J_{vic} = 7.3, CH$_3$C**H**$_2$N); 3.07 (m, 2H, H-1″); 3.15 (m, 2H, H-2″); 3.68 (s, 3H, CH$_3$O-5-C$_6$H$_3$(OMe)$_2$S); 3.72 (s, 3H, CH$_3$O-2-C$_6$H$_3$(OMe)$_2$S); 3.72−3.81 (m, 2H, H-5′); 3.89 (td, 1H, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 2.7, H-4′); 4.36 (ddd, 1H, $J_{3',2'}$ = 5.9, 3.0, $J_{3',4'}$ = 2.7, H-3′); 6.51 (dd, 1H, $J_{1'2'}$ = 8.2, 6.0, H-1′); 6.54 (bs, 2H, NH$_2$); 6.67 (dd, 1H, $J_{4,3}$ = 8.9, $J_{4,6}$ = 3.0, H-4-C$_6$H$_3$(OMe)$_2$S); 6.76 (d, 1H, $J_{6,4}$ = 3.0, H-6-C$_6$H$_3$(OMe)$_2$S); 6.86 (d, 1H, $J_{3,4}$ = 8.9, H-3-C$_6$H$_3$(OMe)$_2$S); 7.24 (s, 1H, H-6); 8.03 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 8.8 (**C**H$_3$CH$_2$N); 25.9 (CH$_2$−1″); 31.5 (CH$_2$−2″); 39.3 (CH$_2$−2′); 45.4 (CH$_3$**C**H$_2$N); 55.7 (CH$_3$O-5-C$_6$H$_3$(OMe)$_2$S); 56.4 (CH$_3$O-2-C$_6$H$_3$(OMe)$_2$S); 65.0 (d, $J_{C,P}$ = 4.6, CH$_2$−5′); 71.6 (CH-3′); 82.3 (CH-1′); 85.5 (d, $J_{C,P}$ = 7.4, CH-4′); 102.2 (C-4a); 110.5 (CH-4-C$_6$H$_3$(OMe)$_2$S); 112.0 (CH-3-C$_6$H$_3$(OMe)$_2$S); 113.6 (CH-6-C$_6$H$_3$(OMe)$_2$S); 113.8 (C-5); 119.5 (CH-6); 126.5 (C-1-C$_6$H$_3$(OMe)$_2$S); 150.6 (C-2-C$_6$H$_3$(OMe)$_2$S); 150.9 (C-7a); 151.7 (CH-2); 153.9 (C-5-C$_6$H$_3$(OMe)$_2$S); 157.8 (C-4). $^{31}P\{^1H\}$ NMR (202.4 MHz, DMSO-d_6): 0.81. MS ESI$^-$: *m*/*z* (%): 525.3 $[M + H]^-$ (100). HRMS: calculated for $[C_{21}H_{25}N_4O_8PS + H]^-$, 525.1214; found, 525.1213.

Preparation of Sulfide-Linked dNTPs by Triphosphorylation: General Procedure C. Sulfide-linked nucleoside **dA^{SR}** (**3**−**7**) was dried at 80 °C for 2 h in vacuo, and after cooling, it was suspended in $PO(OMe)_3$ at 0 °C, and $POCl_3$ (1.2 equiv) was added. The mixture was then stirred at 0 °C for 2 h. Then an ice-cooled solution of $(NHBu_3)_2H_2P_2O_7$ (5 equiv) and Bu_3N (4.16 equiv) in dry DMF (1.5 mL) was added, and the mixture was stirred at 0 °C for 1 h. Then the reaction was quenched by an addition of 2 M aqueous TEAB (triethylammonium bicarbonate, 2 mL) and the solvents were evaporated under vacuum. Products were isolated by HPLC on a C18 column with the use of a linear gradient of 0.1 M TEAB in H_2O to 0.1 M TEAB in H_2O/MeOH (1:1) as eluent. Several codistillations with water and conversion to the sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the final products as white solids.

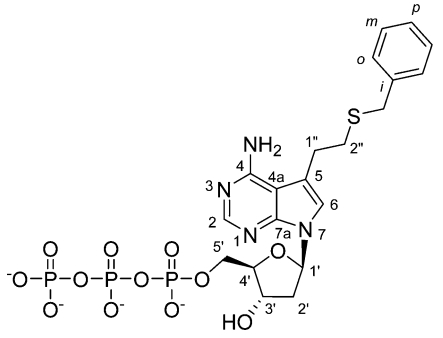
Preparation of Sulfide-Linked dNTPs by Thiol−ene Additions: General Procedure D. Triethylamonium salt of **dA^{V}TP** (**18**) was dissolved in dry methanol under argon. Then, thiol (10 equiv) was added, and the mixture was stirred at room temperature for 20 h. The solvents were evaporated in vacuum, and products were isolated by HPLC on a C18 column with the use of linear gradient of 0.1 M TEAB in H_2O to 0.1 M TEAB in H_2O/MeOH (1:1) as eluent. Several codistillations with water and conversion to the sodium salt form (Dowex 50WX8 in Na^+ cycle) followed by freeze-drying from water gave the final products as white solids.

*7-[2-(Butylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine 5′-O-Triphosphate (**13; dA^{SBu}TP**).*



Compound **13** was prepared according to general procedure C from **3** (42.0 mg, 115 μmol). $PO(OMe)_3$ (1.5 mL) and $POCl_3$ (12.8 μL, 138 μmol) were stirred at 0 °C for 2 h. The product was isolated as a white solid (40.4 mg, 52%). ^1H NMR (600.1 MHz, D_2O): 0.78 (t, 3H, J_{vic} = 7.4, C**H**$_3$CH$_2$CH$_2$CH$_2$S); 1.25 (m, 2H, CH$_3$C**H**$_2$CH$_2$CH$_2$S); 1.43 (m, 2H, CH$_3$CH$_2$C**H**$_2$CH$_2$S); 2.43 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.2, H-2′b); 2.44 (m, 2H, CH$_3$CH$_2$CH$_2$C**H**$_2$S); 2.62 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 8.0, $J_{2'a,3'}$ = 6.2, H-2′a); 2.82 (dt, 1H, J_{gem} = 13.3, $J_{2''b,1''}$ = 7.2, H-2″b); 2.87 (dt, 1H, J_{gem} = 13.3, $J_{2''a,1''}$ = 6.7, H-2″a); 2.98−3.06 (m, 2H, H-1″); 4.13−4.22 (m, 2H, H-5′); 4.24 (tdd, 1H, $J_{4',5'}$ = 4.0, $J_{4',3'}$ = 3.2, $J_{H,P}$ = 1.4, H-4′); 4.72 (dt, 1H, $J_{3',2'}$ = 6.2, 3.2, $J_{3',4'}$ = 3.2, H-3′); 6.58 (dd, 1H, $J_{1'2'}$ = 8.0, 6.2, H-1′); 7.36 (s, 1H, H-6); 8.14 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, D_2O): 15.3 (**C**H$_3$CH$_2$CH$_2$CH$_2$S); 24.0 (CH$_3$**C**H$_2$CH$_2$CH$_2$S); 28.7 (CH$_2$−1″); 33.6, 34.0 (CH$_3$CH$_2$**C**H$_2$CH$_2$S); 34.6 (CH$_2$−2″); 41.6 (CH$_2$−2′); 68.4 (d, $J_{C,P}$ = 5.8, CH$_2$−5′); 74.0 (CH-3′); 85.7 (CH-1′); 87.9 (d, $J_{C,P}$ = 8.8, CH-4′); 104.4 (C-4a); 119.0 (C-5); 123.9 (CH-6); 149.3 (CH-2); 151.1 (C-7a); 156.6 (C-4). $^{31}P\{^1H\}$ NMR (202.4 MHz, D_2O): −22.21 (t, *J* = 19.5 P_β); −10.50 (d, *J* = 19.5, P_α); −9.59 (d, *J* = 19.5, P_γ). MS ESI$^-$: *m*/*z* (%): 445.2 $[M\text{-}2PO_3+H]^-$ (40), 523.2 $[M\text{-}PO_3+2H]^-$ (100), 547.2 $[M\text{-}PO_3+Na+H]^-$ (45). HRMS: calculated for $[C_{17}H_{25}N_4O_{12}P_3S+3H]^-$, 605.0643; found, 605.0634.

*7-[2-(Benzylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine 5′-O-Triphosphate (**14; dA^{SBn}TP**).*

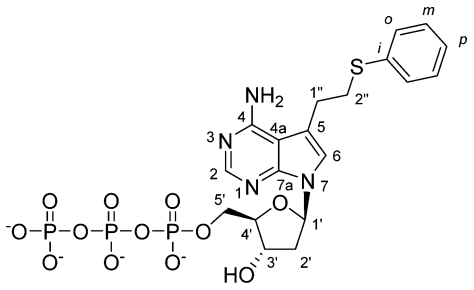


Compound **14** was prepared according to general procedure C from **4** (40.0 mg, 100 μmol), $PO(OMe)_3$ (1.5 mL), and $POCl_3$ (11.2 μL, 120 μmol). The product was isolated as a white solid (45.4 mg, 64%). Alternatively, compound **14** was also prepared according to general procedure D from **dA^{V}TP** (**18**) (23.2 mg, 28.3 μmol) and benzylthiol (33.2 μL, 283 μmol) in 1.5 mL of dry methanol at a yield of 24%. ^1H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.40 (ddd, 1H, J_{gem} = 13.9, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.3, H-2′b); 2.57 (ddd, 1H, J_{gem} = 13.9, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.3, H-2′a); 2.60 (dt, 1H, J_{gem} = 13.8, $J_{2''b,1''}$ = 7.3, H-2″b); 2.68−2.83 (m, 3H, H-1″,2″a); 3.52, 3.55 (2 × d, 2 × 1H, J_{gem} = 13.8, CH$_2$Ph); 4.17 (dd, 2H, $J_{H,P}$ = 5.9, $J_{5',4'}$ = 5.1, H-5′); 4.24 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.3, H-4′); 4.69 (dt, 1H, $J_{3',2'}$ = 6.3, 3.3, $J_{3',4'}$ = 3.3, H-3′); 6.45 (dd, 1H, $J_{1'2'}$ = 7.9, 6.2, H-1′); 7.04−7.17 (m, 6H, H-6, H-*o*,*m*,*p*-Ph); 8.03 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 28.4 (CH$_2$−1″); 33.9 (CH$_2$−2″); 38.4 (CH$_2$Ph); 41.3 (CH$_2$−2′); 68.5 (d, $J_{C,P}$ = 4.8, CH$_2$−5′); 74.0 (CH-3′); 85.4 (CH-1′); 87.7 (d, $J_{C,P}$ = 8.3, CH-4′); 104.5 (C-4a); 118.2 (C-5); 123.5 (CH-6); 129.6 (CH-*p*-Ph); 131.0 (CH-*m*-Ph); 131.3 (CH-*o*-Ph); 141.0 (C-*i*-Ph); 149.9 (CH-2); 151.2 (C-7a); 156.9 (C-4).

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

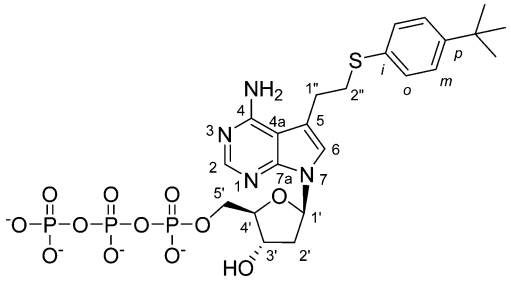
$^{31}P\{^{1}H\}$ NMR (202.4 MHz, D_2O): −22.29 (bdd, J = 19.4, 15.0 P_β); −11.04 (d, J = 19.4, P_α); −9.01 (bd, J = 15.0, P_γ). MS ESI⁻: m/z (%): 479.2 $[M\text{-}2PO_3+H]^-$ (80), 559.2 $[M\text{-}PO_3+2H]^-$ (100), 581.2 $[M\text{-}PO_3+Na+H]^-$ (50). HRMS: calculated for $[C_{20}H_{23}N_4O_{12}P_3S+3H]^-$, 639.0486; found, 639.0477.

*7-[2-(Phenylsulfanyl)ethyl]-2′-deoxy-7-deazaadenosine 5′-O-Triphosphate (**15; dA^{SPh}TP**).*



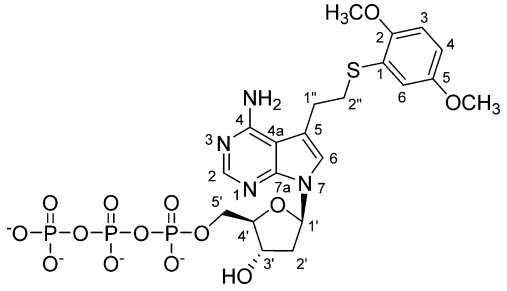
Compound **15** was prepared according to general procedure C from **5** (47.0 mg, 121.6 μmol), $PO(OMe)_3$ (1.5 mL), and $POCl_3$ (11.2 μL, 120 μmol). The product was isolated as a white solid (66.1 mg, 78%). Alternatively, compound **15** was also prepared according to general procedure D from **dA^{V}TP** (**18**) (34.6 mg, 42.2 μmol) and phenylthiol (43.4 μL, 422.2 μmol) in 1.5 mL of dry methanol at a yield of 32%. ^1H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.35 (ddd, 1H, J_{gem} = 13.9, $J_{2'b,1'}$ = 6.1, $J_{2'b,3'}$ = 3.2, H-2′b); 2.48 (ddd, 1H, J_{gem} = 13.9, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.3, H-2′a); 2.87−2.99 (m, 2H, H-1″); 3.12−3.23 (m, 2H, 2″); 4.13−4.19 (m, 2H, H-5′); 4.31 (m, 1H, H-4′); 4.66 (dt, 1H, $J_{3',2'}$ = 6.3, 3.2, $J_{3',4'}$ = 3.2, H-3′); 6.31 (dd, 1H, $J_{1'2'}$ = 7.9, 6.1, H-1′); 6.96 (m, 1H, H-*p*-Ph); 7.01 (m, 2H, H-*m*-Ph); 7.05 (m, 2H, H-*o*-Ph); 7.10 (s, 1H, H-6); 7.93 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 29.4 (CH_2−1″); 36.7 (CH_2−2″); 41.3 (CH_2−2′); 68.5 (d, $J_{C,P}$ = 4.4, CH_2−5′); 73.9 (CH-3′); 85.4 (CH-1′); 87.6 (d, $J_{C,P}$ = 8.2, CH-4′); 104.8 (C-4a); 118.0 (C-5); 123.9 (CH-6); 128.7 (CH-*p*-Ph); 131.2 (CH-*m*-Ph); 131.9 (CH-*o*-Ph); 137.4 (C-*i*-Ph); 149.5 (CH-2); 150.8 (C-7a); 156.8 (C-4). $^{31}P\{^{1}H\}$ NMR (202.4 MHz, D_2O): −22.09 (bdd, J = 18.9, 15.0 P_β); −10.98 (d, J = 18.9, P_α); −8.60 (bd, J = 15.0, P_γ). MS ESI⁻: m/z (%): 465.2 $[M\text{-}2PO_3+H]^-$ (40), 545.1 $[M\text{-}PO_3+2H]^-$ (100), 567.1 $[M\text{-}PO_3+Na+H]^-$ (65). HRMS: calculated for $[C_{19}H_{21}N_4O_{12}P_3S+3H]^-$, 625.0329; found, 625.0319.

*7-{2-[(4-tert-Butylphenyl)sulfanyl]ethyl}-2′-deoxy-7-deazaadenosine 5′-O-Triphosphate (**16; dA^{STBP}TP**).*



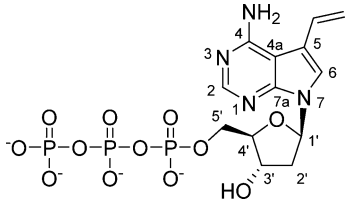
Compound **16** was prepared according to general procedure C from **6** (45.0 mg, 101.7 μmol), $PO(OMe)_3$ (1.5 mL), and $POCl_3$ (11.4 μL, 122.0 μmol). The product was isolated as a white solid (61.5 mg, 81%). ^1H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 1.04 (s, 9H, $(CH_3)_3C$); 2.34, 2.47 (2 × bm, 2 × 1H, H-2′); 3.02 (bm, 2H, H-1″); 3.19 (bm, 2H, 2″); 4.13−4.23 (bm, 3H, H-4′,5′); 4.66 (bm, 1H, H-3′); 6.28 (bt, 1H, $J_{1'2'}$ = 6.8, H-1′); 6.90−6.95 (bm, 4H, H-*o*,*m*-phenylene); 7.16 (s, 1H, H-6); 8.00 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 29.8 (CH_2−1″); 33.3 ($(\mathbf{C}H_3)_3C$); 36.4 ($(CH_3)_3\mathbf{C}$); 36.6 (CH_2−2″); 41.6 (CH_2−2′); 68.5 (d, $J_{C,P}$ = 5.6, CH_2−5′); 73.8 (CH-3′); 85.5 (CH-1′); 87.7 (d, $J_{C,P}$ = 8.3, CH-4′); 104.7 (C-4a); 118.5 (C-5); 124.7 (CH-6); 128.0 (CH-*o*-phenylene); 131.6 (CH-*m*-phenylene); 134.0 (C-*i*-phenylene); 147.9 (CH-2); 150.3 (C-7a); 151.9 (C-*p*-phenylene); 155.6 (C-4). $^{31}P\{^{1}H\}$ NMR (202.4 MHz, D_2O): −22.51 (t, J = 19.1, P_β); −11.05 (d, J = 19.1, P_α); −9.93 (bd, J = 19.1, P_γ). MS ESI⁻: m/z (%): 521.2 $[M\text{-}2PO_3+H]^-$ (30), 601.2 $[M\text{-}PO_3+2H]^-$ (100), 623.2 $[M\text{-}PO_3+Na+H]^-$ (60), 703.2 $[M+Na+2H]^-$ (15). HRMS: calculated for $[C_{23}H_{29}N_4O_{12}P_3S+3H]^-$, 681.0956; found, 681.0945.

*7-{2-[(2,5-Dimethoxyfenyl)sulfanyl]ethyl}-2′-deoxy-7-deazaadenosine 5′-O-Triphosphate (**17; dA^{SDMP}TP**).*



Compound **17** was prepared according to general procedure C from **7** (35.1 mg, 78.6 μmol), $PO(OMe)_3$ (1 mL), and $POCl_3$ (8.8 μL, 94.3 μmol). The product was isolated as a white solid (29.8 mg, 54%). ^1H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.39 (ddd, 1H, J_{gem} = 13.9, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.2, H-2′b); 2.59 (ddd, 1H, J_{gem} = 13.9, $J_{2'a,1'}$ = 7.9, $J_{2'a,3'}$ = 6.4, H-2′a); 3.04 (m, 1H, H-1″b); 3.20 (m, 2H, H-1″a,2″b); 3.46 (m, 1H, H-2″b); 3.62 (s, 3H, CH_3O-5-$C_6H_3(OMe)_2S$); 3.72 (s, 3H, CH_3O-2-$C_6H_3(OMe)_2S$); 4.06 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 5.7, $J_{5'b,4'}$ = 4.8, H-5′b); 4.11 (ddd, 1H, J_{gem} = 11.2, $J_{H,P}$ = 6.4, $J_{5'a,4'}$ = 4.8, H-5′a); 4.18 (td, 1H, $J_{4',5'}$ = 4.8, $J_{4',3'}$ = 3.2, H-4′); 4.70 (dt, 1H, $J_{3',2'}$ = 6.4, 3.2, $J_{3',4'}$ = 3.2, H-3′); 6.35 (dd, 1H, $J_{1'2'}$ = 7.9, 6.2, H-1′); 6.47 (m, 1H, H-6-$C_6H_3(OMe)_2S$); 6.48 (m, 1H, H-4-$C_6H_3(OMe)_2S$); 6.57 (m, 1H, H-3-$C_6H_3(OMe)_2S$); 7.13 (s, 1H, H-6); 7.94 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 30.5 (CH_2−1″); 35.7 (CH_2−2″); 40.6 (CH_2−2′); 58.0 (CH_3O-5-$C_6H_3(OMe)_2S$); 58.6 (CH_3O-2-$C_6H_3(OMe)_2S$); 68.3 (d, $J_{C,P}$ = 5.5, CH_2−5′); 73.9 (CH-3′); 85.1 (CH-1′); 87.5 (d, $J_{C,P}$ = 8.7, CH-4′); 105.8 (C-4a); 114.0 (CH-3-$C_6H_3(OMe)_2S$); 114.2 (CH-4-$C_6H_3(OMe)_2S$); 117.1 (C-5); 120.5 (CH-6-$C_6H_3(OMe)_2S$); 123.3 (CH-6); 125.4 (C-1-$C_6H_3(OMe)_2S$); 152.3 (C-7a); 153.0 (CH-2); 154.1 (C-2-$C_6H_3(OMe)_2S$); 154.6 (C-5-$C_6H_3(OMe)_2S$); 159.6 (C-4). $^{31}P\{^{1}H\}$ NMR (202.4 MHz, D_2O): −20.93 (bs, P_β); −10.20 (d, J = 18.4, P_α); −5.58 (bs, P_γ). MS ESI⁻: m/z (%): 525.3 $[M\text{-}2PO_3+H]^-$ (45), 605.3 $[M\text{-}PO_3+2H]^-$ (80), 627.3 $[M\text{-}PO_3+Na+H]^-$ (100). HRMS: calculated for $[C_{21}H_{25}N_4O_{14}P_3S+3H]^-$, 685.0541; found, 685.0537.

*7-Vinyl-2′-deoxy-7-deazaadenosine 5′-O-Triphosphate (**18; dA^{V}TP**).*[29]



Nucleoside **dA^{V}** (**2**) (200.0 mg, 723.9 μmol) was dried at 80 °C for 2 h. After cooling to r.t., it was suspended in $PO(OMe)_3$ (2.5 mL) at 0 °C, and $POCl_3$ (80.9 μL, 868.6 μmol) was added. The mixture was stirred at 0 °C for 1 h. Then an ice-cooled solution of $(NHBu_3)_2H_2P_2O_7$ (2 g, 3.6 mmol) and tributylamine (719 μL, 3.0 mmol) in dry DMF (6 mL) was added, and the resulting mixture was stirred at 0 °C for 1 h. Then, the reaction was quenched by the addition of 2 M aqueous TEAB (2 mL), and the solvents were evaporated under vacuum. The product was isolated by HPLC on a C18 column with the use of a linear gradient of 0.1 M TEAB in H_2O to 0.1 M TEAB in H_2O/MeOH (1:1) as eluent. Several codistillations with water followed by freeze-drying from water gave the white solid triethylamonium salt of the product (194.8 mg, 33%). NMR and MS data were consistent with the literature.[29]

Primer Extension: 19-Mer Template. PEX reactions with **dA^{SBu}TP**, **dA^{SPh}TP**, **dA^{STBP}TP**, **dA^{SBn}TP**, or **dA^{SDMP}TP** as substrates

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125

were performed in the presence of a DNA polymerase (KOD XL, Vent(exo$^-$) or Pwo). The reaction mixture (20 μL) contained KOD XL DNA polymerase (0.125 U) or Vent (exo$^-$) DNA polymerase (0.100 U), dGTP (16 μM), either dATP or **dA^{SR}TP** (16 μM), 5′-FAM labeled primer$^{248\text{-sh}}$ (0.150 μM), and a 19-mer template (temp^{oligo1A}, 0.225 μM) in buffer (2 μL) supplied by the manufacturer. In the case of Pwo polymerase, 0.200 U of the enzyme and 50 μM of each dGTP and dATP or **dA^{SR}TP** was used. Reaction mixtures were incubated for 30 min at 60 °C. Before gel loading samples were denatured by the addition 20 μL of stop solution (80% [v/v] formamide, 20 mM EDTA, 0.025%, [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol, and Milli-Q water) and heated for 5 min at 95 °C. Reaction mixtures were separated using 12.5% denaturing PAGE. Visualization was performed by fluorescence imaging.

Primer Extension: 31-Mer Template. PEX reactions with a 31-template (temp^{Prb4baseII}) were performed in the same way as described above using KOD XL polymerase (0.250 U), Vent (exo$^-$) polymerase (0.200 U), or Pwo polymerase (0.200 U), dGTP, dCTP, and TTP (200 μM each), dATP, or **dA^{SR}TP** (200 μM), 5′-FAM labeled primer (primer$^{248\text{-sh}}$, 0.150 μM), and a 31-mer template (temp^{Prb4baseII}, 0.225 μM) in buffer (2 μL) supplied by the manufacturer.

Polymerase Chain Reaction. PCR reactions with **dA^{SBu}TP**, **dA^{SPh}TP**, **dA^{STBP}TP**, **dA^{SBn}TP**, or **dA^{SDMP}TP** as substrates were performed using KOD XL polymerase. The PCR reaction mixture (20 μL) contained KOD XL (1 U), dGTP, dCTP, and TTP (160 μM each), either dATP or **dA^{SR}TP** (200 μM), primers (primer^{LT25TH} and primer^{L20}, 2 μM each), and a 98-mer template (temp$^{FVL\text{-}A}$, 0.025 μM) in reaction buffer (2 μL) supplied by the manufacturer. Thirty PCR cycles were run under the following conditions: denaturation for 1 min at 95 °C, annealing for 1 min at 53 °C, extension for 1 min at 72 °C, followed by final extension step of 2 min at 75 °C. Reaction mixtures were than separated by use of a 2% agarose gel with GelRed as an intercalator. Visualization was performed by fluorescence imaging.

Kinetics of Incorporation of dA^{SR}TPs. The PEX reaction mixture (20 μL) contained KOD XL (0.125 U), **dA^{SR}TP** (16 μM), 5′-FAM-labeled primer (primer$^{248\text{-sh}}$, 0.150 μM), and a 16-mer template (temp^{1A_term}, 0.225 μM) in buffer (2 μL) supplied by the manufacturer. Reaction mixtures were incubated at 60 °C in a thermal cycler during 0.1−5 min. Before gel loading, the samples were denatured by the addition 20 μL of stop solution (80% [v/v] formamide, 20 mM EDTA, 0.025%, [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol, and Milli-Q water) and heated for 5 min at 95 °C. Reaction mixtures were separated by using 12.5% denaturing PAGE. Visualization was performed by fluorescence imaging.

Preparation of ONs for MALDI-TOF Analysis: 19- or 31-Mer Template. The reaction mixture (50 μL) contained primer (primer$^{248\text{-sh}}$, 3.2 μM), a biotinylated 19- or 31-mer template (5′-biotinylated temp^{oligo1A} or 5′-biotinylated temp^{Prb4baseII}, 3.2 μM), KOD XL DNA polymerase (0.25 U), dGTP (208 μM), dCTP, and TTP (208 μM each for temp^{Prb4baseII}), and **dA^{SR}TP** (208 μM), in enzyme reaction buffer supplied by the manufacturer (5 μL). The reaction mixture was incubated for 40 min at 60 °C in a thermal cycler. Streptavidin magnetic particle stock solution (50 μL) was washed with binding buffer (3 × 200 μL, 10 mM Tris, 1 mM EDTA, and 100 mM NaCl, pH 7.5). The PEX solution (prepared as described above) and binding buffer (50 μL) were added. The suspension was shaken (1400 rpm) for 30 min at 15 °C. The magnetic beads were collected on a magnet (DynaMag-2) and washed with wash buffer (3 × 200 μL, 10 mM Tris, 1 mM EDTA, 500 mM NaCl, pH 7.5) and water (5 × 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 evaporated to dryness, then dissolved in the mixture of water/acetonitrile (1:1, 5 ul) and analyzed by MALDI-TOF mass spectrometry.

TdT Elongation. The reaction mixture (10 μL) contained TdT (12 U), dATP, **dA^{SBu}TP**, or **dA^{SPh}TP** (200 μM) and 5′-FAM labeled primer (primer$^{248\text{-sh}}$, 0.150 μM) in enzyme reaction buffer (1 μL) supplied by the manufacturer. The reaction mixture was incubated for 1, 1.5, 3, or 21 h at 37 °C in a thermal cycler. Before gel loading samples were denatured by the addition 10 μL of stop solution (80% [v/v] formamide, 20 mM EDTA, 0.025%, [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol, and Milli-Q water) and heated 10 min at 95 °C. Reaction mixtures were separated by using 12.5% denaturing PAGE. Visualization was performed by fluorescence imaging.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02098.

Additional PAGE analyses, copies of NMR, and MALDI spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hocek@uochb.cas.cz.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Academy of Sciences of the Czech Republic (RVO: 61388963 and Praemium Academiae to M.H.), the Czech Science Foundation (14-04289S to M.S. and M.H.), and by Gilead Sciences, Inc. (Foster City, CA, U.S.A.).

■ REFERENCES

(1) Gissot, A.; Camplo, M.; Grinstaff, M. W.; Barthélémy, P. *Org. Biomol. Chem.* **2008**, *6*, 1324−1333.

(2) Patwa, A.; Gissot, A.; Bestel, I.; Barthélémy, P. *Chem. Soc. Rev.* **2011**, *40*, 5844−5854.

(3) Schade, M.; Berti, D.; Huster, D.; Herrmann, A.; Arbuzova, A. *Adv. Colloid Interface Sci.* **2014**, *208*, 235−251.

(4) (a) Liu, H.; Zhu, Z.; Kang, H.; Wu, Y.; Sefan, K.; Tan, W. *Chem. - Eur. J.* **2010**, *16*, 3791−3797. (b) Beales, P. A.; Vanderlick, T. K. *Adv. Colloid Interface Sci.* **2014**, *207*, 290−305.

(5) (a) Langecker, M.; Arnaut, V.; List, J.; Simmel, F. C. *Acc. Chem. Res.* **2014**, *47*, 1807−1815. (b) Dohno, C.; Matsuzaki, K.; Yamaguchi, H.; Shibata, T.; Nakatani, K. *Org. Biomol. Chem.* **2015**, *13*, 10117−10121.

(6) Raouane, M.; Desmaële, D.; Urbinati, G.; Massaad-Massade, L.; Couvreur, P. *Bioconjugate Chem.* **2012**, *23*, 1091−1104.

(7) Fujita, H.; Nakajima, K.; Kasahara, Y.; Ozaki, H.; Kuwahara, M. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 333−336.

(8) (a) Tolle, F.; Brändle, G. M.; Matzner, D.; Mayer, G. *Angew. Chem., Int. Ed.* **2015**, *54*, 10971−10974. (b) Diafa, S.; Hollenstein, M. *Molecules* **2015**, *20*, 16643−16671.

(9) (a) Abe, H.; Abe, N.; Shibata, A.; Ito, K.; Tanaka, Y.; Ito, M.; Saneyoshi, H.; Shuto, S.; Ito, Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 6475−6479. (b) Liu, K.; Zheng, L.; Liu, Q.; de Vries, J. W.; Gerasimov, J. Y.; Herrmann, A. *J. Am. Chem. Soc.* **2014**, *136*, 14255−14262.

(10) Singh, Y.; Murat, P.; Defrancq, E. *Chem. Soc. Rev.* **2010**, *39*, 2054−2070.

(11) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925−1963.

(12) (a) Hottin, A.; Marx, A. *Acc. Chem. Res.* **2016**, *49*, 418−427. (b) Hollenstein, M. *Molecules* **2012**, *17*, 13569−13591.

(13) (a) Hocek, M. *J. Org. Chem.* **2014**, *79*, 9914−9921. (b) Dadová, J.; Cahová, H.; Hocek, M. Polymerase Synthesis of Base-Modified DNA. In *Modified Nucleic Acids*; Nakatani, K., Tor, Y., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp 123−144.

(14) Hoshino, H.; Kasahara, Y.; Fujita, H.; Kuwahara, M.; Morihiro, K.; Tsunoda, S.; Obika, S. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 530−533.

(15) Tateishi-Karimata, H.; Muraoka, T.; Kinbara, K.; Sugimoto, N. *ChemBioChem* **2016**, *17*, 1399−1402.

(16) Baccaro, A.; Marx, A. *Chem. - Eur. J.* **2010**, *16*, 218−226.

(17) Wang, Y.; Tkachenko, B. A.; Schreiner, P. R.; Marx, A. *Org. Biomol. Chem.* **2011**, *9*, 7482−7490.

(18) Ikonen, S.; Macíčková-Cahová, H.; Pohl, R.; Šanda, M.; Hocek, M. *Org. Biomol. Chem.* **2010**, *8*, 1194−1201.
(19) Shaughnessy, K. H. *Molecules* **2015**, *20*, 9419−9454.
(20) Čapek, P.; Cahová, H.; Pohl, R.; Hocek, M.; Gloeckner, C.; Marx, A. *Chem. - Eur. J.* **2007**, *13*, 6196−6203.
(21) Thoresen, L. H.; Jiao, G. S.; Haaland, W. C.; Metzker, M. L.; Burgess, K. *Chem. - Eur. J.* **2003**, *9*, 4603−4610.
(22) Krause, A.; Hertl, A.; Muttach, F.; Jäschke, A. *Chem. - Eur. J.* **2014**, *20*, 16613−16619.
(23) Dadová, J.; Vidláková, P.; Pohl, R.; Havran, L.; Fojta, M.; Hocek, M. *J. Org. Chem.* **2013**, *78*, 9627−9637.
(24) (a) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 1540−1573. (b) Hoyle, C. E.; Lowe, A. B.; Bowman, C. N. *Chem. Soc. Rev.* **2010**, *39*, 1355−1387. (c) Lowe, A. B. *Polym. Chem.* **2010**, *1*, 17−36.
(25) Nair, D. P.; Podgórski, M.; Chatani, S.; Gong, T.; Xi, W.; Fenoli, C. R.; Bowman, C. N. *Chem. Mater.* **2014**, *26*, 724−744.
(26) (a) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron Lett.* **1995**, *36*, 421−424. (b) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron* **1997**, *53*, 3035−3044. (c) Ali, M. M.; Nagatsugi, F.; Sasaki, S.; Nakahara, R.; Maeda, M. *Nucleosides, Nucleotides Nucleic Acids* **2006**, *25*, 159−169. (d) Jones, S. A.; McClean, M. J.; Slater, M. J.; Walker, R. T.; Balzarini, J.; DeClercq, E. *J. Chem. Soc., Perkin Trans. 1* **1987**, 457−464.
(27) (a) Nagatsugi, F.; Kawasaki, T.; Usui, D.; Maeda, M.; Sasaki, S. *J. Am. Chem. Soc.* **1999**, *121*, 6753−6754. (b) Kawasaki, T.; Nagatsugi, F.; Ali, M. M.; Maeda, M.; Sugiyama, K.; Hori, K.; Sasaki, S. *J. Org. Chem.* **2005**, *70*, 14−23. (c) Ali, M. M.; Oishi, M.; Nagatsugi, F.; Mori, K.; Nagasaki, Y.; Kataoka, K.; Sasaki, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 3136−3140.
(28) (a) Overas, A. T.; Bakkestuen, A. K.; Gundersen, L.-L.; Rise, F. *Acta Chem. Scand.* **1997**, *51*, 1116−1124. (b) Liu, F.; Dalhus, B.; Gundersen, L.-L.; Rise, F. *Acta Chem. Scand.* **1999**, *53*, 269−279. (c) Kuchař, M.; Pohl, R.; Votruba, I.; Hocek, M. *Eur. J. Org. Chem.* **2006**, *2006*, 5083−5098. (d) Kuchař, M.; Hocek, M.; Pohl, R.; Votruba, I.; Shih, I.-h.; Mabery, E.; Mackman, R. *Bioorg. Med. Chem.* **2008**, *16*, 1400−1424.
(29) Mačková, M.; Pohl, R.; Hocek, M. *ChemBioChem* **2014**, *15*, 2306−2312.
(30) Seela, F.; Zulauf, M.; Sauer, M.; Deimel, M. *Helv. Chim. Acta* **2000**, *83*, 910−927.
(31) Michelson, A. M.; Orkin, S. H. *J. Biol. Chem.* **1982**, *257*, 14773−14782.
(32) (a) Kumar, A.; Tchen, P.; Roullet, F.; Cohen, J. *Anal. Biochem.* **1988**, *169*, 376−382. (b) Cho, Y.; Kool, E. T. *ChemBioChem* **2006**, *7*, 669−672. (c) Hollenstein, M.; Wojciechowski, F.; Leumann, C. J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4428−4430. (d) Kobayashi, T.; Takezawa, Y.; Sakamoto, A.; Shionoya, M. *Chem. Commun.* **2016**, *52*, 3762−3765.
(33) Horáková, P.; Macíčková-Cahová, H.; Pivoňková, H.; Špaček, J.; Havran, L.; Hocek, M.; Fojta, M. *Org. Biomol. Chem.* **2011**, *9*, 1366−1371.
(34) During the preparation of this manuscript, a related paper on functionalization of a dNTP with protein was published based on thiol-ene addition of sulfanylalkyl-linked dUTP with a maleimide-linked protein: Welter, M.; Verga, D.; Marx, A. *Angew. Chem., Int. Ed.* **2016**, *55*, 10131−10135.

DOI: 10.1021/acs.joc.6b02098
J. Org. Chem. 2016, 81, 11115−11125