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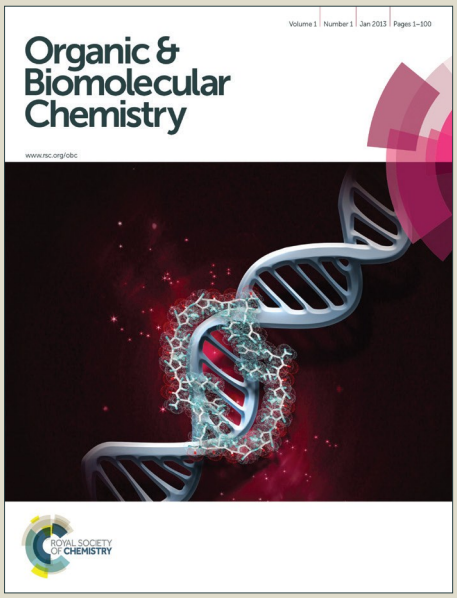

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ARTICLE

Copper-mediated arylsulfanylations and arylselanylations of pyrimidine or 7-deazapurine nucleosides and nucleotides

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Synthesis of 5-arylsulfanyl- or 5-arylselanylpyrimidine and 7-arylsulfanyl- or 7-arylselanyl-7-deazapurine nucleosides and nucleotides was developed by Cu-mediated sulfanylations or selanylations of the corresponding 5-iodopyrimidine or 7-iodo-7-deazapurine nucleosides or nucleotides with diaryldisulfides or -diselenides. The reactions were also applicable for direct modifications of 2'-deoxycytidine triphosphate and the resulting 5-arylsulfanyl or 5-arylselanyl-dCTP served as substrates for polymerase synthesis of modified DNA bearing arylsulfanyl or arylselanyl groups in the major groove.

Introduction

DNA molecules bearing modifications in major groove find diverse applications mainly in bioanalysis and chemical biology.[1] 5-Substituted pyrimidine and 7-substituted 7-deazapurine 2'-deoxyribonucleoside 5'-*O*-triphosphates (dNTPs) are good substrates for DNA polymerases in enzymatic synthesis of base-modified DNA.[2,3] Modified dNTPs are mostly synthesized by triphosphorylation of corresponding modified nucleosides[4] but this approach can fail in case of some reactive modifications not compatible with the triphosphorylation methodology. Therefore, direct methods of functionalization of dNTPs are desirable but are inherently difficult due to lability of dNTPs which are prone to hydrolysis. So far, the only reported reactions suitable for modification of dNTPs were aqueous cross-coupling reactions of halogenated dNTPs,[3,5] thiol-maleimide addition,[6] some amide-forming reactions of 5-aminoalkylethynyl-dUTP,[7] hydrazone-formation,[8] Diels-Alder[9] and CuAAC click reaction.[10] The Suzuki-Miyaura cross-coupling reaction with arylboronic acids[11] and the Sonogashira reactions with terminal acetylenes[12] are the most general and useful reactions used in the synthesis of base-modified dNTPs. In addition, several examples of the Heck coupling[13] with acrylates, as well as the Stille reaction[14] with aryl- or alkenylstannanes were recently reported. To the best of our knowledge, no method for direct attachment of a heteroatom to dNTPs has been published.

5-Alkylsulfanyl- or arylsulfanyl-pyrimidine nucleosides were reported to inhibit thymidylate kinase[15] and slightly destabilized DNA duplexes,[16] whereas saturated 5-phenylsulfanyl-thymidine analogues were used[17] as radical precursors for photochemical generation of thymine in DNA. Some 7-arylsulfanyl-7-deazaadenosine analogues displayed[18] weak cytostatic effects, while the corresponding 7-S-substituted 7-deazaguanine derivatives have never been reported. 5-Selenylated pyrimidine nucleotides inhibit thymidylate synthase[19] and have been utilized[20] for modification of DNA or RNA for X-ray crystallography and 5-(phenylselenylmethyl)uracil was used[21] as a T radical precursor for DNA crosslinking. Also related 5-(phenyltelluranyl)uracil nucleoside has been prepared[22] and, after incorporated to DNA, used for X-ray and STM imaging. However, no selenylated 7-deazapurines are known so far. Therefore, we report here the synthesis of arylsulfanyl and arylselanyl derivatives of pyrimidine and 7-deazapurine nucleosides and nucleotides and their potential for polymerase incorporation to DNA.

Results and Discussion

Synthesis

Previously, 5-(alkylsulfanyl)pyrimidine bases or nucleosides were prepared by alkylation of 5-mercaptouracil,[23] reactions of toxic 5-(chloromercuri)pyrimidines with disulfides,[24] or more recently by Pd-catalyzed coupling of 5-bromopyrimidine derivatives with thiols,[25] whereas 7-arylsulfanyl-7-deazapurines were prepared by Cu-mediated S-H sulfenylations.[18] The 5-selenylated pyrimidines were prepared by Mn-mediated C-H selenylations[20] or electrophilic aromatic selenylation.[26]

Inspired by the works of Taniguchi[27] on copper-catalyzed reactions of diaryldisulfides or diaryldiselenides with iodoarenes, we started our study by testing of reactions of unprotected halogenated nucleosides.[5] The reaction conditions were first tested on 5-iodo-2'-deoxycytidine (**dC^{I}**) in reaction with diphenyldisulfide. In our hands, the Cu-catalyzed (10 mol. % of CuI) reactions in presence or in the absence of Mg[27] gave

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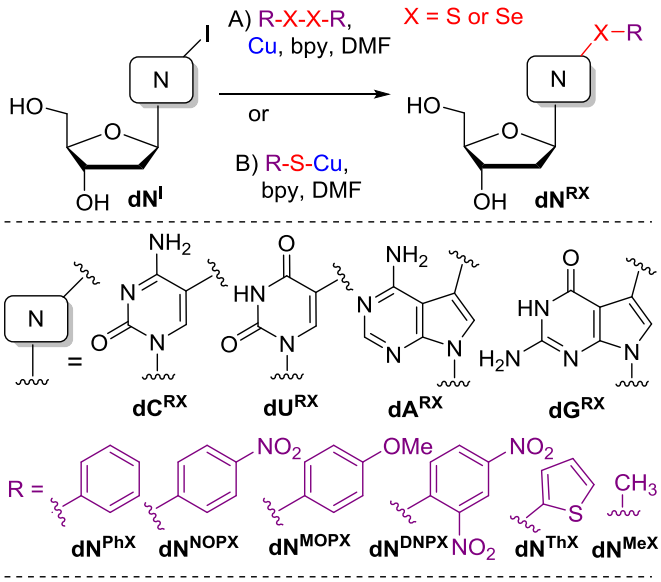
complex mixtures of products. Therefore, we used stoichiometric amounts of copper powder in presence of 2,2'-bipyridine (bpy). The reactions were performed at 80-110°C in DMF (Scheme 1). Under these conditions (Method A), the desired 5-phenylsulfanyl-2'-deoxycytidine was formed as major product (in addition to small amounts of dehalogenated 2'-deoxycytidine) and isolated in good yield of 58%. Similar conversion and yield was achieved when using pre-generated phenylsulfanylcuprate (Method B).



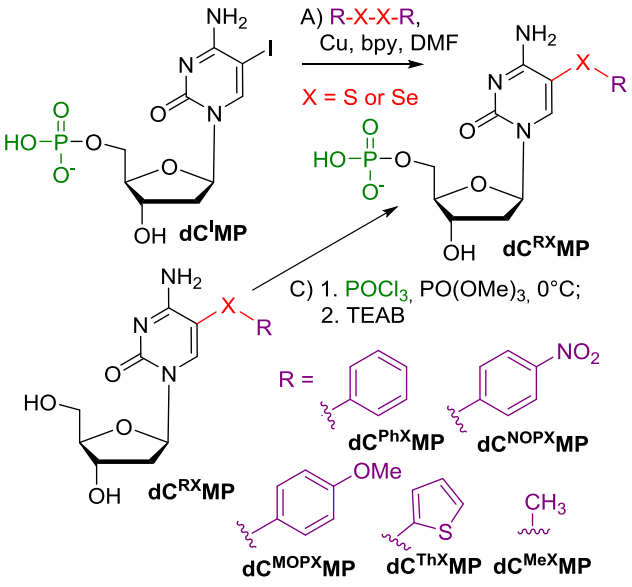
Product	Method	Yield
dC^{PhS}	A	58%
dC^{PhS}	B	56%
dC^{NOPS}	A	50%
dC^{MOPS}	A	21%
dC^{DNPS}	A	28%
dC^{ThS}	A	46%
dC^{PhSe}	A	50%
dC^{MeSe}	A	18%

Product	Method	Yield
dU^{PhS}	B	47%
dU^{ThS}	A	26%
dU^{PhSe}	A	24%
dU^{MeSe}	A	11%
dA^{PhS}	B	50%
dA^{PhSe}	A	32%
dA^{MeSe}	A	14%
dG^{PhS}	B	39%
dG^{PhSe}	A	45%

Scheme 1. Sulfanylations and selanylations of nucleosides

The same reaction of nucleoside **dC^{I}** (Method A) was then performed with a small series of diaryldisulfides to obtain the corresponding 5-(4-nitrophenyl)sulfanyl (**dC^{NOPS}**), 5-(4-methoxyphenyl)sulfanyl (**dC^{MOPS}**), 5-(2,4-dinitrophenyl)sulfanyl (**dC^{DNPS}**) and 5-(2-thienylsulfanyl)- (**dC^{ThS}**) 2'-deoxycytidines in moderate yields (21-50%). The reaction (Method A) with diphenyldiselenide gave the 5-(phenylselanyl)cytosine nucleoside **dC^{PhSe}** in good 50% yield, whereas the corresponding 5-(methylselanyl)C nucleoside **dC^{MeSe}** was only obtained in low 18% yield.

Then we tested other iodinated nucleosides (Scheme 1). The reaction of 5-iodo-2'-deoxyuridine (**dU^{I}**) with PhSCu (Method B) provided the 5-substituted **dU^{PhS}** nucleoside in 47%, whereas the reactions with dithienyldisulfide or diselenides (Method A) gave the other corresponding 5-arylsulfanyl- or phenyl- or methylselanyl uracil nucleosides (**dU^{ThS}**, **dU^{PhSe}** and **dU^{MeSe}**) in low yields. The reactions of 7-iodo-7-deazaadenine **dA^{I}** and -7-deazaguanine **dG^{I}** nucleosides with PhSCu (Method B) gave the 7-(phenylsulfanyl)deazapurine nucleosidies **dA^{PhS}** and **dG^{PhS}** in acceptable 50 or 39% yields, whereas the reactions with diphenyldiselenide furnished the corresponding phenylselanyl nucleosides **dA^{PhSe}** and **dG^{PhSe}** in moderate yields. Again, the reaction with dimethyldiselenide gave very low conversion and the **dA^{MeSe}** was isolated only in 14%. Apparently the reactivity of dimethyldiselenide is very low and the methylsulfanylation is of very limited synthetic applicability.



Product	Method	Yield	Method	Yield
dC^{PhS}MP	A	5%	C	42%
dC^{NOPS}MP	A	7%	C	27%
dC^{MOPS}MP	A	0%	C	22%
dC^{ThS}MP	A	45%	C	-
dC^{PhSe}MP	A	21%	C	48%
dC^{MeSe}MP	A	0%	C	48%

Scheme 2. Sulfanylations and selanylations of **dC^{I}MP**

Next, we tested the reactions of nucleotides and started with stable nucleoside 5'-*O*-monophosphates (dNMPs). The model iodinated **dC^{I}MP** was tested in reactions with diaryldisufides or diselenides (Scheme 2, Method A). Most of these reactions gave very low conversions and only two products, **dC^{ThS}MP** and **dC^{PhSe}MP** were isolated in acceptable yields. On the other hand, the phosphorylation of the 5-arylsulfanyl- or arylselanyl-cytosine nucleosides gave the desired modified nucleotides in better yields (22-48%).

Finally, we tested the reactions for direct modification of hydrolytically labile dNTPs (Scheme 3). Thus the iodinated triphosphate dC^{I}TP was reacted with PhSCu (Method B) to give the desired 5-(phenylsulfanyl)-dCTP (**dC^{PhS}TP**) in low 7% yield. Better conversions were achieved when using reactions with

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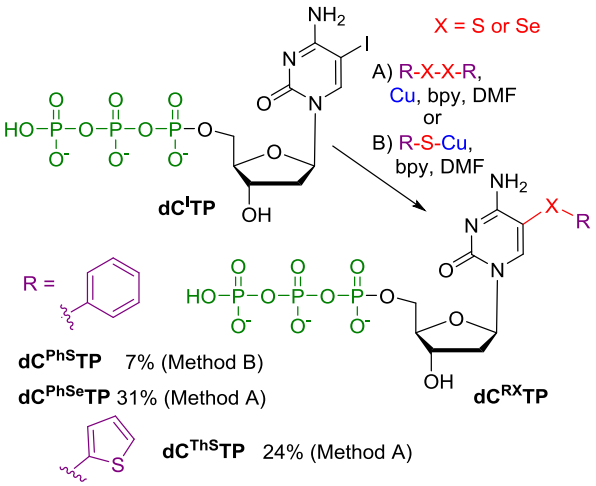
diaryldisulfides or diselenides (Method A). The desired **dC^{ThS}TP** and **dC^{PhSe}TP** were obtained in good yields of 24 and 31% (which are fully comparable to typical yields of cross-coupling reactions of dNTPs[11-14]).



Scheme 3. Sulfanylations and selanylations of dNTPs

Polymerase incorporation of modified nucleotides

The three new 5-S- or Se-linked dNTPs (**dC^{PhS}TP**, **dC^{ThS}TP** and **dC^{PhSe}TP**) were then tested as substrates for DNA polymerases. At first we tested them in primer extension (PEX) reaction with either KOD XL, Vent(exo-) or Pwo polymerases, 15-mer Primer$^{248\text{-}sh}$ and a 19-mer template temp^{oligo1C} (for sequences, see Table 1). Figure 1 shows the PAGE analysis of the PEX reactions. While KOD XL and Vent(exo-) polymerases gave quite clean bands of the 19-mer oligonucleotide (ON) products bearing one modified **dC^{RX}** nucleotide, Pwo gave a mixture of the full-lenths and a truncated product.

Table 1: List of ON sequences used in this study

Oligo	Sequence
Primer$^{248\text{-}sh}$	5'-CATGGGCGGCATGGG-3'
temp^{oligo1C}	5'-CCCGCCCATGCCGCCCATG-3'
temp^{Prb4baseII}	5'-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3'
primer^{LT25TH}	5'-CAAGGACAAAATACCTGTATTCCTT-3'
primer^{L20}	5'-GACATCATGAGAGACATCGC-3'
temp$^{FVL\text{-}A}$	5'-GACATCATGAGAGACATCGCCTCTGGGCTAATAGGACTACTT CTAATCTGTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGGT ATTTTGTCCTTG-3'

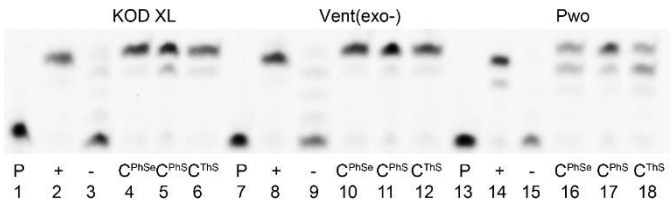


Figure 1: PEX reactions with temp^{oligo1C} using KOD XL, Vent(exo-) or Pwo polymerase. Lanes 1,7,13, P: primer; +: products of PEX with natural dNTPs; -: products of PEX with dTTP, dATP, dGTP; lanes 4-6, 10-12 and 16-18, C^{RX}: products of PEX with dTTP, dATP, dGTP and functionalized **dC^{RX}TP**

Then we tested the same nucleotides (**dC^{PhS}TP**, **dC^{ThS}TP** and **dC^{PhSe}TP**) in a more challenging PEX reaction using a 31-mer template temp^{Prb4baseII} (Figure 2). This PEX reactions leads to a 31-mer DNA containing four modified **dC^{RX}** nucleotides. KOD XL was found the best polymerase which gave clean full-length products in all three cases, whereas the other two enzymes gave less clean products containing minor amounts of truncated products. The PEX products were characterized by MALDI-TOF analysis (Table 2).

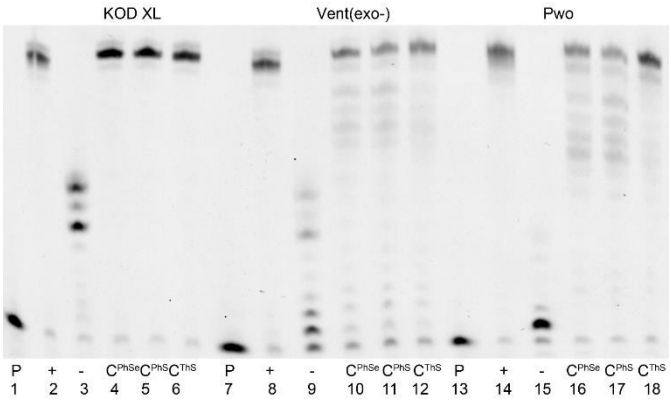


Figure 2: PEX reactions with temp^{Prb4baseII} using KOD XL, Vent(exo-) or Pwo polymerase. Lanes 1,7,13, P: primer; +: products of PEX with natural dNTPs; -: products of PEX with dTTP, dATP, dGTP; lanes 4-6, 10-12 and 16-18, C^{RX}: products of PEX with dTTP, dATP, dGTP and functionalized **dC^{RX}TP**

Table 2: MALDI-TOF data of modified oligodeoxyribonucleotides

ssDNA	M (calc.) (Da)	M (found) [M or M+H]$^+$ (Da)
ON^{4ThS}	10073.2	10075.1
ON^{4PhS}	10049.3	10050.8
ON^{4PhSe}	10241.1	10240.2

Finally, we tested the nucleotides (**dC^{PhS}TP**, **dC^{ThS}TP** and **dC^{PhSe}TP**) in PCR amplification using a 98-mer template (temp$^{FLV\text{-}A}$). Figure 3 shows that all three dNTPs were good substrates of KOD XL polymerase in PCR reaction and gave the corresponding full-length amplified products (double-stranded DNA with modification in both strands). The yield of PCR with **dC^{PhSe}TP** was further improved by addition of Mg^{2+} or higher concentration of the modified nucleotide (see Figures S1-S3 in ESI).

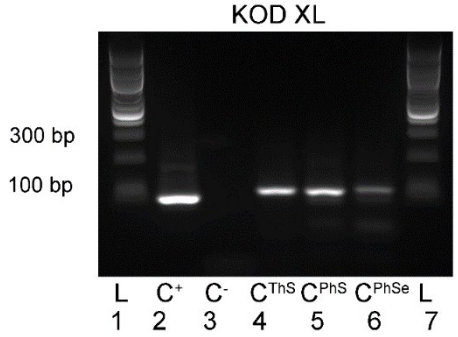


Figure 3: PCR experiments using KOD XL DNA polymerase. Lanes 1,7 , L: ladder ; lane 2, C^+: products of PEX with natural dNTPs; lane 3, C^-: products of PEX with dTTP, dATP, dGTP; lanes 4-6, C^{RX}: products of PCR with dTTP, dATP, dGTP and functionalized **$dC^{RX}TP$**.

Conclusions

In conclusion, we developed a new method for direct functionalization of 5-iodopyrimidine and 7-iodo-7-deazapurine nucleosides and nucleotides based on Cu-mediated arylsulfanylation or aryl/alkylselenylation. The reactions are even applicable for modification of fragile halogenated dNTPs. The S- or Se-modified dNTPs are good substrates for DNA polymerases and can be used as building blocks for enzymatic synthesis of modified ONs or DNA.

In this way, an aryl substituent can be attached to the nucleobase (in nucleoside, nucleotides or DNA) through a flexible sp^3-hybridized sulfide or selenide linkage, which in principle offers possibility for further transformations (e.g. oxidations). The arylsulfanyl or arylselenyl group can also serve as a radical precursor and the selenyl substituents can be used for X-ray crystallography of nucleic acids. The aryl group can be functionalized (e.g. NO_2 or MeO groups) so the approach can be potentially used for redox[28] or fluorescent[29] labelling of nucleic acids. Research along these lines is on-going.

Experimental

For full Experimental part, procedures and characterization of all compounds, see ESI.

Acknowledgements

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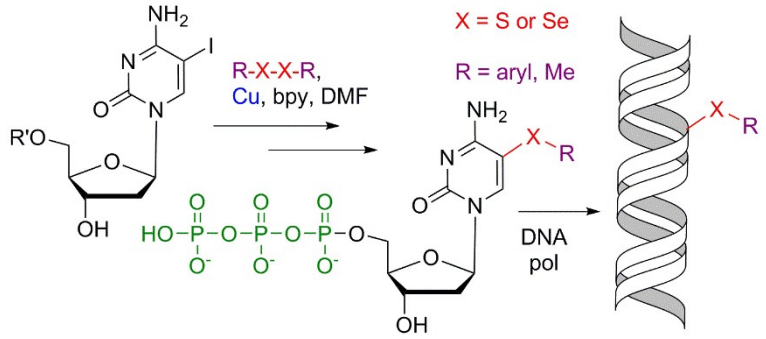

Nucleosides or nucleotides were modified by Cu-mediated arylsulfanylations or -selanylations and used in enzymatic synthesis of DNA bearing arylsulfanyl or arylselanyl groups.