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

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The syntheses of 5-arylsulfanyl- or 5-arylselenylpyrimidine and 7-arylsulfanyl- or 7-arylselenyl-7-deazapurine nucleosides and nucleotides were developed by the Cu-mediated sulfanylations or selenylations 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′-deoxyctydine triphosphate and the resulting 5-arylsulfanyl or 5-arylselenyl-dCTP served as substrates for the polymerase synthesis of modified DNA bearing arylsulfanyl or arylselenyl groups in the major groove.

Introduction

DNA molecules bearing modifications in the major groove have found 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 the enzymatic synthesis of base-modified DNA.2–5 Modified dNTPs are mostly synthesized by the triphosphorylation of the corresponding modified nucleosides6 but this approach can fail in the 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 the lability of dNTPs which are prone to hydrolysis. So far, the only reported reactions suitable for modification of dNTPs have been the aqueous cross-coupling reactions of halogenated dNTPs,6–8 thiol–maleimide addition,6 some amide-forming reactions of 5-α-alkynylalkynyl-dUTP,7 hydrazone-formation,8 Diels–Alder9 and the CuAAC click reaction.10 The Suzuki–Miyaura cross-coupling reaction with arylboronic acids11 and the Sonogashira reactions with terminal acetylenes12 are the most general and useful reactions used in the synthesis of base-modified dNTPs. In addition, several examples of the Heck coupling13 with acrylates, as well as the Stille reaction14 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 kinase15 and slightly destabilized DNA duplexes,16 whereas saturated 5-phenylsulfanylthymidine analogues were used17 as radical precursors for photocatalytic generation of thymine in DNA. Some 7-arylsulfanyl-7-deazaadenosine analogues displayed18 weak cytostatic effects, while the corresponding 7-S-substituted 7-deazaguanine derivatives have never been reported. 5-Selenylated pyrimidine nucleotides inhibit thymidylate synthase19 and have been utilized20 for modification of DNA or RNA for X-ray crystallography and 5-(phenylselenylmethyl)uracil was used21 as a T radical precursor for DNA crosslinking. Also the related 5-(phenyltelluranyl)uracil nucleoside has been prepared22 and, after incorporation into DNA, it was used for X-ray and STM imaging. However, no selenylated 7-deazapurines have been known so far. Therefore, we report here the synthesis of the arylsulfanyl and arylselenyl derivatives of pyrimidine and 7-deazapurine nucleosides and nucleotides and their potential for polymerase incorporation into 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,\textsuperscript{23} whereas 7-arylsulfanyl-7-deazapurines were prepared by Cu-mediated S–H sulfenylation.\textsuperscript{18} The 5-selenylated pyrimidines were prepared by Mn-mediated C–H selenylations\textsuperscript{20} or electrophilic aromatic selenylation.\textsuperscript{26} 

Inspired by the work of Taniguchi\textsuperscript{27} on the copper-catalyzed reactions of diaryldisulfides or diaryldiselenides with iodoarenes, we started our study by testing the reactions of unprotected halogenated nucleosides.\textsuperscript{5} The reaction conditions were first tested on 5-iodo-2′-deoxycytidine (dC\textsuperscript{i}) in reaction with diphenylsulfide. The Cu-catalyzed (10 mol\% of CuI) reactions in the presence or absence of Mg\textsuperscript{27} gave complex mixtures of products. Therefore, we used stoichiometric amounts of copper powder in the 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 the major product (in addition to small amounts of dehalogenated 2′-deoxycytidine) and isolated in a good yield of 58%. A similar conversion and yield were achieved when using pre-generated phenylsulfanyl-cuprate (Method B).

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

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

Next, we tested the reactions of nucleotides and started with stable nucleoside 5′-O-monophosphates (dNMPs). The model iodinated dC\textsuperscript{MP} was tested in reactions with diaryldisulfides or diselenides (Scheme 2, Method A). Most of these...
reactions gave very low conversions and only two products, dCThSMP and dCPhSeMP, 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 dCITP was reacted with PhSCu (Method B) to give the desired 5-(phenylsulfanyl)-dCTP (dCPhSTP) in low 7% yield. Better conversions were achieved when using reactions with diaryldisulfides or diselenides (Method A). The desired dCThSTP and dCPhSeTP were obtained in good yields of 24 and 31% (which are fully comparable to the typical yields of the cross-coupling reactions of dNTPs).

Polymerase incorporation of modified nucleotides

The three new 5-S- or Se-linked dNTPs (dCPhSTP, dCThSTP and dCPhSeTP) were then tested as substrates for DNA polymerases. At first we tested them in a primer extension (PEX) reaction with KOD XL, Vent(exo-) or Pwo polymerases, 15-mer primer248-sh and a 19-mer template tempoligo1C (for sequences, see Table 1). Fig. 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 dCRX nucleotide, Pwo gave a mixture of the full-lengths and a truncated product.

Then we tested the same nucleotides (dCPhSTP, dCThSTP and dCPhSeTP) in a more challenging PEX reaction using a 31-mer template tempPrb4baseII (Fig. 2). This PEX reaction leads to a 31-mer DNA containing four modified dCRX nucleotides. KOD XL was found to be 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).

Finally, we tested the nucleotides (dCPhSTP, dCThSTP and dCPhSeTP) in PCR amplification using a 98-mer template (tempFLV-A). Fig. 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 dCPhSeTP was further improved by the addition of Mg2+ or a

| Oligo  | Sequence                                      |
|--------|----------------------------------------------|
| Prime248-sh | 5’-CATGGGCGGCGATGGG-3’                        |
| Temp oligo1C     | 5’-CCCCGCCATGGCGCCCATG-3’                     |
| Temp Prb4baseII | 5’-CTAACGATGACGCTACAGCCATGGCGCCCATG-3’       |
| Prime1725TH      | 5’-CAAGGACAAATACCTGCTATCCTCT-3’              |
| Prime2720        | 5’-GACATGACGAGACGAGCCGACGGATTGGCTATAGGA-3’   |
| Temp FLV-A       | 5’-GGAAATACAGGTATTTTGTTCGCTTG-3’             |

Table 1 List of ON sequences used in this study
higher concentration of the modified nucleotide (see Fig. S1–S3 in the ESI†).

Conclusions

In conclusion, we developed a new method for the direct functionalization of 5-iodopyrimidine and 7-ido-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 the enzymatic synthesis of modified ODNs or DNA.

In this way, an aryl substituent can be attached to the nucleobase (in nucleoside, nucleotides or DNA) through a flexible sp3-hybridized sulfide or selenide linkage, which in principle offers a 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. NO2 or MeO groups) so the approach can be potentially used for redox28 or fluorescent29 labelling of nucleic acids. Research along these lines is ongoing.

Experimental

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

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