

SYNTHESIS OF DNA-OLIGONUCLEOTIDES DAMAGED BY ARYLAMINE-MODIFIED 2'-DEOXYGUANOSINE

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□ *C8-Arylamine-dG adducts bearing a labile N-formamidinium group at the exocyclic amino function were converted into their corresponding 5'-O-DMTr-3'-O-phosphoramidite-C8-arylamine-dG derivatives. These compounds were used for the automated synthesis of site-specifically modified oligonucleotides. These oligonucleotides were characterized by ESI-MS and enzymatic digestion and studied for their CD properties and T_m values.*

Keywords DNA-damage; aromatic amines; carcinogenicity; Pd-cross-coupling; C8-adducts

INTRODUCTION

Exposure to chemical carcinogens can occur from environmental or work conditions, diet, smoking, and endogenous processes. Poly- and monocyclic aromatic amines belong to the class of chemical carcinogens that are known to form covalent adducts with DNA. Chemically damaged DNA (by electrophiles) may be the reason for the induction of chemical carcinogenesis.^[1] If these covalently bonded modifications are not repaired, they might compromise the fidelity of DNA replication, leading to mutations and possibly cancer.^[2,3]

The arylamines require a metabolic activation leading to ultimate carcinogens, which are arylnitrenium ions. The predominant reaction of the arylnitrenium ion is the C8-position of 2'-deoxyguanosine (dG) leading to the corresponding adduct as the major product.^[4,5] Our interest is related to DNA-adducts of monocyclic aromatic amines that act as so-called borderline carcinogens like aniline or 4-methylaniline which are often used as pharmacophores and can be found in cigarette smoke.^[6]

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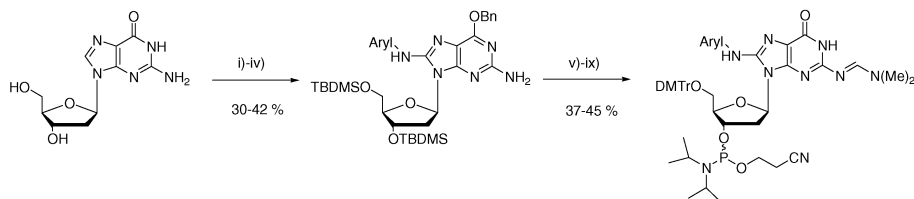
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To study the biochemical effects and the structure of “damaged” oligonucleotides a strategy for site-specific incorporation of dG-carcinogen adducts is needed. So far, modified oligonucleotide strands have been prepared by post-synthetic treatment of oligonucleotides with electrophilic amination reagents. However, the yields were very low (1–5%).^[7,8] Due to the low yields, this procedure, therefore, is only applicable for oligonucleotide sequences containing one dG. More suitable would be a strategy for a site-specific incorporation of modified dG using the phosphoramidite approach.

RESULTS

Recently, we published a highly efficient synthesis for the C8-arylamine-dG adducts using palladium cross-coupling, their conversion into the corresponding phosphoramidites and the synthesis of site-specifically modified oligonucleotides.^[9] For DNA-synthesis a protecting group for the exocyclic amino function is necessary. In the past we used the regular *iso*Butyryl (*i*Bu) group. After oligonucleotide synthesis deprotection of the amino function was done in concentrated ammonia solution within 16 hours at 55°C. Because of the sensitivity of the arylamine-dG adducts to alkaline conditions a much faster deprotection is preferable to increase the yields of the modified oligonucleotides. Therefore, the more labile formamidine protecting group was introduced. Adducts of seven different aromatic amines (aniline, 4-methoxyaniline, 4-methylaniline, 4-cyanoaniline, 3,5-dimethylaniline, 4-aminobiphenyl, and 2-aminofluorene) have been prepared in 60–91% yield. Subsequently, the adducts were converted into the *N*²-formamidine-protected phosphoramidites. The synthesis is summarized in Figure 1.

Using the phosphoramidites a series of modified oligonucleotides bearing C8-arylamine-dG adducts have been prepared using standard oligonucleotide synthesis. For the incorporation of the modified phosphoramidites three coupling cycles were used with a coupling time of 500 seconds. Cleavage of the oligonucleotides from the solid support (CPG) and deprotection



i) NBS, H₂O, rt, 15 minute ii) TBDMSCl, imidazole, pyridine, rt, 1 hour ; iii) PhCH₂OH, PPH₃, DIAD, 1, 4-dioxane, rt, 1 hour ; iv) arylamine, Pd₂(dba)₃ (10 mol%), *rac* BINAP (30 mol%), K₃PO₄, 1,2-dimethoxyethane, 80°C, 48–72 hour ; v) Pd/C, MeOH, rt, 1–24 hour ; vi) Et₃N·3HF, Et₃N, CH₂Cl₂/THF 1:1, rt, 3 hour ; vii) dimethylformamide-diethylacetate, pyridine, rt, 16 hour ; viii) DMTCl, pyridine, rt, 3.5 hour ; ix) *bis*-(di-*isopropylamino*)-2-cyanoethyl-phosphine, DCl, CH₃CN/CH₂Cl₂ 1:1, rt, 1 hour.

FIGURE 1 Synthesis of the arylamine-adduct phosphoramidites.

was done with aqueous ammonia within 4 hours at 40°C. β -Mercaptoethanol was added to prevent a possible oxidative cleavage of the adducts under alkaline conditions. Purification of the modified oligonucleotides has been performed by HPLC following a DMTr-off protocol and a RP-C18-column using triethylammonium acetate buffer (pH 6.9)/acetonitrile gradients. Due to the change to the more labile protecting group the yields of the modified oligonucleotides were 4- to 5-fold higher as compared to the *i*Bu-route. The oligonucleotides have been studied for purity by HPLC analysis and were characterized by ESI-mass spectroscopy and enzymatic digestion.

T_m values of a self-complementary strand (5'-TATATCXATATA-3' and a mixed 30mer-oligonucleotide (5'-AAA TXA ACC TAT CCT CCT TCA GGA CCA ACG-3') against the complementary strand were measured. The T_m value of the unmodified self-complementary strand was found to be 33°C. A considerable decrease in the T_m was found when dG was replaced by an arylamine-dG adduct: In the case of 4-methoxyaniline the T_m drops to 19.1°C, with a 4-aminobiphenyl-adduct to 20.0°C. In both cases a second T_m was found (66–69°C) probably a result of a hairpin structure.^[9] The unmodified 30mer-oligonucleotide showed a T_m value of 66°C. For the modified oligonucleotides the values differ from 63.5–64.1°C. Again, one modification was responsible for a loss of thermal stability of the duplex. Interestingly, the effects of the strong carcinogens 4-aminobiphenyl and 2-aminofluorene were found to be comparable to the borderline carcinogens.

Further structural effects of the incorporation of the adducts into oligonucleotides were studied using CD-spectroscopy. However, no difference in the CD-spectra was observed for the oligonucleotides bearing the adduct compared to the unmodified cases. All curves point to B-type DNA duplexes.

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