First Synthesis of C8-Arylamine Adducts of 2′-Deoxyadenosine and Incorporation of the Phosphoramidite into an Oligonucleotide

Maike I. Jacobsen, Chris Meier*

Department of Chemistry, Organic Chemistry, Faculty of Mathematics, Informatics and Natural Sciences, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany
Fax +49(40)428382495; E-mail: chris.meier@chemie.uni-hamburg.de
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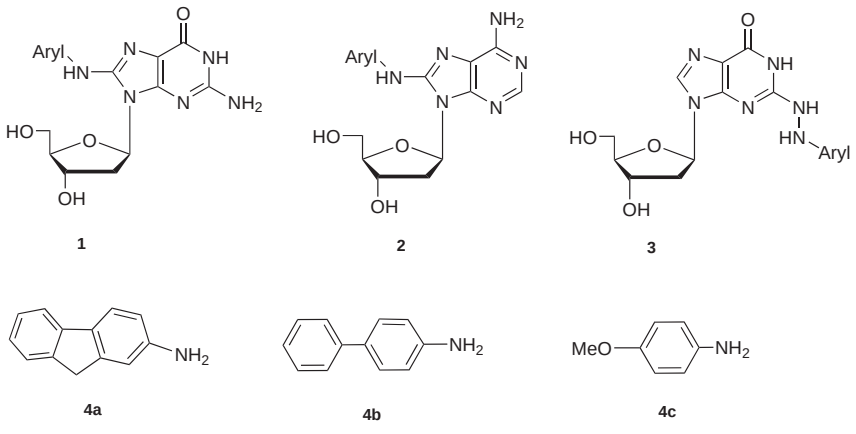
Abstract: The synthesis of C8-adducts of 2′-deoxyadenosine **7a**,**b** with carcinogenic arylamines *p*-anisidine **4c** and 4-aminobiphenyl **4b**, their conversion into the phosphoramidites and successful incorporation into oligonucleotides are described.

Key words: Pd-cross coupling, amination, nucleoside adducts, phosphoramidites, oligonucleotides

Covalent modification of DNA by electrophiles alters the structure as well as the fidelity of DNA replication. If these DNA damages are not repaired, they may lead to mutations and finally may play an important role in the induction of chemical carcinogenesis.[1] Poly- and monocyclic aromatic amines belong to the class of chemical carcinogens that form covalently bonded adducts with DNA after metabolic activation.[2,3] The ultimate carcinogen is an arylnitrenium ion generated by cytochrome P450 oxidation of the arylamine to the hydroxylamine, followed by esterification and solvolysis.[4] The predominant site of reaction is the C8-position of 2′-deoxyguanosine (dG) leading to so-called C8-adducts like **1** (about 80% of the adducts found in in vivo studies).[5] Moreover, the corresponding C8-arylamine adducts of 2′-deoxyadenosine **2** have been found in these studies to a minor extent (>10%). In addition, N^2-adducts of dG **3** have been identified. In the past, several studies discussed the biochemical consequences of C8-arylamine-dG-adducts 'damaged' by carcinogens like 2-aminofluorene **4a**, 4-aminobiphenyl **4b** or *p*-anisidine **4c** (Figure 1).[6]

C8-Arylamine adducts are known to be the most persistent DNA-adducts.[3] In contrast to dG-adducts, no reports have been published so far on how dA-adducts behave in biochemical processes and how dA-adducts contribute to the possible induction of chemical carcinogenesis. To study the mutagenic effects, structure and DNA-repair of these lesions, an efficient synthesis of adducted dA-nucleoside phosphoramidites would be the prerequisite for the access to site-specifically modified oligonucleotides. In contrast to C8-dG adducts,[7] no methodology has been reported either for the chemical synthesis of the arylamine dA-adduct phosphoramidites or the post-synthetic oligonucleotide modification. Post synthetic procedures as reported for C8-arylamine-dG modified oligonucleotides might be possible in principle but only oligonucleotides bearing one modified C8-dA are accessible by this strategy in extremely low chemical yields.[8]

In 1999, Lakshman[9] and Johnson[10] introduced C−N bond formation by palladium-catalyzed reactions in the synthesis of N^2-aryl-2′-deoxyguanosine and N^6-aryl-2′-deoxyadenosine adducts. Schoffers[11] used comparable procedures



1 2 3 4a 4b 4c

Figure 1 C8-arylamine-dG adduct **1**, C8-arylamine-dA adduct **2** and N^2-arylamine adducts of dG **3** found in in vivo studies and three carcinogens **4a**,**b**,**c**

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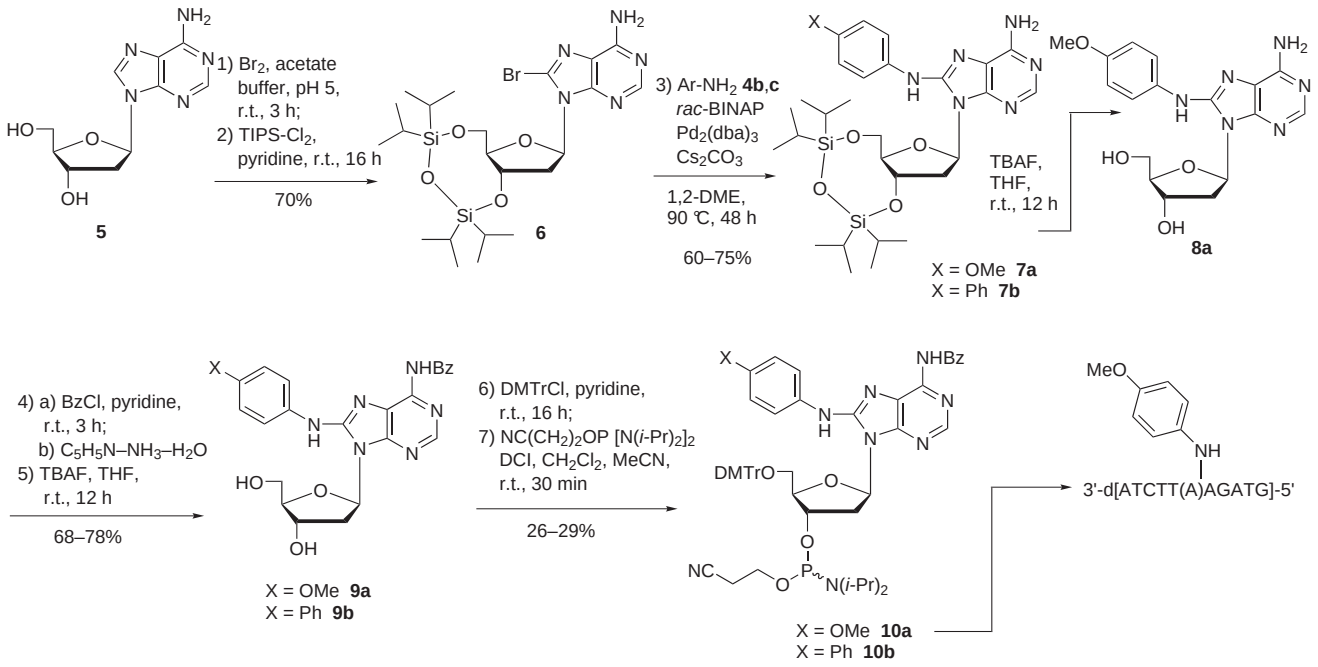
for the preparation of C8-arylamine-adenosine adducts. However, neither phosphoramidites nor oligonucleotides were prepared. We reported on the first synthesis of phosphoramidites of C8-arylamine-dG adducts.[12] Here we report on a highly convincing synthesis of C8-arylamine-2′-deoxyadenosine adducts, the first efficient conversion into the corresponding 3′-phosphoramidites and their site-selective incorporation into oligonucleotides.

Starting from 2′-deoxyadenosine (dA; **5**), bromination in acetate buffer (pH 5), led to the formation of 8-Br-dA.[13] In contrast to dG, the reaction can be accomplished in large scales in good yields. In the case of dG, bromination was achieved with NBS. However, this method failed in the case of dA. 8-Br-dA was then protected by 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2) to give 3′,5′-protected 8-Br-dA **6** in excellent yield (98%, Scheme 1).[14] In the key step, the formation of the C8-arylamine dA adducts **7a**,**b**, the C−N bond was formed with the aromatic amine in the presence of $Pd_2(dba)_3$ (10 mol%), *rac*-BINAP (30 mol%) and Cs_2CO_3 as base.[15,16] As arylamines the borderline carcinogen *p*-anisidine (**4c**) and the strong carcinogen 4-aminobiphenyl (**4b**) were used. C8-Arylamine adducts **7** were isolated in 70% (**7a**) and 60% yield (**7b**). However, lower amounts of the catalyst also led to lower yields of the adducts (Scheme 1). The fully deblocked adduct **8a** was accessible from **7a** by desilylation using tetra(*n*-butyl)ammonium fluoride (TBAF) in tetrahydrofuran.

This proves that 2′-deoxyadenosine arylamine adducts can now be prepared as reference compounds for analytical purposes.

Because the exocyclic amino group of adenine should be protected during oligonucleotide synthesis, the regularly used *N*-benzoyl group was introduced (BzCl in pyridine) in 73% (X = OMe) and 87% yields (X = Ph), respectively. Treating this material with TBAF in tetrahydrofuran led to the deprotection of the 2′-deoxyribose hydroxyl groups in almost quantitative yields (compounds **9a**,**b**). Then the 5′-dimethoxytrityl (DMTr) group was introduced. The yields in these reactions were surprisingly moderate (42% and 46%). As side products, 3′,5′-di-DMTr-dA adducts were isolated in about 30% yield. Attempts to optimize the regioselectivity failed for unknown reasons. Finally, 5′-DMTr-protected adducts were converted into the phosphoramidites using 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite and 4,5-dicyanoimidazole (DCI).[17,18] The phosphoramidites **10a**,**b** were isolated after chromatography on silica gel in 69% and 62% yields, respectively. To prove the suitability of these compounds to work as building blocks in the automated DNA-synthesis, the phosphoramidite **10a** was incorporated into a mixed DNA sequence (Scheme 1). For the incorporation of amidite **10a** the standard coupling protocol of the DNA synthesizer was used. In contrast to the corresponding dG adducts,[19] the coupling efficiency of *p*-anisidine-dA phosphoramidite (**10a**) was almost quantitative [>97%; the same stands for the following phosphoramidite (+1 position)]. After deprotection with ammonia, the oligonucleotide was purified by RP18-silca gel column chromatography using triethylammonium buffer (pH 6.9)–acetonitrile gradients. The final product was characterized by ESI–MS spectrometry and the sequence was confirmed by an enzymatic degradation assay and analysis of the ratio of the four nucleosides (Figure 2).[20]



Scheme 1 Synthesis of phosphoramidites **10a**,**b** and the incorporation of **10a** into an oligonucleotide

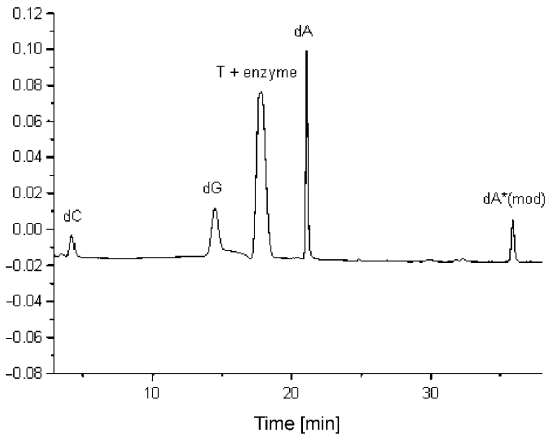


Figure 2 Enzyme degradation assay of the oligonucleotide ATCTT(A*)AGATG

In conclusion, we have developed a complete synthesis of C8-arylamine-2′-deoxyadenosine phosphoramidites **10a** and **10b** that are suitable for standard automated DNA-synthesis. Due to this method site-selective incorporation of such adducts could be performed in any sequence for the first time. Now, the effects of chemically modified dA-bases within oligonucleotides can be studied and compared to the effects of the corresponding dG adducts. This may provide new significant insights into the importance of this type of DNA damage for the possible induction of the chemical carcinogenesis. Moreover, fully unprotected C8-arylamine-dA adducts were prepared which can be used in analytical studies as reference compounds. Work along this field is currently underway in our laboratories.

Acknowledgment

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References and Notes

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(15) N^6-Benzoyl-8-*N*-(4-methoxyphenylamino)-3′,5′-*O*-[1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl]-2′-deoxyadenosine (**7a**) and N^6-benzoyl-8-*N*-(4-aminobiphenyl)-3′,5′-*O*-[1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl]-2′-deoxy-adenosine (**7b**): N^6-Bz-8-bromo-3′,5′-*O*-(TIPDS)-2′-dA **6** (500 mg, 0.87 mmol), Cs_2CO_3 (426 mg, 1.31 mmol), tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$; 80.0 mg, 87.0 μmol], racemic-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP; 163 mg, 0.26 mmol) and *p*-anisidine (**4c**; 215 mg, 1.74 mmol) or 4-aminobiphenyl (**4b**; 295 mg, 1.74 mmol) were solubilized in anhyd 1,2-dimethoxyethane (30 mL) in an inert gas atmosphere and stirred at 90 °C until the reaction was complete (TLC analysis). After cooling to r.t., sat. $NaHCO_3$ solution (1 mL) was added. After addition of brine (10 mL) the layers were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL) and once with a mixture of brine (10 mL) and H_2O (2 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. Purification by chromatography on silica gel, eluting with 20% EtOAc in hexane afforded **7a** (374 mg, 0.61 mmol; 70%) and **7b** (345 mg, 0.52 mmol; 60%) as light-yellow foams.
(16) **7a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (s, 1 H), 7.48 (m, 2 H), 7.41 (br s, 1 H), 6.88 (m, 2 H), 6.31 (dd, *J* = 3.8, 7.8 Hz, 1 H), 5.34 (br s, 2 H), 4.90 (dd, *J* = 7.9, 15.2 Hz, 1 H), 4.16 (dd, *J* = 3.4, 9.2 Hz, 1 H), 3.98 (dd, *J* = 5.0, 12.5 Hz, 1 H), 3.90 (ddd, *J* = 3.4, 4.9, 7.0 Hz, 1 H), 3.79 (s, 3 H), 3.06 (ddd, *J* = 3.6, 8.1, 13.4 Hz, 1 H), 2.59 (ddd, *J* = 7.9, 13.4 Hz, 1 H), 0.89–1.13 (m, 28 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 155.8, 152.5, 149.4, 132.5, 121.5, 117.4, 114.5, 85.3, 83.8, 70.5, 61.9, 55.7, 38.8, 17.0–17.6, 12.6–13.5. HRMS (FAB): *m*/*z* [M + H]$^+$ calcd for $C_{29}H_{46}N_6O_5Si_2$: 615.3147; found: 615.3155. **7b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1 H), 7.78 (br s, 1 H), 7.69 (m, 2 H), 7.58 (m, 4 H), 7.44 (m, 2 H), 7.33 (m, 1 H), 6.35 (dd, *J* = 3.8, 7.5 Hz, 1 H), 5.59 (s, 2 H), 4.88 (dd, *J* = 7.9, 15.2 Hz, 1 H), 4.16 (dd, *J* = 3.6, 12.6 Hz, 1 H), 4.04 (dd, *J* = 4.8, 12.5 Hz, 1 H), 3.95 (ddd, *J* = 3.0, 4.4, 7.3 Hz, 1 H), 3.07 (ddd, *J* = 3.6, 8.0, 13.4 Hz), 2.59 (ddd, *J* = 7.8, 13.4 Hz, 1 H), 0.91–1.12 (m, 28 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.7, 152.4, 149.7, 148.3, 140.9, 132.5, 121.5, 117.4, 114.5, 85.3, 83.8, 70.5, 61.9, 55.7, 38.8, 17.0–17.6, 12.6–13.5. HRMS (FAB): *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{48}N_6O_4Si_2$: 661.3354; found: 661.3377.
(17) N^6-Benzoyl-8-*N*-(4-methoxyphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyadenosine-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (**10a**) and N^6-benzoyl-8-*N*-(4-aminobiphenyl)-5′-*O*-dimethoxytrityl-2′-deoxy-adenosine-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphor-amidite (**10b**): N^6-Bz-8-*N*-(4-methoxyphenylamino)-5′-*O*-DMTr-2′-dA (150 mg, 0.19 mmol) and N^6-Bz-8-*N*-(4-aminobiphenyl)-5′-*O*-DMTr-2′-dA (150 mg, 0.18 mmol), respectively, were dissolved in anhydrous CH_2Cl_2 (3 mL) and anhydrous MeCN (3 mL) in an inert gas atmosphere and

treated subsequently with a solution of 4,5-dicyanoimidazole in MeCN (0.25 M, 1.5 mL, 0.38 mmol) and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (86 mg, 0.29 mmol). After stirring for 30 min at r.t., the reaction was stopped by adding of MeOH (0.5 mL). The solution was diluted with CH_2Cl_2 (50 mL) and washed with 5% aq $NaHCO_3$ followed by brine. The organic layer was dried and concentrated to dryness. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2, 2% Et_3N and 0–3% MeOH. The products were redissolved in CH_2Cl_2 and further washed with H_2O to give **10a** (130 mg, 0.13 mmol; 69%) and **10b** (116 mg, 0.11 mmol; 62%) as light-yellow solids.

(18) **9a**: 2 diastereomers + 2 rotamers. ^{1}H NMR (400 MHz, C_6D_6): δ = 9.55 (m, 2 H), 9.18 (s, 1 H), 9.16 (s, 1 H), 8.90 (m, 2 H), 8.67 (s, 1 H), 8.66 (s, 1 H), 8.29 (s, 1 H), 8.27 (s, 1 H), 7.91 (m, 2 H), 7.82 (m, 5 H), 7.75 (m, 3 H), 7.64–7.70 (m, 4 H), 7.54–7.62 (m, 6 H), 7.43–7.53 (m, 8 H), 7.32 (m, 12 H), 7.32 (m, 5 H), 7.08–7.16 (m, 8 H), 6.87–7.06 (m, 22 H), 6.64–6.80 (m, 24 H), 5.99–6.13 (m, 2 H), 5.91 (m, 2 H), 5.25 (m, 2 H), 5.07 (m, 4 H), 4.50–4.64 (m, 2 H), 4.41 (m, 2 H), 3.81–3.93 (m, 4 H), 3.61–3.75 (m, 4 H), 3.44–3.62 (m, 16 H), 3.22–3.43 (m, 36 H), 2.28–2.53 (m, 4 H), 1.68–2.00 (m, 12 H), 1.11−1.23 (m, 48 H). ^{13}C NMR (101 MHz, C_6D_6): δ = 165.9, 165.1, 159.2, 159.2, 159.9, 154.1, 152.6, 151.1, 150.1, 149.4, 146. 1, 145.9, 145.3, 144.2, 142.0, 136.9, 136.8, 136.0, 135.8, 135.0, 132.7, 132.6, 130.9, 130.8, 130.5, 128.6, 127.1, 127.0, 124.0, 123.4, 121.6, 121.5, 117.8, 117.6, 115.4, 114.6, 113.6, 113.5, 111.7, 86.9, 86.8, 86.0, 85.4, 82.5, 82.3, 64.9, 63.4, 63.2, 59.2, 59.1, 59.0, 58.8, 58.6, 55.0, 54.9, 54.9, 43.6, 43.6, 37.5, 37.2, 30.5, 30.2, 24.8, 24.8, 24.7, 20.2, 20.1, 20.1. ^{31}P NMR (202 MHz, C_6D_6): δ = 149.20, 148.95, 148.84, 148.20 (0.30:0.33:0.20:0.17). HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{24}H_{24}N_6O_5$: 1001.4093; found: 1001.4120. **9b**: 2 diastereomers + 2 rotamers. ^{1}H NMR: (400 MHz, C_6D_6): δ = 9.87 (m, 2 H), 8.94 (s, 1 H), 8.92 (s, 1 H), 8.85 (s, 1 H), 8.84 (s, 1 H), 8.60 (m, 2 H), 8.44 (s, 1 H), 8.42 (s, 1 H), 8.24 (s, 1 H), 8.21 (s, 1 H), 8.03 (m, 4 H), 7.92 (m, 4 H), 7.86 (m, 4 H), 7.69 (m, 12 H), 7.49–7.63 (m, 20 H), 7.40 (m, 12 H), 7.32 (m, 12 H), 7.22 (m, 10 H), 7.04–7.18 (m, 12 H), 6.97 (m, 2 H), 6.85 (m, 8 H), 6.77 (m, 10 H), 5.87–6.03 (m, 2 H), 5.17–5.34 (m, 2 H), 4.99–5.15 (m, 2 H), 4.54–4.66 (m, 2 H), 4.41 (m, 2 H), 3.88 (m, 4 H), 3.74 (m, 2 H), 3.57–3.66 (m, 10 H), 3.31 (m, 12 H), 3.30 (m, 12 H), 3.00–3.16 (m, 8 H), 2.28–2.62 (m, 4 H), 1.70–1.97 (m, 10 H), 1.01–1.10 (m, 48 H). ^{13}C NMR (101 MHz, C_6D_6): δ = 159.2, 149.7, 141.1, 136.0, 135.9, 134.9, 132.6, 131.9, 130.8, 130.6, 129.0, 129.0, 128.7, 128.6, 127.9, 127.1, 127.0, 119.7, 117.6, 117.0, 116.3, 113.6, 113.5, 87.0, 86.8, 85.8, 59.2, 59.0, 58.0, 58.0 54.8, 45.4, 45.3, 43.7, 43.6, 24.8, 24.8, 24.7, 24.6, 23.7, 22.9, 22.8, 22.8, 22.8, 20.6, 20.1, 20.1, 19.5, 19.4. ^{31}P NMR (202 MHz, C_6D_6): δ = 149.37, 149.11, 148.69, 148.38 (0.31:0.37:0.17:0.15). MS (FAB): *m*/*z* $[M + H]^+$ calcd for $C_{29}H_{26}N_6O_4$: 1025.4480; found: 1025.4.

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(20) Enzyme degradation: Oligonucleotide (3 μg) was dissolved in a NaOAc buffer (100 μL, pH 5.3, 30 mM). $ZnSO_4$ solution (5 μL, 20 mM) and the nuclease P1 (3 units, from penicillium citrinum; Roche Nr. 91095923/30) were added and incubated for 4 h at 37 °C. Then a tris(hydroxymethyl)aminomethane–HCl buffer (20 μL; pH 8.7; 50 mM) and 4 units of the alkaline phosphatase (Roche Nr. 49048126/15) were added and incubated overnight at 37 °C.