Highly Efficient Synthesis of a Phosphoramidite Building Block of C8-Deoxyguanosine Adducts of Aromatic Amines

Chris Meier,* Sonja Gräsl

Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany
Fax +49(40)428384324; E-mail: chris.meier@chemie.uni-hamburg.de

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Abstract: The synthesis of 5′-*O*-DMTr-3′-*O*-phosphoramidite-C8-arylamine-dG adducts **11** and **12** is described. The compounds are potential building blocks for the automated synthesis of site-specifically modified oligonucleotides. The C8-adducts were synthesized by a palladium-catalyzed cross-coupling reaction.

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

Covalent alteration of DNA by electrophiles may be the reason for the induction of chemical carcinogenesis.[1] If these covalently bonded modifications are not repaired, they compromise the fidelity of DNA replication, leading to mutations and possibly cancer. 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 corresponding hydroxylamine, followed by esterification and solvolysis.[4] The predominant site of reaction is the C8-position of 2′-deoxyguanosine (dG) **1**, although N^2-adducts have also been isolated as minor products (Figure 1). To properly study the mutagenic effects, structure and DNA-repair of these lesions, an efficient synthesis of the adducted nucleoside phosphoramidites would be the prerequisite for the access to site-specifically C8-dG modified oligonucleotides. So far, only post-synthetic oligonucleotide modification has been reported in low yields.[5] However, only singly modified oligonucleotides were accessible by this strategy, e.g. nucleotide strands containing one C8-dG adduct of 2-aminofluorene (AF).[6]

In contrast, our interest is related to DNA-adducts of so-called borderline carcinogens like toluidine and anisidine.[7] We report here on a highly efficient synthesis of the corresponding C8-adducts using a palladium-catalyzed cross-coupling reaction and the first synthesis of the corresponding 3′-phosphoramidites.

The synthesis of C8-arylamine-dG adducts by electrophilic amination has been reported in low yields[8,9] and thus this approach was unsuitable for the phosphoramidite synthesis. Moreover, direct nucleophilic substitution of protected 8-Br-dG with arylamines was unsuccessful due to depurination.[10]

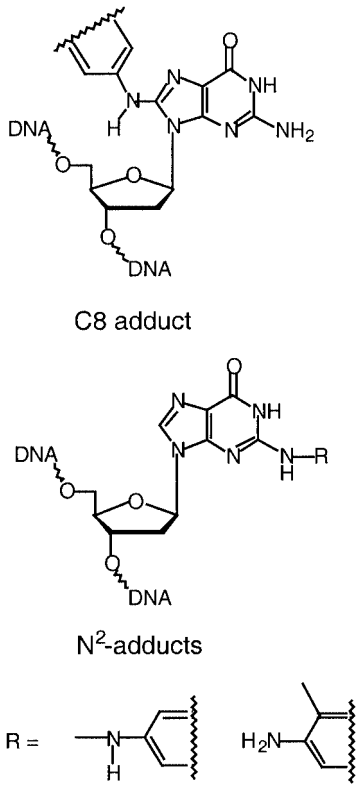

Figure 1 C8- and N^2-adducts of chemical carcinogens and 2′-dG **1**

Recently, C-N bond formation with palladium catalysts (Buchwald–Hartwig reaction)[11] was introduced by Lakshman[12] and Johnson[13] for the synthesis of N^6- and N^2-adducts of adenosine. While this work was in progress, Rizzo published the coupling of a heterocyclic food mutagen (IQ) and a few aromatic amines to the C8-position of 2′-deoxyguanosine[14] and Schoffers prepared C8-arylamine adduct of tris-*O*-TBDMS-adenosine.[15] However, their approach needs the use of the strong base LiHMDS and NaO-*t*-Bu and/or protecting group chemistry that is not compatible with conditions of automated oligonucleotide synthesis. Moreover, no attempts have been made to prepare the corresponding phosphoramidites and in order to get suitable DNA-building blocks, both approaches would need long reaction sequences.

Our key reaction step was also a Pd-catalyzed cross-coupling reaction of a 8-bromo-dG derivative with the aroma-

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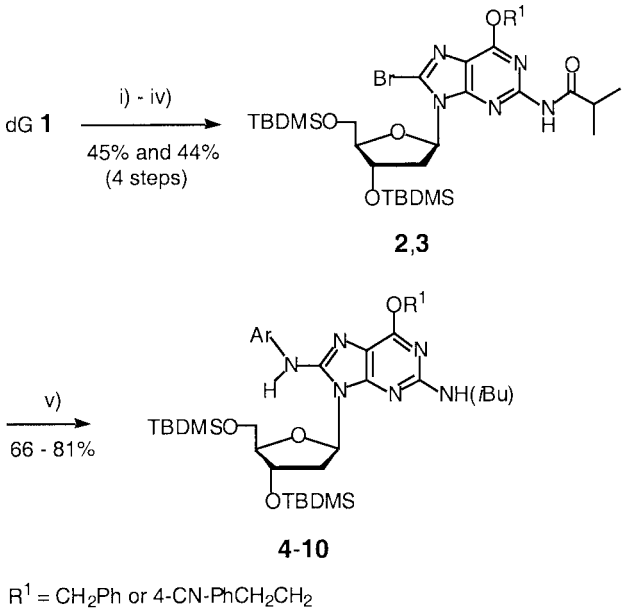
tic amine. From initial studies it became apparent that the O^6-position and the exocyclic 2-amino group of the guanine moiety as well as the hydroxy groups of the 2′-deoxyribose should be blocked during the cross-coupling reaction. Although Buchwald and others reported the N-arylation of amides,[16] we decided to introduce the usually used N^2-isobutyryl (*i*-Bu) group. Thus, N^2-*i*-butyryl-O^6-benzyl-8-bromo-3′,5′-*O*-(*t*-butyldimethylsilyl)-2′-deoxyguanosine **2** was used as starting material. Moreover, we introduced the 4-cyanophenylethyl protecting (CPE) group to the O^6-position[17] instead of the benzyl (Bn) residue. This group can be removed after the oligonucleotide synthesis by DBU treatment and thus would avoid a further deprotection step prior to the phosphoramidite synthesis. Hence, also N^2-*i*-butyryl-O^6-(4-cyanophenyl)ethyl-8-bromo-3′,5′-*O*-TBDMS-2′-deoxyguanosine **3** was prepared as a second starting material. Protected dG-derivatives **2** and **3** were synthesized starting from 2′-dG **1** by NBS-bromination to yield 8-bromo-dG in 78% yield.[18] *O*-Silylation with TBDMS-chloride[19] lead to 8-Br-3′,5′-*O*-TBDMS-dG (83% yield). O^6-Protection with benzylalcohol (Bn) or 4-cyanophenylethanol (CPE)[20] and DIAD/PPh_3 was achieved in 72% and 75% yield, respectively. Finally, the exocyclic amino group was blocked by treatment with *i*-butyrylchloride to give key intermediates **2** and **3** in 96% and 89% yield (Scheme 1). Fully protected 8-bromo-dG **2** was then subjected to the cross-coupling reaction. In contrast to the work reported before,[14] 10 mol% $Pd(dba)_3$ and 30 mol% *rac*-BINAP instead of 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl was used as catalyst in 1,2-dimethoxyethane (1,2-DME).[21] Further, 1.5 equivalents of K_3PO_4 were added as base.[22] Two equivalents of aniline, toluidine, anisidine, 4-cyanoaniline, 2-aminofluorene and 4-aminobiphenyl were used. After heating the reaction mixtures to 80 °C for 50 hours, silica gel chromatography gave the C8-arylamine adducts **4**–**9** in yields between 73% and 80% (Scheme 1; Table).[23] Only the acceptor-substituted 4-cyanoaniline gave slightly lower yields (**7**, 66%). The yields reported here are considerable higher than reported so far.[14,15] These high yields were obtained using the monoprotected N^2-*i*-Bu-dG derivative. In one experiment we also studied the aminoarylation with N^2-bis(*i*-Bu)-8-bromo-dG. However, yields of the C8-toluidine adduct were considerably lower as described above and a mixture of the bis(*i*-Bu) and the mono(*i*-Bu) adduct was isolated. Obviously, the conversion of the amino group into a guanidin-type moiety is sufficient to prevent a side reaction of the *i*-Bu-amide.[16]

The same reaction protocol has been applied to the second 8-bromo-dG derivative **3**. Using toluidine, 81% of the dG-adduct **10** was isolated after 43 hours without cleavage of the O^6-CPE-protecting group. It should be added that no depurination side products have been observed both starting from **2** or **3**.

As a representative reaction, the O^6-Bn protected toluidine adduct **5** was first desilylated by tetrabutylammonium fluoride (TBAF) in 97% yield and subsequent Pd



Scheme 1 Synthesis of the fully protected C8-arylamine adducts **4**–**10**. i) NBS, H_2O, r.t., 15 min; ii) TBDMSCl, imidazole, pyridine, r.t., 1 h; iii) $PhCH_2OH$ (2 equiv) or 4-CN-$PhCH_2CH_2OH$ (3 equiv), PPh_3, DIAD, 1,4-dioxane, r.t., 1 h; iv) *i*-butyrylchloride, pyridine, r.t, 1 h; v) $Pd_2(dba)_3$ (10 mol%), *rac*-BINAP (30 mol%), K_3PO_4, arylamine (2 equiv), 1,2-DME, 80 °C, 50 h.

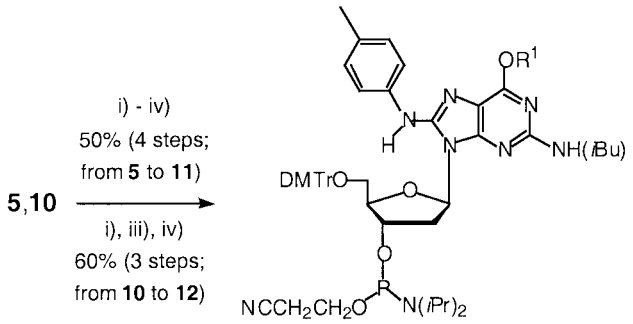
(black) debenzylation (83% yield) gave the O^6,3′,5′-O-unblocked intermediate. This material was 5′-*O*-dimethoxytritylated in 72% yield and further converted into the 5′-*O*-DMTr-3′-*O*-phosphoramidite **11** (85% yield, Scheme 2).[24] Neither during the introduction of the DMTr group nor the phosphitylation reaction a side reaction at the N^8-atom took place. The same strategy has been followed for the O^6-CPE adduct **10**: 3′,5′-*O*-desilylation, 5′-*O*-dimethoxytritylation and phosphitylation gave the target phosphoramidite **12**.[25]

The overall yield of the amidites **11** and **12** were 16% and 21% for the 9- and 8-step synthetic procedure, respectively.

In conclusion, we have accomplished a high yielding synthetic procedure for the access to C8-arylamine-dG adducts using palladium-catalyzed C-N bond formation. The obtained yields are superior to the yields reported so

Table Yields of C8-Arylamine dG Adducts **4**–**10**

Adduct **4**–**10**	R^1	Ar	Chem. yield [%]
4	Bn	Ph	77
5	Bn	4-Me-Ph	75
6	Bn	4-MeO-Ph	76
7	Bn	4-CN-Ph	66
8	Bn	2-fluorenyl	73
9	Bn	4-biphenyl	80
10	CPE	4-Me-Ph	81



Scheme 2 Synthesis of the 5′-*O*-DMTr-3′-*O*-phosphoramidites **11**, **12**. i) *n*-Bu_4NF (3 equiv), THF, r.t., 4 h; ii) Pd black, formamide, 1,4-cyclohexadiene, EtOAc/EtOH/MeOH, r.t., 6 h; iii) DMTrCl (1.5 equiv), DMAP, pyridine, r.t., 12 h; iv) β-cyanoethyl-di(*i*-propyl)aminochlorophosphine (1.5 equiv); DIPEA, CH_2Cl_2, 0 °C, 30 min.

far. These adducts were converted into two potential monomeric phosphoramidite building blocks in high yields that should meet the conditions for automated DNA-oligonucleotide synthesis. This opens the possibility to incorporate dG-adducts site-specifically into oligonucleotides and/or DNA. Work along this route is currently underway in our laboratories.

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(23) Amination of N^2-*i*-Butyryl-O^6-benzyl-8-bromo-3′,5′-bis(*t*-butyldimethylsilyl)-2′-deoxyguanosine: 450.0 mg (0.61 mmol) 8-Bromo-2′-deoxyguanosine, 156.0 mg (0.73 mmol) K_3PO_4, 56.1 mg (61.0 μmol) tris(dibenzylideneacetone)dipalladium(0) (Pd_2dba_3), 114.4 mg (0.18 mmol) racemic-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) and 131.2 mg (1.22 mmol) of *p*-toluidine was solubilized in 15 mL dry 1,2-DME in an inert atmosphere and stirred at 80 °C until the reaction was complete (TLC analysis). After cooling to r.t., 1 mL of sat. sodium bicarbonate solution was added. After addition of 10 mL of brine the layers were separated and the aq layer was extracted three times with 10 mL of ethyl acetate. The combined organic layers were washed twice with 10 mL of brine and once with a mixture of 10 mL brine and 2 mL water. The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. Purification by chromatography on silica gel, eluting with 20% ethyl acetate in hexane afforded 350 mg (75%) of the desired product as a light-yellow foam.
(24) N^2-*i*-Butyryl-8-*N*-(4-methylphenylamino)-*O*3′-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)phosphinyl]-*O*5′-dimethoxytrityl-2′-deoxyguanosine: 200.0 mg (0.27 mmol) of N^2-*i*-butyryl-8-*N*-(4-methylphenylamino)-*O*5′-dimethoxytrityl-2′-deoxyguanosine were dissolved in 7 mL dry CH_2Cl_2 and treated subsequently with 234 μL (1.34 mmol) of DIPEA and 113 μL (0.51 mmol) of (2-cyanoethoxy)-(*N*,*N*-diisopropylamino)-chlorophosphine. After stirring for 1 h at r.t., the reaction was stopped by adding 0.5 mL of methanol. The solution was diluted with 50 mL of CH_2Cl_2 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/acetonitrile and CH_2Cl_2/methanol to give 215.7 mg (85%) as a light-yellow solid.
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