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Palladium-Catalyzed Direct *N*-Arylation of Nucleosides, Nucleotides, and Oligonucleotides for Efficient Preparation of dG−N^2 Adducts with Carcinogenic Amino-/Nitroarenes

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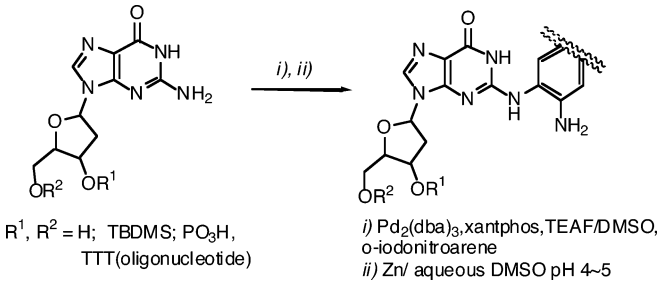

A method for direct palladium-catalyzed *N*-arylation reaction of nucleobases was developed for the convenient synthesis of DNA adducts with carcinogenic compounds. Using xantphos as the phosphine ligand and tetraethylammonium fluoride as the base in DMSO, several *o*-iodonitroarenes could be efficiently coupled with 2′-deoxyguanosine, 2′-deoxyadenosine, and 2′-deoxycytidine. The presence of a 3′-phosphate group in the deoxyribose moiety was found to be compatible with this *N*-arylation reaction; further, oligonucleotides could serve as substrates. The facile nitroreduction of the coupling compounds (**12**) yielded 2′-deoxyguanosin-N^2-ylarylamine adducts, which are known to be biologically important. Compound **12** was easily converted to phosphoramidite derivatives, allowing the preparation of site-specific modified oligonucleotides with arylamine after the nitroreduction.

Introduction

Aromatic amino and nitro compounds, which are common mutagens/carcinogens widely distributed in the environment, covalently bind to DNA after metabolic activation; this is recognized as the crucial first step in mutagenesis/carcinogenesis.[1] In mammalian cells, the manner of DNA adduct formation with these classes of carcinogens is very similar; aminoarenes are oxidatively activated by the P450 species, whereas nitroarenes are reductively activated by coenzymes of P450 such as NADPH.[1] Both of these reactions yield hydroxyamines that are esterified by acetyl- or sulfotransferases to form *O*-esterified hydroxyaminoarenes. The spontaneous fission of the N−O bond generates highly active arylnitrenium ions to attack the DNA molecules. Generally, the arylnitrenium ions are believed to directly attack N7 of 2′-deoxyguanosine (dG); this results in the subsequent transfer of the arylamine at the N7 position of dG to the C8 position, forming *N*-(deoxyguanosin-8-yl)arylamine, i.e., dG−C8 adduct **1**. Carbocations that are resonance stabilized from the arylnitrenium ions also attack dG to form 2′-deoxyguanosin-N^2-yl-arylamine (dG-N^2 adduct **3**) wherein the C atom located at the ortho position of the amino group often attaches covalently to the N^2 position of dG.[1d−f] Biologically, the efficiency of amino-/nitroarene-derived dG−N^2 adduct formation is generally lower than that of the formation of the corresponding dG−C8 adducts. However, the in vivo efficiency of the DNA repair enzymes in the repair of dG−N^2 adducts is less than that in the repair of dG−C8 adducts. Thus, dG−N^2 adducts have attracted significant interest in genotoxicity studies.[2] In addition, although it is generated to a far lesser extent, 2′-deoxyadenosine (dA) serves as a target for the arylnitrenium ions, forming 2′-adenosin-8-ylaminoarene (dA−C8 adduct **2**) and 2′-adenosin-6-ylaminoarene (dA−N^6 adduct **4**), which are analogous to the dG adducts (Scheme 1).[1]

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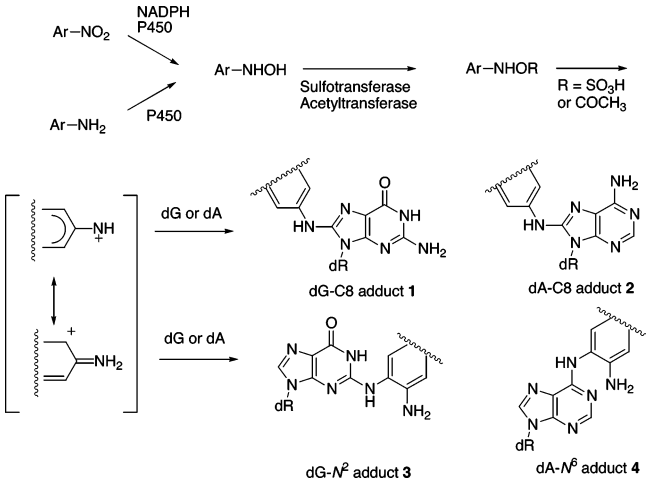
SCHEME 1. Metabolic Activation and DNA Adduct Formation of Amino-/Nitroarenes



The DNA adducts of carcinogenic amino-/nitroarenes are classically obtained by a simple electrophilic amination reaction[3] and can now also be prepared using the Buchwald−Hartwig *N*-arylation strategy.[4] The classical conditions, however, have certain limitations,[3] whereas it has been demonstrated that the Buchwald−Hartwig palladium (Pd)-catalyzed arylamination reaction is widely applicable for the generation of a variety of DNA adducts, including dG−C8 **1**, dG−N^2 **3**, dA−C8 **2**, and dA−N^6 **4** adducts.[4] Johnson's group demonstrated examples of the preparation of some dG−N^2-type **3** and dA−N^6-type **4** adducts by the Buchwald−Hartwig arylamination reaction using Pd(0)/2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) systems with Cs_2CO_3.[5] This system was later applied to the preparation of several N^2-modified dG derivatives.[6] The N^2 adducts of dG **3** with nitropyrene and also with an imidazoquinoline-type mutagen could be synthesized in a very similar manner.[7] Some related PAH-derived dG and dA adducts were initially prepared using Lakshman's Pd(0) with a series of biphenylphosphine ligands or BINAP system.[8] Nitrous acid-mediated cross-linking nucleosides have also been described.[9] *N*,*O*-Protected 8-bromonucleosides were found to readily undergo an arylamination reaction of carcinogenic arylamines under conditions of Pd(0)/BINAP with lithium hexamethyldisilazide (LiHMDS), potassium *tert*-butoxide (tBuOK), or K_3PO_4, yielding dG−C8 adduct **1** derived from carcinogenic heterocyclic amines by subsequent deprotection.[10] Initially, Schoffer et al.[11a] reported that the protection of the N^6 atom of adenosine derivatives in the arylamination reaction was not required for the preparation of adenosine−C8−PAH adducts, and Elmquist et al. later utilized the same technique for the preparation of dG−C8 adducts **1**.[11b] We have already demonstrated that a Pd(0)/xantphos system works well in the arylamination reaction for both suitably protected 8-aminonucleosides with bromoarenes and for protected 8-bromonucleosides with heterocyclic amines.[12] Generally, the phosphoramidite derivatives of these DNA adducts could be obtained on a large scale by the Pd-catalyzed amination strategy for the production of site-specific adducted oligonucleotides that are useful tools for the physicochemical and biological studies investigating the fundamental mechanisms underlying mutation as the initial step in carcinogenesis.[11b,13]

In these arylamination studies, it was necessary to protect the OH groups of the ribose moiety in all of the reactions in order to enhance solubility of nucleosides in organic solvents; in particular, the amide moiety at the N1 position of dG required protection to remove the unfavorable acidic proton that may have obstructed arylamination. This protection/deprotection strategy leads to easy separation, but inevitably adds two steps to synthetic procedures, reduces the overall yields, and sometimes requires expensive chemicals for protection.

While studying the synthesis of dG−C8 adducts using the Pd(0)/xantphos system, we obtained evidence that activated halogenated arenes might enable the direct preparation of DNA adducts, i.e., coupling to nucleosides without protective groups is feasible. Moreover, once this direct coupling reaction of nucleosides has occurred, nucleotides and oligonucleotides could also serve as substrates for the arylamination reaction. To our knowledge, there have been no previous studies on the direct arylamination of nucleotides and oligonucleotides without protective groups. In this paper, we report the development of a successful method for the direct arylamination of nucleosides, nucleotides, and oligonucleotides, and its application in the syntheses of dG−N^2 adducts with amino-/nitroarenes and site-specific modified oligonucleotides.

Results and Discussion

Arylamination of *O*-Protected dG. To select the most effective catalytic system for the following direct arylamination

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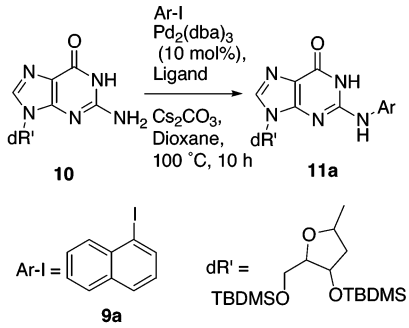
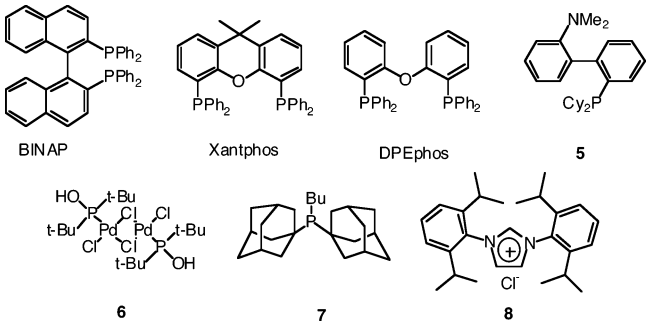
SCHEME 2. Effect of Phosphine Ligand on the Synthesis of 11a[a]



Entry	Ligand	Conv (%)[1]	Yield (%)[2]
a	BINAP	64	55
b	xantphos	74	65
c	DPEphos	55	46
d	**5**	trace	0
e	**6**	0	0
f	**7**	0	0
g	**8**	0	0

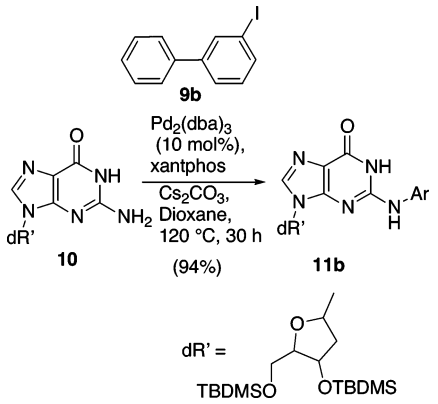
[a] Key: (1) Conversion after 10 h reaction time; (2) isolation yield.

CHART 1. Ligands Tested in This Study

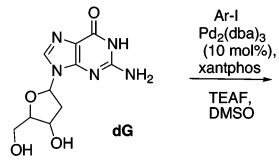


coupling reaction, 1-iodonaphthalene **9a** and 3′,5′-*O*-TBDMS-dG **10** were considered as the initial substrates, and this combination was tested with several phosphine ligands and $Pd_2(dba)_3$ to determine the optimal conditions for catalytic *N*-arylation (Scheme 2). Without O^6-protection, the arylamination reaction with bromoarenes was slow, as expected; however, this sluggishness might be overcome by using iodoarenes since they are generally recognized to be more reactive than the corresponding bromoarenes. The ligands screened were BINAP, xantphos, DPEphos, 2-(dicyclohexylphosphino)-2′-(*N*,*N*-dimethylamino)biphenyl **5**, Popd2 **6**, butyldi-1-adamantylphosphine **7**, and 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride **8**, as illustrated in Chart 1, that are commonly used or were newly discovered in a Pd-catalyzed C−N bond forming process.[8d,14,15] The screening reactions were carried out under standard conditions of $Pd_2(dba)_3$ (10 mol %), the ligand (30 mol %),

SCHEME 3. Arylamination Reaction of *O*-TBDMS-dG with 3-Iodobiphenyl



SCHEME 4. Direct Pd-Catalyzed Arylamination with DG and Selected Iodoarenes[a]



Entry	Ar-I	Temp (°C)	Yield (%)[1]	Entry	Ar-I	Temp (°C)	Yield (%)[1]
a	**9a**	100	22 (19)[2]				
b	**9c**	90	84	e	**9f**	120	0
c	**9d**	90	80	f	**9g**	120	33
d	**9e**	90	73 (64)[2]				

[a] Key: (1) isolated yield; (2) Cs_2CO_3 was used.

and Cs_2CO_3 (2 equiv to the nucleoside derivatives) in dioxane at 100 °C for 10 h (Scheme 2).

Dioxane was selected over other inert solvents such as toluene because the protected dG solubilizes better in dioxane. No reaction occurred in the control experiment without the Pd catalyst/ligands. The reactions employing ligands **5**−**8** also failed. BINAP and xantphos yielded moderate conversion as did DPEphos to a lesser extent. Xantphos and BINAP demonstrated a similar activity in the conversion of the starting materials, although the former was somewhat superior to the latter with regard to the isolation yields. The Pd(0)/xantphos system also proved to be efficient for the coupling of 3-iodobiphenyl **9b** at a higher reaction temperature of 120 °C, although 3-bromobiphenyl did not yield any catalyzed reaction products under the same reaction conditions (Scheme 3).

Direct Arylamination of dG for the Preparation of dG−*N*² Adducts. The system that was optimized for *O*-protected dG **10** and **9a** was applied for the direct arylamination of dG without any protective groups. However, only poor conversion was achieved, probably due to the sparing solubility of dG in dioxane. DMSO, the next choice as a more polar solvent with greater solubilization activity, was found to produce N^2-aryl nucleoside **12a** at low yields (Scheme 4).

Changing the base to tetraethylammonium fluoride (TEAF) also yielded **12a** with a similar efficiency. It was encouraging,

(14) For an adamantylphosphine ligand, see: (a) Ehrentraut, A.; Zapf, A.; Beller, M. *J. Mol. Catal.* **2002**, *182/183*, 515. For xantphos, see: (b) Ali, M. H.; Buchwald, S. L. *J. Org. Chem.* **2001**, *66*, 2560. (c) Yin, J.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 6043. For Popd2, see: (d) Li, G. Y.; Zheng, G.; Noonan, A. F. *J. Org. Chem.* **2001**, *66*, 8677. For an imidazol type ligand, see: (e) Grassa, G. A.; Viciu, M. S.; Huang, J.; Nolan, S. P. *J. Org. Chem.* **2001**, *66*, 7729. For DPEphos, see: (f) Belfield, A. J.; Brown, G. R.; Foubister, A. J. *Tetrahedron* **1999**, 11399.

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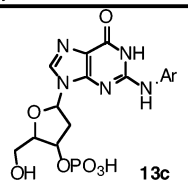
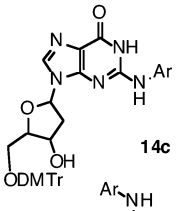
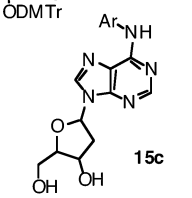
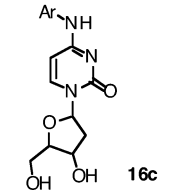
however, that the use of more activated *o*-iodonitrobenzene with the Pd(0)/xantphos/TEAF system produced the corresponding N^2-nitrobenzene-dG **12c**, which can be used as a precursor for the generation of the desired dG-N^2-aniline adduct **19c** in a moderate yield. Generally, Cs_2CO_3 has a coupling efficiency similar to that of TEAF. However, due to its ease of handling during the isolation steps, TEAF is superior. With TEAF in DMSO, the modified nucleoside derivatives were highly soluble in general organic solvents such as $CHCl_3/CH_3OH$ making it easy to apply the derivatives to silica gel column chromatography, although some of the compounds were isolated as tetraethylammonium (TEA) salts. To our knowledge, this is the first demonstration that TEAF can act as the base in Pd-catalyzed arylamination. It should be noted that this Pd(0)/xantphos/TEAF system could not be used with other solvents such as THF. When the same catalyst and base load were used in THF, no conversion of the starting materials was observed. Another quaternary ammonium salt, tetraethylammonium bromide, did not yield coupling compound **12** in DMSO.

The direct coupling of dG and a series of iodoarenes **9d**−**g** was carried out to determine the utility of the reaction. Targets were chosen so as to yield dG−N^2 adducts of carcinogenic amino-/nitroarenes or their precursors. Ortho-substituted iodonitroarenes containing 3-iodo-4-nitrobiphenyl **9d** and 1-iodo-2-nitronaphthalene **9e** could couple within 4 h at 90 °C at a good yield. *O*-Acetylamide iodobenzene **9f** gave no reaction products even at 120 °C, whereas 6-acetylamide-1-iodopyrene **9g** gave dG-N^2-yl-6-acetylaminopyrene **12g** at moderate yields; this is a deacetylation derivative that is known to be generated in the reaction of the ultimate mutagenic forms of 1-nitropyrene with dG.[7a,16] The other nucleosides dA and dC also gave the corresponding adducts modified with nitrobenzene, i.e., **15c** and **16c**, respectively, at excellent yields in the presence of Pd(0)/xantphos/TEAF (Table 1, entries c and d). It is noteworthy that the coupling reaction with dC proceeded even at room temperature. The Pd(0)/xantphos/Cs_2CO_3 system in dioxane worked well for the *N*-arylation of dA as observed by TLC analyses; however, due to the sparing solubility of the reaction products in commonly used organic solvents, the isolation was tedious and resulted in a low isolation yield.

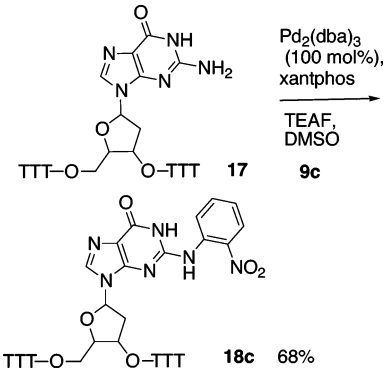
Given the success of the Pd(0)/xantphos/TEAF system for the direct *N*-arylation of nucleosides, its potential application with nucleotides was explored. The nucleotide, 2′-deoxyguanosine-3′-phosphate (2′-dGp), was coupled with *o*-iodonitrobenzene to yield 2′-deoxyguanosin-N^2-ylnitrobenzene **13c** in 61% yield (Table 1, entry a). In DNA samples, a DNA adduct with a 3′-phosphate group is an essential substrate that is used for quantitative determination by ^{32}P-postlabeling analysis.[17] Authentic samples of this type of nucleotide have only been obtained by a nitrenium ion-mediated reaction with 2′-dGp derivatives or a post-phosphorylation reaction with the corresponding modified nucleosides.[18,19] The oligonucleotide TTTGTTT **17** could also be coupled efficiently by using the Pd(0)/xantphos/TEAF system, although 100 mol % of Pd(0) was loaded in 100 μL of DMSO due to the technical difficulty of weighing the correct amount for a small-scale reaction (300 μmol) and to prevent the cleavage of phosphate bonds during the prolonged heating conditions at 90 °C (Scheme 5). The reaction was completed within 1 h with 100% conversion, and the isolated yield of the modified oligonucleotide **18c** after HPLC was estimated to be 68% using UV absorbance at 260 nm. Although the reaction with mixed sequence oligonucleotides containing dA an dC has not been tested yet, dA and dC in oligonucleotides are expected to couple with iodoarenes because amino groups of dA and dC are known to be more nucleophilic than those of dG. Regarding the Pd(0)-catalyzed coupling

TABLE 1. Arylamination Reaction of Nucleosides with *o*-Iodonitrobenzene

Entry	Product[a]	Temp (°C)	Yield (%)
a	 **13c**	90	61
b	 **14c**	90	92
c	 **15c**	90	87
d	 **16c**	RT	98

[a] Ar = *O*-nitrobenzene. Reactions were performed with nucleotide/nucleoside (1 mmol), *o*-iodonitrobenzene (2 mmol), $Pd_2(dba)_3$ (0.1 mol), xantphos (0.2 mol), and TEAF (2 mmol).

SCHEME 5. Arylamination of Oligonucleotide TTTGTTT with *o*-Iodonitrobenzene



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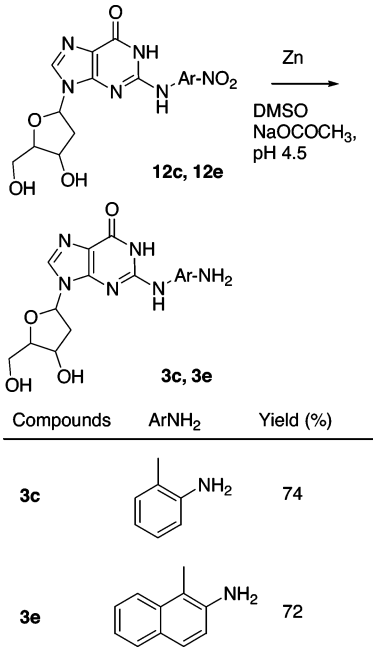
SCHEME 6. Nitroreduction of 12c and 12e

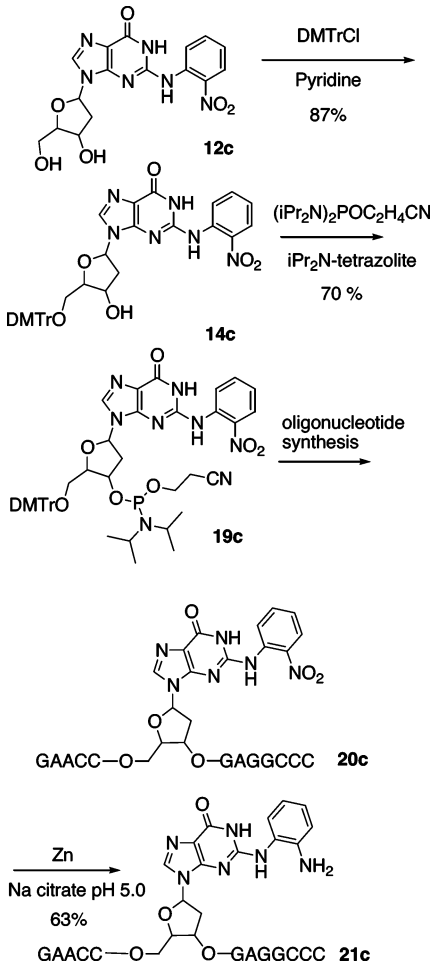


reaction, the Sonogashira reaction of protected oligonucleotides was previously performed by Khan and Grinstaff.[20]

Synthesis of dG−N^2 Adduct and Its Application for the Synthesis of Site-Specific Modified Oligonucleotides. Since the Pd/xantphos system displayed high reactivity with *o*-iodonitroarenes, we were interested in the synthesis of authentic dG−N^2−arylamine adduct **3** specimens that are found in biological samples. The direct reduction of the nitro group of **12c** and **12e** would yield the N^2−arylamine adducts of dG **3**. We tested several nitroreduction systems including Pd/C with H_2, cyclohexadiene with Pd black, $Na_2S_2O_4$, and Zn dust. Among them, an efficient reduction to the desired amine was observed in the system of cyclohexadiene in DMF with Pd black heated at 90 °C and with Zn dust in a 1:1 mixture of DMSO and 500 mM CH_3COONH_4 at pH 4.5. Treatment of **12c** with aqueous $Na_2S_2O_4$ in aqueous DMSO also resulted in rapid reduction of a nitro group but always involved the sulfonylation products of the resulting aromatic amine, as confirmed by liquid chromatography−mass spectrometry (LC−MS) techniques. Pd/C with H_2 gas at atmospheric pressure did not cause the conversion of the starting materials. The Zn/500 mM CH_3COONH_4 system at pH 4.5 proved superior to the cyclohexadiene/Pd black system because the former reaction proceeded under mild conditions without heating and was completed within 1 h at room temperature. The desired **3c** compound was purified by passing the filtrate of the reaction mixture through a C18 RP-silica gel column and eluting with a water/MeOH gradient. This reduction procedure could be applied to another nitro derivative **3e** (Scheme 6).

The nitrophenyl-dG derivative**12c** could be used directly for general oligonucleotide synthesis by the phosphoramidite approach (Scheme 7). Compound **12c** could be converted to the 5′-dimethoxytriphenylmethyl (DMTr) derivative **14c** in the general manner of 4,4′-dimethoxytriphenylmethyl chloride (DMTrCl) in pyridine. Compound **14c** was also obtained efficiently by the direct coupling reaction between 5′-DMTr-dG and iodonitroarene **9c** under the conditions of $Pd(OAc)_2$/xantphos/Cs_2CO_3 in dioxane at 90 °C, resembling the conditions for *O*-silyl protected dG (Table 1, entry b). In this case, $Pd(OAc)_2$ was used instead of $Pd_2(dba)_3$. When $Pd_2(dba)_3$ was employed as the Pd source, the reaction gave rise to some colored impurities as well as the desired compounds that could not be separated by silica gel chromatography. The dimethoxytrityrated N^2−nitrophenyl-dG adduct **14c** was phosphitylated by using the 2-cyanoethyltetraisopropylphosphorodiamidite/tetrazole system in CH_2Cl_2. Careful workup with silica gel chromatography gave the desired 5′-DMTr-N^2-2-nitrophenyl-2′-deoxyguanosine phosphoramidite **19c**. The amidite **19c** was then subjected to a DNA synthesizer with a slight modification where the coupling time of the modified amidite was changed to 15 min. The coupling efficiency was generally higher than 80% as determined by UV monitoring during tritanol elimination. Oligonucleotide 13mer **20c** modified with nitrobenzene was thus obtained purified by HPLC. The UV spectrum of the oligonucleotide showed absorbance at 400−500 nm, which was derived from the nitrobenzene moiety at the N^2 position. MS analysis of this modified oligonucleotide showed a peak of *m*/*z* 4088. Enzymatic hydrolysates of the oligonucleotide clearly indicated the presence of N^2−nitrophenyl-dG **12c**. The nitro group in oligonucleotide **20c** was effectively converted to an amino group by using Zn dust in Na citrate at pH 5. The Zn/CH_3COONH_4 system worked well for the nitro reduction; however, some minor HPLC peaks were always observed,

SCHEME 7. Synthesis of Oligonucleotide Containing dG-N^2 Adduct 3c



(20) Khan, S. I.; Grinstaff, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 4704.

probably due to the formation of hydroxyamine derivatives from insufficient nitro reduction, whereas overnight treatment with Zn in Na citrate buffer resulted in the complete reduction of the nitro group in oligonucleotide **20c**. Liquid chromatography electrospray ionization tandem mass spectrometry (LC−ESI−MS) indicated that the nitrophenyl oligonucleotide **20c** was converted to aminophenyl oligonucleotide **21c** whose enzymatic hydrolysates also showed the presence of N^2−aminophenyl-dG **3c**. The synthesis of site-specific modified oligonucleotides with the dG−N^2 adduct was recently reported by Johnson's group; they used the phosphoramidite derivatives of acetylaminophenyl-dG or trifluoroacetylaminophenyl-dG followed by the alkali deprotection of the trifluoroacetyl moiety.[14] The system using nitro groups as the protective group of the dG−N^2 adduct is now an efficient alternative tool for the synthesis of site-specific modified oligonucleotides.

Conclusion

Here, we described the efficient preparation of dG−N^2 adducts from carcinogenic aromatic amino/nitro compounds via the direct *N*-arylation reaction. This methodology will be applied for a wide variety of dG−N^2 adducts and also to dA−N^6 DNA adducts; in particular, it will be used for the preparation of site-specific adducted oligonucleotides with dG−N^2 adducts during solid-phase oligonucleotide synthesis.

Experimental Section

Typical Procedure for the Coupling of Silyl-Protected 2′-Deoxyguanosine 10 with Iodoarene 9. Compound **10** (1 mmol), $Pd_2(dba)_3$ (0.1 mmol), phosphine ligand (0.3 mmol), iodoarene **9** (2 mmol), and Cs_2CO_3 (2 mmol) were dissolved in 3 mL of dioxane and stirred at 100 °C for 10 h. The reaction mixtures were then evaporated, and the residues were dissolved in $CHCl_3$, subjected to column chromatography on silica gel, and eluted using a step gradient of MeOH in $CHCl_3$. The fractions containing the desired products were combined, evaporated, and finally dried under high vacuum to remove traces of the solvent.

3′,5′-Di-*tert*-butyldimethylsilyl-N^2-naphthalen-1-yl-2′-deoxyguanosine (11a): ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H), 8.84 (s, 1H), 8.03 (d, J = 6.6 Hz, 2H), 7.92−7.87 (m, 2H), 7.67 (d, J = 8.1 Hz, 1H), 7.56−7.47 (m, 2H), 7.41 (t, J = 7.9 Hz, 1H), 6.06 (t, J = 6.7 Hz, 1H) (s, 1H), 4.28 (br s, 1H), 3.71 (br s, 1H), 3.47 (d, J = 5.4 Hz, 1H), 3.11 (d, J = 5.1 Hz, 1H), 2.65−2.55 (m, 1H), 2.16−2.07 (s, 1H), 0.81 (m, 9H), 0.77 (m, 9H) 0.05 (s, 6H), −0.09 (m, 6H); ^{13}C NMR ($CDCl_3$) 156.3, 150.1, 149.3, 136.3, 133.6, 133.2, 128.3, 126.6, 125.9, 125.3, 124.1, 121.4, 118.8, 118.3, 87.1, 82.5, 72.2, 62.7, 25.7, 25.6, 17.9, 17.6, −4.7, −4.9, −5.4; FAB-HRMS (nitrobenzyl alcohol/nba) *m*/*z* [M + Na^+] calcd for $C_{61}H_{74}N_9O_6Si_2Na$ 644.3064, found 644.3123.

3′,5′-Di-*tert*-butyldimethylsilyl-N^2-biphenyl-3-yl-2′-deoxyguanosine (11b): ^{1}H NMR ($CDCl_3$) δ 12.24 (br s, 1H), 9.96 (br s, 1H), 8.31 (br s, 1H), 8.15−7.97 (m, 1H), 7.87−7.72 (m, 3H), 7.42−7.25 (m, 6H), 6.30−6.21 (m, 1H), 4.55−4.45 (m, 1H), 4.02−3.95 (m, 1H), 3.80−3.65 (m, 2H), 2.55−2.25 (m, 2H), 0.99−0.87 (m, 18H), 0.15−0.02 (s, 9H); ^{13}C NMR ($CDCl_3$) 158.7, 150.3, 149.5, 141.0, 140.8, 139.5, 139.1, 136.0, 128.6, 128.3, 128.2, 127.1, 126.9, 122.4, 120.9, 120.2, 118.9, 118.7, 118.4, 118.2, 87.6, 84.0, 72.0, 62.8, 40.7, 31.5, 25.9, 25.7, 22.6, 18.3, 17.9, 14.1, −4.59, −4.62, −4.73, −4.75, −5.37, −5.47; FAB-HRMS (nba) *m*/*z* [M + Na^+] calcd for $C_{34}H_{49}N_5O_4Si_2Na$ 670.3220, found 670.3261.

Typical Procedure for the Direct *N*-Arylation of Nucleosides with Iodoarene 9. Nucleoside (1 mmol), $Pd_2(dba)_3$ (0.1 mmol), xantphos (0.3 mmol), iodoarene **9** (2 mmol), and TEAF (2 mmol) were dissolved in 3 mL of DMSO and stirred at the temperature indicated in the Results and Discussion. The reactions were monitored by TLC, and upon completion, the reaction mixtures were dissolved in a $CHCl_3$−MeOH solution, directly subjected to column chromatography on silica gel, and eluted with a step gradient of MeOH in $CHCl_3$. The fractions containing the desired products were combined, evaporated, and finally dried under high vacuum to remove traces of the solvent.

***N*^{2}-Naphthalen-1-yl-2′-deoxyguanosine (12a):** ^{1}H NMR (DMSO-d_6) δ 10.9 (s, 1H), 8.89 (s, 1H), 8.13−8.08 (m, 2H), 8.03 (s, 1H), 7.96 (dd, J = 7.8 Hz, 1.0, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.62−7.51 (m, 3H), 6.10 (t, J = 6.8 Hz, 1H), 5.24 (d, J = 4.1 Hz, 1H), 4.84 (t, J = 5.5 Hz, 1H), 4.23 (td, J = 6.2 Hz, 3.2 Hz, 1H), 3.76 (dd, J = 7.8 Hz, 4.6 Hz, 1H), 3.40 (tt, J = 16.3 Hz, 5.7 Hz, 1H), 2.59−2.51 (m, 1H), 2.18 (dq, J = 13.2 Hz, 3.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) 156.4, 150.1, 149.4, 136.5, 133.6, 133.2, 128.3, 126.5, 126.0, 125.7, 124.1, 121.3, 118.9, 118.3, 87.6, 82.8, 70.5, 61.6; FAB-HRMS (nba) *m*/*z* [M + H^+] calcd for $C_{20}H_{20}N_5O_4$ 394.1515, found 394.1492.

***N*^{2}-2-Nitrobenzen-1-yl-2′-deoxyguanosine (12c):** ^{1}H NMR (DMSO-d_6) δ 11.8 (s, 1H), 9.60 (s, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.11−8.07 (m, 2H), 7.74 (t, J = 5.9 Hz, 1H), 7.29 (t, J = 6.7 Hz, 1H), 6.09 (t, J = 6.7 Hz, 1H), 5.25 (d, J = 3.7 Hz, 1H), 4.86 (t, J = 5.2 Hz, 1H), 4.30 (br s, 1H), 3.78 (q, J = 3.9 Hz, 1H), 3.46 (tt, J = 16.2 Hz, 5.2 Hz, 1H), 2.57−2.51(m, 1H), 2.26−2.18 (m, 1H); ^{13}C NMR (DMSO-d_6) 156.4, 148.4, 148.3, 139.0, 136.9, 134.6, 133.2, 125.3, 123.9, 123.4, 119.2, 87.6, 83.0, 70.5, 61.5; FAB-HRMS (nba) (*m*/*z*) [M + H^+] calcd for $C_{16}H_{17}N_6O_6$ 389.1210, found 389.1225.

***N*^{2}-4-Nitrobiphenyl-3-yl-2′-deoxyguanosine (12d):** ^{1}H NMR (DMSO-d_6) δ 12.06 (br s, 1H), 9.88 (br s, 1H), 8.85 (s, 1H), 8.22 (dd, J = 8.8 Hz, 1.0, 1H), 8.17 (d, J = 1.0 Hz, 1H), 7.78 (d, J = 7.8 Hz, 2H), 7.60−7.53 (m, 3H), 7.46 (t, J = 7.0 Hz, 1H), 6.24 (t, J = 6.7 Hz, 1H), 5.30 (d, J = 3.2 Hz, 1H), 4.91 (t, J = 4.9 Hz, 1H), 4.28 (s, 1H), 3.85 (s, 1H), 3.30 (s, 2H), 2.63−2.55 (m, 1H), 2.28−2.20 (m, 1H), −0.87 (m, 18H), 0.15−0.02 (s, 9H); ^{13}C NMR (DMSO-d_6) 156.5, 148.5, 148.7 146.1, 137.6, 136.7, 136.6, 134.3, 129.3, 129.0, 127.0, 126.4, 121.1, 120.9, 119.3, 87.9, 82.7, 70.7, 61.6; FAB-HRMS (nba) *m*/*z* [M + H^+] calcd for $C_{22}H_{21}N_6O_6Si_2Na$ 465.1522, found 465.1488.

***N*^{2}-2-Nitronaphthalen-1-yl-2′-deoxyguanosine (12e):** ^{1}H NMR (DMSO-d_6) δ 8.30 (d, J = 8.3 Hz, 1H), 8.11 (dd, J = 7.9 Hz, 1.1 Hz, 1H), 8.02 (s, 2H), 7.95 (s, 1H), 7.78−7.69 (m, 2H), 5.79 (t, J = 6.8 Hz, 1H), 5.10 (d, J = 3.2 Hz, 1H), 4.71 (br s, 1H), 4.00 (br s, 1H), 3.18 (br s, 2H), 2.31−2.22 (m, 1H), 1.98 (dq, J = 13.1 Hz, 3.3 Hz, 1H), −0.87 (m, 18H), 0.15−0.02 (s, 9H); ^{13}C NMR (DMSO-d_6) 150.6, 149.3, 141.6, 136.3, 135.1, 129.3, 128.7, 128.3, 127.9, 126.6, 124.8, 120.7, 118.2, 87.6, 82.8, 70.5, 61.6; FAB-HRMS (nba) *m*/*z* [M + H^+] calcd for $C_{20}H_{19}N_6O_6$ 439.1366, found 439.1329.

***N*^{2}-(8-Acetylamidopyren-1-yl)-2′-deoxyguanosine (12g):** ^{1}H NMR (DMSO-d_6) δ 10.35 (s, 1H), 8.59 (d, J = 8.5 Hz, 1H), 8.40−8.10 (m, 8H), 6.09 (t, J = 7.0 Hz, 1H), 5.19 (br s, 1H), 4.82 (br s, 1H), 4.21 (m, 1H), 3.76 (m, 1H), 3.16 (tm, 2H), 2.60 (m, 1H), 2.49 (s, 3H), 2.18 (m, 1H); ^{13}C NMR (DMSO-d_6) 168.9, 156.6, 153.5, 150.7, 126.2, 135.1, 131.5, 128.2, 127.5, 126.3, 125.9, 125.1, 124.8, 124.5, 123.4, 123.2, 122.6, 122.1, 118.4, 116.5, 87.5, 82.7, 82.4, 70.6, 61.6, 14.1; FAB-HRMS (nba) *m*/*z* [M + Na^+] calcd for $C_{28}H_{24}N_6O_5Na$ 547.1706, found 547.1590.

***N*^{6}-2-Nitrobenzen-1-yl-2′-deoxyadenosine (15c):** ^{1}H NMR (DMSO-d_6) δ 10.6 (s, 1H), 8.60 (s, 1H), 8.43 (d, J = 8.3 Hz, 1H), 8.39 (d, J = 0.5 Hz, 1H), 8.11 (d, J = 8.3 Hz, 1H), 7.77 (t, J = 7.4 Hz, 1H), 7.31 (t, J = 7.7 Hz, 1H), 6.42 (t, J = 6.8 Hz, 1H), 5.34 (d, J = 4.1 Hz, 1H), 5.06 (t, J = 5.6 Hz, 1H), 4.46−4.40 (m, 1H), 3.89 (dd, J = 7.2 Hz, 4.3 Hz, 1H), 3.66−3.60 (m, 1H), 3.56−3.49 (m, 1H), 2.81−2.72 (m, 1H), 2.33 (dq, J = 13.2 Hz, 3.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) 151.4, 150.8, 149.4, 141.6, 139.7, 134.6, 133.6, 125.3, 124.3, 123.5, 120.8, 87.9, 83.9, 70.7, 61.6; FAB-HRMS (nba) *m*/*z* [M + H^+] calcd for $C_{16}H_{17}N_6O_5$ 373.1260, found 373.1237.

***N*^{6}-2-Nitrobenzen-1-yl-2′-deoxycytidine (16c):** ^{1}H NMR (DMSO-d_6) δ 9.90 (s, 1H), 7.92 (d, J = 8.1 Hz, 2H), 7.64 (br s, 2H), 7.30 (br s, 1H), 6.07 (t, J = 8.1 Hz, 1H), 5.98 (br s, 1H), 5.16 (t, J = 4.1 Hz, 1H), 4.93 (t, J = 5.1 Hz, 1H), 4.15 (dt, J = 8.5 Hz, 3.4 Hz, 1H), 3.73 (d, J = 3.2 Hz, 1H), 3.50 (ddd, J = 21.7 Hz, 12.0 Hz, 4.8 Hz, 2H), 2.15−2.05 (m, 1H), 1.98−1.87 (m, 1H); ^{13}C NMR (DMSO-d_6) 153.9, 142.7, 141.9, 133.7, 131.5, 126.6, 124.8, 94.8, 87.4, 85.0, 70.2, 61.2, 48.5; FAB-HRMS (nba) m/z [M + Na^+] calcd for $C_{15}H_{16}N_4O_6Na$ 371.0967, found 373.0968.

Typical Procedure for the Coupling of Nucleotide with 9c. Nucleotide (0.04 mmol), $Pd_2(dba)_3$ (0.02 mmol), xantphos (0.03 mmol), **9c** (0.08 mmol), and TEAF (0.08 mmol) were dissolved in 1 mL of DMSO and stirred at 90 °C for 4 h. After the reactions were completed as judged from the TLC analyses, 5 mL of water followed by 4 mL of $CHCl_3$ were added to the reaction mixtures. The aqueous layer was subjected to column chromatography on octadecyl silica (ODS) and eluted using a step gradient of MeOH in water. The fractions containing the desired products were combined, evaporated, and finally dried under high vacuum to remove traces of the solvent.

***N*^{2}-2-Nitrobenzen-1-yl-2′-deoxyguanosine-3′-phosphate (13c):** ^{1}H NMR (DMSO-d_6) δ 8.12 (d, J = 8.1 Hz, 1H), 8.01 (s, 1H), 8.00 (d, J = 6.8 Hz, 1H), 7.70 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 6.01 (t, J = 6.7 Hz, 1H), 4.75 (br s, 1H), 3.99 (q, J = 3.3 Hz, 1H), 3.55−3.43 (m, 1H), 3.18 (q, J = 7.1 Hz, 8H), 2.60−2.52 (m, 1H), 2.45−2.37 (m, 1H), 1.13 (t, J = 6.6 Hz, 12H); ^{13}C NMR (DMSO-d_6) 158.54, 150.0, 148.7, 139.6, 135.9, 134.4, 133.7, 125.1, 123.9, 122.8, 119.0, 86.5, 82.6, 74.1, 61.8, 51.3, 7.10; ^{31}P NMR (DMSO-d_6) 1.38; FAB-HRMS (nba) m/z [M + H^+] calcd for $C_{16}H_{18}N_6O_9P$ 469.0872, found 469.0848.

Direct *N*-Arylation of Oligonucleotide 17 with 9c. In a screw-capped vessel containing 0.3 μmol of oligonucleotide **17** was added 100 μL of a premixed solution of **9c** (1.2 μmol), $Pd_2(dba)_3$ (0.3 μmol), xantphos (0.6 μmol), and TEAF (1.2 μmol) in DMSO. The reaction was allowed to proceed at 90 °C for 1 h until the complete conversion to **17** was confirmed by HPLC analysis. The reaction was extracted with $CHCl_3$, and the aqueous layer was subjected to HPLC under the following conditions: column, Cosmosil ODS AR II (4.6 × 250 mm); eluent, linear gradient of 0%-40% CH_3CN in 0.25% TEA−AcOH over 20 min at a flow rate of 1.0 mL/min to yield **18c**: ESI-MS m/z $[M - 2H]^{2-}$, 1106.2; $[M - 3H]^{3-}$, 737.4; $[M - 4H]^{4-}$, 553.1.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-2-nitrobenzen-1-yl-2′-deoxyguanosine (14c): Procedure A. Five milliliters of dioxane was added to a reaction vessel containing 5′-*O*-(4,4′-dimethoxytrityl)-2′−deoxyguanosine (0.5 mmol), $Pd(OAc)_2$ (0.05 mmol), Cs_2CO_3 (1 mmol), xantphos (0.15 mmol), and **9c** (1 mmol) The reaction mixture was stirred for 4 h at 90 °C and evaporated; the residue was then purified by column chromatography. The fractions containing the desired products were combined, evaporated, and finally dried under high vacuum to remove traces of the solvent.

Procedure B. To a solution of **12c** (0.2 mmol) in pyridine was added 0.3 mmol of 4,4′-dimethoxytrityl chloride After the reaction was stirred at room temperature for 4 h, another aliquot of 4,4′-dimethoxytrityl chloride (0.3 mmol) was added. After a 6 h reaction, triethylamine (3 mL) and MeOH (3 mL) were added to the solution. The mixture was then evaporated and applied to a chromatographic column, affording the desired compound (79%): ^{1}H NMR ($CDCl_3$) δ 10.2 (s, 1H), 8.33 (s, 1H), 7.83 (s, 1H), 7.63 (br s, 1H), 7.32−7.02 (m, 11H), 6.82 (br s, 1H), 6.70 (d, J = 8.1 Hz, 4H), 6.21 (br s, 1H), 4.52 (br s, 1H), 4.12 (br s 1H), 3.71 (br s, 1H), 3.64 (s, 6H), 3.31 (br s, 1H), 3.23 (br s, 1H), 2.72−2.45 (m, 2H); ^{13}C NMR ($CDCl_3$) 158.2, 148.9, 147.9, 144.3, 136.9, 135.5, 135.4, 134.8, 133.8, 129.8, 129.0, 127.9, 127.6, 126.7, 125.4, 122.5, 119.0, 113.0, 86.3, 85.6, 83.8, 72.0, 64.1, 63.6, 40.6, 30.9, 29.2; FAB-HRMS (nba) m/z [M + H^+] calcd for $C_{37}H_{35}N_6O_8$ 691.2516, found 691.2411.

3′-*O*-[(*N*,*N*-Diisopropylamino)-2-cyanoethoxyphosphinyl]-5′-*O*-(4,4-dimethoxytrityl)-*N*^{2}-2-nitrobenzen-1-yl-2′-deoxyguanosine (19c). A premixed solution of *N*,*N*-diisopropylaminotetrazolide (1.2 equiv) and 2-cyanoethyltetraisopropylphosphorodiamidite (1.2 equiv) was added to a solution of **14c** (80 mg) in CH_2Cl_2 (5 mL). The reaction was stirred for 2 h, and the solvent was then removed. The residue was purified by column chromatography on a neutral silica gel column and eluted with chloroform containing 2% triethylamine to yield **19c** (70%): ^{1}H NMR ($CDCl_3$) δ 10.6 (s, 1H), 8.06 (s, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 12.0 Hz, 1H), 7.31 (t, J = 6.3 Hz, 2H), 7.22−7.07 (m, 7H), 6.88 (br s, 1H), 6.70 (t, J = 6.3 Hz, 4H), 6.17 (t, J = 5.4 Hz, 1H), 4.53 (br s, 1H), 4.16 (br s 1H), 3.87−3.44 (m, 10H), 3.30−3.20 (m, 2H), 2.75−2.25 (m, 4H), 1.25−1.00 (m, 12H); ^{13}C NMR ($CDCl_3$) 158.3, 149.6, 144.3, 135.7, 135.5, 135.4, 134.8, 134.0, 129.9, 128.0, 127.9, 127.8, 127.4, 126.8, 125.2, 123.6, 122.2, 119.5, 117.5, 117.3, 113.0, 86.3, 85.5, 85.3, 83.7, 73.8, 63.6, 58.9, 55.2, 45.6, 43.3, 40.3, 31.6, 24.6, 22.6, 20.2, 14.1, 8.79; ^{31}P NMR ($CDCl_3$) 149.0, 148.6; FAB-HRMS (nba) m/z [M + H^+] calcd for $C_{46}H_{52}N_8O_9P$ 891.3594, found 891.3452.

Solid-Phase Synthesis of Oligonucleotide 20c. Oligonucleotide 5′-d(GAACC-**9c**-GAGGCCC) **20c** was prepared on a 1 μmol scale by using general isobutyryl- or benzoyl-protected cyanoethylphosphoramidites and modified phosphoramidite **19c**. The manufacturer's standard synthesis protocol was followed, except for the prolonged coupling reaction time of 15 min. The deprotection of the oligonucleotides was achieved by treatment with concentrated ammonia and 0.25 M mercaptoethanol at 50 °C for 17 h. The purification was performed using a Sep-Pak C18 column followed by HPLC (column, Unison US-C18 (3.0 × 150 mm); column temperature, 50 °C; eluent, linear gradient of 3%-40% acetonitrile over 30 min at a flow rate of 0.5 mL/min) to yield **20c** (27%, MALDI-MS analysis: [M − 1] calcd for **20c** 4088.72, found 4088.94). Nucleotide digestion yielded **20c** along with three normal nucleotides in the correct stoichiometric ratio. HPLC conditions: column, Cosmosil AR-II ODS column (4.6 × 250 mm); eluent, 50 mM ammonium acetate gradient of 2%-10% over 18 min, acetonitrile gradient of 10%-100% over 15 min, and isocratic elution for 10 min.

Typical Procedure of Nitroreduction of 20c. To an aqueous solution (100 μL) of **21c** (10 ng) in 200 mM Na citrate at pH 5.0 was added 3 mg of Zn dust. The reaction was allowed to proceed for 24 h, and the product was subjected to HPLC under the following conditions: column, Xterra MS C8 (4.6 × 50 mm); eluent: linear gradient of 0%-34% CH_3OH in 0.25% TEA adjusted to pH 7.0 with CH_3COOH at a flow rate of 0.5 mL/min to yield **21c** (63%).

Typical Procedure of Nitroreduction of 9. To a solution of **9** (0.1 mmol) in 2 mL of DMSO/500 mM $NaCOOCH_3$ (1:1) was added 50 mg of Zn dust. The color of the solution disappeared immediately. After confirmation of completion of the reaction by TLC ($CHCl_3$/MeOH = 5:1), the filtrate was directly subjected to column chromatography on an ODS column and eluted using a step gradient of MeOH/water.

***N*^{2}-2-Aminobenzen-1-yl-2′-deoxyguanosine (3c):** ^{1}H NMR (DMSO-d_6) δ 10.6 (s, 1H), 7.96 (s, 1H), 7.93 (s, 1H), 7.38 (d, J = 7.3 Hz, 1H), 6.91 (t, J = 7.2 Hz, 1H), 6.76 (d, J = 7.3 Hz, 1H), 6.60 (t, J = 7.2 Hz, 1H), 6.04 (t, J = 7.0 Hz, 1H), 5.23 (d, J = 3.4 Hz, 1H), 4.94 (s, 2H), 4.83 (t, J = 4.8 Hz, 1H), 4.26 (br s, 1H), 3.76 (dd, J = 7.1 Hz, 4.1 Hz, 1H), 3.43 (ddd, J = 22.6 Hz, 12.0 Hz, 5.0 Hz, 1H), 2.58−2.51 (m, 1H), 2.17 (dq, J = 13.2 Hz, 2.0, 1H); ^{13}C NMR (DMSO-d_6) 156.6, 150.7, 150.0, 142.0, 135.9, 125.5, 125.0, 123.1, 117.6, 116.4, 115.8, 87.6, 82.6, 70.7, 61.7; FAB-HRMS (nba) m/z [M + H^+] calcd for $C_{16}H_{19}N_6O_4$ 359.1467, found 359.1447.

***N*^{2}-2-Aminonaphthalen-1-yl-2′-deoxyguanosine (3f):** ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H), 7.89 (s, 1H), 7.69 (d, 1H), 7.61 (d, 1H), 7.54 (d, 1H), 7.32 (dt, J = 10.5 Hz, 3.8, 1H), 7.15−7.12 (m, 1H), 7.09 (d, J = 8.8 Hz, 1H), 5.84 (br s, 1H), 5.41 (s, 2H), 5.09

(s, 1H), 4.70 (s, 1H), 4.36 (t, J = 5.0 Hz, 1H), 4.07 (s, 1H), 3.63 (s, 1H), 3.50−3.34 (m, 1H), 3.26 (s, 1H), 2.42−2.35 (m, 1H), 2.05−1.95 (m, 1H); ^{13}C NMR (DMSO-d_6) 156.6, 152.0, 150.5, 143.3, 135.5, 132.3, 127.8, 126.7, 126.3, 121.0, 120.3, 118.8, 117.3, 87.5, 82.2, 70.7, 61.6, 55.9; FAB-HRMS (nba) m/z [M + H$^+$] calcd for $C_{20}H_{21}N_6O_4$ 409.1624, found 409.1577.

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Supporting Information Available: General experimental information; ^{1}H NMR and ^{13}C NMR for compounds **11a**,**b**, **12a**,**c**−**e**,**g**, **13c**, **14c**, **15c**, **16c**, **3c**,**e**, and **19c**; and ^{31}P spectra for **13c** and **19c**; mass spectra of **17** and **18c**; HPLC profiles of the coupling reaction of **17** yielding **18c** and enzymatic hydrolysates of **17** and **18c** and nitro reduction of **21** yielding **22c** and enzymatic hydrolysates of **21** and **22c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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