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Synthesis of oligonucleotides containing bulky adducts at guanine N^2 via the phosphoramidite of O^2-triflate-O^6-NPE 2′-deoxyxanthosine

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Abstract

Oligodeoxynucleotides bearing bulky adducts at guanine N^2 have been prepared by a postoligomerization strategy in which oligonucleotides containing a highly reactive O^2-trifluoromethanesulfonyl-O^6-(*p*-nitrophenethyl) 2′-deoxyxanthosine residue were reacted with (±)-10β-amino-7,8,9,10-tetrahydro-benzo[*a*]pyrene-7β,8α,9α-triol and related compounds.

Keywords: nucleic acids; nucleosides; polycyclic aromatic compounds; purines.

The synthesis of oligonucleotides bearing site- and stereospecific adducts of carcinogens has been an active field of investigation in recent years. Such adducted oligonucleotides are needed for biological and structural studies, the goal of which is understanding the basis of chemical carcinogenicity. Several synthetic strategies have been developed for the preparation of these oligonucleotides. The direct reaction of oligonucleotides with reactive metabolites such as aflatoxin B_1 epoxide[1] or benzo[*a*]pyrene diol epoxide (BPDE)[2] is the simplest and can be highly efficient in cases where there are a very limited number of target sites in the oligonucleotide, e.g. one or two guanines. In other cases the desired adduct can be prepared at the nucleoside stage and converted to phosphoramidite to be used in oligonucleotide synthesis[3–5] or a phosphoramidite containing a reactive substituent can be introduced into the oligonucleotide and the adduct introduced by a postoligomerization reaction.[6,7] We have exploited the third strategy successfully for a number of adducts; e.g. this approach works well for adenine adducts of BPDE if 6-fluoropurine nucleoside is used as the reactive electrophile in reaction with aminotriol **1** (prepared by opening of BPDE with ammonia).[8] However, progress in the synthesis of oligonucleotides containing adducts of bay-region diol epoxides at guanine N^2 has not moved as fast. In particular, the problem of preparing NMR quantities of oligonucleotides containing such adducts in sequences containing multiple guanines has not been fully solved. Initially, we explored the use of 2-fluoro-2′-deoxyinosine (or an O^6-protected derivative), but had difficulty achieving reproducible

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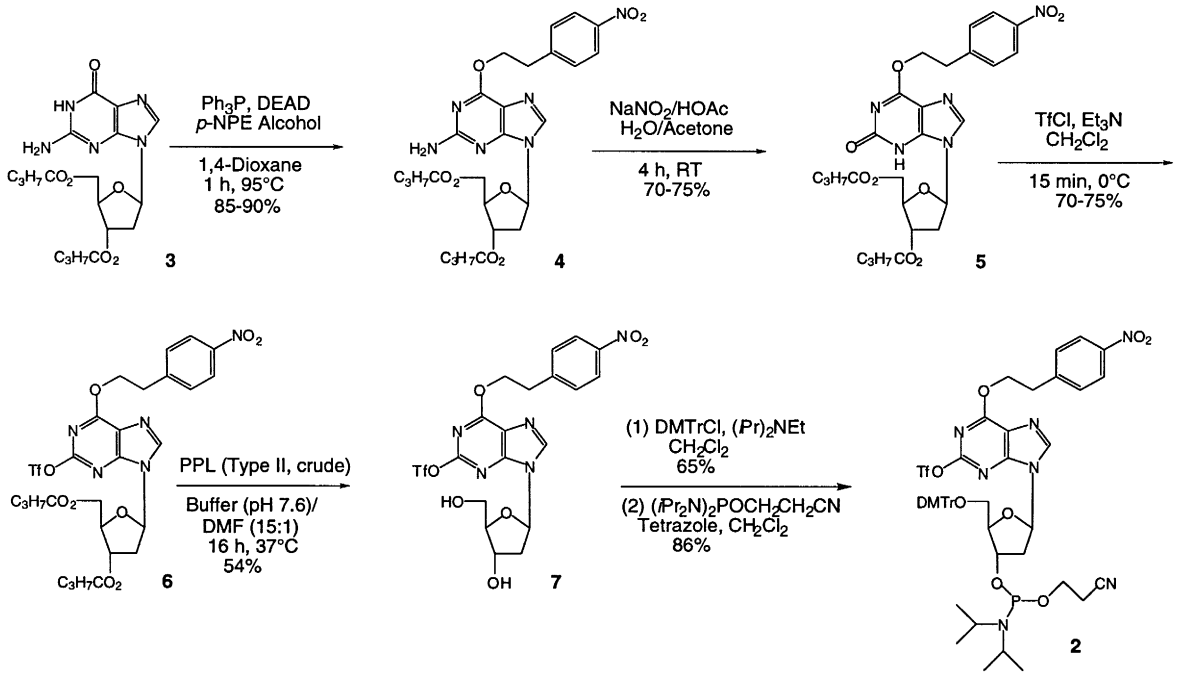
results with **1**. Reaction at the nucleoside level proceeded satisfactorily but postoligomerization reaction required more vigorous reaction conditions (80°C, 9 days) than we cared to use. The successful use of 2-fluoro-2′-deoxyinosine in the synthesis of several BPDE-adducted oligonucleotides has recently been reported, although the yield varied widely with the sequence.[4,9] We chose to explore the obvious approach of using a more reactive leaving group at C-2. Steinbrecher et al. have shown that O^6-protected 2′-deoxyxanthosine with a triflate at the 2 position is more reactive than the corresponding 2-fluoro compound and have synthesized a wide variety of N^2-substituted guanine derivatives using this approach.[10,11] Although their yield in the reaction of the triflate with the tri-*O*-acetyl derivative of aminotriol **1** was very low (5%), we felt that it would be worthwhile to look further at the reactivity of the triflate. We now report the successful synthesis of the phosphoramidite of O^2-triflate-O^6-(*p*-nitrophenethyl) 2′-deoxyxanthosine (**2**), the use of **2** in the synthesis of oligonucleotides containing multiple guanines and postoligomerization reactions of these oligonucleotides with hindered aminotriol **1** and related compounds.

The triflate phosphoramidite was prepared from 3′,5′-*O*-dibutyryl[12] (or the diacetyl analog) 2′-deoxyguanosine (**3**) (Scheme 1) which was protected at the O^6 position by Mitsunobu alkylation with *p*-nitrophenethyl alcohol.[13–15] Conversion of protected 2′-deoxyguanosine **4** to deoxyxanthosine **5** was initially attempted with pentyl or *t*-butyl nitrite[10] but the aqueous diazotization procedure of Eritja[16] was found to be more reproducible. Protected **5** was converted to 2-triflate **6** with trifluoromethanesulfonyl chloride and Et_3N[10] in 70–75% yield. Removal of the acyl groups was accomplished with porcine pancreatic lipase (PPL)[17] to give **7** in 54% yield (75–80%, based on recovered starting material); the dibutyrate was more satisfactory than the diacetate for this step, being a better substrate for PPL. Conversion of **7** to phosphoramidite **2** was carried out by the usual methodology.[18–21]

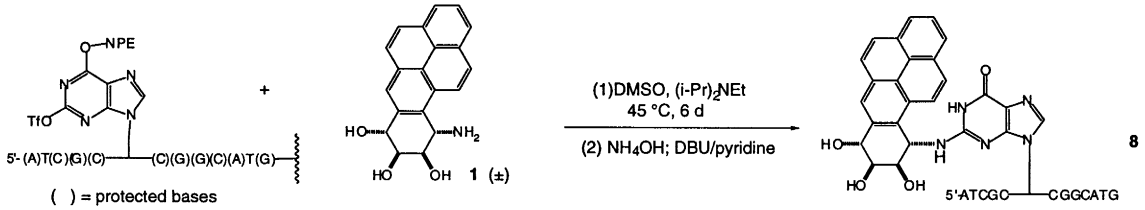


Scheme 1.

Before continuing with oligonucleotide synthesis a brief NMR study (^1H and ^{19}F) was done comparing the reactivity of aminotriol **1** with triflate **7** and the analogous 2-fluoro compound which clearly showed that the triflate was more reactive than the 2-fluoro compound under the same experimental conditions (DMSO, $(i\text{-Pr})_2NEt$, 45°C). After 7 days, **7** had almost completely disappeared (with concomitant forma-

tion of product) whereas the 2-fluoro compound was largely unreacted. The stability of phosphoramidite **2** toward the DNA synthesizer reagents (3% TCA/CH_2Cl_2, tetrazole, I_2/pyridine, *t*-BuPAC anhydride/1-Me imidazole) was also examined and appeared to be satisfactory within the time frame for 1-μmol syntheses of reasonably short oligonucleotides (11–16-mers).

Phosphoramidite **2** was used for the synthesis of a 13-mer, 5′-ATCGC-*X*-CGGCATG-3′ (**8**, Scheme 2). Normal automated synthesis conditions were employed (PerSeptive Biosystems Model 8909 DNA synthesizer, *t*-BuPAC amidites) except that a long coupling (15 min) cycle was used for introduction of **2**. Coupling yields of ~80% were seen for introduction of **2**, based on trityl monitoring and recovered failure sequences. Manual coupling was also explored for this step. It did not give improved yields nor did extending the machine coupling time to 60 min. The matrix-bound protected oligonucleotide was reacted with racemic aminotriol **1** in anhydrous DMSO containing $(i\text{-Pr})_2$NEt for 6 days at 45°C. Following deprotection (concd NH_4OH, 60°C, 1 h, then DBU/pyridine, rt, 1–3 days), oligonucleotide **8** was passed through a Sephadex G-25 column and purified by HPLC on a phenylhexyl column (Phenomenex, Torrance, CA; 0.1 M triethylammonium acetate, pH 7.0/MeOH). The oligonucleotides containing the diastereomeric adducts were easily separated with the 7*S*,8*R*,9*S*,10*R* isomer eluting first. The oligomers were characterized by capillary gel electrophoresis, enzymatic hydrolysis[8] and mass spectroscopy (negative-ion MALDI-TOF, calculated for $(M–H)^-$: 4275.6; found for the (7*S*,8*R*,9*S*,10*R*) isomer, 4276.0; (7*R*,8*S*,9*R*,10*S*) isomer, 4275.5). Stereochemistry of adducts was assigned by CD spectroscopy and by HPLC comparison of adducted nucleosides in enzymatic digests with adducted nucleoside standards of known stereochemistry. 11-Mers, including the G-rich *N-ras* 12 sequence, 5′-GGCAG*G*TGGTG-3′ adducted at the underlined position with aminotriol **1** or the analogous aminotriols derived from the bay-region (BRBA) and non-bay-region (nBRBA) *anti*-diol epoxides of benz[*a*]anthracene[22] were prepared similarly, except that a first HPLC purification was carried out on NPE-protected product for the BPDE and nBRBA sequences. Typical yields of purified oligomers ranged from 6 A_{260} units/diastereomer/1 μmol cassette for nBRBA to 4–5 A_{260} units/diastereoisomer for the benzo[*a*]pyrene-adducted oligonucleotides. The major byproducts seen in the reactions were failure sequences and full-length sequence containing 2′-deoxyxanthosine from hydrolysis of the triflate (which occurred primarily during treatment with NH_4OH during workup). Structural and biological studies are now in progress with these oligonucleotides.



Scheme 2.

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17. To compound **6** (5 g, 7.2 mmol) in DMF (100 mL) was added 0.05 M sodium phosphate buffer (1500 mL), pH 7.6, containing 0.01 M NaCl and 0.001 M $CaCl_2$. PPL (100 g, Sigma L-3126) was added in portions with stirring. The suspension was heated at 37°C for 16–18 h. The suspension was centrifuged and compound **7** was extracted from the precipitate with MeOH. TLC (CH_2Cl_2:MeOH, 9:1) R_f **6**, 0.95; **7**, 0.35; monobutyrates, 0.80, 0.85; deacylated **5**, 0.2.
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21. Phosphoramidite **2**: ^{1}H NMR (CH_3CN-d_3) δ 8.22 (s, 1H, H8), 8.14 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 7.54 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 7.34–7.30 (m, 2H, DMTr), 7.23–7.16 (m, 7H, DMTr), 6.77–6.69 (m, 4H, DMTr), 6.35 (t, 1H, *J*=6.8 Hz, H1′), 4.84 (t, 2H, *J*=6.6 Hz, C*H*$_2$CH$_2$PhNO$_2$), 4.76 (m, 1H, H3′), 4.20 (m, 1H, H4′), 3.80–3.58 (m, 8H, DMTr, C*H*$(CH_3)_2$), 3.33–3.20 (m, 4H, CH_2C*H*$_2$PhNO$_2$, H5′, H5′′), 2.67–2.60 (m, 1H, H2′), 2.53–2.43 (m, 5H, C*H*$_2$C*H*$_2$CN, H2′′), 1.16 (m, 12H, CH(C*H*$_3$)$_2$). ^{19}F NMR (CH_3CN-d_3) δ −72.91. ^{31}P NMR (CH_3CN-d_3) δ 150.19, 150.11. HRMS-FAB$^+$ (NBA) calcd for $C_{49}H_{53}F_3N_7O_{12}PS$ (M+H)$^+$ 1052.3241, found 1052.3220. Compound **4**: ^{1}H NMR: ($CDCl_3$) δ 8.12 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 7.74 (s, 1H, H8), 7.45 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 6.26 (dd, 1H, *J*=8.0+6.0, H1′), 4.93 (br s, 2H, NH_2), 5.39 (dt, 1H, *J*=6.2+2.4 Hz, H3′), 4.69 (t, 2H, *J*=6.8 Hz, C*H*$_2$CH$_2$PhNO$_2$), 4.43 (dd, 1H, *J*=11.5+4.3 Hz, H5′), 4.34 (dd, 1H, J=16.6+4.8 Hz, H5′′), 4.31 (ddd, 1H, *J*=6.8+4.6+2.4 Hz, H4′), 3.24 (t, 2H, *J*=7.0 Hz, CH_2C*H*$_2$PhNO$_2$), 2.94 (ddd, 1H, *J*=14.2+8.0+6.3 Hz, H2′), 2.49 (ddd, 1H, *J*=14.2+6.0+2.4 Hz, H2′′), 2.33 (t, 2H, *J*=7.4 Hz, CH_2), 2.29 (t, 2H, *J*=7.4 Hz, CH_2), 1.68 (p, 2H, *J*=7.4 Hz, CH_2), 1.60 (p, 2H, *J*=7.4 Hz, CH_2), 0.95 (t, 3H, *J*=7.5 Hz, CH_3), 0.90 (t, 3H, *J*=7.5 Hz, CH_3). HRMS-FAB$^+$ (NBA) calcd for $C_{26}H_{33}O_8N_6$ 557.2360 (M+H)$^+$, found 557.2344. Compound **5**: ^{1}H NMR: ($CDCl_3$) δ 8.20 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 7.96 (s, 1H, H8), 7.53 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 6.38 (t, 1H, *J*=7.0 Hz, H1′), 5.33 (p, 1H, *J*=3.0 Hz, H3′), 4.81 (t, 2H, *J*=6.8 Hz, C*H*$_2$CH$_2$PhNO$_2$), 4.39 (m, 3H, H4′, H5′, H5′′), 3.26 (t, 2H, *J*=6.8 Hz, CH_2C*H*$_2$PhNO$_2$), 2.65 (m, 2H, H2′, H2′′), 2.34 (m, 4H, 2×CH_2), 1.65 (septet, 4H, *J*=7.3 Hz, 2×CH_2), 0.94 (q, 6H, *J*=7.3 Hz, 2×CH_3). HRMS-FAB$^+$ (NBA) calcd for $C_{26}H_{33}O_9N_5$ (M+H)$^+$ 558.2199, found 558.2200. Compound **6**: ^{1}H NMR: ($CDCl_3$) δ 8.21 (s, 1H, H8), 8.18 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 7.49 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 6.38 (dd, 1H, *J*=7.7 Hz, 6.0 Hz, H1′), 5.39 (dt, 1H, *J*=6.2+2.5 Hz, H3′), 4.85 (t, 2H, *J*=6.8 Hz, CH_2C*H*$_2$PhNO$_2$), 4.36 (m, 3H, H4′, H5′, H5′′), 3.32 (t, 2H, *J*=6.8 Hz, C*H*$_2$CH$_2$PhNO$_2$), 2.83 (ddd, 1H, *J*=14.2+7.7+6.4 Hz, H2′), 2.65 (ddd, 1H, *J*=14.1+5.9+2.7 Hz, H2′′), 2.37 (t, 2H, *J*=7.5 Hz, CH_2), 2.30 (t, 2H, *J*=7.5 Hz, CH_2), 1.70 (p, 2H, *J*=7.5 Hz, CH_2), 1.64 (p, 2H, *J*=7.5 Hz, CH_2), 0.98 (t, 3H, *J*=7.5 Hz, CH_3), 0.93 (t, 3H, *J*=7.5 Hz, CH_3). ^{19}F NMR: ($CDCl_3$) δ −73.21 (s). HRMS-FAB$^+$ (NBA) calcd for $C_{27}H_{31}O_{11}N_5F_3S$ (M+H)$^+$ 690.1693, found 690.1718. Compound **7**: ^{1}H NMR (DMSO-d_6) δ 8.71 (s, 1H, H8), 8.17 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 7.62 (d, 2H, *J*=8.7 Hz, $PhNO_2$), 6.32 (t, 1H, *J*=6.5 Hz, H1′), 5.37 (d, 1H, *J*=4.4 Hz, 3′-OH, D_2O-exch), 4.92 (t, 1H, *J*=5.4 Hz, 5′-OH, D_2O-exch), 4.84 (t, 2H, *J*=6.5 Hz, C*H*$_2$CH$_2$PhNO$_2$), 4.38 (m, 1H, H3′), 3.86 (m, 1H, H4′), 3.57–3.46 (m, 2H, H5′, H5′′), (CH_2C*H*$_2$PhNO$_2$ under H_2O), 2.68 (ddd, 1H, *J*=13.1+6.3+6.3 Hz, H2′), 2.35 (ddd, 1H, *J*=14.1+6.3+3.8 Hz, H2′′). ^{19}F NMR (DMSO-d_6) δ −72.45 (s). LRMS-FAB$^+$ (glycerol/TFA/DMSO) calcd for $C_{19}H_{19}F_3N_5O_9S$ (M+H)$^+$ 550.4, found 550.1, 572.1 (M+Na)$^+$, 434.0 (NPE-Xan-OTf+H)$^+$. HRMS-FAB$^+$ (glycerol/TFA/DMSO) calcd for $C_{19}H_{19}F_3N_5O_9S$ 550.0855 (M+H)$^+$, found 550.0867.
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