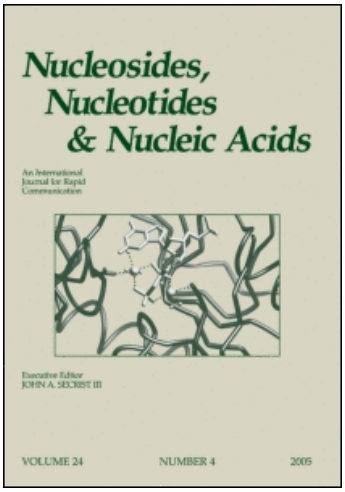

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AN IMPROVED METHOD FOR THE PREPARATION OF THE PHOSPHORAMIDITES OF MODIFIED 2′-DEOXYNUCLEOTIDES: INCORPORATION OF 8-OXO-2′-DEOXY-7*H*-GUANOSINE INTO SYNTHETIC OLIGOMERS

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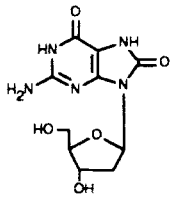
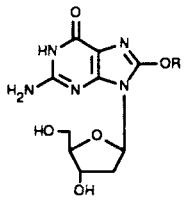
Abstract: 3′-O-(Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-O-(4,4′-dimethoxytrityl)-N^2-isobutyryl-8-oxo-2′-deoxy-7*H*-guanosine (7) was synthesized and used for the introduction of an 8-oxo-2′-deoxy-7*H*-guanosine residue into a DNA oligomer by means of automated synthesis. A modification of the preparation of the phosphoramidite markedly improves the coupling efficiency in the oligomer synthesis in this and several other cases that were tested.

The incorporation of 8-oxo-2′-deoxy-7*H*-nucleosides[1] into DNA oligomers is important for studies on genetic toxicity. Such modified bases are known to occur in DNA that has been exposed to certain carcinogenic substances or to ionizing radiation.[2-6] The biological role of the 8-oxo-2′-deoxy-7*H*-guanosine lesion has been studied by Kuchino *et al.*[7] who reported that DNA templates containing this base are misread by the Klenow fragment of *E. coli* DNA polymerase I both at the altered site and at adjacent residues. Their method of introducing this lesion into DNA oligomers involved the automated incorporation of an 8-methoxy-2′-deoxyguanosine (2) residue followed by post-synthetic conversion of the methoxy group to the desired oxo substituent. They accomplished the latter transformation by treatment with triethylamine in aqueous N, N-dimethylformamide after the general ammonia deprotection step. However, in our studies with 8-methoxy-2′-deoxyguanosine (2)

itself, neither of these steps proved effective in generating the corresponding 8-oxo-2′-deoxy-7*H*-guanosine (**1**). Equally ineffective was the use of aqueous $Et_3N/Et_3N{\cdot}HCl$. Thus, we approached the problem of incorporation of the 8-oxo-2′-deoxy-7*H*-guanosine residue into DNA by a different procedure.



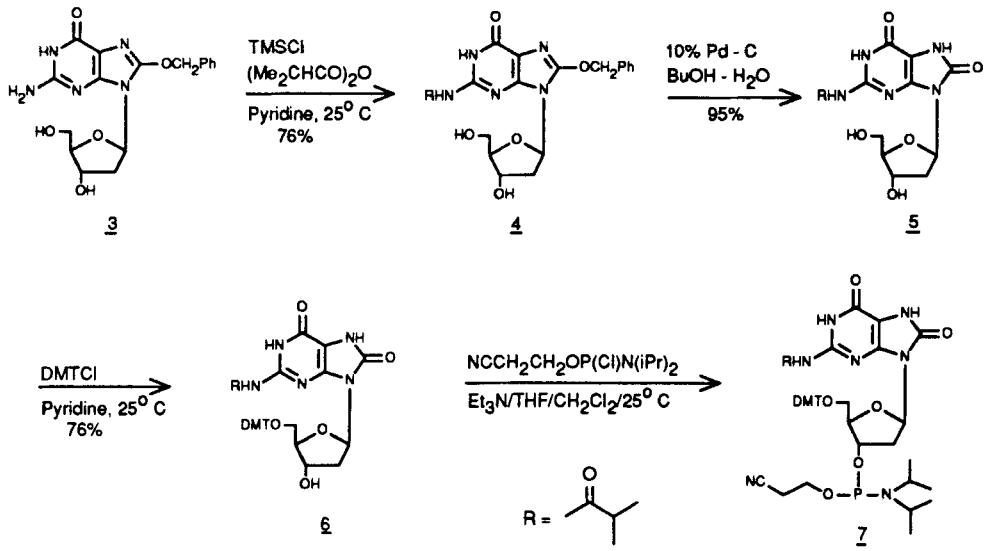
1



2 R = CH_3
3 R = CH_2Ph

Recognizing that the 8-oxo group of **1** is part of a urea system it seemed unlikely that any protection of the oxygen atom (cf. thymidine) was necessary during oligomer synthesis. Therefore, we synthesized the desired intermediate **7** from **3** according to the *Scheme 1*. The required benzyloxy derivative **3** was prepared according to the literature[8, 9] and then converted to **4** using the transient protection method.[10] On catalytic hydrogenation **4** led to **5**, which in turn was converted to the 4,4′-dimethoxytrityl (DMT) derivative **6**.[11] Although **6**[12] could be converted to the desired phosphoramidite **7** (88%, crude yield) using the standard procedure[13 - 16] (including an aqueous work-up), the product proved difficult to purify.[17] Interestingly, despite the knowledge that the phosphoramidites of 2′-deoxy-nucleosides undergo partial hydrolysis (5-20%) during aqueous work-up, this method of phosphoramidite isolation seems to have persisted in the literature.[15] In our case, attempted chromatographic purification of crude **7** on silica gel led to very substantial decomposition. An alternate method of phosphoramidite purification,[16, 18] namely precipitation from hexane at -78° C, when applied to **7** gave a material that resulted in rather poor coupling efficiency (60%) during oligomer synthesis. We have now found that when the phosphoramidites are prepared under completely anhydrous conditions, excellent coupling yields can be achieved. The following procedure for the preparation of anhydrous **7** is typical of the general method.[19]

Experimental Procedure: To a mixture of 5′-O-(4, 4′-dimethoxytrityl)-N^2-isobutyryl-8-oxo-2′-deoxy-7*H*-guanosine (**6**) (0.58 g, 0.88 mmol, dried rigorously over P_2O_5 *in*

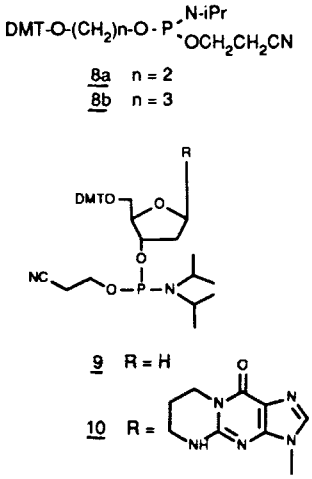


Scheme 1

vacuo for three days) and triethylamine (0.28 mL, 2.0 mmol, dried over NaH and then distilled from Na under nitrogen prior to reaction) in dry tetrahydrofuran (THF)-methylene chloride (5mL; 1:1) under nitrogen was added 2-cyanoethyl N,N-diisopropyl chlorophosphoramidite (American Bionetics) (0.23 g, 0.97 mmol). The progress of the reaction was monitored by thin layer chromatography (TLC) and no starting material was observed after 30 minutes. The mixture was then filtered under nitrogen atmosphere to remove triethylamine hydrochloride which was washed once with dry THF. The combined filtrates were evaporated to dryness at reduced pressure, and the residual viscous material was triturated with dry benzene. The solution was filtered to remove residual traces of $Et_3N{\cdot}HCl$. The filtrate was evaporated to dryness, and the process repeated. The high purity of the resulting glassy product (7) was evident from the ^{31}P NMR ($CDCl_3$) spectrum which showed peaks only for the two diastereomers in 1:1 ratio at δ 146.91 and δ 146.76 ppm [chemical shifts are reported relative to an external standard of $P(OCH_3)_3$]; FAB/MS (-ve ion, thioglycerol matrix): m/z 854 [$(M-H)^-$, 12%], 654 (loss of phosphoramidite, 14%), 236 [(Heterocyclic base-H)$^-$, 7%].

When this method was used for the synthesis of a series of known phosphoramidites (8-10) derived respectively from ethylene and propylene glycols,[20]

the abasic site model[20, 21] and the acrolein adduct model,[22] they were obtained in essentially quantitative yield. No deterioration of these substances could be observed when stored under dry nitrogen at -20°C, and even after four months coupling yields were reproducible. In fact, in automated syntheses of DNA oligomers these phosphoramidites could not be distinguished, in terms of coupling efficiency (>98%), from the standard commercially available phosphoramidites derived from the normal DNA nucleosides. However, it should be noted that in common with uric acid and 8-aminoaryl guanine derivatives, 8-oxo-2′-deoxy-7*H*-guanosine (1) is very sensitive to oxygen under alkaline conditions.[23] During ammonia deprotection of oligomers containing this residue, it is therefore necessary to add an antioxidant such as 2-mercaptoethanol to prevent degradation and strand cleavage. A study of the oxidation of 1 in alkali will be reported later.



In conclusion, the phosphoramidites of abnormal nucleoside derivatives and related compounds are best prepared under scrupulously dry conditions if high coupling yields are to be realized when they are used in the automated synthesis of oligomeric DNA. The use of this procedure allows 8-oxo-2′-deoxy-7*H*-guanosine to be incorporated directly into oligomeric DNA with high efficiency.

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of oligo-2′-deoxynucleotides. We would also like to thank Professor A.P. Grollman and Dr. S. Shibutani for their interest in this work.

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1. Strictly speaking these compounds should be named as purine nucleoside derivatives. However, for convenience in referring to them we have elected to use the 8-oxo-2′-deoxy-7*H*-guanosine terminology.
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12. Compound **6**, like many of the 5′-O-DMT-2′-deoxynucleosides that we have synthesized appears to retain water tenaciously (perhaps as a partial hydrate) and needs rigorous drying before phosphoramidite preparation is attempted.
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19. Satisfactory physical data were obtained for all new substances described in this paper. Data for representative products are as follows.

(**4**). TLC (CH_2Cl_2/MeOH 4:1): R_f 0.72; m.p. 220° C; UV (MeOH): 293, 258; ^1H NMR (300 MHz, DMSO-d_6) δ 12.03 (1H, NH, *s*), 11.52 (1H, NH, *s*), 7.52 - 7.31 (5H, aryl, *m*), 6.17 (1H, H-C(1´), *t*, J = 7.05 Hz), 5.48 (2H, -CH_2-Ph, *s*), 5.20 (1H, HO-C(3´), *d*, J = 3.90 Hz), 4.70 (1H, HO-C(5´), *t*, J = 5.55 Hz), 4.26 (1H, H-C(3´), *m*), 3.72 (1H, H-C(4´), *m*), 3.44 - 3.38 (2H, H-C(5´), *m*), 2.90 (1H, H-C(2´), *m*), 2.76 (1H, -CH-CO-N, *m*), 2.06 (1H, H-C(2´), *m*), 1.12 (6H, C-$(Me)_2$, *d*, J = 6.90 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.67, 154.56, 153.29, 148.43, 147.84, 136.35, 129.31, 129.22, 129.07, 115.79, 88.20, 82.57, 71.98, 71.58, 62.78, 36.88, 35.53, 19.69. FAB/MS (+ve ion, thioglycerol matrix): m/z 444 [$(M+H)^+$, 7%], 328 [(Heterocyclic base+2H)$^+$, 9%], 274 (17%), 232 (51%), 181 (44%).

(**5**). TLC (CH_2Cl_2/MeOH 4:1): R_f 0.56; m.p. 230° C (dec.); UV (MeOH): 301, 265; ^1H NMR (300 MHz, DMSO-d_6) δ 12.14 (1H, NH, *s*), 11.59 (1H, NH, *s*), 11.28 (1H, NH, *s*), 6.09 (1H, H-C(1´), *t*, J = 6.60 Hz), 5.17 (1H, HO-C(3´), *d*, J = 2.40 Hz), 4.73 (1H, HO-C(5´), *m*), 4.37 (1H, H-C(3´), *m*), 3.75 (1H, H-C(4´), *m*), 3.59 - 3.44 (2H, H-C(5´), *m*), 3.07 (1H, H-C(2´), *m*), 2.74 (1H, -CH-CO-N, *m*), 1.98 (1H, H-C(2´), *m*), 1.11 (6H, C-$(Me)_2$, *d*, J = 8.10 Hz). FAB/MS (+ve ion, thioglycerol matrix): m/z 354 [$(M+H)^+$, 8%], 238 [(Heterocyclic base+2H)$^+$, 15%].

(**6**). TLC (CH_2Cl_2/MeOH 4:1): R_f 0.78; m.p. 164 - 166° C; UV (MeOH): 302, 265, 201; ^1H NMR (300 MHz, DMSO-d_6) δ 12.13 (1H, NH, *s*), 11.43 (1H, NH, *s*), 11.24 (1H, NH, *s*), 7.34 - 6.76 (13H, aryl, *m*), 6.14 (1H, H-C(1´), *t*, J = 6.05 Hz), 5.16 (1H, HO-C(3´), *d*, J = 4.50 Hz), 4.44 (1H, H-C(3´), *m*), 3.91 (1H, H-C(4´), *m*), 3.72 and 3.71 (6H, OCH_3, 2*s*), 3.32 and 3.06 (2H, H-C(5´), 2*m*), 2.98 (1H, H-C(2´), *m*), 2.74 (1H, -CH-CO-N, *m*), 2.12 (1H,

H-C(2′), *m*), 1.12 (6H, C-(Me)$_2$, *d*, J = 6.60 Hz). FAB/MS (-ve ion, thioglycerol matrix): m/z 654 [(M-H)$^-$, 100%], 584 (28%), 352 (12%), 236 [(Heterocyclic base-H)$^-$, 30%].

(7). TLC (CH_2Cl_2/MeOH 8:1): R_f 0.73; ^{1}H NMR (300 MHz, $CDCl_3$): two diastereomers, δ 11.75 - 11.13 (3H, 3NH, *br s*), 7.34 - 6.73 (13H, aryl, *m*), 6.12 (1H, H-C(1′), 2*t*, two diastereomers), 4.61 (1H, H-C(3′), *m*), 4.03 (3H, H-C(4′) and -O-CH_2-C, *m*), 3.71 (6H, OCH_3, *s*), 3.45 (2H, -N-CH(iPr)$_2$, *m*), 3.34 - 3.92 (2H, H-C(5′), *m*), 2.76 (2H, -CH_2-CN, *t*, J = 6.0 Hz), 2.63 (1H, -CH-CO-N, *m*), 2.31 (2H, H-C(2′), *m*), 1.26 (12H, -CH_3 of (iPr)$_2$, *dd*), 1.15 (6H, -CO-C(Me)$_2$, *dd*). ^{31}P NMR (121 MHz, $CDCl_3$) δ 146.63, 146.50 (two diastereomers). FAB/MS (-ve ion, thioglycerol matrix): m/z 854 [(M-H)$^-$, 12%], 654 (14%, loss of phosphoramidite), 236 [(Heterocyclic base-H)$^-$, 7%].

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