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TRICYCLIC "OXADIAZAPHOSPHORINE OXIDE" GUANOSINES: A NEW STRATEGY TO THE PROTECTION OF GUANINE BASES DURING RNA SYNTHESIS

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Abstract: A novel strategy for simultaneously protecting the lactam and N2-amino functions of guanine nucleosides is reported. N2-benzoylated guanosines were readily converted into tricyclic "oxadiazaphosphorine oxide" guanosines upon reaction with N,N-diisopropylphosphoramidous dichloride followed by oxidation with t-butyl hydroperoxide. The tricyclic guanine derivative is reconverted readily to the natural guanine aglycone upon treatment with ethylenediamine/ethanol (1:4, 1 min, r.t.).

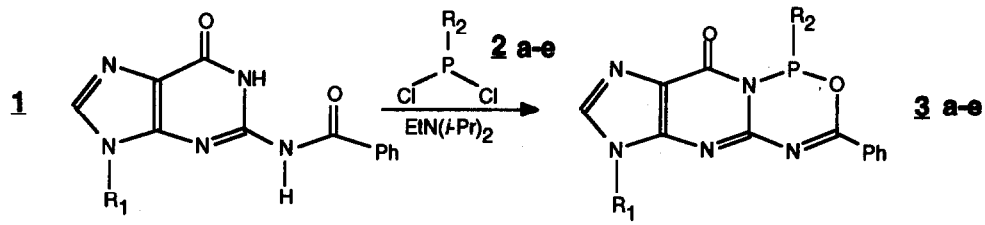
Guanine bases are notoriously susceptible to reaction at the 6-oxygen with a variety of sulfonylating, phosphorylating, phosphitylating, and acylating reagents.[1] Precautionary measures which reverse these side reactions during oligonucleotide synthesis may minimize, but cannot completely eliminate these modifications.[2] This is particularly true in the synthesis of long oligomers where repeated exposure of the guanine residues to coupling reagents leads to an increase in base modification with increasing chain length. To avoid side-reactions at guanine residues during synthesis of oligonucleotides, several protecting groups for the O6-position of guanine have been developed. To date, substituted ethyl groups which are cleaved by β-elimination reactions are the most popular, *e.g.*, the p-nitrophenylethyl[3] and β-cyanoethyl groups[4] are the most widely used.

In this report we describe the synthesis of tricyclic guanosine derivatives containing a novel oxadiazaphosphorine oxide ring and demonstrate their usefulness as "O6-N1, N2"-protected guanosines in the solid-phase synthesis of oligoribonucleotides. The strength of these derivatives is their stability to general conditions used in current methods for RNA synthesis, yet their extremely rapid transformation into natural guanosine at ambient temperature.

Certain guanosines acylated at N2 (*e.g.*, **1**) were reported by Damha and Ogilvie to react rapidly with N,N-diisopropylphosphoramidous dichloride (**2a**) and N,N-diisopropylethylamine to produce tricyclic guanosine derivatives in high yields (Scheme 1).[5] Specifically, guanosine **3a**, obtained from **1** in 96% yield, was carefully and thoroughly characterized (UV, ^{31}P, ^{13}C, ^{15}N-NMR; properties, Table 1). This derivative served as a "protected" guanosine since it reconverted readily to **1** upon treatment with 3% trichloroacetic acid/chloroform (20 °C). While such protection may be of some utility for guanine nucleosides, it is unlikely that it could be used for oligonucleotide synthesis in which cyclic removal of trityl protecting groups with trichloroacetic acid is used during the course of the synthesis. Analogous tricyclic derivatives obtained from N2-isobutyryl and N2-acetylguanosine were even less stable toward the acidic treatment.

As a means to evaluate the properties of this new class of guanosine analogues, we prepared a variety of other oxadiazaphosphorines. Generally, variation in the tricyclic structure was introduced by changing the phosphitylating reagent (Scheme 1). Among the derivatives **3b-e**, the tetramethylpiperidyl derivative **3b** was the only derivative which was stable for chromatography on silica gel (48% yield from **1**; properties, Table 1). Its stability to trichloroacetic acid/chloroform solutions, however, was about the same as found for **3a** (3% TCA, $t_{1/2}$ *ca.* 1 min, 20 °C). Derivatives **3c**, **3d**, and **3e** were extremely sensitive to water and

SCHEME 1



their isolation was unsuccessful. Nevertheless, they could be detected and characterized by monitoring the course of the phosphitylation reactions by NMR (Table 1). For example, when **2e** (14 μL, 0.12 mmol) was added to a solution of nucleoside **1** (0.10 mmol) and N,N-diisopropylethylamine (0.75 mmol) in dry deuterated chloroform (0.30 mL), ^{31}P-NMR showed complete reaction within 2 min at ambient temperature. The spectrum displayed no traces of unreacted **2e** (181.4 ppm) but only two signals at 97.7 and 97.3 ppm in a ratio of 1:1. These chemical shifts are characteristic of oxadiazaphosphorine derivatives (Table 1) and have been tentatively assigned as the diastereomeric derivative **3e**. Support for this assignment has been provided by ^{13}C-NMR [**1**, -HN2^{13}COPh, δ 167.1 ppm. **3e**, -N2=^{13}C(Ph)-O-^{31}P-, **isomer one**: δ 157.7 ppm, $^{2}J^{13}C$-^{31}P= 7.0 Hz ; **isomer two**: δ 157.6 ppm, $^{2}J^{13}C$-^{31}P= 7.0 Hz). Addition of methanol (10 μL, 25 mmol) into the NMR tube resulted in the immediate and quantitative conversion of the phosphorus species at *ca.* 97 ppm into trimethylphosphite (141.2 ppm, a product of the -N1-P(OMe)-O-C=N2- group reacting with methanol). Analysis of the resulting mixture by TLC showed the presence of only one UV-absorbing species identified as the starting material **1**. The lability of derivatives **3d** and **3e** explains why such modified guanine compounds have never been isolated from earlier chlorophosphite syntheses. Furthermore, the reaction of N2-acylated guanine bases with alkylphosphorodichloridites may account for the low yields of guanine-rich oligonucleotides obtained in previous phosphorodichloridite syntheses.[6] To the best of our knowledge, these tricyclic derivatives were first detected by Letsinger *et al.* but their structures were not elucidated.[7]

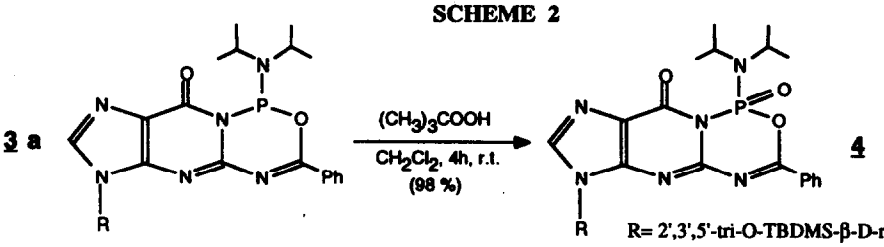
Oxidation of **3a** (1 mmol) by *t*-butyl hydroperoxide (40 mmol) in CH_2Cl_2 (1 h, r.t.) afforded oxadiazaphosphorine-P(V)-oxide derivative **4** as a yellow solid in 98% yield (Scheme 2, properties Table 1). Unlike **3a**, derivative **4** was stable to 3% trichloroacetic acid/dichloroethane treatment (r.t., 2 h). Furthermore, no detectable modification of **4** was observed (TLC, UV) when it was treated with the following reagents at r.t. for 16 h: (i) 0.1 M iodine in THF/pyridine/water (75:20:2, v/v/v); (ii) 5% acetic anhydride/ 5% 2,6-lutidine/ 8% N-methylimidazole in THF; (iii) 0.5 M tetrazole in acetonitrile; (iv) 0.5 M 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole/ 1 M N-methylimidazole in pyridine. Treatment of **4** with excess ethylenediamine (EDA)/ethanol (1:4, v/v) at room temperature, however, resulted in complete cleavage of the oxadiazaphosphorine oxide ring within 1 sec at 20 °C to give **6** in quantitative yield (Scheme 3). By comparison, N2-benzoyl-2',3',5'-tri-O-TBDMS-guanosine (**1**) and N2-(dimethylamino)methylene-2',3',5'-tri-O-TBDMS-guanosine were transformed into **6** by 1:4 EDA/ethanol with a half-life of *ca.* 7 h and 10 min, respectively.

TABLE 1. PROPERTIES OF TRICYCLIC OXAPHOSPHORINE GUANOSINES

Compound	λ max, nm (95% EtOH)	^{31}P-NMR ($CDCl_3$)	FAB-MS MH^+, $(B+2H)^+$	R_f (TLC) silica gel
1	237, 258, 265, 296	-	730, 256	0.50[a]
3a	262, 344, 356, 328sh, 377sh, 408sh	93.7, 93.5	859, 385	0.83[a]
3b	260, 340, 360, 329sh, 380sh, 408sh	114.2, 113.6	899, 425	0.45, 0.41[b]
3c	c	80.0, 79.9	c	c
3d	c	101.8, 101.0	c	c
3e	c	97.7, 97.3	c	c
4	263, 343, 360, 326sh, 382sh, 407sh	-7.4	875, 401	0.70, 0.65[a]
6	256, 275sh	-	626, 152	0.04[a]
9	234, 259, 265, 298	-	774, 256	0.32[a]
10	276, 344, 360, 326sh, 380sh, 406sh	-8.1, -8.3	918, 401	0.63, 0.54[a]
11	273, 344, 361, 327sh, 382sh, 408sh	152.3, 152.0, 150.3, 149.5 -7.32, -7.37, -7.42, -7.44	1016, 401	0.63, 0.54[a]

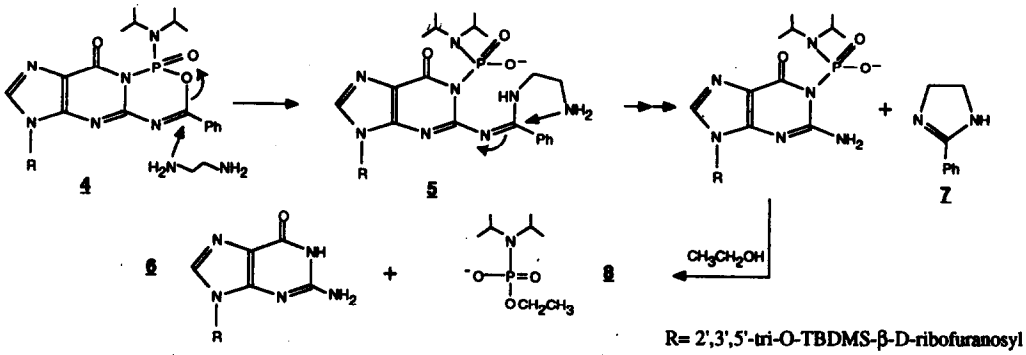
a. 3:7 ether/CH_2Cl_2; b. 1:11 ether/CH_2Cl_2; c. decomposes upon work-up (see text).

SCHEME 2



At least two factors appear to be responsible for the extremely fast and quantitative conversion of the tricyclic oxadiazaphosphorine-P(V)-oxide system into the natural guanine aglycone: (a) the facile cleavage of cyclic imidate esters (in our case, a cyclic imido-phosphorodiamidate) by nitrogen nucleophiles,[8] and (b) the entropically favoured cyclization reaction of the resulting benzamidine intermediate **5** (Scheme 3). The identification of 2-phenylimidazoline (**7**) and phosphoramidate **8** by NMR and FAB-MS substantiates our proposed mechanism.

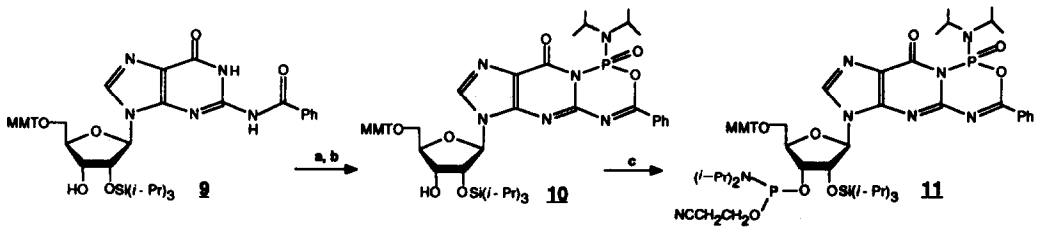
SCHEME 3



To determine the applicability of the oxadiazaphosphorine oxide protecting group to RNA synthesis, the tridecamer r(AGAAGGGAGAGGG) (**12**) was prepared on a 1.0 μmol scale by the normal solid-phase protocol using rA^{Bz} monomer[9] and the tricyclic rG monomer **11** (preparation, Scheme 4). At the end of the synthesis, the LCAA-CPG beads were treated with 1:1 EDA/ethanol (12 h, r.t.) to simultaneously cleave

oxadiazaphosphorine oxide rings, benzoyl and β-cyanoethyl groups, and the oligomer from the support. Removal of the TBDMS groups with 1.0 M TBAF/THF (0.5 mL, r.t., 10 h) afforded 22 A(260) units of the crude oligomer. Conventional purification by polyacrylamide gel electrophoresis (PAGE) and reverse-phase

SCHEME 4



[a, $Cl_2PN(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, 10 min, r.t., 73%; b, t-BuOOH, CH_2Cl_2, 4h, r.t., 78%; c, $ClP(OCH_2CH_2CN)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NEt$, 5h, r.t., 83%]

on C18-SEP-PAK™ afforded the 13-mer, 6.8 A(260) units (31 %). For comparison, an RNA oligomer with the same sequence was prepared under the same conditions, except that rG^{iBu} monomer[9] was used instead of **11** (30 % yield). The two oligomers were the same, as shown by PAGE and thermal dissociation (Tm 77 °C, 800 mM NaCl) of complexes formed with a complementary oligoribonucleotide, r(CCCUCUCCCUUCU). In addition, enzymatic hydrolysis (snake venom phosphodiesterase and alkaline phosphatase) of the oligomer derived from tricyclic guanine bases afforded the ribonucleosides in the expected ratio.

The tridecamer r(UGUGUGUCUUUAU) (**13**) was also prepared on a 0.2 μmol scale using rG **11** and rA^{dmf}, rC^{iBu}, rU monomers.[10] In this case, the support was sequentially treated with 1:4 EDA/ethanol (10 min, r.t.), ethanol (2 min wash; r.t.), and 3:1 conc. NH_4OH/ethanol (12 h, r.t.). Evaporation of the ammoniacal solution, treatment with 1.0 M TBAF/THF (0.4 mL, r.t., 12 h), and purification of the oligomer as described above afforded **13** in 23% yield.

In summary, RNA oligomers assembled with G-oxaphosphorine oxide **11** in conjunction with standard ribonucleoside phosphoramidite reagents can be deprotected rapidly and under very mild conditions. This improved methodology not only eliminates reactions at guanine 6-oxygen and unwanted chain cleavage, but should also allow the synthesis of oligoribonucleotide analogues containing base-sensitive moieties.

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References

1. Beaucage, S. *Tetrahedron* **1992**, *48*, 2223-2311, and references therein.
2. Eadie, J.S.; Davidson, D.S. *Nucl. Acids Res.* **1987**, *15*, 8333-49.
3. Himmelsbach, F.; Schulz, B.S.; Trichtinger, T.; Charubala, R., Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59.
4. Gaffney, B.L.; Marky, L.A.; Jones, R.A. *Tetrahedron* **1984**, *40*, 3.
5. Damha, M.J.; Ogilvie, K.K. *J. Org. Chem.* **1986**, *51*, 3559-60.
6. Ogilvie, K.K.; Schifman, A.L.; Penney, C.L. *Can. J. Chem.* **1979**, *57*, 2230.
7. Letsinger, R.L.; Groody, E.P.; Lander, N.; Tanaka, T. Tetrahedron **1984**, *40*, footnote 13, pp. 143.
8. Patai, S. (ed.), *The Chemistry of Amidines and Imidates*, John Wiley and Sons, 1975.
9. Damha, M.J.; Ogilvie, K.K. *Oligoribonucleotide Synthesis - The Silyl-Phosphoramidite Method,* in *Synthetic Chemistry of Oligonucleotides and Analogs,* Agrawal, S. (ed.), The Humana Press, *in press.*
10. Vinayak; R. Anderson, P.; McCollum, C.; Hampel, A. *Nucl. Acids Res.* **1992**, *20*, 1265-69.

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