Tetrahedron Letters Vol. 21, pp 3899 - 3902 0040-4039/80/1001-3899$02.00/0


# NEW GUANOSINE DERIVATIVES: FACILE $O^6$-PHOSPHORYLATION, THIOPHOSPHINYLATION SULFONYLATION AND SILYLATION OF GUANOSINE DERIVATIVES BY 4-DIMETHYLAMINOPYRIDINE CATALIZED REACTION

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Summary: Appropriately protected guanosine derivatives were successfully converted to the corresponding $O^6$-substituted guanosine derivatives by treatment with dialkyl- or diaryl-phosphoryl halides, dialkyl- or diaryl-phosphinothioyl halides, arenesulfonyl chlorides, and trialkylsilyl chlorides.

Introduction of new methodologies into nucleotide chemistry has dramatically facilitated the chemical synthesis of defined oligonucleotides.[1] In fact, recent developments by use of arenesulfonylazoles and new protecting groups of nucleotidic functional groups have led to satisfactory results considerably on the aspects of the reaction time and the coupling yield of oligonucleotides. However, there have been still observed complicated side reactions during the chain elongation of oligomers. These side reactions are not clear even now but this problem has often been encountered, especially in the condensation between guanosine-containing fragments. In the ribo-series, these side reactions occur to an appreciable extent. A few years ago, Reese[2] reported that appropriately protected guanosine derivatives were unexpectedly sulfonylated with arenesulfonyl chlorides in pyridine to afford $O^6$-substituted guanosine derivatives. The result suggests strongly that during the elongation reactions of oligonucleotide chain unavoidable side reactions ofguanine residue might occur at the $O^6$-position. Though the $O^6$-phosphorylated speacies have been discussed as side products, they have never been characterized since they were thought to be too unstable to be isolated from the reaction mixture.

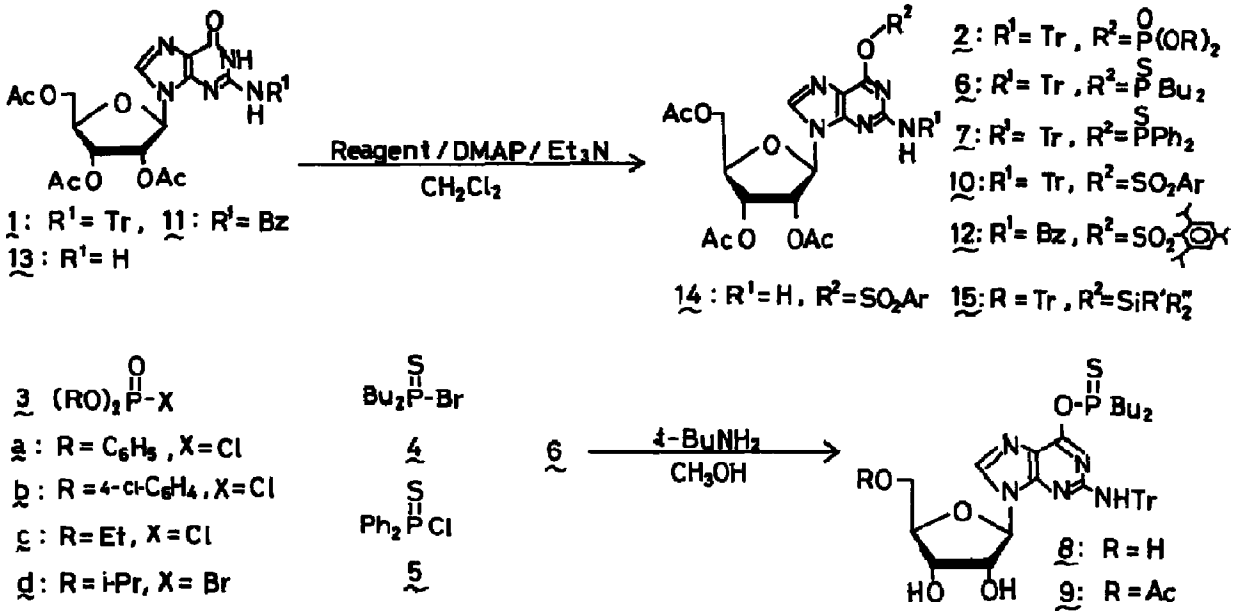
In this paper, we wish to report the isolation of $O^6$-phosphorylated guanosine derivatives and related electrophilic reactions such as sulfonylation and silylation by use of 4-dimethylaminopyridine known as a strongly effective catalyst for acylation reactions.[3,4]

In the present experiments, 2',3',5'-tri-O-acetyl-$N^2$-tritylguanosine (1) was chosen as a substrate. It was found that 1 was readily phosphorylated at the $O^6$-position under the conditions described below to give $O^6$-phosphorylated guanosine derivatives (2): Two mmol of 1 was treated with 2.94 mmol of an

approprite phosphorylating reagent (3a, 3b, 3c, or 3d) and 3.3 mmol of triethylamine in the presence of 0.2 mmol of 4-dimethylaminopyridine[3,4] in 30 mL of methylene chloride at room temperature for 12 h. After removal of the solvent in vacuo, the residue was chromatographed on silica gel (benzene/ethyl acetate) to afford the corresponding $O^6$-phosphorylated derivative (2a, 2b, or 2d) in 30-40% yield. The $O^6$-phosphorylated products were found to be essentially formed quantitatively by monitorization of the products on tlc. However, the partial decomposition of 2 took place during workup. In the case of 2c, it could be detected on the tlc as the single fluorescent spot, but it was too unstable to be purified by repeated silica-gel column chromatography. The bright blue fluorescence is characteristic of $O^6$-dialkylphosphorylated guanosine derivatives such as 2c and 2d, while 2a and 2b do not have such fluorescence. The structures of the isolated compounds were confirmed by their elemental analyses, ultraviolet spectra, and nmr spectra.[5] As one can expect the instability of the $O^6$-phosphorylated guanosine derivatives, they were quickly dephosphorylated by treatment with aqueous pyridine to give the parent nucleoside.

On the other hand, phosphinothioyl halides (4) and (5) were employed in place of the phosphoryl halides in order to introduce the phosphinothioyl groups into the $O^6$-position of guanosine. Consequently, the corresponding $O^6$-phosphinothioyl guanosine derivatives (6) and (7) were successfully isolated in 98% and 60% yields, respectively. These products were found to be stable during workup and also stable in aqueous pyridine for one day. Especially, the former product, 6, has a clean blue fluorescence and the dibutylphosphinothioyl group was relatively stable under the conditions for removal of acetyl groups. For example, when 1.59 mmol of 6 was treated with 16.4 mmol of t-butylamine in 12 mL of methanol at room temperature for 15 min, the completely deacetylated product (8) and 5'-O-monoacetyl product (9) were obtained in 50% and 10% yields, respectively. On the other hand, treatment of 6 with 60% formic acid gave quantitatively 2',3',5'-tri-O-acetylguanosine.

Furthermore, this DMAP/$Et_3N$/$CH_2Cl_2$ system could be also applied to the $O^6$-sulfonylation and silylation of guanosine derivatives. Sulfonylation of 1 with toluenesulfonyl chloride (TsCl), mesitylenesulfonyl chloride (MsCl), and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) occured very smoothly and cleanly under similar conditions to give the $O^6$-sulfonylated products (10) in 75-87% yields. The $O^6$-sulfonylation of $N^2$-benzoyl-2',3',5'-tri-O-acetylguanosine (11) with TPS also readily proceeded to give the corresponding $O^6$-sulfonylated product (12) in 78% yield. On the other hand, when $N^2$-unprotected 2',3',5'-tri-O-acetylguanosine (13) was treated with TsCl, MsCl, and TPS, the $O^6$-sulfonylation took place selectively to afford the corresponding $O^6$- sulfonates (14) in 73-78% yields. The $O^6$-sulfonylated derivatives of guanosine have proved to be useful synthetic intermediated for the transformation of guanosine




derivatives.[2b] Therefore, the above system provides quite useful method for the synthesis of this type of compounds because the reaction is very clean and rapid to give the sulfonylated products in satisfactory yields. Selective deprotection of the trityl group of 10 could be performed by using 80% acetic acid. For example, treatment of 10 with 80% acetic acid in dioxane/water (4:1, v/v) at room temperature for 1 h gave 14 in quantitative yield.

Finally, we examined the silylation of 1 using, t-butyldimethylsilyl chloride and t-butyldiphenylsilyl chloride. As a consequence, the silylation was essentially quantitative in each case. The t-butyldimethylsilylated product (15a: R'=t-Bu, R"=Me) was detected on tlc but was not stable enough to be isolated by silica-gel column chromatography. On the other hand, the t-butyldiphenylsilylated derivative (15b: R'=t-Bu, R"=Ph) could be isolated in 98% yield. When the latter product was dissolved in aqueous pyridine, the t-butyldiphenylsilyl group was feasibly removed from 15a to give 1.

The results of the above experiments indicate that introduction of suitable protecting groups into the $O^6$-position of guanosine moiety might provide a promising way for the prevention of side reactions encountered in oligonucleotide synthesis and also even in nucleoside and nucleotide chemistry.

Quite recently, Reese[8] has reported the reaction of 1-arenesulfonyl-3-nitro-1,2,4-triazoles and nucleoside base residues.

References and Note

1) a) E. Ohtsuka, T. Tanaka, S. Tanaka, and M. Ikehara, J. Am. Chem. Soc., 100, 4580 (1978); b) E. Ohtsuka, T. Tanaka, and M. Ikehara, ibid., 100, 8210 (1978); c) J. Stawinski, T. Hozumi, S. A. Narang, C. P. Bahl, and R. Wu,

Nucleic Acids Res., 4, 353 (1977); d) W. L. Sung, H. M. Hsiung, R. Brousseau, J. Michniewicz, R. Wu, and S. A. Narang, ibid., 7, 2199 (1979); e) R. Crea, A. Kraszewski, T. Hirose, and K. Itakura, Proc. Natl. Acad. Sci. USA., 75, 5765 (1978); f) J. H. van Boom, P. M. J. Burgers, R. Crea, G. van der Marel, and G. Wille, Nucleic Acids Res., 4, 747 (1977); g) J. H. van Boom, P. M. J. Burgers, G. van der Marel, C. H. M. Verdegaal, and G. Wille, ibid., 4, 1047 (1977); h) R. Arentzen, C. A. A. van Boeckel, G. van der Marel, J. H. van Boom, Synthesis, 137 (1979); i) R. W. Adamiak, E. Biala, K. Grzeskowiak, R. Kierzek, A. Kraszewski, W. T. Markiewicz, J. Okupniak, J. Stawinski, and M. Wiewirowski, Nucleic Acids Res., 5, 1889 (1978); j) H. S. Sista, R. T. Loder, and M. H. Caruthers, ibid., 6, 2583 (1979); k) K. K. Ogilvie, and N. Y. Theriault, Can. J. Chem., 57, 3140 (1979).

2) a) P. K. Bridson, W. Markiewicz, and C. B. Reese, Chem. Comm., 447 (1977); b) idem., 791 (1977).

3) Very recently, Hernandez has reported that DMAP is an effective catalyst in t-butyldimethylsilylation and trirylation: S. K. Chaudhary, and O. Hernandez, Tetrahedron Lett., 99 (1979).

4) H. Vorbrüggen, Angew. Chem. Intern. Ed., 17, 569 (1978).

5) 2a) Rf (benzene/AcOEt, 4:1, v/v) 0.51; $\lambda_{max}^{dioxane}$ 291, 261 nm, $\lambda_{min}^{dioxane}$ 277, 245 nm; NMR($CDCl_3$): δ 1.93(3H, s), 2.08(6H, br. s), 4.13(3H, br. s), 5.13-5.55(3H, m) 6.08(1H, br. s, $^2$NHTr), 7.18(25H, br. s), 7.7(1H, s); Anal. Calcd for $C_{47}H_{42}O_{11}N_5P$: C, 63.86; H, 4.80; N, 7.92%. Found: C, 64.06; H, 4.78; N, 7.61%. 2b): Rf 0.65; $\lambda_{max}^{dioxane}$ 292, 257 nm, $\lambda_{min}^{dioxane}$ 286, 250 nm; NMR($CDCl_3$): δ 1.97(3H, s), 2.10(6H, s), 4.17(3H, br. s), 5.17-5.63(3H, m), 6.27(1H, br. s, $^2$NHTr), 7.20(23H, m), 7.60(1H, s); Anal. Calcd for $C_{47}H_{40}O_{11}N_5Cl_2P$: C, 55.02; H, 3.90; N, 6.83; Cl, 6.94%. Found: C, 55.04; H, 3.98; N, 6.46; Cl, 6.48%. 2c): Rf 0.23. Other physical data could not be obtained because of its instability. 2d): Rf 0.31; $\lambda_{max}^{dioxane}$ 291, 257 nm, $\lambda_{min}^{dioxane}$ 277, 244 nm; NMR($CDCl_3$): δ 1.38(6H, d, J=6Hz), 1.96(3H, s), 2.08 (6H, s), 4.16(3H, br. s), 4.16(3H, br. s), 4.92(2H, m), 5.20-5.63(3H, m), 6.56(1H, s, $^2$NHTr), 7.23(15H, br. s), 7.70(1H, s); Anal. Calcd for $C_{41}H_{46}O_{11}N_5P$: C, 60.36; H, 5.68; N, 8.58%. Found: C, 60.29; H, 5.69; N, 8.53%.

6) The structures of 6, 7, 10, 12, 14, and 15 were confirmed by their elemental analyses, ultraviolet spectra, and NMR spectra. These products have a moderately broad singlet at δ 6.0-6.5 ($^2$N-proton) which disappears after $D_2O$ exchange (see ref. 2a). In the case of the $O^6$-sulfonylated compounds, $\lambda_{max}^{dioxane}$ lies between 298-303 nm except 12 ($\lambda_{max}^{dioxane}$ 276 nm). The thiophosphinylated products have $\lambda_{max}^{dioxane}$ at 299-300 nm.

7) Physical data of all products obtained here will be shortly reported elsewhere.

8) C. B. Reese and A. Ubásawa, Tetrahedron lett., 21, 2265 (1980).

(Received in Japan 27 June 1980)