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4-CHLOROPHENYL 5-CHLORO-8-QUINOLYL HYDROGEN PHOSPHATE: A USEFUL PHOSPHORYLATING AGENT FOR GUANOSINE 3'-PHOSPHOTRIESTER

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5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-benzoylguanosine smoothly reacts with 4-chlorophenyl 5-chloro-8-quinolyl hydrogen phosphate in the presence of 8-quinolinesulfonyltetrazolide (QS-te) to give 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-benzoylguanosine 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphate in high yield without a side product such as O^6-phosphorylated guanosine.

The useful synthetic procedures for the synthesis of oligonucleotides have been recently developed, and they have been applied successfully to the chemical synthesis of the fragments of genes of somatostatin[1], human insulin[2], human hormone[3], lactose operon[4], and human interferon[5]. However, it has been shown by a few workers[6] that the synthesis of oligonucleotides containing the guanosine unit by the phosphotriester approach has still a synthetic problem, i.e., unfavorable formation of O^6-substituted guanosine derivatives. Recently, we have also observed the O^6-phosphorylated guanosine derivative during the phosphorylation of 3'-hydroxyl group of 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-benzoylguanosine 5d by using 4-chlorophenyl 5-chloro-8-quinolyl phosphorotetrazolide 2. In order to explore the reaction of 5d with 2, we tried the reaction of 2 with simplified guanosine derivative such as 2',3',5'-tri-O-acetyl-N^2-benzoylguanosine. To a dry THF solution (8 ml) of 4-chlorophenyl 5-chloro-8-quinolyl phosphorotetrazolide 2 (1.5 mmol) prepared according to previously discribed procedure[7] was added 2',3',5'-tri-O-acetyl-N^2-benzoylguanosine (1.2 mmol) (Rf=0.05, benzene:acetone=8:2, solvent A). After 15 min, the corresponding O^6-phosphorylated guanosine 3 (Rf=0.3, solvent A) was found to be formed almost quantitatively by tlc-analysis, but it could not be purified by a silica gel column chromatography (benzene/acetone) because of partial decomposition. Analysis of the fluorescent spot of 3 (Rf=0.3, solvent A) on tlc by UV spectroscopy revealed a higher wavelength maximun at 235 nm which is characteristic of 5-chloro-8-quinolyl phosphate[8]. Furthermore, 3 was rapidly hydrolyzed with aqueous pyridine to give 2',3',5'-tri-O-acetyl-N^2-benzoylguanosine and 4-chlorophenyl 5-chloro-8-quinolyl phosphate. From the above fact, it is clear

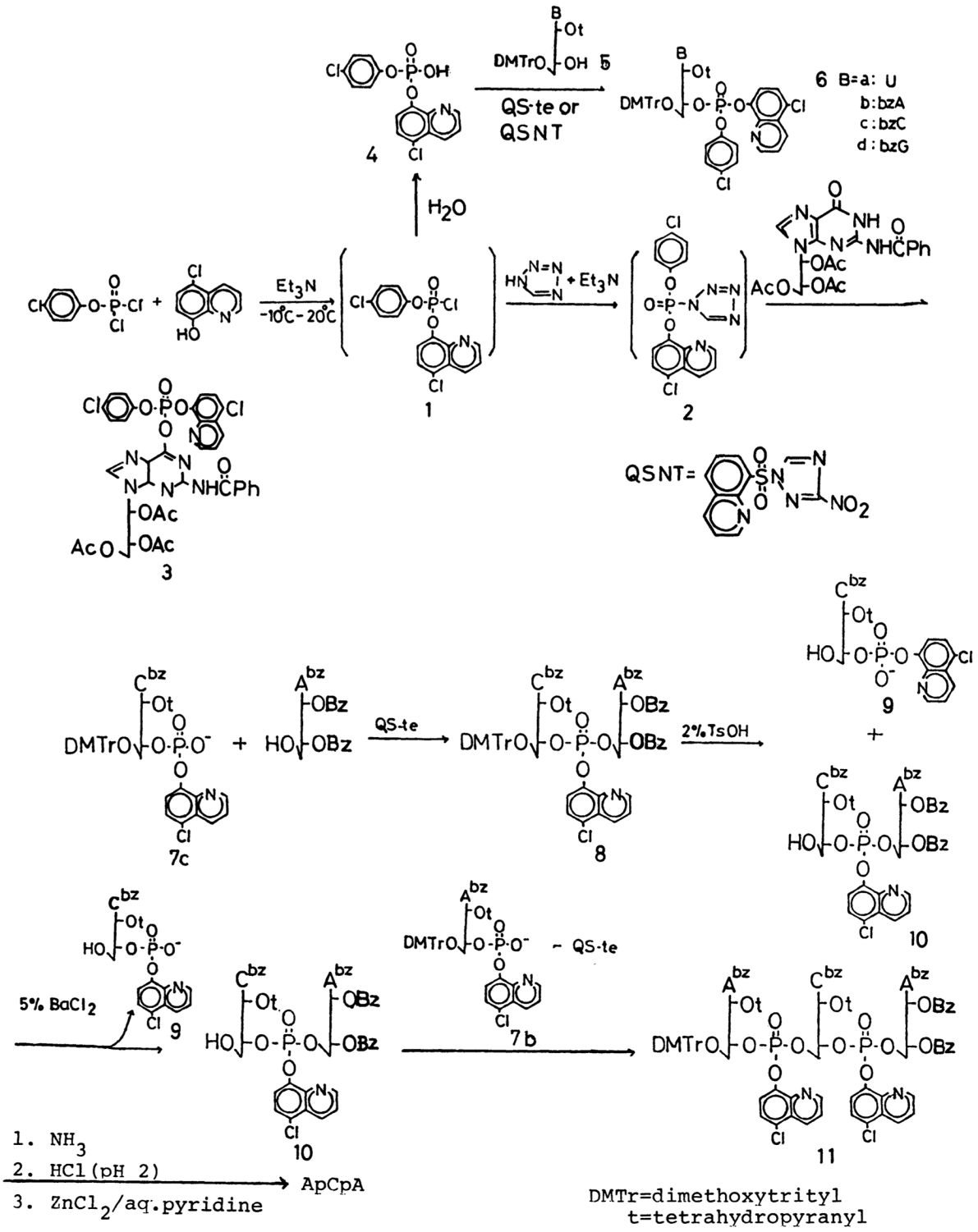
that such a side reaction leads to diminished yield of 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-benzoylguanosine 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphate 6d. We have therefore undertaken the development of new phosphorylating agents for guanosine in phosphorylation.

We examined to investigate the possibility of the phosphorylation of 3'-hydroxyl group of 5d using 4-chlorophenyl 5-chloro-8-quinolyl hydrohen phosphate 4 in the presence of 8-quinolinesulfonyltetrazolide (QS-te). The phosphorylating agent, 4 was prepared as follows: To a solution of 4-chlorophenyl phosphorodichloridate (1.66 ml, 10 mmol) in dry THF (75 ml) was added dropwise a solution of 5-chloro-8-hydroxyquinoline (1.79 g, 10 mmol) in dry THF (25 ml) during 30 min at -10°C; subsequently a dry THF (10 ml) solution of triethylamine (1.66 ml, 12 mmol) and the reaction mixture was gradually warmed to room temperature. After 45 min, this solution was added dropwise to aqueous pyridine (50%) (500 ml) during 5 h at 0°C. After removal of solvent, the residual solid was recrystallized from acetonitrile-water to give 3.41 g (87%) of 4: mp 108-110°C; Rf=0.81 (iso-PrOH-conc.NH_4OH-H_2O, 7:1:2); Anal. Calcd for $C_{15}H_{10}NO_4PCl_2.H_2O$: C, 46.47; H, 3.11; N, 3.60%. Found: C, 46.45; H, 3.08; N, 3.95%. The phosphorylating agent 4 (299 mg, 0.6 mmol) thus obtained was allowed to react with 5d (309 mg, 0.4 mmol) in the presence of QS-te (313 mg, 1.2 mmol) in dry pyridine (3 ml) for 2 h. After completion of the reaction, the reaction mixture was quenched with ice-water and extracted with methylene chloride (3 X 15 ml). The combined organic extracts were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in a small amount of methylene chloride and chromatographed on a silica gel column. The desirable nucleoside 3'-phosphotriester 6d was isolated in 90% yield (405 mg) by eluting the column with methylene chloride-methanol (95:5). It is noteworthy that O^6-phosphorylated guanosine derivative was not observed during the phosphorylation reaction. Furthermore, the reaction of 5d with 4 in the presence of 8-quinolinesulfonyl-3-nitro-1,2,4-triazole (QSNT)[9] as a new coupling agent also proceeded rapidly to give the corresponding guanosine 3'-phosphotriester 6d in an excellent yield of 87%. However, the use of 8-quinolinesulfonyl chloride (QS)[10] in place of QS-te gave poorer yeild of 6d.

By this method, other nucleoside 3'-phosphotriesters, 6a, 6b, and 6c were ontained in 95%, 92%, and 90% yields, respectively.[11]

Next, the rapid synthesis of trinucleotide, ACA for the 3'- and 5'-reiterated terminal sequences of Rous Sarcoma Virus 35S RNA[12] using 6 was examined. The phosphotriesters, 6b-c were treated with 1M-N^1,N^1,N^3,N^3-tetramethylguanidium salt of 2-pyridinaldoxime in a mixture of dioxane and water (1:1) for 16 h at 20°C to give the phosphodiester, 7b-c in quantitative yields.[11] The phosphodiester 7c (1.5 mmol) thus obtained was condensed with N^6,2',3'-O-tribenzoyladenosine (486 mg, 0.8 mmol) by using QS-te (990 mg, 3.75 mmol) in dry pyridine (4 ml). After 1.5 h, the reaction mixture was quenched with ice-water, extracted with methylene chloride, concentrated in vacuo, and coevaporated with toluene. The residue was treated with 2% p-toluenesulfonic acid in a mixture of methylene chloride and methanol (7:3) (92 ml) for 15 min at 0°C.[8] The reaction mixture was washed with phosphate buffer (1.0M, pH 7.5) (3 X 20 ml) and water. The detritylated phosphodiester 9

was removed from the reaction mixture by extraction with 5% $BaCl_2$ in methylene chloride. The 5'-hydroxyl dinucleotide 10 was precipitated from a mixture of n-hexane and ether (10:1) and used for the next coupling reaction without further purification. The dinucleotide 10 thus obtained was treated with 7b (1.2 mmol) in the presence of QS-te (792 mg, 3.0 mmol) in dry pyridine (4 ml) for 2 h. The reaction mixture was quenched with ice-water and extracted with methylene chloride.



The methylene chloride extract was washed with water, and evaporated in vacuo. The residue was dissolved again in a small amount of methylene chloride and chromatographed on a silica gel coulmn. The fully protected trinucleotide, ACA, 11 was isolated in 69% (1.22 g) yield, based on N^6,2',3'-O-tribenzoyladenosine by eluting the coulmn with a stepwise gradient of methanol (0-5%) in methylene chloride. The fully protected trinucleotide 11 was completely deblocked by treatment with concentrated ammonia for 5 h at 50°C, followed by 0.01N hydrochloric acid for 20 h at 20°C and zinc chloride in aqueous pyridine for 24 h at room temperature.[8] The deblocked trinucleotide, ACA was obtained in 85% yield after chromatography using Toyo Roshi No,514 paper (n-PrOH-conc.NH_4OH-H_2O, 55:10:35). The purity of ApCpA was checked by PE and HLPC on Finepak C_{18} as well as hydrolysis with nucleoase P1 to A, pC, and pA in a ratio 1.00:1.04:1.01.

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9. 8-Quinolinesulfonyl-3-nitro-1,2,4-triazole (QSNT) was prepared as follows: A THF (2 ml) solution of triethylamine (1.4 ml, 10 mmol) was added to a stirred suspension of 8-quinolinesulfonyl chloride (2.28 g, 10 mmol) and 3-nitro-1,2,4-triazole (1.14 g, 10 mmol) in THF (20 ml) with ice cooling. After 2h, the precipitate was removed by filtration, and the filtrate was evaporated in vacuo. The crystalline residue was dissolved in methylene chloride, and the solution was washed with water. The solution was dried over Na_2SO_4, filtered, and evaporated in vacuo. The resulting crystalline product was washed with a small amount of benzene, yielding 2.6 g (85%) of white powder. mp 227-229°C. Anal. Calcd for $C_{11}H_7N_5O_4S$: C,43.28; H, 2.31; N, 22.94%. Found: C, 43.13; H, 2.31; N, 22.78%. QSNT was found to be stable crystalline solid.
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