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3,4-DIMETHOXYBENZYL GROUP: A NEW PROTECTING GROUP FOR THE GUANOSINE RESIDUE DURING OLIGONUCLEOTIDE SYNTHESIS

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The protection of the O^6-amide group of deoxyguanosine with the 3,4-methoxybenzyl group is described. This protecting group could be introduced effectively and was readily removed with DDQ. This was used to demonstrate the synthesis of the oligodeoxyribonucleotide.

KEYWORDS———protecting group; 3,4-dimethoxybenzyl group; deoxyguanosine; oligodeoxyguanylate synthesis

Various side reactions of guanine residue during the phosphorylation and condensation steps have been reported in recent years.[1] Several methods for the O^6-protection of guanosine have been reported by Reese,[2] Hata,[3] Jones,[4] and Pfleiderer[5]. The protecting groups proposed so far are, however, unstable in alkali conditions.

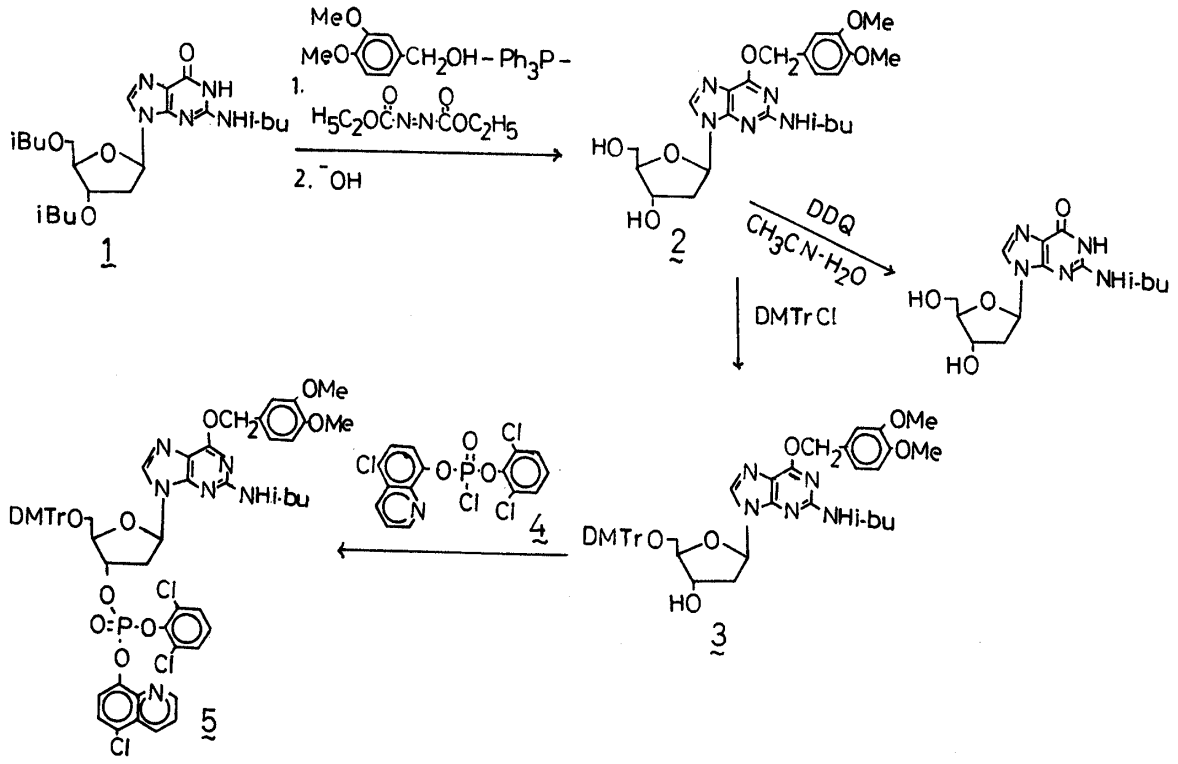
In this paper, we report a useful protecting group, 3,4-dimethoxybenzyl group, for the protection of the O^6-amide group of deoxyguanosine. This is stable in acid and alkali, and removable by treatment with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[6] in CH_3CN-H_2O (4:1, v/v).

We first investigated the effect of the 3,4-dimethoxybenzyl group of the O^6-amide of the deoxyguanosine derivative (1) by use of the procedure reported by Pfleiderer.[5] 3',5'-O-N^2-Triisobutyryldeoxyguanosine (1) (2.68 g, 6.0 mmol) was treated with 3,4-dimethoxybenzyl alcohol (5.01 g, 30 mmol) in the presence of triphenylphosphine (4.33 g, 16.5 mmol) and diethyl azodicarboxylate (2.61 g, 15 mmol) in THF (60 ml) at room temperature. After 20 h, TLC analysis showed complete conversion of starting material 1 into a high Rf product. The reaction mixture was quenched with ice water (5 ml), extracted with CH_2Cl_2 (60 ml X 2), and washed with 5% $NaHCO_3$ solution. The organic layer was dried with Na_2SO_4 and evaporated in vacuo. The residue was dissolved in a mixture of EtOH (30 ml) and pyridine (10 ml) and the solution was treated with 2N NaOH (28 ml) at 0°C for 15 min. The reaction mixture was neutralized with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (50 ml), and washed with 5% $NaHCO_3$ solution and with water. The organic layer was dried with Na_2SO_4 and evaporated under reduced pressure to give an oil. The oil was dissolved in a small amount of CH_2Cl_2 and subjected to silica gel column chromatography. The appropriate fractions (eluted with a stepwise gradient of MeOH (0-2%) in CH_2Cl_2) were evaporated to give the corresponding O^6-3,4-dimethoxybenzyl-N^2-isobutyryldeoxyguanosine (2), which was isolated (3.69 g, 78%) as a white soild by precipitation from hexane: mp 107-109°C; Rf 0.33 (CH_2Cl_2-MeOH, 9:1, v/v); UV λ max (MeOH) nm: 265, 230; ^{1}H-NMR(DMSO-d_6): δ9.02(1H, s, NH), 8.21(1H, s, C_8-H),

6.64(3H, d, ArH), 6.01(1H, t, $J_{1',2'}$=6.0Hz, $C_{1'}$-H), 5.30-4.61(3H, m, $C_{3'}$-H, $C_{3'}$-OH, and $C_{5'}$-OH), 4.21(2H, br s, $ArCH_2$), 3.70(1H, m, $C_{4'}$-H), 3.51(6H, s, OCH_3), 3.39(2H, br s, $C_{5'}$-H), Anal. Calcd for $C_{23}H_{29}N_5O_7 \cdot 2H_2O$: C, 52.76; H, 6.35; N, 13.38. Found: C, 53.17; H, 6.45; N, 13.28.

The 3,4-dimethoxybenzyl group was found to be quite stable for 1 day in 80% AcOH and also in conc. ammonia. Recently, Okikawa et al. reported[7] that the 4-methoxybenzyl group of alcohol is readily removed with DDQ in CH_2Cl_2-H_2O (18:1, v/v) under neutral conditions. Therefore, 2 was treated with DDQ (2 mol eq) in CH_2Cl_2-H_2O (18:1, v/v) at room temperature. However, the reaction did not proceed effectively. Thus, after 6 h, N^2-isobutyryldeoxyguanosine was obtained in 84% yield, and 12% of 2 was recovered. It is now found that CH_3CN-H_2O (4:1, v/v) is much more effective as solvent for the DDQ dehydrogenation. The use of CH_3CN-H_2O shortened dramatically the time for removal of 3,4-dimethoxybenzyl group as compared with CH_2Cl_2-H_2O.

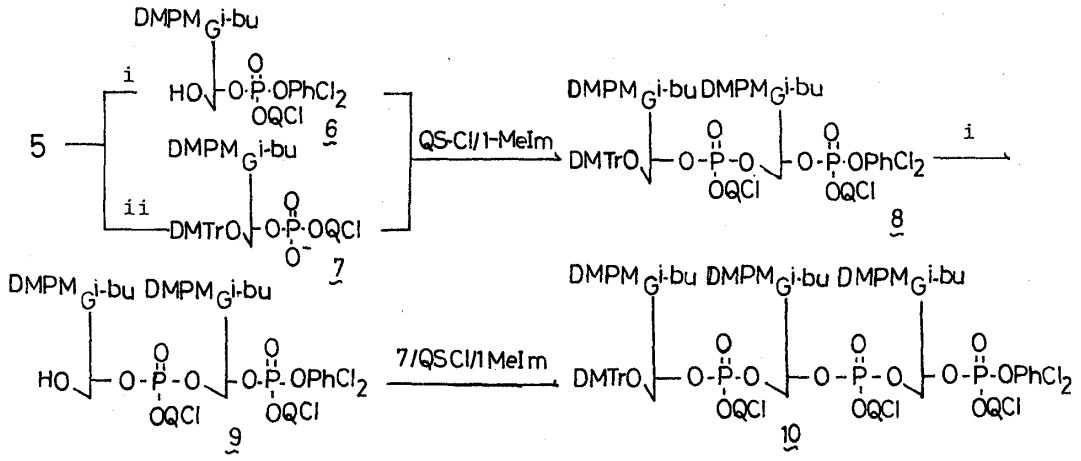
The compound 2 (789 g, 1 mmol) was tritylated with dimethoxytrityl chloride (441 mg, 1.3 mmol) in dry pyridine for 3 h. After the usual workup, chromatography on silica gel afforded the tritylated product 3[8] (744 mg, 82%). The tritylated compound 3 (789 mg, 1.0 mmol) was allowed to react with 2,6-dichlorophenyl 5-chloro-8-quinolyl phosphorochloridate (4)[9] prepared from 2,6-dichlorophenyl phosphodichloridate (558 mg, 2.0 mmol) and 5-chloro-8-hydroxyquinoline (293 mg, 2.2 mmol) in the presence of 1-methylimidazole (0.31 ml, 4.0 mmol) in dry THF (10 ml) at room temperature for 1 h. The reaction mixture was quenched



with ice-water (1 ml), extracted with CH_2Cl_2 (25 ml X 2), and washed with water. The organic layer was dried with Na_2SO_4 and evaporated in vacuo. The residue was chromatographed on silica gel to give the corresponding mononucleotide unit (5)[10] (1.06 g, 90%).

The utility of this new protecting group, 3,4-dimethoxybenzyl group, can be demonstrated in the following synthesis of d-GpGpGp (10). According to the previous paper,[10] the unit 5 (708 mg, 0.6 mmol) was treated with 3% Cl_3CCOOH in CH_3CN-MeOH (95:5, v/v) at room temperature for 3 min to give the corresponding 5'-hydroxyl component 6 (483 mg, 92%). On the other hand, removal of the 2,6-dichlorophenyl group from 5 (841 mg, 0.75 mmol) was performed by treatment with pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v, 10 ml) at room temperature for 1.5 h. The mixture was extracted with CH_2Cl_2 (15 ml), washed with 5% $NaHCO_3$, dried over Na_2SO_4, and evaporated in vacuo. The phosphodiester 7 thus obtained was treated with 6 (439 mg, 0.5 mmol) in the presence of QS-Cl[11] (273 mg, 1.50 mmol) and 1-methylimidazole (0.12 ml, 1.55 mmol) in dry pyridine. The reaction was completed in 1 h and the usual workup gave the dimer (8) (821 mg, 91%). The yield was very increased and the undesirble side reactions in the condensation reaction were not observed. Similarly, treatment of 8 (820 mg, 0.43 mmol) with 3% Cl_3CCOOH afforded the detritylated product (9) (624 mg, 92%), whereas mild treatment of 5 (580 mg, 0.5 mmol) with pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v) gave the phosphodiester 7. A solution of both compounds 9 (401 mg, 0.4 mmol) and 7 in dry pyridine was treated with QS-Cl (228 mg, 1.0 mmol) and 1-methylimidazole (0.16 ml, 1.0 mmol) for 1 h. The fully protected trinucleotide (10) was obtained in 93% (941 mg) yield after separation by silica gel column chromatography.

Deprotection of all the protecting groups from 10 was performed as follows: 1) To a solution of 10 (12.9 mg, 5 μmol) in CH_3CN-H_2O (4:1, v/v, 0.5 ml) was added DDQ (4.5 mg, 20 μmol). The mixture was stirred at room temperature for 1 h and



1. DDQ
2. pyridine-H_2O-t-$BuNH_2$(8:1;!)
3. zinc acetate (aqu. pyridine)
4. conc. ammonia
5. 80% AcOH

→ d GpGpGp

i=3% Cl_3CCOOH (CH_2NO_2-MeOH, 95:5)$_2$
ii=pyridine-H_2O-t-$BuNH_2$(8:1:1)
DMPM=3,4-dimethoxybenzyl

The solution was concentrated in vacuo. At this stage the 3,4-dimethoxybenzyl group was removed. 2) The residue was treated with pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v) at room temperature for 2 h to remove the 2,6-dichlorophenyl group. 3) The solution was concentrated and the residue was treated with zinc acetate (107 mg, 500 μmol) in aqueous pyridine at room temperature for 24 h.[13] The solution was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with aqueous pyridine. 4) The filtrate was concentrated in vacuo and the residue was treated with conc. ammonia at 60°C for 6 h. 5) The solution was concentrated and 80% AcOH was added. After 15 min, the solution was coevaporated with water. The deblocked trimer, d-GpGpGp was isolated in 85% yield after chromatographic separation using Whatman 3MM paper with n-PrOH-conc. ammonia-water (55:35:10, v/v). The deblocked trimer was completly degraded by spleen phosphodiesterase to give a single spot of d-Gp.

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10) mp 107-109°C; UV λmax (MeOH) nm: 282(sh), 270, 236; Rf 0.72 (CH_2Cl_2-MeOH, 9:1, v/v). Anal. Calcd for $C_{58}H_{54}N_6O_{12}PCl_3$: C, 59.82; H, 4.67; N, 7.23. Found: C, 60.09; H, 4.74; N, 7.38.

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