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DIPHENYLCARBAMOYL AND PROPIONYL GROUPS: A NEW COMBINATION OF PROTECTING GROUPS FOR THE GUANINE RESIDUE

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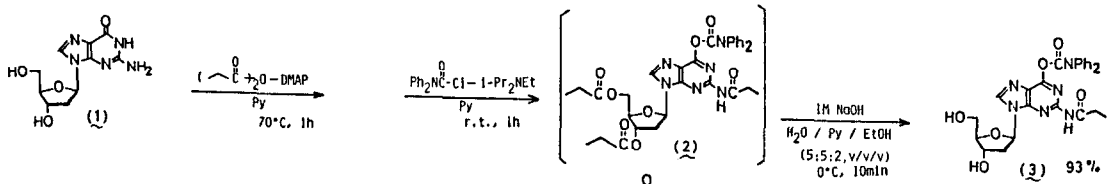
Summary: Protection of the guanine residue with the O^6-diphenylcarbamoyl and N^2-propionyl groups is described. These protecting groups could be readily introduced and removed simultaneously. They were used to demonstrate the synthesis of the deoxyguanylate dimer in high yield.

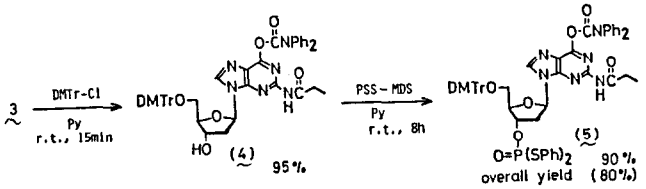
As one of the recent developments in oligonucleotide synthesis, exploration of some protecting groups have been achieved[1] to overcome the crucial problem which resulted from the undoubted reactive site of the guanine residue.[2] These groups have been introduced into the guanine residue in unsatisfactory yields through several-step reactions or at the final stage of the nucleotide-unit synthesis. Moreover, removal of the conventional N^2-isobutyryl group on the guanine residue required relatively long time.

Recently, we found that the diphenylcarbamoyl (DPC) group was most promissing as a protecting group for the O^6-amide group of the guanine residue in the ribo-series.[3] We applied this protecting group to the deoxyribo-series and employed the propionyl (Pro) group which is more base-labile than the isobutyryl group for protecting the 2-amino group.

We first investigated introduction of the DPC and Pro groups on the guanine residue: Deoxyguanosine (1) was treated first with propionic anhydride (5 equiv.)--N,N-dimethylaminopyridine (DMAP) (1 equiv.) in dry pyridine at 70°C for 1 h, then with diphenylcarbamoyl chloride (2.0 equiv.)—ethyldiisopropylamine (1.5 equiv.) in dry pyridine at room temperature for 1 h, and finally with 2M NaOH-EtOH-pyridine (5:5:2, v/v/v) at 0°C for 10 min. The 3',5'-free hydroxyl derivative (3)[4] could be purified in 93% yield by flash chromatography on silica gel. Scheme I reveals that DMAP was very effective for acylation of the 2-amino group and the DPC group was directly introduced without isolation of (2). Furthermore, the DPC and N^2-Pro groups survived under the basic conditions employed for hydrolysis of the 5'- and 3'-O-Pro groups. Next, the fully protected unit (5)[5] was obtained in the usual manner[1c,6] as shown in Scheme II with no loss of the guanine protecting groups.



Scheme I



Scheme II

In order to measure the life time of the DPC and N^2-Pro groups under the deprotective conditions, 5'-O-DMTr-derivative (4) was treated with conc. ammonia-pyridine (2:1, v/v) at room temperature and the rates of removal were estimated by tlc. Table I shows that the DPC group is more labile than the Pro gorup.

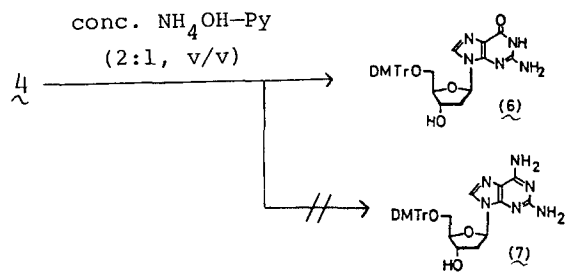
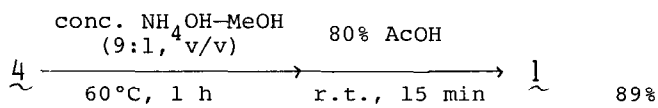


Table 1 The life time of the DPC and Pro groups of 4

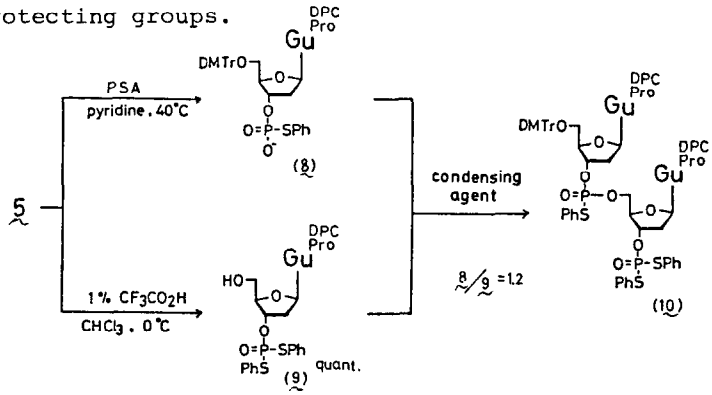
	DPC group	Pro group
$t_{1/2}$	20 min	3 h
t_∞	2 h	10 h

Then, 4 was treated with conc. ammonia—methanol (9:1, v/v) at 60°C for 1 h followed by treatment with 80% AcOH at room temperature for 15 min to give deoxyguanosine 1 in 89% yield without formation of the 2,6-diamino-purine derivative (7).[2a] In contrast to these results, the isobutyryl group required 6 h for its complete removal under the same conditions.



The availability of the new combination of the DPC and Pro groups can be demonstrated in the following synthesis of d-GpGp. According to our methodology,[1c,6] the unit 5 was treated with phosphinic acid (PSA) (30 equiv.) — triethylamine (20 equiv.) in dry pyridine at room temperature for 15 min. On the other hand, treatment of 5 with 1% trifluoroacetic acid (TFA) in dry $CHCl_3$ at 0°C for 3 min gave the 5'-hydroxyl component (9) quantitatively. The 3'-phosphate component (8) and 5'-hydroxyl component 9 were coupled by using mesitylene-1,3-disulfonyl chloride (MDS)[6b] (3 equiv.) and 3-nitro-1,2,4-triazole (NT) (3 equiv.) at room temperature. The condensation reaction was completed in 20 min and the usual workup gave the

desired dimer (10)[7] in 95% yield. The yield was dramatically increased and the inevitable side reactions in the conventional methods were not observed at all. In addition, 8 and 9 were coupled by using mesitylenesulfonyl chloride (3 equiv.) and 1-methylimidazole (MeIm) (3 equiv.) according to the effective condensing method recently reported by Efimov.[8] The reaction was completed in 10 min and the dimer 10 was also obtained in 94% yield without damage of the guanine protecting groups.



Deprotection of the fully protected dimer 10 was performed smoothly as follows: 1) 0.2 M NaOH—dioxane (1:1, v/v) for 10 min at room temperature to remove the internucleotidic PhS group and one of the two PhS groups at the 3'-terminal phosphate; 2) conc. ammonia—methanol (9:1, v/v) for 3 h at 60°C to remove the DPC and Pro groups; 3) 30 equiv. of silver acetate in pyridine—water (2:1, v/v) for 14 h at room temperature to remove the remaining PhS group at the 3'-terminal phosphate; 4) 80% AcOH for 15 min at room temperature to remove the 5'-dimethoxytrityl group. Thus, d-GpGp was isolated in 81% yield after chromatographic separation using Whatman 3MM paper with i-PrOH—conc. ammonia—water (6:1:3, v/v/v). The deblocked dimer was completely degraded by spleen phosphodiesterase to give a single spot of d-Gp.

In addition to the successful protection of the guanine residue during the dimer building reactions, introduction of the DPC group improves the solubility and chromatographic properties of its derivatives. Moreover, its presence on tlc plate is readily detected as a blue colored spot after spraying an acidic solution followed by heating. Both the DPC and Pro groups can be removed by treatment with conc. ammonia which is the conventional deprotective condition for the exo-amino acyl groups of other nucleoside bases. An effective combination of the DPC and Pro groups is a promissing protective fashion for the guanine residue in oligonucleotide synthesis of both the deoxyribo- and ribo-series.

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References and Notes

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3) The manuscript is in preparation and will shortly be reported elsewhere.

4) ^{1}H-nmr($CDCl_3$/TMS): δ 1.24(t, 3H, J=9Hz, CH_3), 2.09-2.60(4H, H-2' and CH_2), 3.60(2H, H-5'), 3.95(1H, H-4'), 4.61(3H, H-3' and OH-3',5'), 6.23(1H, H-1'), 7.10-7.38(10H, ArH), 8.17(s, 1H, H-8), 9.03(1H, NH). Anal. Calcd for $C_{26}H_{26}N_6O_6$ 7/10H_2O: C, 58.80; H, 5.20; N, 15.82%. Found: C, 58.59; H, 5.00; N, 15.75%.

5) ^{1}H-nmr($CDCl_3$/TMS): δ 1.12, 1.14(t, 3H, J=9Hz, CH_3), 2.42-2.69(3H, H-2' and CH_2), 2.80-3.10(1H, H-2'), 3.37(d, 2H, J=4.5Hz, H-5'), 3.69(s, 6H, OCH_3), 4.20(1H, H-4'), 5.32-5.53(1H, H-3'), 6.22(t, 1H, J=7.0Hz, H-1'), 6.73(d, 4H, J=9Hz, ArH), 7.15-7.51(29H, ArH), 8.01(s, 1H, H-8), 8.19(s, 1H, NH). Anal. Calcd for $C_{59}H_{53}N_6O_9S_2P$: C, 65.30; H, 4.92; N, 7.74; S, 5.91%. Found: C, 65.14; H, 5.80; N, 7.63; S, 5.57%.

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7) ^{1}H-nmr($CDCl_3$/TMS): δ 1.05-1.26(6H, CH_3), 2.30-2.66(6H, H-2' and CH_2), 2.86-3.44(6H, H-2',5'), 3.68(s, 6H, OCH_3), 4.30, 4.38(2H, H-4'), 5.37(2H, H-3'), 6.16(2H, H-1'), 6.71(d, 4H, J=9Hz, ArH), 7.93, 7.96, 7.99, 8.01(2H, H-8), 8.93, 9.08(2H, NH). Anal. Calcd for $C_{91}H_{82}N_{12}O_{16}S_3P_2$: C, 62.18; H, 4.70; N, 9.56%. Found: C, 61.93; H, 4.72; N, 9.42%.

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