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A New Type of Protection Mode for the Guanine Residue by Using 1,2-Diisobutyryloxyethylene Group

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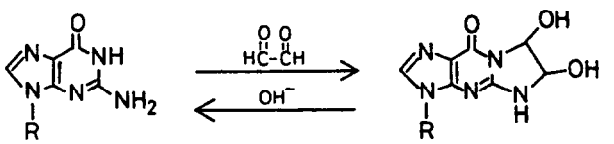
Summary: A new protecting group, 1,2-diisobutyryloxyethylene group, has successfully been used for protection of the guanine residue in the synthesis of oligodeoxyguanylates.

In current oligodeoxyribonucleotide synthesis, the most serious problem is the side reaction resulting from the remaining reactive sites of base residues, which caused the difficult isolation of the desired oligomer with low yield. Reese[1] and we[2] have noticed that N^2-acylguanine residues should be protected restrictly since they are mainly responsible for such side reactions. Reese[3] has recently reported that the O^6-amide of guanine can be protected with o-nitrophenyl group. This group was, however, introduced into the guanine residue in unsatisfactory yield through four-step reactions. More recently, Jones[4] has reported some O^6-protecting groups which can be removed by β-elimination.

In this paper, we report a useful method for protection of the guanine residue utilizing guanine-glyoxal addition followed by acylation of the resulting diol.

It is well known that guanine and related compounds were modified specifically with glyoxal under defined conditions to afford 1:1 adducts like 1 which revert to the starting materials at pH 11.[5] This guanine-selective modification has widely been applied to structural and enzymatic studies of both DNA and RNA.[5,6]



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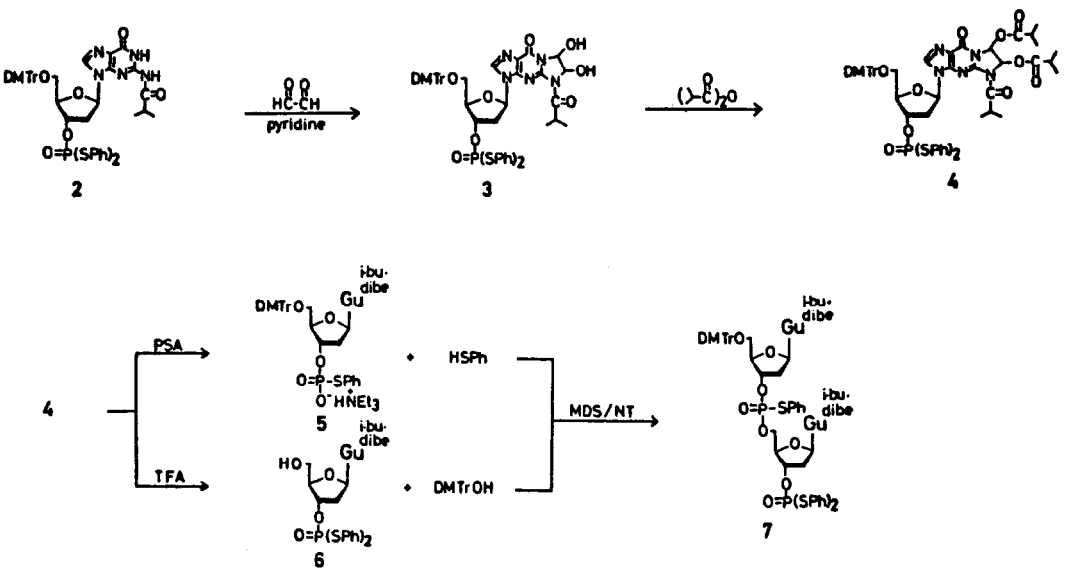
We found that S,S-diphenyl N^2-isobutyryl-5'-O-dimethoxytritylguanosine 3'-phosphorodithioate (2)[7] was converted readily into a 1:1 adduct (3) when 2 (2 mmol) was mixed with glyoxal (40 mmol)[8] in pyridine and coevaporated with dry pyridine several times. The adduct 3 was converted to the diisobutyryl ester (4)[9] without isolation of 3 by in situ treatment with isobutyric anhydride (12.4 mL) in pyridine (20 mL) for 1 h. The mixture was diluted with pyridine (10 mL), quenched with ice (10 g), and kept at 0°C for 30 min. The solution was transferred into a separatory funnel with $CHCl_3$ and washed with water, 5% $NaHCO_3$, and again water.



During the extraction the excess glyoxal and isobutyric acid were separated into the aqueous layers. The organic extract was dried over Na_2SO_4, evaporated in vacuo, and chromatographed on silica gel with hexane-CH_2Cl_2 to give 4 as a foam in 97%. The NMR spectrum[9] of 4 suggests that only one stereoisomer of the adduct was formed or two isomers were highly superimposed, so that 4 appeared as a single spot on TLC. The utility of this new type of protecting group, 1,2-diisobutyryloxyethylene (DIBE) group, can be demonstrated in the following synthesis of d-GpGp. According to our approach previously reported[10] the deoxyguanosine unit 4 (0.25 mmol) was treated with phosphinic acid (PSA)(18 mmol)[11] in pyridine (6 mL)–triethylamine (14.7 mmol)[12] at 40°C for 20 min. The excess phosphinate was easily removed from the $CHCl_3$ solution by extraction with water and then 0.2 M $Et_3NH_2CO_3$ solution. The $CHCl_3$ solution containing the diester (5) and thiophenol was used in the next condensation without further purification. On the other hand, treatment of 4 (0.25 mmol) with 2% trifluoroacetic acid (TFA) in $CHCl_3$ (10 mL) at 0°C for 8 min followed by washing with 5% $NaHCO_3$ solution gave a mixture of the hydroxyl component (6) and dimethoxytritylcarbinol. During the above treatments, the DIBE group remained intact. The mixtures, obtained by treatments of 4 with PSA and TFA, respectively, were combined, mixed with 3-nitro-1,2,4-triazole or 1H-tetrazole (0.5 mmol), coevaporated with dry pyridine several times, and dissolved in dry pyridine (3 mL). Mesitylene-1,3-disulfonyl chloride (MDS)(0.5 mmol)[10] was added to the mixture. The reaction was complete within 1 h. Thus, the usual workup gave the fully protected deoxyguanosine dimer (7) in 86 or 82% yield. The yields were extremely high compared with the conventional methods nevertheless the ratio of both the components 5 and 6 was stoichiometric. The presence of thiophenol did not affect the condensation between 5 and 6. In our long experi-

Table I. Conditions and Results of the Synthesis of Oligodeoxyguanylates

starting material[a] (mmol)	removal of PhS group[b] temp. (°C)	time (min)	removal of DMTr group[c] Rf value of hydroxyl comp. CH_2Cl_2-MeOH, 9:1, v/v	condensation azole[d] (mmol)	MDS (mmol)	time (min)	product comp. No.	yield %	Rf value (CH_2Cl_2-MeOH) 9:1 v/v	20:1 v/v	ratio of total 3'-protons to CH_3-O protons of DMTr group[e]	(calcd)
4 (0.25)	40	20	0.52	Te(0.50)	0.50	60	7	86	0.77	0.33	3.1	(3.0)
4 (0.25)	40	20		NT(1.0)	0.50	30	7	82		0.38		
7 (0.09)	40	15	0.55	NT(0.36)	0.18	60	8	81	0.77	0.13	1.6	(1.5)
8 (0.03)	25	30	0.60	NT(0.12)	0.06	40	9	71	0.77	0.05	0.74	(0.75)

[a] Each for the diester or the hydroxyl component.

[b] A pyridine solution of PAS (2-3 M) containing triethylamine (0.82 equiv) was used.

[c] Time required for removal of DMTr group was 8 min in each case.

[d] Te and NT refer to 1H-tetrazole and 3-nitro-1H-1,2,4-triazole, respectively.

[e] Calculated by 100 NH_2 ^{1}H-NMR.

ence, we know that phosphotriesters of PhS-P(O)$(OR)_2$ can not be synthesized by the reaction of dialkyl phosphates $(RO)_2P(O)(OH)$ with thiophenol in the presence of previously known condensing agents. In a similar manner, a fully protected deoxyguanosine tetramer (8: DMTr[Gp(SPh)$^{ibu\cdot dibe}$]$_4$SPh) or octamer (9: DMTr[Gp(SPh)$^{ibu\cdot dibe}$]$_8$SPh) was synthesized in high yield from a 1:1 mixture of the diester and the hydroxyl component derived from 7 or 8 (see Table I).

All the phenylthio groups could be removed from the dimer 7 by treatment with silver acetate (50 equiv per one phosphate) in pyridine-H_2O (2:1, v/v) at 50°C for 9 h followed by the subsequent treatments with conc. NH_3-pyridine (5:1, v/v) at 50°C for 4 h and then with 80% acetic acid at room temperature for 15 min. Thus, dGpGp[13] was isolated in 91% yield. Similarly, d-$(Gp)_4$[13] and d-$(Gp)_8$[13] were isolated in 72% and 70% yields, respectively, from 8 and 9.[14]

Khorana[14] reported that the characterization of oligodeoxyguanylates [d-$(pG)_n$] by using enzyme degradation is difficult because they form the unusual multistrand structure which resists to enzymes such as venom phosphodiesterase and bacterial phosphomonoesterase. Similar phenomena have been reported in the case of guanine-rich RNA fragments.[15-17] In fact, the dimer and the tetramer (30 OD each) were degraded very slowly with a large excess amount of spleen phosphodiesterase (Boehringer, 60 μg) to give d-Gp[18] after incubation in 0.05 M ammonium acetate (pH 6.5) at 37°C for 5 days. The octamer was extremely resistant to the enzyme and degraded to d-Gp only to the extent of 50% under the same conditions.

In conclusion, the new type of double protection mode described here would provide a promising method for the synthesis of guanine-rich oligonucleotides because the DIBE group could be introduced in virtually quantitative yield into the guanine residue under neutral conditions and it was removed very easily under the usual conditions where N-acyl protecting groups were removed.

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7) The synthesis of 2 will be shortly reported elswhere.
8) Commercially available glyoxal (30% aqueous solution) was concentrated and co-evaporated with pyridine several times. After the residual oil was dissolved in pyridine and a small amount of insoluble material was filtered, dry glyoxal was stocked as the pyridine solution (ca. 1-3 M) in a refrigerator at -20°C. This solution can be used for 4 months.
9) NMR($CDCl_3$):δ1.04(m, 18, $C(CH_3)_2$), 2.40-2.88(m, 5, 2'-H and $CH(CH_3)_2$), 3.40(m, 2, 5'-H), 3.78(s, 6, OCH_3), 5.35(m, 1, 3'-H), 6.25(m, 1, 1'-H), 6.81(s, 1, SCHS), 6.82(d, 4, J=9Hz, ArH), 6.87(s, 1, SCHS), 7.10-7.68(m, 19, ArH), 7.87 (s, 1, 8-H). Satisfactory elemental analyses were obtained for compounds 2, 4, 6, and 7.
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12) S. Honda, et al. unpublished works.
13) d-GpGp: Rf(Whatman 3 MM, relative to d-pG) 0.34(Solv. I: iPrOH-conc. NH_3-H_2O, 6:1:3), 0.84(Solv. II: nPrOH-conc. NH_3-H_2O, 55:10:35); UVmax 252, 270(sh) nm (pH 7.0), 256 nm(pH 1.5), 260 nm(pH 10). UVmin 223 nm(pH 7.0), 232 nm(pH 1.5), 235 nm(pH 10). Abs250/270=1.49. These changes of UV spectra in the range of pH 1.5-10 were well compatible with those of d-Gp. d-$(Gp)_4$: Rf 0.50(Solv. II); UVmax 252, 270(sh) nm, UVmin 224 nm. Abs250/270=1.56. d-$(Gp)_8$: Rf 0.02 (Solv. II); UVmax 252, 270(sh) nm, UVmin 224 nm. Abs250/270=1.57. The yields were calculated by using the ε values of 2.4×10^4, 4.6×10^4, and 8.0×10^4 at 252 nm for d-$(Gp)_2$, d-$(Gp)_4$, and d-$(Gp)_8$, respectively, where the hypochromicities were estimated to be 12, 15 and 20%, respectively.
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18) When such excess enzyme was used for prolonged period, d-Gp was further converted to d-G and inorganic phosphate to a considerable degree (ca. 10-15%) by the action of a contaminated other enzyme. Control treatment of d-Gp with the enzyme gave d-G to the same extent.

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