6. W. C. Jin, G. Y. Ryu, and J. H. Shin, *Bull. Korean Chem. Soc.*, **10**, 591 (1989).
7. (a) P. v. R. Schleyer and G. W. Van Dine, *J. Am. Chem. Soc.*, **88**, 2321 (1966); (b) Y. F. Rhodes and V. G. Difate, *J. Am. Chem. Soc.*, **94**, 7582 (1972), and references cited therein; (c) G. A. Olah and G. Liang, *J. Am. Chem. Soc.*, **96**, 6794 (1974).
8. H. C. Brown, "*The Nonclassical Ion Problem*" Plenum Press; New York, 1977.
9. P. G. Gassman and A. F. Fentiman, *J. Am. Chem. Soc.*, **92**, 2549 (1981).
10. W. V. R. Doering, *Tetrahedron*, **21**, 25 (1965).

Development of New Protecting Groups for Guanine Residue in Oligodeoxyribonucleotide Synthesis

Byung Jo Moon* and Kyung Lan Huh

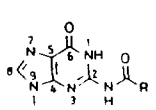
Department of Biochemistry, College of Natural Sciences, Kyungpook National University, Taegu 702-701. *Received November 14, 1990*

Attempts were made to develop new protecting groups for 1,6-lactam function of 2-N-acyl guanine in oligodeoxyribonucleotide synthesis. Several acyl groups, aryl groups, and carbamoyl groups were tested. Dimethylcarbamoyl and phenylacetyl groups are shown to be a good combination for guanine residue. 6-O-Di-methylcarbamoyl-2-N-pheylacetyl-2'-deoxyguanosine have been successfully used in the synthesis of d[AAGCTT], which is Hind III recognition sequence.

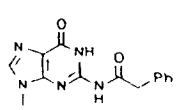
Introduction

In the most common oligonucleotide synthetic route, the phosphotriester method,[1,2] the choice is to block each reactive center because of the poor selectivity of the reagents used, the need for high yield and purity, and the difficult separations often encountered. Since Khorana and his coworker[3] introduced the use of N^2-isobutyryl group for guanine residue [as in (1)], almost every worker followed Khorana's original initiative. But it is well known that 2-N-acyl guanine is susceptible to side rections during oligonucleotide synthesis,[4,5] Therefore, attemts were made to develop new protecting groups for 1,6-lactam function of 2-N-acyl guanine and the number of different protecting groups were introduced by several workers.[6-9] Despite several protecting groups were introduced, because of unsatisfactory yield of preparation of building block, relatively long time or strong reaction condition for deprotection, or depurination, we still need to develop better protecting groups for guanine residue in oligodeoxyribonucleotide synthesis.

Recently, diphenylcarbamoyl (DPC) group was introduced by Hata and his coworkers for the O^6-amide group of guanine residue in the ribo-series[10] and in the deoxyriboseries.[11] They applied DPC group in combination with propionyl group for protecting the 2-amino group.



(1)



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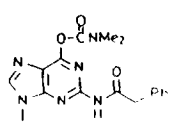

(3)

We favor the use of phenylacetyl group for protection of the 2-amino group [as in (2)], since the group is stable under all the reaction conditions used in the synthesis of oligodeoxyribonucleotide and is more base labile than conventional isobutyryl or isopropyl group. When we used DPC group with phenylacetyl group, we found quite a lot of depurination through the preparation of protected deoxyguanosine. To find out the appropriate protecting group of the O^6-amide group of 2-N-phenylacetyl-2'-deoxyguanosine, we investigated several acyl groups, aryl groups, and carbamoyl groups.

In this paper, we have shown that dimethylcarbamoyl (DMC) group is the most promising group for protection of O^6-amide group of 2-N-phenylacetyl-2'-deoxyguanosine [as in (3)]. We applied this protecting group for the synthesis of d[AAGCTT], which is Hind III recognition sequence.

Results and Discussion

We first investigated introduction of acyl and carbamoyl groups on the guanine residue of deoxyguanosine (Figure 1) by using modification of the reported procedure.[11] Without protection of 3',5'-hydroxyl group, 2-N-phenylacetyl-2'-deoxyguanosine was allowed to react with acyl chlorides or carbamoyl chlorides to give 6-O-acyl or 6-O-carbamoyl-2-N-phenylacetyl-2'-deoxyguanosine. As shown in Table 1, most of the chlorides gave reasonably good yield. DMC group was introduced in very good yield. When we introduced DPC group on the guanine residue of deoxyguanosine, we found that depurination occurred in substantial amount. Especially when DPC group was first introduced on 3'-5'-Di-O-methoxyacetyl-2-N-phenylacetyl-2'-deoxyguanosine followed deproection of dimethoxyacetyl groups with 4M-methanolic ammonia, the depurinated compound 6-O-diphenylcarbamoyl-2-N-phenylacetylguanine was isolated as major product.

Some acyl groups were also tested. We undertook synthesis of 6-O-trimethylbenzoyl-2-N-phenylacetyl-2'-deoxyguanosine

Table 1. Reaction Time, Yields, and R_f of 6-O-acyl or 6-O-carbamoyl-2-N-phenylacetyl-2'-deoxyguanosine

R	Reation time	Yield (%)	R_f[a]
MS	5 h	68 %	0.44
DcBz	1 h	72 %	0.40
DMC	1 h	85 %[b]	0.28
	50 min	80 %[c]	
DPC	50 min	60 %[b]	0.40
	50 min	16 %[c]	

[a]TLC solvent system: $CHCl_3$-EtOH (90; 10, v/v), [b]Compound (4) was reacted with the chloride, [c]Compound (5) was reacted with the chloride.

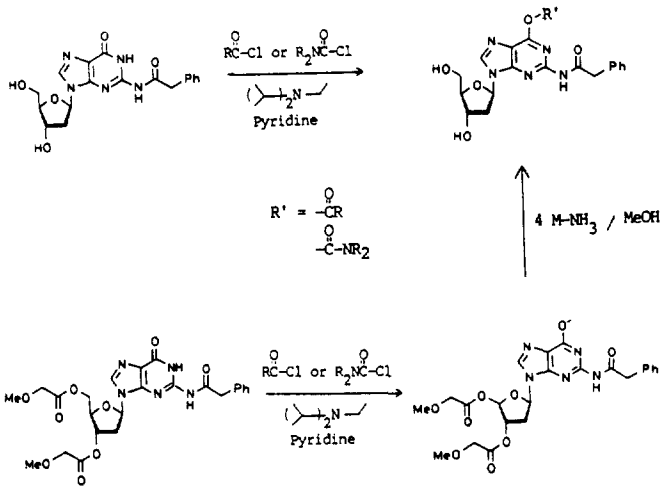

Figure 1. Synthesis of 6-O-Acyl or 6-O-Carbamoyl-2-N-phenylacetyl-2'-deoxy guanosine.

and 6-O-(2,5-dichlorobenzoyl)-2-N-phenylacetyl-2'-deoxyguanosine. These compounds could be synthesized in reasonably good yield. Aryl-protecting groups such as 3,5-dichlorophenyl and 3-chlorophenyl were used successfully for protection of guanine residue in oligonucleotide synthesis by Reese and his coworkers.[8] These groups were shown to be stable under all the reaction conditions used in the oligonucleotide synthesis and removed under the conditions used for the unblocking of internucleotide linkages with N^1, N^1, N^3, N^3-tetramethylguanidinium 2-nitrobenzaldehyde oximate.[12] But one of remaining problem is unsatisfactory yields through several-step reactions in the introduction of these groups on guanine residue. Considering this problems, we favor the use of carbamoyl or acyl groups for protection of guanine residue.

In order to investigate the stabilities of 6-O-protected-2-N-phenylacetyl-2'-deoxyguanosine, the deoxyguanosine derivatives were tested under various conditions which are applied in oligodeoxyribonucleotide synthesis. As shown in Table 2, DMC and 3-chlorophenyl groups are stable enough to be used in oligodeoxyribonucleotide synthesis. When we treated DCBz and DPC derivatives with phenyl dihydrogen phosphate, we found the degradation of compounds which seemed to be unblocked or depurinated products by TLC analysis.

Table 2. Stabilities 6-O-protected 2'-Deoxyguanosine Derivatives

R	PDHP[a]	80% AcOH[b]	K_2CO_3/ aq.dioxane	Pyridine	c-NH_4OH[c]
MS	100 %[d]	100 %[d]	stable	stable	100 %
DCBz	10 %	stable	stable	stable	100 %
DMC	stable	stable	stable	stable	100 %
DPC	15 %[d]	stable	stable	stable	100 %
NPE	60 %	stable	stable	stable	stable
3-ClPh	stable	stable	stable	stable	stable

The figures in the table mean the percentage of removal of each protecting group., [a]Depixylation condition (8 eq. PDHP to the correspond substrate, 10 min reaction reaction time., [b]AcOH-H_2O, 4:1, 30 min., [c]aqueous ammonia (d 0.88) overnight No modified deoxyguanosine derivative was observed by HPLC, [d]Depurination was observed instead of removal of the protecting group.

When 6-O-trimethylbenzoyl-2-N-phenylacetyl-2'-deoxyguanosine was treated with acids, surprisingly it degraded completely to depurinated products. We do not have an explanation for this acid lability of trimethylbenzoyl deoxyguanosine derivative. Nitrophenylethyl (NPE) group[7] which is another promising group was introduced and tested stabilities under various conditions. When NPE group, which has been known to be more stable than is required, was combinated with phenylacetyl group, depurination was observed (Table 2).

Considering protecting group criteria, which are ease of introduction, stability under conditions applied in oligonucleotide synthesis, and high yield cleavage under mild conditions, we found that DMC group was the most promising group in combination with phenylacetyl group for the protection of guanine of deoxyguanosine.

The availability of DMC group in combination with phenylacetyl group was demonstrated in the following synthesis of d[AAGCTT], which is Hind III recognition sequence. The standard benzoyl group was used for the protection of the N^6- and N^4-exocyclic amino groups of 2'-deoxyadenosine and 2'-deoxycytidine, respectively. The hexamer was made by the stepwise procedures from 3'-end in solution using phosphotriester method with MSNT as a condensing agent. The protected hexamer was prepared in 70% yield. Deprotection of the protected nucleotide was performed by the reported procedure.[12] The unblocked material was passed through a Sephadex G-15 column and was then chromatographed on DEAE Sephadex A-25 [linear gradient from 0.001-1.0 M triethylammonium bicarbonate (pH 7.5)]. The appropriate fractions were combined and were analyzed by HPLC. The product was homogeneous on HPLC (Figure 2) and gave rise to the expected products on enzymatic digestion.

Experimentals

Protected nucleosides and their phophotriesters were purified by column chromatography on Kiesel 60H. Thin layer chromatography was carried out on Merck HPTLC plates that were eluted with chloroform-methanol mixure. ^{1}H-NMR spectra of the protected nucleosides were measured with a Bruker 250-MHz spectrometer.

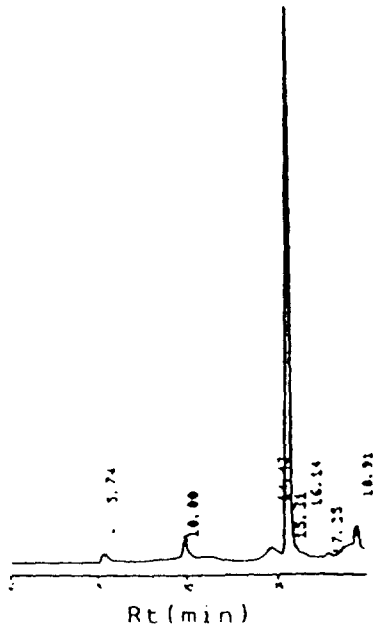

Figure 2. HPLC analysis of d[AAGCTT]. The chromatography was carried out on a 4.6×250 mm column of ODS with a flow of 1.5 m*l*/min at 25℃ using a gradient of 6-20% acetonitrile in 0.1 M triethylammonium acetate buffer.

General procedures for preparation of 6-O-acyl or 6-O-carbamoyl-2-N-phenylacetyl-2′-deoxyguanosine. Acyl chloride (2 mmol) or carbamoyl chloride (2 mmol) and diisopropylethylamine (0.52 m*l*, 3 mmol) were added to a stirred solution of 2-N-phenylacetyl-2′deoxyguanosine[8] (0.35 g, 1 mmol) in dry pyridine (4 m*l*) at room temperature. After 50 min to 5 h (Table 1), to the reaction mixure was added methanol (1 m*l*) and the solution was stirred for 30 min. The products were poured into saturated $NaHCO_3$ solution (30 m*l*) and the mixture was extracted with chloroform (2× 30 m*l*). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting glass was purified by short-column chromatography ($CHCl_3$-EtOH, 92:8, v/v). The glass was precipitated in light petroleum (bp. 30-40℃). Some Physical constants for 6-O-acyl or 6-O-carbamoyl-2-N-phenylacetyl-2′-deoxyguanosine are given in Table 1.

6-O-Dimethylcarbamoyl-2-N-phenylacetyl-2′-deoxyguanosine (Found: C, 54.9; H, 5.2; N, 17.9. $C_{21}H_{24}N_6O_6$ requires C, 55.3; H, 5.5; N, 18.4%), λ_{max}(95% EtOH) 228, λ_{min} 240 nm: ^{1}H NMR(DMSO-d_6) α 2.32(1H, m), 2.76(1H, m), 2.96 (3H, s), 3.19(3H, s), 3.57(2H, m), 3.89(3H, m), 4.87(2H, m), 5.35(1H, t), 6.4(1H, m), 7.2-7.46(5H, m), 8.61(1H, s), 10.97(1H, s).

6-O-Diphenylcarbomoyl-2-N-phenylacetyl-2′-deoxyguanosine. ^{1}H-NMR (DMSO-d_6) δ 2.35(1H, m), 2.76(1H, m), 3.57(2H, m), 3.82(2H, s), 3.87(1H, m), 4.45(1H, m), 4.92(1 H, t, J=6.0 Hz), 5.38(1H, d, J=4.0 Hz), 6.37(1H, t, J=7.4 Hz), 7.2-7.4(7H, m), 7.4-7.6(8H, m), 8.67(1H, s), 11.0(1H, s).

6-O-Trimethylbenzoyl-2-N-phenylacetyl-2′-deoxyguanosine. ^{1}H-NMR (DMSO-d_6) δ 2.32(1H, m), 2.35(1H, m), 2.50(6H, s), 2.70(1H, m), 3.82(2H, s), 3.90(1H, m), 4.96(1H, m), 4.92(1H, t, J=5.5 Hz), 5.36(1H, d, J=5.0 Hz), 6.41(1H, t, J=7.0 Hz), 7.05(2H, s), 7.2-7.4(5H, m), 8.72(1H, s), and 11.07(1H, s).

6-O-(2,6-Dichlorobenzoyl)-2-N-phenylacetyl-2′-deoxyguanosine. ^{1}H-NMR (DMSO-d_6) δ 2.35(1H, m), 2.81(1 H, m), 3.59(2H, m), 3.81(2H, s), 3.82(1H, m), 3.47(1H, m), 4.93(1H, t, J=5.5 Hz), 5.37(1H, d, J=4.8 Hz), 6.48(1H, t, J=6. 6 Hz), 7.2-7.5(5H, m), 7.6-7.8(3H, m), 8.85(1H, s), and 11.10(1 H, s).

Protected Hexanucleotide $dA_pdA_pdG_pdC_pdT_pdT$. Triethylammonium [5′-O-pixyl thymidine-3′-(2-chlorophenyl phosphate)] (0.217 g, 0.275 mmol) was coupled with 3′-O-benzoylthymidine (0.087 g, 0.25 mmol) by using MSNT (0.250 g, 0.825 mmol) in dry pyridine (2 m*l*) at room temperature. After 30 min, $NaHCO_3$ solution (0.75 m*l*) was added to the reaction mixture and the reaction mixture was stirred for 3 min. The reaction mixture was extracted with chloroform and chloroform layer was washed with TEAB buffer (0.1 M, pH=7.5). The solution was dried ($MgSO_4$) and the solvent evaporated in vacuo to give protected dinucleotide as glass. After removal of the pixyl group from Px-dT_pdT-Bz with phenyl dihydrogen phosphate (PDHP) in 5% ethanol in chloroform (10 min, 25℃), the protected dinucleotide was purified by short column chromatography over silica gel, eluting with 5% ethanol in chloroform (73.0% yield). The protcted dinucleotide was coupled with triethylammonium [5′-O-pixyl-4-N-benzoyl-2′-deoxycytidine-3′-O-(2-chlorophenyl phosphate)] (0.247 g, 0.275 mmol) as described above to give protected trinucleotide Px-dC_pdT_pdT-Bz (85.0% yield). After removal of the pixyl group and purification by short column chromatography as described above, HO-dC_pdT_pdT-Bz was extended by stepwise reaction to give the title compound dA_pdA_p-$dG_pdC_pdT_pdT$ (35% yield) using triethylammonium [5′-O-pixyl-2-N-phenylacetyl-6-O-dimethylcarbamoyl-2′-deoxyguanosine-3′-O-(2-chlorophenyl phosphate)] and triethylammonium [5′-O-pixyl-6-N-pivaloyl-2′-deoxyadenosine-3′-O-(2-chlorophenyl phosphate)] as building blocks.

Unblocking of fully-protected hexanucleotide. To a magnetically stirred solution of the substrate (0.0072 g, 0.0025 mmol) in pyridine (1 m*l*) was added acetic anhydride. After 2 h, methanol (0.4 m*l*) was added and the solution was stirred for 30 min. $CHCl_3$ was added to the solution and the solution was washed with $NaHCO_3$ solution. The organic layer was dried and evaporated to glass. The glass was dissolved in dioxane (0.3 m*l*). To the solution was added syn-*p*-nitrobenzaldoxime (0.021 g, 0.125 mmol), N^1, N^1, N^3, N^3-tetramethylguanidine (0.013 g, 0.113 mmol) and water (0.3 m*l*) at room temperature. After 24 h, aqueous NH_3 (d, 0.88, 3 m*l*) was added and the solution was stirred. After 24 h, the products were concentrated under reduced pressure and then acidified to pH 5 with 0.01 M-HCl. The resulting solution was extracted with $CHCl_3$ and ether. The aqueous layer was then concentrated under reduced pressure. The unblocked material was passed through a Sephadex G-15 and was chromatographed on DEAE Sephadex A-25. The column was eluted with triethyl ammonium bicarbonate buffer (pH 7.5, linear gradient from 0.001-1.0 M).

Enzymatic Hydrolysis. To a solution of the oligomer (*ca.* 1-2 O.D. units) in H_2O (0.01 m*l*) was added a solution of snake venom diesterase (20 μ*l*) in Tris HCl buffer (pH 9, 0.01 M respect to $MgCl_2$, 0.1 m*l*). The solution was maintained at 37℃. After 10 h, a suspension of alkaline phosphatase (10 μ*l*, 4 mg/m*l*) was added to the above solution and the solution was maintained for another 2 h at 37℃. The

products were analyzed by reverse-phase HPLC. The chromatography was carried out on a Jones APEX ODS colum with a flow of 1.6 ml/min at 25℃. The column was eluted with 5% CH_3CN in 0.1 M triethylammonium acetate buffer.

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References

1. S. A. Narang, *Tetrahedron*, **39**, 3 (1983).
2. C. B. Reese, *Tetrahedron*, **34**, 3143 (1979).
3. P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6162 (1958).
4. C. B. Reese and A. Ubasawa, *Tetrahedron Lett.*, **21**, 2265 (1980).
5. R. Pon, M. Damha, and K. Ogilvie, *Nucleic Acids Res.*, **13**, 6447 (1985).
6. T. Trichtinger, R. Charubala, and W. Pfleiderer, *Tetrahedron Lett.*, **24**, 211 (1983).
7. B. L. Gaffney and R. A. Jones, *Tetrahedron Lett.*, **23**, 2257 (1982).
8. C. B. Reese and P. A. Skone, *J. Chem. Soc., Perkin Trans.*, *1*, 1263 (1984).
9. S. S. Jones, C. B. Reese, S. Sibanda, and A. Ubasawa, *Tetrahedron Lett.*, **22**, 4755 (1981).
10. T. Kamimura, M. Tsuchiya, K. Urakami, K. Koura, M. Sekine, K. Shinozaki, K. Miura, and T. Hata, *J. Am. Chem. Soc.*, **106**, 4552 (1984).
11. T. Kamimura, M. Tsuchiya, K. Koura, M. Sekine, and T. Hata, *Tetrahedron Lett.*, **24**, 2775 (1983).
12. C. B. Reese, R. C. Titmas, and L. Yau, *Tetrahedron Lett.*, 2727 (1978).

Chain Ordering Effects in the Nematic-Isotropic Phase Transition of Polymer Melts

Han Soo Kim, Hyungsuk Pak*, and Song Hi Lee†

Department of Chemistry, Seoul National University, Seoul 151-742
†Department of Chemistry, Kyungsung University, Pusan 608-736. *Received November 19, 1990*

A statistical theromdynamic theory of theromtropic main-chain polymeric liquid crystalline melts is developed within the framework of the lattice model by a generalization of the well-known procedure of Flory and DiMarzio. According to the results of Vasilenko *et al.*, the theory of orientational ordering in melts of polymers containing rigid and flexible segments in the main chain is taken into account. When the ordering of flexible segments in the nematic melt is correlated with that of rigid mesogenic groups, the former is assumed to be given as a function of the ordering of rigid mesogenic cores. A free energy density that includes short-range packing contributions is formulated. The properties of the liquid-crystalline transiton are investigated for various cases of the system. The results calculated in this paper show not only the order-parameter values but also the first-order phase transition phenomena that are similar to those observed experimentally for the thermotropic liquid-crystalline polymers and show the transitional entropy terms which actually increase upon orientational ordering. In the orientational ordering values, it is shown that mesogenic groups, flexible segments, and gauche energy (temperature) may be quite substantial. Finally, by using the flexibility term, we predict the highly anisotropic mesophase which was shown by Vasilenko *et al.*

Introduction

Liquid crystalline polymers are largely characterized by the thermotropic mesophases and the lyotropic ones. The hydrocarbon chains in amphiphilic mesophases, such as lipid monolayer and bilayer, are highly ordered near the interfacial plane of the polar head groups. These hydrocarbon chains exhibit the lyotropic liquid crystalline polymers. On the other side, thermotropic liquid crystalline polymers have their mesogenic elements separated by flexible or semiflexible spacer groups, which exhibit anisotropic-isotropic phase transition temperature considerably lower than that required for thermal degradation of the macromolecules. Thermotropic liquid crystalline polymers have several different mesophases according to the molecular configurations-nematic, smectic, and cholesteric polymers.

Not until 1981 was, a paper published by Matheson and Flory[1] in which the liquid crystalline transition in a solution of macromolecules containing rigid rods, freely rotating joints between the rods, and flexible segments was considered on the basis of the well-known lattice method.[2,3] Vasilenko, Khokhlov, and Shibaev (VKS)[4] have extended the Matheson-Flory lattice model treatment to include the induced ordering of the flexible spacer segments in the anisotropic melt. They show that the self-consistent orientational field due to the liquid crystalline order affects the flexible chain segments as well as the rigid groups, leading to straightening and stiffening of the former. The ordering of flexible segments, accompanying the formation of the liquid crystalline phase, is also indicated by some of the experimental results.[5-7] A statistical thermodynamic theory of thermotropic liquid crystalline polymers was developed, by application and extension