Communications to the Editor

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SPECIFIC REMOVAL OF 5-CHLORO-8-QUINOLYL PROTECTING GROUP OF INTERNUCLEOTIDIC BONDS

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5-Chloro-8-quinolyl protecting group of internucleotidic bonds was efficiently removed by treatment with zinc acetate or 2-pyridinecarboxaldehyde oxime at room temperature. On the other hand, alkaline hydrolysis of phosphotriester intermediates with free 3'-hydroxyl function (2a) leads to the formation of 3'-3' in addition to 3'-5'-internucleotidic bonds.

KEYWORDS———phosphate protecting group; 5-chloro-8-quinolyl phosphate; deprotection; zinc acetate; 2-pyridinecarboaldehyde oxime

We have previously reported[1] that 5-chloro-8-quinolyl group could be effectively used for the protection of internucleotidic bonds in the synthesis of oligoribonucleotides by the phosphotriester approach. Nussbaum et al.[2] have more recently described the advantageous points of 8-quinolyl group compared with 4-chlorophenyl group as protecting groups of internucleotidic bonds in the synthesis of oligodeoxyribonucleotides by the phosphotriester approach. Thus, 8-quinolyl group was removed by treatment with aqueous ammonia for 2 days at 27°C from internucleotidic bonds. However, we observed the formation of by-products during the removal of 5-chloro-8-quinolyl protecting group from dithymidylic acid derivative (2a) by treatment with either aqueous ammonia or hydroxide ion.

In this paper, we wish to report a more efficient and general method for removal of 5-chloro-8-quinolyl protecting group of internucleotidic bonds and provide evidence for the formation of by-products by alkaline hydrolysis of 2a.

The 5-chloro-8-quinolyl-, 8-quinolyl-, and 4-chlorophenyl-protected dithymidylic acid derivatives (1a-c) were all prepared according to the procedures reported.[3,4,5]

We first examined the selective removal of 3'-O-acetyl group from the fully protected dinucleotides (1a-c). When the fully protected dinucleotides (1a-c) (0.2 mmol) were treated with 2N-NaOH (2 ml) in a mixture of pyridine and water (1:1, v/v) at 0°C, only minimal loss of 5-chloro-8-quinolyl and 8-quinolyl groups and extensive loss of 4-chlorophenyl group are observed (Table I). This result indicates that the quinolyl groups are stable to alkaline than 4-chlorophenyl group, and that they can be used for elongation of the chain in the 3'-direactions.

Next, we examined the removal of 5-chloro-8-quinolyl group from internucleotidc bond of 2a by the treatment with either hydroxide ion or aqueous ammonia.[6]

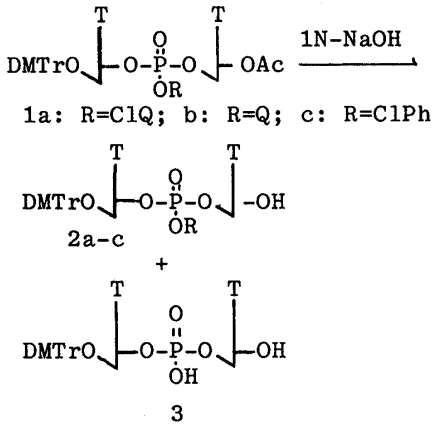
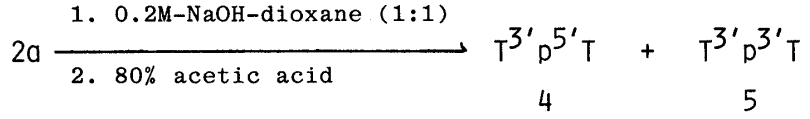


Table I. Selective Removal of 3'-Acetyl Group from DMTrTp(R)TOAc (1) Using 1N-NaOH[a)]

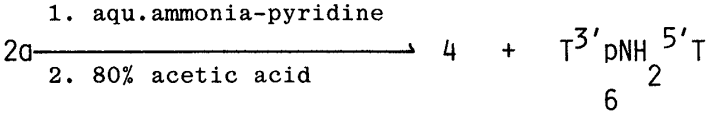
R	Time(min)	2(%)	3(%)
a) ClQ	15	98	2
	30	64	36
	60	10	90
b) Q	15	94	6
	30	65	35
	60	7	93
c) ClPh	15	13	79
	25	7	83
	30	-	86

a) Reactions were at 0°C and analyzed by chromatography on TLC.

The compound 2a (0.1 mmol) was treated with 0.2M-NaOH-dioxane (1:1, v/v) (4 ml) and the mixture was kept at 22°C for 3 h. The solution was neutralized with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in 80% acetic acid (5 ml) and the solution was kept at room temperature for 10 min. After removal of acetic acid in vacuo, the residue was dissolved in water (2 ml) and extracted with ether for removal of trityl alcohol and 5-chloro-8-hydroxyquinoline. The solution was applied on Whatmann 3MM paper and developed with solvent (2-propanol-conc.ammonia-water, 7:1:2 v/v) to afford TpT (Rf 0.43, 91.2%). When TpT (96 OD) thus obtained was incubated with spleen phosphodiesterase, TpT was recovered in 20% (19.2 OD) along with T (38.8 OD) and Tp (40.4 OD). The result clearly indicates that the formation of 3'-3' isomer takes place during alkaline hydrolysis of the intermediate 3'-hydroxyl derivative 2a. Thus, the 3'-3' isomer probably forms through a cyclization of the intermediate 3'-hydroxyl derivative 2a.[6a-b)]



On the other hand, treatment with aqueous ammonia-pyridine (1:1, v/v) at room temperature for 36 h followed by 80% acetic acid gave thymidylyl (3'-5') thymidine (4) (t=4.0 min, 85%) and the corresponding phosphoramidate (6) (t=6.0 min, 10%) by HPLC analysis (Fig. 1). The phosphoramidate (6) was formed by nucleophilic attack of NH_3 on the phosphotriester bonds.[6d-e)]



The above results indicate that alkaline solution and aqueous ammonia are not so effective for the removal of 5-chloro-8-quinolyl protecting group from internucleotidic bonds.

In a previous paper,[1)] we reported the mild and selective removal of 5-chloro-

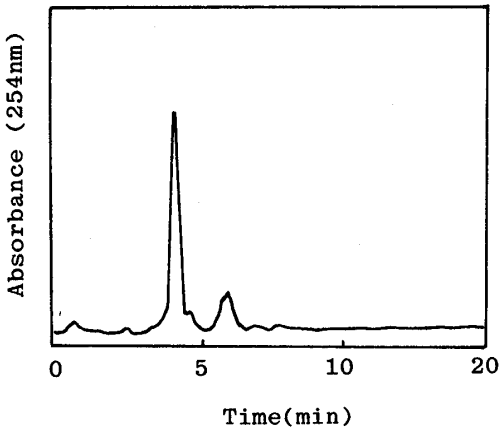


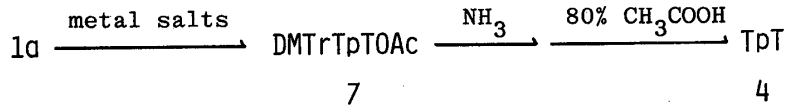
Fig. 1. 2a was treated with aqueous ammonia for 36 h followed by 80% acetic acid. The nucleotide products were analyzed by HPLC using Raidal PAK uBONDAPAK C_{18} column with a gradient 10% to 15% acetonitrile in 0.1M aqueous triethylammonium acetate; flow rate, 1 ml/min.

8-quinolyl protecting group from internucleotidic bonds by treatment with zinc chloride (50 equiv.) in aqueous pyridine at room temperature for 24 h. It is now found that zinc acetate is a much more effective than zinz chloride. Zinc chloride has a very low solubility in aqueous pyridine so that the reaction takes place in heterogeneous phase and requires a large excess of zinc chloride. Among the metal salts tested using 1a, zinc acetate was superior to zinc chloride, cupric chloride, cupric acetate, and nickel chloride (Table II). When the mixture obtained by selective removal of 5-chloro-8-quinolyl group from internucleotidic bonds with zinc acetate was analyzed using reverse phase HPLC, internucleotidic bond cleavage was not observed (less than 2-3%) and the expected product was DMTrTpTOAc (7). Further, the dimethoxytrityl group of 1a is stable under the condition for complete removal of the 5-chloro-8-quinolyl group from internucleotidic bonds. The compound 7 was further treated with aqueous ammonia-pyridine (1:1, v/v) for 4 h followed by treatment of 80% acetic acid for 10 min. The unblocked compound, TpT (4) was obtained by paper chromatography (Whatmann 3MM) in 93% yield.[7)]

Table II. Selective Removal of 5-Chloro-8-quinolyl Group from 1a Using Metal Salts[a)]

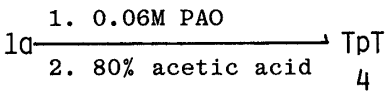
Metal salts	mol metal salts/mol 1a	Time (h)	7 (%)	1a (%)
$Zn(CH_3CO_2)_2$	8	28	98	-
$ZnCl_2$	50	24	95	-
$Cu(CH_3CO_2)_2$	8	48	76	21
$CuCl_2$	8	48	61	35
$NiCl_2$	8	48	70	23

a) These recations were carried out in pyridine-water (9:1, v/v) at room temperature and analyzed by chromatography on TLC.



Further, 2-pyridinecarboxaldehyde oxime was found to be very effective for

removal of 5-chloro-8-quinolyl group from internucleotidic bonds. The fully protected dinucleotide 1a (0.1 mmol) was treated with 0.06M-N^1,N^1,N^3,N^3-tetramethylguanidium salt of 2-pyridinecarboxaldehyde oxime in a mixture of dioxane and eater (2:1, v/v) (4 ml) at room temperature for 12 h. The solution was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with 50% aqueous pyridine. The filtrate was evaporated in vacuo, and the residue was 80% acetic acid for 10 min. The mixture was evaporated in vacuo and coevaporated with water and then with pyridine. The residue was dissolved in pyridine and applied on Whatmann 3MM paper and developed with solvent (2-propanol-conc.ammonia-water, 7:1:2, v/v) to afford TpT (4) (Rf 0.45, 90%). The dimer, 4, was completely degraded by spleen phosphodiesterase to give a ratio of Tp:T (1.03:1.00). In this reaction, 5-chloro-8-quinolyl group was removed by treatment with PAO from internucleotidic bonds along with acyl groups.



Thus, it is clearly demonstrated that 5-chloro-8-quinolyl group can be removed very effectively by treatment with zinc acetate or 2-pyridinecarboxaldehyde oxime from internucleotidic bonds, whereas treatment with either alkaline solution or aqueous ammonia for the removal of 5-chloro-8-quinolyl group from phosphotriester intermediates with free 3'-hydroxyl group leads to the formation of by-products.

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7) The dimer, TpT (4) was treated with spleen phosphodiesterase. It was completely degraded to T and Tp (1.00:1.07).

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