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A Comparative Study on Phosphate Protecting Groups in Oligonucleotide Synthesis by the Phosphotriester Approach[1)]

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The efficiencies of various of phosphate protecting groups in the synthesis of internucleotidic bonds by the phosphotriester approach have been studied. We found that in the coupling reaction using alkyl groups as phosphate protecting groups of internucleotidic bonds, extensive sulfonation of the 5′-hydroxyl group of nucleoside occurred, whereas when aryl groups were used in place of alkyl groups, the sulfonation did not occur.

Keywords——oligonucleotide synthesis; phosphate protecting group; coupling agents; internucleotidic bonds; phosphotriester synthesis

In the chemical synthesis of oligonucleotides by the phosphotriester approach, various protecting groups for the internucleotidic phosphates have been offered and examined.[2)] Among these protecting groups, the substituted phenyl[2d,e)] and trichloroethyl groups[2b,c)] are most widely used in the synthesis of oligonucleotides by the phosphotriester approach. On the other hand, a few workers[3)] have utilized the methyl group in applying the Letsinger phosphite procedure[4)] to methyl phosphorodichloridite in the synthesis of oligonucleotides. However, Weiwiorowski reported[5)] a low yield (60%) in the synthesis of dithymidilic acid using an alkyl group such as an ethyl group as the internucleotidic phosphate protecting group in the phosphate procedure. More recently, it was also demonstrated in this laboratory that diribonucleotides containing alkyl groups were synthesized from nucleoside alkyl phosphodiesters and nucleoside in the presence of 8-quinolinesulfonyltetrazolide (QS-te).[6)] During work on diuridylic acid synthesis using alkyl groups as an internucleotidic phosphate protecting group, we observed that the yields of diuridylic acid are quite low, and sulfonation at the 5′-hydroxyl of uridine is very high. From these facts, the choice of phosphate protecting group in the phosphodiesters requires careful consideration.

In this paper we report the synthesis of diribonucleotide (3) from nucleoside alkyl- or nucleoside aryl-phosphodiesters (1) and nucleoside (2) in the presence of coupling agents. As coupling agents, five compounds were tested, *i.e.*, QS-te, 8-quinolinesulfonyl chloride (QS),[7)] 2,4,6-triisopropylbenzenesulfonyltetrazolide (TPS-te),[8)] 2,4,6-triisopropylbenzenesulfonyl chloride (TPS),[9)] and mesitylenesulfonyltriazolide (MS-t).[2e)]

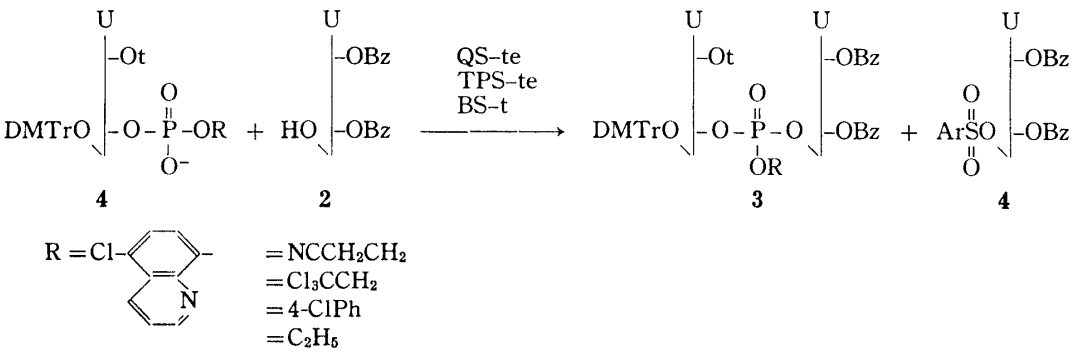


Chart 1

t=tetrahydropyranyl, QS-te=8-quinolinesulfonyltetrazolide.

Table I. Reaction Conditions for the Synthesis of Dinucleotide Monophosphates [DMTrUtp(OR)U(OBz)$_2$] (3)

Reaction No.	DMTrUtpOR	Coupling agent	Time (h)	Isolated yield (%) of 3	2	4
1	5-ClC$_9$H$_5$N-8-	QS-te	1	92	—	—
2	4-ClPh-	QS-te	1	94	—	—
3	5-ClC$_9$H$_5$N-9-	TPS-te	2	89	—	4
4	NCCH$_2$CH$_2$-	QS-te	20	38	29	20
5	Cl$_3$CCH$_2$-	QS-te	20	72	14	11
6	C$_2$H$_5$-	QS-te	20	52	22	24
7	C$_2$H$_5$-	MR-t	48	46	48	Trace
8	5-ClC$_9$H$_5$N-8-	QS	24	81	7	4
9	5-ClC$_9$H$_5$N-9-	TPS	24	70	4	15

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-alkyl- and aryl phosphates (**1**) (0.3 mmol) were treated with 2′,3′-O-dibenzoyluridine (**2**) (0.2 mmol) in the presence of coupling agents (0.6 mmol) in dry pyridine (2 ml) for the times listed in Table I. After usual work-up, the reaction mixtures were chromatographed on a silica gel column; the results are shown in Table I. As shown in Table I, the rates of coupling reaction using nucleoside aryl phosphosiesters (react. No. 1,2) and QS-te are twenty times as fast as using nucleoside alkyl phosphodiesters (react. No. 4—6). Also the degree of sulfonation at the 5′-hydroxyl group is much higher for nucleoside alkyl phosphodiesters than for nucleoside aryl phosphodiesters. Inspection of the sulfonation at the 5′-hydroxyl group in the coupling reaction using nucleoside alkyl phosphodiesters indicated that the degree of sulfonation was dependent upon the alkyl subsituent on the phosphorus atom, *i.e.*, ethyl, 2-cyanoethyl, and 2,2,2-trichloroethyl groups. In the case of nucleoside aryl phosphodiesters, no sulfonated product was observed. On the other hand, when the coupling reactions were carried out in the presence of QS and TPS for 24 h, the corresponding sulfonated products were obtained in 4% and 15% yields, respectively. Thus, the degree of sulfonation was slightly higher than with the tetrazole derivatives (QS-te and TPS-te), but was much lower than with TPS.

MS-t is known to be a very weak sulfonating agent.[2e] We carried out the condensation of nucleoside ethyl phosphodiester and **2** in the presence of MS-t. The rate of coupling reaction was very slow (Table I) but only a trace of sulfonated product was observed (react. No. 7.). However, the extremely slow coupling reaction makes the use of this reagent questionable for the synthesis of internucleotidic bonds.

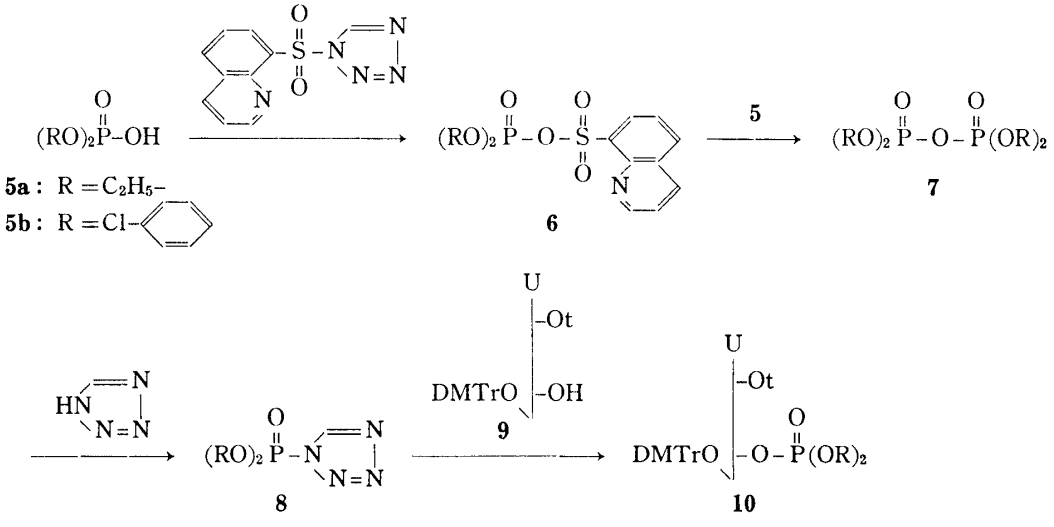
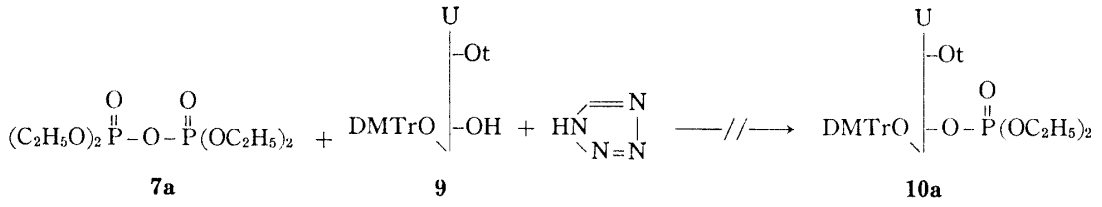


Chart 2



7a **9** **10a**

Chart 3

These observations suggest that the slow rate of the internucleotidic bond formation using nucleoside alkyl phosphodiesters was caused by the lower reactivity of the phosphorus atom in the active phosphorylating species. To investigate the different inductive effects of alkyl and aryl substituents, we carried out the condensation of diethyl phosphate (**5a**) and bis(4-chlorophenyl) phosphate (**5b**) with 5′-*O*-dimethoxytrityl-2′-*O*-tetrahydropyranyluridine (**9**) in the presence of QS-te. When **9** (1 mmol) was treated with diethyl phosphate (**5a**) (1.5 mmol) in the presence of QS-te (3 mmol) in dry pyridine for 20 h, the corresponding 5′-*O*-dimethoxyrityl-2′-*O*-tetrahydropyranyluridine 3′-diethyl phosphate (**10a**) was not obtained and 90% of **9** was recoverd. Therefore, the phosphorylation of **9** was attempted by the use of tetraethyl pyrophosphate (**7a**) in the presence of tetrazole, but the phosphorylation of **9** did not proceed. On the other hand, when bis(4-chlorophenyl) phosphate (**5b**) was used in place of **5a**, the corresponding 5′-*O*-dimethoxytrityl-2′-*O*-tetrahysdropyranyluridine 3′-bi(4-chlorophenyl) phosphate (**10b**) was obtained in 90% yield. These results were quite expected since tetraethyl pyrophosphate (**7a**) is known as a poor phosphorylating agent.[10] Thus, the low yield of nucleoside alkyl phosphodiester was caused by the lower reactivity of the phosphorylating species (tetrapyrophosphate).

In conclusion, it is noted that alkyl groups are not suitable as the protecting group of internucleotidic bonds in the synthesis of oligonucleotides by the phosphotriester approach, whereas aryl groups can be effectively so used.

Experimental

All general methods for thin layer chromatography (TLC), chromatography on silica gel, paper chromatography, paper electrophoresis, and enzyme analyses are described in Refs 5 and 7. HPLC was carried out on a Finepak C_{18} column, which was eluted with 10% acetonitrile in triethylammonium acetate (pH 7.0). 5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine 3′-(5-chloro-8-quinolyl),[5] (4-chlorophenyl),[11] (ethyl),[12] (2,2,2-trichloroethyl),[13] and (2-cyanoethyl)[14] phosphates were prepared by modifications of the published procedures.

The structures of the fully protected dinucleoside monophosphates were established by complete digestion with nuclease P1 after complete removal of the protecting groups. In the case of 5′-*O*-dimethoxytrityl-2′-*O*-tetrahydropyranyluridyl (3′-ethyl-5′) 2′,3′-*O*-dibenzoyluridine, the structure was proved by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) after removal of the dimethoxytrityl group.

General Procedure for the Synthesis of Dinucleotide Monophosphates (3) DMTrUtp(OR)U(OBz)$_2$——The phosphodiester (**1**) (0.3 mmol) was combined with 2′,3′-*O*-dibenzoyluridine (**2**) (90 mg, 0.2 mmol), rendered anhydrous by coevaporation with anhydrous pyridine three times, and then treated with the coupling agent (QS-te, QS, TPS-te, TPS, or MS-t, 0.6 mmol) in anhydrous pyridine (3 ml) at room temperature. After 1—48 h, the reaction mixture was quenched with ice-water, the extracted with methylene chloride (3×30 ml). The methylene chloride layer was washed with 0.1 M triethylammonium bicarbonate (pH 7.5) (3×10 ml), and then water (2×10 ml), dried over anhydrous sodium sulfate, filtered, and evaporated to a gum. The gum was dissolved in benzene and applied on a silica gel column (3×8 cm). The column was eluted with a stepwise gradient of acetone (0—10%) in benzene. The results are shown in Table I.

5′-*O*-8-Quinolinesulfonyl-2′,3′-*O*-dibenzoyluridine (4a)——2′,3′-*O*-Dibenzoyluridine (**2**) (452 mg, 1.0 mmol), 8-quinolinesulfonyl chloride (QS) (441 mg, 2.0 mmol), and triethylamine (0.28 ml, 2.0 mmol) were taken up in anhydrous tetrahydrofurane (THF) (8 ml) solution at room temperature. After 6 h, ice-water was added and after a further period of 1 h, the material was extracted with methylene chloride (3×50 ml). The methylene chloride was washed with water (2×30 ml), then dried over anhydrous sodium sulfate, filtered, and evaporated to a gum. The gum was dissolved in methylene chloride and applied on a silica gel column (3×10 cm). The column was eluted with a stepwise gradient of methanol (0—5%) in methylene chloride.

Crystallization of the purified material from *n*-hexane gave **4a** (641 mg, 98%) as a crystalline solid, mp 107—109°C. *Anal.* Calcd for $C_{32}H_{25}N_3O_{10}S$: C, 59.72; H, 3.91; N, 6.53. Found: C, 59.59; H, 4.09; N, 6.41. Rf=0.75 (benzene/acetone, 1: 1 v/v). UV λ_{max}^{MeOH} nm: 260, 230; λ_{min}^{MeOH} nm: 250.

5′-*O*-2,4,6-Triisopropylbenzenesulfonyl-2′,3′-*O*-dibenzoyluridine (4b)——2′,3′-*O*-Dibenzoyluridine (**2**) (452 mg, 1.0 mmol), 2,4,6-trisopropylbenzenesulfonyl chloride (TPS) (605 mg, 2.0 mmol), and triethylamine (0.28 ml, 2.0 mmol) were taken up in anhydrous THF (8 ml) solution at room temperature for 3 h and then worked up as above. The product was fractionated by column chromatography on a silica gel. Recrystallization of the product from benzene gave **4b** (667 mg, 92%) as a crystalline solid, mp 83—85°C. *Anal.* Calcd for $C_{35}H_{39}N_2O_{10}S$: C, 61.82; H, 5.78; N, 4.12. Found: C, 61.53; H, 5.97; N, 3.95. Rf=**0.76** (benzene/acetone, 1: 1 v/v). UV λ_{max}^{MeOH} nm: 282 (sh), 260, 233; λ_{min}^{MeOH} nm: 250.

Phosphorylation of 5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine by Means of Diethyl Phosphate (5a)——5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine (**9**) (631 mg, 1.0 mmol) was rendered anhydrous by coevaporation with anhydrous pyridine three times and the residue was dissolved in anhydrous pyridine (5 ml). Diethyl phosphate (**5a**) (220 mg, 1.5 mmol) and QS-te (391 mg, 3.0 mmol) were added to the pyridine solution. After 3 h, TLC indicated that the starting material **9** was unchanged (Rf=0.63 in 10% MeOH–CH_2Cl_2). The reaction mixture was quenched with ice-water, then extracted with methylene chloride (3×30 ml). The methylene chloride layer was washed with water (2×10 ml), dried over anhydrous sodium sulfate, filtered, and evaporated to a gum. The gum was dissolved in methylene chloride and applied on a silica gel column. The column was eluted with a gradient of methanol (0—5%) in methylene chloride. The appropriate fractions were concentrated to give **9** (567 mg, 90%) as a crystalline solid by precipitation from *n*-hexane–ether (95: 5 v/v). Rf=0.65 (CH_2Cl_2/MeOH, 9: 1 v/v). UV λ_{max}^{MeOH} nm: 265, 237 (sh); λ_{min}^{MeOH} nm: 250. NMR ($CDCl_3$): δ 7.80 (1H, d, $J_{5,6}$=8 Hz, C_6–H), 7.53 (13H, m, Ar), 6.10 (1H, d, $J_{1',2'}$=5.6 Hz), 5.45 (1H, d, $J_{5,6}$=8 Hz, C_5–H), 4.96 (1H, s, acetal), 4.41—4.15 (3H, m, C_2'–H, C_3'–H, or C_4'–H), 3.80 (6H, s, CH_3O–), 1.65 (6H, br, C-methylene).

Phosphorylation of 5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine by Means of Tetraethyl Pyrophosphate (7a)——5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine (**9**) (631 mg, 1.0 mmol) was rendered anhydrous by coevaporation with anhydrous pyridine three times and the residue was dissolved in anhydrous pyridine (5 ml). Tetraethyl pyrophosphate (**7a**) (435 mg, 1.5 mmol) and tetrazole (105 mg, 1.5 mmol) were added to the pyridine solution. After 3 h, the mixture was worked up in the same manner as described for the diethyl phosphate reaction. **9** was recovered: 548 mg (87%).

5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine 3′-Bis(4-chlorophenyl) Phosphate (10b)——5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranyluridine (**9**) (631 mg, 1.0 mmol) was combined with bis(4-chlorophenyl) phosphate (**5b**) (479 mg, 1.5 mmol), rendered anhydrous by coevaporation with anhydrous pyridine three times, and then treated with OS-te (391 mg, 3.0 mmol) in anhydrous pyridine (5 ml). After 1 h, the reaction mixture was quenched with ice-water, then extracted with methylene chloride (3×30 ml). The methylene chloride layer was washed with 0.1 M triethylammonium bicarbonate (pH 7.5) (2×10 ml), and then water (2×10 ml), dried over anhydrous sodium sulfate, filtered, and evaporated to a gum. The gum was dissolved in methylene chloride and applied on a silica gel column. The column was eluted with a stepwise gradient of methanol (0—2%) in methylene chloride. The product **10b** was precipitated with *n*-hexane from its solution in methylene chloride. The yield of **10b** was 660 mg (90%). mp 93—96°C. *Anal.* Calcd for $C_{47}H_{44}Cl_2O_{12}N_2P$: C, 60.65; H, 4.77; N, 3.01. Found: C, 61.16; H, 5.36; N, 2.78. Rf=0.68 (CH_2Cl_2/MeOH, 9: 1 v/v). UV λ_{max}^{MeOH} nm: 260, 233; λ_{min}^{MeOH} nm: 255.

References and Notes

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