8-Quinolyl Esters of Nucleoside 5'-Phosphates. Part 2.[1] Application to the Synthesis of Oligodeoxyribonucleotides bearing a 5'-Phosphate End Group

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8-Quinolyl nucleoside 5'-phosphates were easily prepared in high yields by the reaction of 8-hydroxyquinoline with nucleoside 5'-phosphates in the presence of di-2-pyridyl diselenide and triphenyl phosphite. The 8-quinolyl group was effective as a protecting group for the 5'-phosphate systems of nucleotides in the synthesis of deoxyribo-oligonucleotides.

PROTECTED oligodeoxyribonucleotides bearing 5'-phosphate end groups are required as intermediates for the synthesis of polydeoxyribonucleotides by frequent condensation. For their production several phosphate protecting groups have been examined.[2] We have briefly reported[1] that the 8-quinolyl group (q) can be used; it can be removed smoothly by treatment with copper(II) chloride in dimethyl sulphoxide–water (5 : 1 v/v). We now describe details of the synthesis of oligodeoxyribonucleotides by this method.

Synthesis of Nucleoside 5'-(8-Quinolyl Phosphates).—The reaction of 8-hydroxyquinoline (5 mmol) with 3'-*O*-acetylthymidine 5'-phosphate (pT_dOAc) (0.5 mmol) was carried out in the presence of di-2-pyridyl diselenide [$(pySe)_2$] (2.5 mmol) and triphenyl phosphite [$(PhO)_3P$] (2.5 mmol) as condensing agents[3] at room temperature for 12 h. Thymidine 5'-(8-quinolyl phosphate) (qpT_d) was obtained in 91% yield after treatment with 2N-sodium hydroxide at 0 °C for 10 min.

TABLE 1

Preparation of nucleoside 5'-(8-quinolyl phosphate)s (1)

Starting materials			Conditions				
Nucleotide	(mmol)	8-Hydroxyquinoline (mmol)	Pyridine (ml)	$(PhO)_3P$–$(pySe)_2$ (mmol)	Time (h)	Products (1)	Yield (%)
d-pTOAc	0.5	5.0	2.5	2.5	12	qpT_d	91
d-pA^{bz}OAc	1.0	10.0	5.0	5.0	12	qpA_d^{bz}	90
d-pG^{iby}OBu	0.5	5.0	2.5	2.5	12	qpG_d^{iby}	94
d-pC^{mp}OAc	0.5	5.0	2.5	2.5	16	qpC_d^{mp}	93
d-pU	1.0	10.0	5.0	5.0	12	qpU_d	90

In a similar manner, 8-quinolyl esters of N^6-benzoyldeoxyadenosine 5'-phosphate (qpA_d^{bz}), N^2-isobutyryldeoxyguanosine 5'-phosphate (qpG_d^{iby}), deoxyuridine 5'-phosphate (qpU_d), and N^4-*p*-methoxyphenyl deoxycytidine 5'-phosphate (qpC_d^{mp}) were obtained as shown in Table 1.

Synthesis of Dinucleotides.—The coupling of qpT_d (0.1 mmol) with $pdA^{bz}OAc$ (0.2 mmol) was carried out in dry pyridine in the presence of $(pySe)_2$ (1.0 mmol) and $(PhO)_3P$ (1.0 mmol) at room temperature for 2 days. The reaction was quenched with water and the mixture was kept at room temperature overnight. It was then treated with 2N-sodium hydroxide to remove the 3'-*O*-acetyl group. After neutralization with Dowex 50W-X2 resin (pyridinium form), the solution was concentrated to small volume. It was chromatographed on a

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[2] G. M. Tener, *J. Amer. Chem. Soc.*, 1961, 83, 829; A. F. Cook, M. J. Holman, and A. L. Nussbaum, *ibid.*, 1965, **87**, 2513; A. Franke, F. Eckstein, K. H. Scheit, and F. Cramer, *Chem. Ber.*, 1968, **101**, 944; E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, *J. Amer. Chem. Soc.*, 1970, **92**, 3441; S. A. Narang, O. S. Bhanot, J. Goodchild, R. H. Wightman, and S. K. Dheer, *ibid.*, 1972, **94**, 6183; R. L. Agarwal, M. Fridkin, E. Jay, and H. G. Khorana, *ibid.*, 1973, **95**, 2020; T. Hata, I. Nakagawa, and Y. Nakada, *Tetrahedron Letters*, 1975, 467;

[3] H. Takaku, Y. Shimada, Y. Nakajima, and T. Hata, *Nucleic Acids Res.*, 1976, 3, 1233.

TABLE 2

Synthesis of protected dinucleotides (2) †

Compound (1) (mmol)	3′-*O*-Acylnucleotide (mmol)	$(pySe)_2$–$(PhO)_3P$ (mmol)	Dinucleotide	Yield (%)
qpT_d 0.1	$pA_d^{bz}OAc$ 0.2	1.0	$qp_dTpA_d^{bz}$	63
qpT_d 1.0	$pG_d^{iby}OBu$ 2.0	10.0	$qpT_dG_d^{iby}$	62
qpT_d 0.2	pT_dOAc 0.4	2.0	qpT_dpT_d	72
qpT_d 0.17	$pC_d^{mp}OAc$ 0.34	1.7	$qpT_dC_d^{mp}$	55
qpA_d^{bz} 0.1	$pA_d^{bz}OAc$ 0.2	1.0	$qpA_d^{bz}Pa_d^{bz}$	64
qpA_d^{bz} 0.1	pT_dOAc 0.2	1.0	$qpA_d^{bz}PT_d$	69
qpU_d 0.5	pU_dOAc 1.0	2.5	qpU_dpU_d	64

† Reactions carried out in dry pyridine at room temperature.

DEAE cellulose column with a linear gradient (0—0.5M) of triethylammonium hydrogen carbonate (TEAB) in 50% ethanol. A mixture of $qpT_dpA_d^{bz}$ and qpT_d was eluted. The solvent was removed by addition and evaporation of pyridine and the residue was further applied to a column of Sephadex G-25 (superfine) [4] eluted with TEAB (0.1M) solution. First, the dinucleotide, $qpT_dpA_d^{bz}$, was eluted. In a similar manner, the other dinucleotide derivatives were obtained as shown in Table 2.

In the above reaction, 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPS) can be also used as the coupling agent in place of $(pySe)_2$ and $(PhO)_3P$.

Synthesis of Trinucleotides.—Trinucleotide derivatives were synthesized by coupling of dinucleotide derivatives (2) prepared as described in the above experiment with 3′-*O*-acetylnucleoside 5′-phosphates.

For example, when $qpT_dpA_d^{bz}$ (0.15 mmol) was treated with $pA_d^{bz}OAc$ (0.3 mmol) in the presence of $(pySe)_2$ (1.5 mmol) and $(PhO)_3P$ (1.5 mmol) in dry pyridine (1.5 ml) at room temperature for 2 days, $qpT_dpA_d^{bz}pA_d^{bz}$ was obtained.

In a similar manner, trinucleotide derivatives (3) such as $qpU_dpU_dpU_d$, $qpT_dpT_dpT_d$, $qpT_dpG_d^{iby}pT_d$, $qpT_dpA_d^{bz}pA_d^{bz}$, and $qpT_dpA_d^{bz}pC_d^{mp}$ were synthesized as described in the Experimental section.

TABLE 3

Degradation of trinucleotides (4) by snake venom phosphodiesterase

Compound	Nucleotides	Ratios of nucleotides: Predicted	Found
$pT_dpT_dpT_d$	pT_d		
$pT_dpA_dpA_d$	$pT_d : pA_d$	1 : 2	1 : 1.96
$pT_dpG_dpT_d$	$pT_d : pG_d$	2 : 1	2.01 : 1
$pT_dpA_dpC_d$	$pT_d : pA_d : pC_d$	1 : 1 : 1	0.98 : 1 : 0.94
$pU_dpU_dpU_d$	pU_d		

Removal of the 8-Quinolyl Group from the Trinucleotides.—A trinucleotide derivative, qpT_d-$pA_d^{bz}pA_d^{bz}$ (0.075 mmol) was treated with copper(II) chloride (0.075 mmol) in Me_2SO–H_2O (5 : 1 v/v; 15 ml) at 45 °C for 5 h. The mixture was concentrated to dryness *in vacuo* and the residue was dissolved in water. The aqueous solution was washed with chloroform to remove 8-hydroxyquinoline–copper complex. The trinucleotide $pT_dpA_d^{bz}pA_d^{bz}$ was obtained in 81% yield, homogeneous on paper chromatography and paper electrophoresis. The structure was supported by u.v. spectra before and after removal of the benzoyl groups on the adenine residues. The trinucleotide, $pT_dpA_dpA_d$ was obtained in 94% yield based on $pT_dpA_d^{bz}pA_d^{bz}$ after treatment with methanolic ammonia and degraded with snake venom phosphodiesterase to give pT_d and pA_d in the ratio 1.0 : 1.96.

Similarly, several trideoxyribonucleotides were obtained. Spectral properties and chromatographical data are given in Table 4.

TABLE 4

Spectral properties and chromatographic data

Compound	$\lambda_{max.}(H_2O)$ ($\varepsilon \times 10^{-3}$)/nm (pH 7)	R_F Value [a] (A)	(B)	(C)	R_m Value [b, c] (pH 8.0)
pT_d	267(9.6)	0.17	0.33	0.48	1.00
pT_dOAc	267(9.6)		0.45		0.98
qpT_d	266(9.6), 237	0.50	0.87		0.48
qpT_dpT_d	267(18.3), 234	0.40	0.78		0.65
$qpT_dpT_dpT_d$	267(25.5), 233	0.38	0.69		
$pG_d^{iby}OBu$	259(16.7)		0.58		
qpG_d^{iby}	254(16.7), 234		0.90		0.50
$qpT_dpG_d^{iby}$	258(25.0), 235		0.78		0.70
$qpT_dpG_d^{iby}pT_d$	260(34.3), 233		0.69		
$pC_d^{mp}OAc$	300(22.4), 234		0.56		
qpC_d^{mp}	300(22.4), 234		0.91		0.40
$qpT_dpC_d^{mp}$	301(22.4), 275sh, 234		0.74		0.60
$pA_d^{bz}OAc$	280(18.8)		0.50		
qpA_d^{bz}	280(18.8), 234		0.89		0.49
$qpT_dpA_d^{bz}$	280(25.3), 234		0.73		0.66
$qpA_d^{bz}pA_d^{bz}$	280(35.0), 234		0.78		0.58
$qpT_dpA_d^{bz}pA_d^{bz}$	281(42.8), 232		0.55		0.63
$qpT_dpA_d^{bz}pC_d^{mp}$	285(40.6), 300sh, 238		0.66		0.73
pU_dOAc	260(10.0)		0.34		
qpU_d	260(10.0), 234	0.63	0.91		0.45
qpU_dpU_d	261(19.1), 235	0.46	0.75		0.63
$qpU_dpU_dpU_d$	261(28.3), 234	0.35	0.70		
$pT_dpG_d^{iby}pT_d$	260(34.3)		0.19		
$pT_dpA_d^{bz}pA_d^{bz}$	280(42.8)		0.24		
$pT_dpA_d^{bz}pC_d^{mp}$	283(40.6), 300sh, 238		0.23		
$pU_dpU_dpU_d$	260(28.3)			0.17	1.03
$pT_dpT_dpT_d$	267(25.4)			0.19	1.07
$pT_dpG_dpT_d$	262(29.4)			0.11	1.09
$pT_dpA_dpA_d$	259(35.0)			0.24	1.09
$pT_dpA_dpC_d$	264(28.0)			0.22	1.12

[a] Paper chromatography performed by descending technique with Toyo Roshi No. 51 paper in solvent system as described in Experimental section. [b] Paper electrophoresis; R_m refers to the mobility relative to pT. [c] Phosphate buffer (0.05M) used as solvent.

EXPERIMENTAL

Paper chromatography was performed by the descending technique with Toyo Roshi Nos. 51 and 51A papers. Solvent systems used were: (A) propan-2-ol–concentrated ammonia–water (7 : 1 : 2 v/v); (B) ethanol–M-ammonium

[4] S. A. Narang, J. J. Michniewicz, and S. K. Dheer, *J. Amer. Chem. Soc.*, 1969, **91**, 936; S. A. Narang and S. K. Dheer, *Biochemistry*, 1969, **8**, 3443.

acetate (pH 7.5) (7 : 3 v/v); (C) propan-1-ol–concentrated ammonia–water (55 : 10 : 35 v/v); and (D) saturated ammonium sulphate–propan-2-ol–water (79 : 19 : 2 v/v).

Paper electrophoresis was performed in potassium phosphate buffer (0.05M; pH 8.0) by a commercially available apparatus capable of giving a potential of 1 200 V.

Pyridinium salts of the protected nucleotides, pT_d-OAc,[5] $pC_d^{mp}OAc$,[6] $pA_d^{bz}OAc$,[7] $pG_d^{iby}OBu$,[8] and pU_dOAc * were prepared by literature procedures. Dry pyridine was prepared by distillation after treatment with toluene-*p*-sulphonyl chloride and dried over calcium hydride. $(pySe)_2$ was prepared as described previously.[3] $(PhO)_3P$ was purified by distillation before use. Phosphorus-containing compounds were detected as blue spots on paper chromatograms by spraying with the Hanes–Isherwood reagent [9] followed by u.v. irradiation.[10] Avecel-cellulose t.l.c. plates (Funakoshi), DEAD cellulose (Brown, Seikagaku Kogyo), and Sephadex G-25 (superfine) (Pharmacia Fine Chem.) were purchased commercially.

Deoxyribonucleoside 5′-(8-Quinolyl Phosphates) (1) (*General Procedure*).—A mixture of 3′-*O*-acylnucleoside 5′-phosphate (1 mmol) and 8-hydroxyquinoline (10 mmol) was rendered anhydrous by addition and evaporation of dry pyridine and finally dissolved in dry pyridine (5 ml). $(pySe)_2$ (5 mmol) and then $(PhO)_3P$ (5 mmol) were added with exclusion of moisture. The mixture was kept at room temperature for 12 h; water (2 ml) was then added and the mixture was shaken at room temperature for 12 h. The solution was concentrated to dryness and the residue was treated with 2N-sodium hydroxide at 0 °C for 10 min. It was neutralised with Dowex 50W-X2 resin (pyridinium form). After removal of the resin, the solution was concentrated *in vacuo*. The residue was coevaporated with dry pyridine and then was added dropwise to a large amount of ether–n-hexane (1 : 1 v/v). The precipitate was collected by centrifuge and washed twice with n-hexane. After removal of n-hexane *in vacuo*, the nucleoside 5′-(quinolyl phosphate) (1) was obtained as a white powder.

Reaction conditions and yields are summarised in Table 1.

Synthesis of Protected Dinucleotides (*General Procedure*).—A mixture of nucleoside 5′-(8-quinolyl phosphate) (0.1 mmol) and 3′-*O*-acylnucleoside 5′-phosphate (0.2 mmol) was rendered anhydrous by addition and evaporation of dry pyridine and finally dissolved in dry pyridine (1 ml). $(pySe)_2$ (0.5 mmol) and then $(PhO)_3P$ (0.5 mmol) were added with continuous stirring. The mixture was kept at room temperature for 2 days. After removal of the acyl group with 2N-sodium hydroxide at 0 °C for 10 min, the solution was neutralised by addition of Dowex 50W-X2 resin (pyridinium form). The resin was filtered off and the filtrate was coevaporated with toluene *in vacuo* at 20 °C to remove pyridine. It was chromatographed on a DEAE cellulose column (carbonate form; 52 × 2.5 cm) with a linear gradient (0—0.5M) of TEAB in 50% ethanol. A mixture of dinucleotide (2) and (1) was eluted. The eluate was concentrated with addition of pyridine and further applied to a Sephadex G-25 (superfine) column (50 × 2.5 cm). The column was treated with 0.1M-TEAB buffer (pH 7.1). First, the dinucleotide (2) was eluted. Reaction conditions and results are summarised in Table 2.

Synthesis of Trinucleotides.—$pT_dpT_dpT_d$. To a solution of qpT_dpT_d (0.1 mmol) and pT_dOAc (0.2 mmol) in dry pyridine (0.5 ml), were added $(pySe)_2$ (1.0 mmol) and $(PhO)_3P$ (1.0 mmol) at room temperature and the mixture was stirred continuously for 2 days. The reaction was quenched with water and the mixture was kept at room temperature for 12 h. The solution was concentrated to dryness *in vacuo*. The residue was treated with 2N-sodium hydroxide at 0 °C for 10 min and then neutralised with Dowex 50W-X2 resin (pyridinium form). After removal of the resin, the filtrate was concentrated *in vacuo* to a small volume. It was chromatographed on a DEAE cellulose column (carbonate form; 50 × 3.0 cm). Elution was performed by a linear gradient of 0—0.5M-TEAB in 50% ethanol. A mixture of $qpT_dpT_dpT_d$ and qpT_dpT_d was eluted. The eluate was concentrated with addition of pyridine and applied to a Sephadex G-25 (superfine) column (70 × 2.5 cm). Elution was performed with 0.1M-TEAB buffer (pH 7.1). First, $qpT_dpT_dpT_d$ was eluted (63% yield).

The $qpT_dpT_dpT_d$ (0.05 mmol) was dissolved in Me_2SO–H_2O (5 : 1 v/v; 5 ml), and copper(II) chloride (6.7 mg, 0.05 mmol) was added. The solution was kept at 45 °C for 5 h. It was then concentrated *in vacuo* and the residue was dissolved in water (10 ml). The aqueous solution was washed with chloroform (2 × 10 ml) to remove the 8-hydroxyquinoline–copper complex. The aqueous layer was concentrated to a small volume and applied to a paper chromatogram. [solvent (B)] $pT_dpT_dpT_d$ was separated (0.045 mmol, 90% estimated spectrophotometrically).

Spectral properties and chromatographic data of qpT_d-pT_dpT_d and $pT_dpT_dpT_d$ are listed in Table 4.

$pT_dpA_d^{bz}pA_d^{bz}$. To a solution of $qpT_dpA_d^{bz}$ (0.15 mmol) and $pA_d^{bz}OAc$ (0.3 mmol) in dry pyridine (1.5 ml) were added $(pySe)_2$ (1.5 mmol) and $(PhO)_3P$ (1.5 mmol) at room temperature. The mixture was stirred for 2 days and the reaction was quenched with water. The mixture was kept at room temperature overnight. After the usual work-up, $qpT_dpA_d^{bz}pA_d^{bz}$ was isolated in 62% yield by the same procedure as described for $qpT_dpT_dpT_d$. The 8-quinolyl group was removed from the trinucleotide (0.075 mmol) by treatment with copper(II) chloride (10.1 mg, 0.075 mmol) as in the above experiment to give $pT_dpA_d^{bz}pA_d^{bz}$ in 81% yield (see Table 4).

$pT_dpG_d^{iby}pT_d$. To a solution of $qpT_dpG_d^{iby}$ (0.6 mmol) and pT_dOAc (1.2 mmol) in dry pyridine (6 ml) were added $(pySe)_2$ (6.0 mmol) and $(PhO)_3P$ (6.0 mmol) at room temperature. The mixture was stirred continuously at room temperature for 2 days. The reaction was quenched by addition of water and the mixture was kept at room temperature overnight. After the usual work-up, $qpT_dpG^{iby}pT_d$ was isolated in 43% yield by the same procedure as described for $qpT_dpT_dpT_d$. $qpT_dpG_d^{iby}pT_d$ (0.16 mmol) was treated with copper(II) chloride (21.4 mg, 0.16 mmol) to give $pT_dpG_d^{iby}pT_d$ in 90% yield by the same procedure as for $pT_dpT_dpT_d$ (see Table 4).

$pT_dpA_d^{bz}pC_d^{mp}$. To a solution of $qpT_dpA_d^{bz}$ (0.23 mmol) and $pC^{mp}OAc$ (0.46 mmol) in dry pyridine (2.6 ml) were

* pU_dOAc was prepared by modification of the procedure for pT_dOAc described by Khorana.[5]

[5] P. T. Gilham and H. G. Khorana, *J. Amer. Chem. Soc.*, 1958, **80**, 6212; H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *ibid.*, 1961, **83**, 686.

[6] H. Schaller and H. G. Khorana, *J. Amer. Chem. Soc.*, 1963, **85**, 3828.

[7] S. A. Narang, T. M. Jacob, and H. G. Khorana, *J. Amer. Chem. Soc.*, 1967, **89**, 2167.

[8] H. Buchi and H. G. Khorana, *J. Mol. Biol.*, 1972, **72**, 251.

[9] C. S. Hanes and F. A. Isherwood, *Nature*, 1949, **164**, 1107.

[10] R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, 1951, **193**, 405.

added $(pySe)_2$ (2.3 mmol) and $(PhO)_3P$ (2.3 mmol) at room temperature and the mixture was stirred for 2 days. The reaction was quenched with water and the mixture was kept at room temperature overnight. After the usual work-up, $qpT_dpA_d^{bz}pC_d^{mp}$ was isolated in 58% yield by the same procedure as for $qpT_dpT_dpT_d$. $qpT_dp_d^{bz}ApC_d^{mp}$ (0.1 mmol) was treated with copper(II) chloride (13.4 mg, 0.1 mmol) to give $pT_dpA_d^{bz}pC_d^{mp}$ in 81% yield (see Table 4).

$pU_dpU_dpU_d$. To a solution of qpU_dpU_d (0.5 mmol) and pU_dOAc (1.0 mmol) in dry pyridine (5 ml) were added $(pySe)_2$ (5.0 mmol) and $(PhO)_3P$ (5.0 mmol) at room temperature. The mixture was stirred for 2 days. The reaction was quenched with water and the mixture was kept at room temperature overnight. After the usual work-up, $qp_dUpU_dpU_d$ was isolated in 74% yield by the same procedure as for $qp_dTpT_dpT_d$. $qpU_dpU_dpU_d$ (0.1 mmol) was treated with copper(II) chloride (13.4 mg, 0.1 mmol) to give $pU_dpU_dpU_d$ in 95% yield (see Table 4).

Removal of the Protecting Groups on Nucleoside Bases from (4) *and Enzymic Degradation of Trinucleotides.*—The protecting groups of nucleoside bases such as adenine, guanine, and cytosine were removed by treatment with an excess of methanolic ammonia at room temperature for 2 days. The solution was then concentrated to dryness *in vacuo*. Each unprotected trinucleotide (*ca.* 10 OD_{260} units) was dissolved in H_2O (120 μl) and treated with snake venom phosphodiesterase (1 mg ml^{-2}) (10 μl) in the presence of M-$(NH_4)_2HCO_3$ (20 μl) at 37 °C for 12 h.[11] The incubated solution was chromatographed on Toyo Roshi No. 51 paper in solvents (C) and (D). Each spot was extracted with water (pH 7) and u.v. absorption spectra were recorded. The results are listed in Table 3.

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[11] M. Ikehara, S. Uesugi, and J. Yano, *J. Amer. Chem. Soc.*, 1974, **96**, 4966.