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Studies on Transfer Ribonucleic Acids and Related Compounds. XVII.[1] Studies on Protecting Groups of Trisubstituted Phosphates in the Synthesis of Ribooligonucleotides

Eiko Ohtsuka, Hisayo Tsuji, Tetsuo Miyake, and Morio Ikehara

Faculty of Pharmaceutical Sciences, Osaka University[2]

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Various protecting groups were introduced to the phosphate of 2′-O-benzoyluridine 3′-phosphate. Stability and selective removal of these groups from the trisubstituted phosphate were investigated and nucleotides with trisubstituted phosphate were used for the synthesis of dinucleotides by condensation with a properly protected mononucleotide.

Keywords——cyanoethyl phosphate of 2′-O-benzoyluridine; trichloroethyl phosphate of 2′-O-benzoyluridine; photolysis of *o*-nitrobenzyl phosphate; phosphoroanilidates of nucleotide esters

Synthesis of ribooligonucleotides with defined sequence remains a crucial problem for studies on structure-function relationship of nucleic acids. The fragment condensation technique has been shown to be one useful method in the synthesis of ribo- and deoxyribopolynucleotides.[3] Aromatic amidates have been used as protecting groups for the phosphate in the synthesis of ribooligonucleotide blocks which were suitable for fragment condensation.[4] Oligonucleotides with a 3′-phosphate have become useful substrate for ribonucleic acid (RNA) ligase.[5] To improve the method for the synthesis of this type of compound introduction of various substituents to the 3′-phosphate of 2′-O-benzoyluridine was investigated. Condensation of the mononucleotide (I) (Chart 1) with the nucleoside (II) proceeds better than the corresponding reaction which involves the protected nucleotide (III).[6] The 3′-phosphate

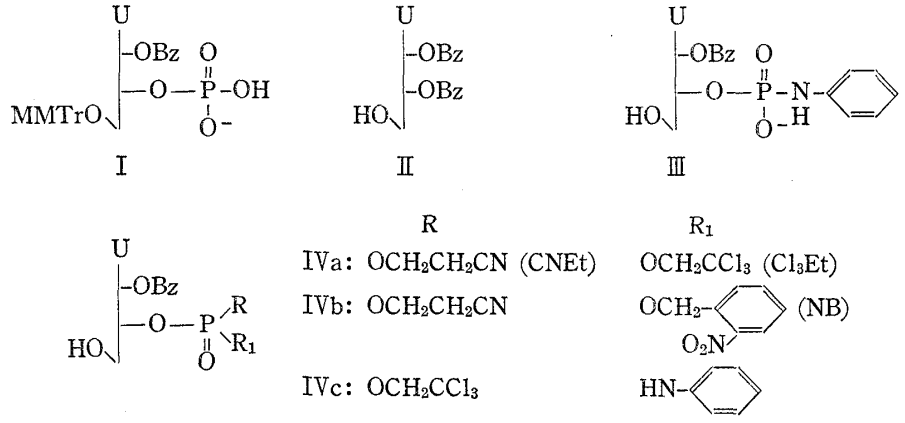

Chart 1

1) Part XVI: E. Ohtsuka, S. Tanaka, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **25**, 949 (1977).
2) Location: *133–1 Yamadakami, Suita, Osaka, 565, Japan.*
3) *a*) M. Ikehara, *Accounts of Chem. Res.*, **7**, 92 (1974); *b*) H. Kössel and H. Seliger, "Progress in the Chemistry of Organic Natural Products," Vol. 32, ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, Wien, 1975.
4) *a*) E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, *J. Am. Chem. Soc.*, **92**, 3441 (1970); *b*) E. Ohtsuka, M. Ubasawa, S. Morioka, and M. Ikehara, *ibid.*, **95**, 4725 (1973); *c*) E. Ohtsuka, K. Fujiyama, M. Ohashi, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **24**, 570 (1976).
5) J.W. Cranston, R. Silber, V.G. Malathi, and J. Hurwitz, *J. Biol. Chem.*, **249**, 7447 (1974).
6) E. Ohtsuka, T. Sugiyama, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **23**, 2257 (1975).

in III could be fully protected by esterification or amidate formation to prevent side reactions during condensation. Three types of trisubstituted uridine 3′-phosphate (IV) were synthesized and the conditions for selective removal of the substituents was investigated. These fully substituted nucleotides were used in the synthesis of dinucleotides to test their utilities as starting materials.

2′-O-Benzoyluridine 3′-(β-Cyanoethyl, β′,β′,β′-Trichloroethyl)phosphate (IVa)

The β-cyanoethyl (cyanoethyl) group has been used most commonly in deoxyribooligonucleotide synthesis as a protecting group for phosphomonoesters.[3,7] Söll and Khorana synthesized 2′-O-benzoyluridine 3′-cyanoethylphosphate and found that ammoniacal removal of the cyanoethyl group competed with that of the 2′-O-benzoyl group. The free 2′-hydroxyl group attacks phosphorus atom to give the 2′,3′-cyclic phosphate.[8] It is also well known that removal of the cyanoethyl group from the ester of nucleoside 5′-phosphate requires rather strong alkaline treatment.[9] On the other hand dicyanoethylated thymidine 5′-phosphate was converted easily to the monocyanoethylated product at pH 9.[7] From this evidence it seemed reasonable to introduce only one cyanoethyl group to uridine 3′-phosphate and to cover the third dissociation with an alkaline stable group so that the cyanoethyl group could be removed from the trisubstituted phosphate in a partial deprotection step. The β,β,β-trichloroethyl (trichloroethyl) group was used as the alkaline stable group.[10] 2′-O-Benzoyluridine 3′-(cyanoethyl trichloroethyl) phosphate (IVa) was synthesized by condensing 5′-monoethoxytrityl-2′-O-benzoyluridine 3′-phosphate[11] (I) with a 7 fold excess of trichloroethanol using a 3 fold excess of dicyclohexylcarbodiimide (DCC) in pyridine for 15 hr at room temperature, and then with a 10 fold excess of cyanoethanol using a 10 fold excess of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS). Deoxynucleoside 3′(cyanoethyl, trichloroethyl) phosphates have been synthesized by Catlin, *et al.*[10b] The product was characterized by thin-layer chromatography (TLC) and its ultraviolet (UV) spectral properties. The yield of the triester was *ca.* 70%. The monomethoxytrityl group was removed by treatment of 80% acetic acid for 5 hr. The cyanoethyl group was stable either in this treatment or in anhydrous pyridine for 5 days but could be removed with dilute ammonia (pH 9.0) in aqueous pyridine within 10 min. Removal of the trichloroethyl group of IVa was attempted both by treatment with Zn/pyridine–acetic acid[12] or with a Zn-Cu couple in dimethyl formamide (DMF).[13] The former treatment gave a few percent of the desired reaction product after 2 hr whereas the latter yielded 2′-O-benzoyluridine 3′-cyanoethyl phosphate (74%). The rest of the product was the decyanoethylated diester. Treatment of 2′-O-benzoyluridine 3′-trichloroethyl phosphate under the same conditions resulted in complete removal of the trichloroethyl group but 17% of the 2′-O-benzoyl group was also removed. It was also observed that the N-benzoyl group of cytidine and adenosine was cleaved by the Zn-Cu couple.[14] It seems that treatment with this metal couple is not suitable for synthesis where acylated oligonucleotide blocks are involved.

2′-O-Benzoyluridine 3′-(Cyanoethyl, *o*-Nitrobenzyl)phosphate (IVb)

In an alternative approach the *o*-nitrobenzyl group was then investigated instead of the trichloroethyl group. This photosensitive group has been used for amino acid and carbohy-

7) E. Ohtsuka, M. Moon, and H.G. Khorana, *J. Am. Chem. Soc.*, **87**, 2956 (1965).
8) D. Söll and H.G. Khorana, *J. Am. Chem. Soc.*, **87**, 360 (1965).
9) G.M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
10) *a*) F. Eckstein, *Chem. Ber.*, **100**, 2236 (1967); *b*) J.C. Catlin and F. Cramer, *J. Org. Chem.*, **38**, 245 (1973).
11) R. Lohrmann, D. Söll, H. Hayatsu, E. Ohtsuka, and H.G. Khorana, *J. Am. Chem. Soc.*, **88**, 819 (1966).
12) *a*) J. Smrt, *Collection Czech. Chem. Commun.*, **37**, 1870 (1972); *b*) *Idem, ibid.*, **38**, 3642 (1973).
13) E. LeGoff, *J. Org. Chem.*, **29**, 2048 (1964).
14) T. Tanaka, Unpublished experiments.

drate protection.[15] 2′,3′-O-Isopropyrideneuridine 5′-(*o*-nitrobenzyl)phosphate was shown to be cleaved with UV irradiation at wavelengths longer than 305 nm.[16] For the synthesis of IVb, 5′-O-monomethoxytrityl-2′-O-benzoyluridine 3′-phosphate (I) was reacted with *o*-nitrobenzyl alcohol and DCC at room temperature for 2 days. As shown in chart 2 the

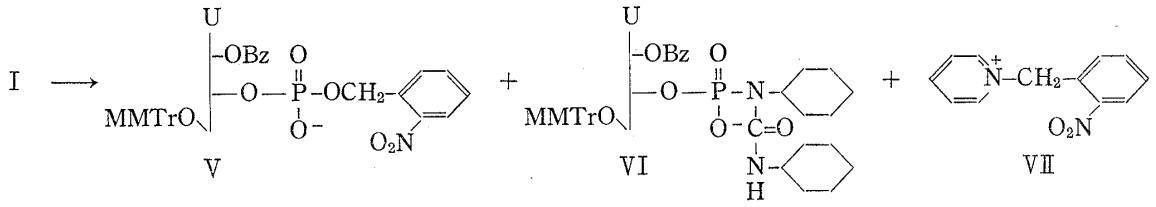

Chart 2

o-nitrobenzyl ester (V) was obtained in a yield of 78% and the phosphorylurea (*p*(DCU)) (VI), was detected as a side product (22%). VI was characterized by cyclization after removal of the protecting groups.[17] A pyridinium compound VII was detected in paper electrophoresis. (Table I) The *o*-nitrobenzyl ester (V) was identified by UV irradiation after detritylation

Table I. Paper Chromatography and Electrophoresis

	Rf Solvent A	*Rf* Solvent B	Relative mobility pH 7.5
C	0.56	0.69	0
C>p	0.48	0.49	0.61
Cp	0.21	0.22	1
Cp(CNEt)	0.56		0.59
C^{Bz}(OBz)p–(CNEt)			0.33
U	0.52	0.67	0
U>p	0.45	0.54	0.67
Up	0.19	0.22	1
Up(DCU)		0.88	0.34
Up–(CNEt)		0.62	0.57
Up–(Cl_3Et)	0.49	0.65	0.57
Up–oNB	0.66	0.71	0.43
U(OBz)p–(CNEt)			0.49
U(OBz)p–(Cl_3Et)			0.48
U(OBz)–p(NB)			0.36
CpUp	0.1		1.01
CpUp–(NB)	0.4		0.65
CpUp–(Cl_3Et)	0.22		0.77
VII		0.72	−1.01

and deacylation to give uridine 3′-phosphate. Treatment of V with DCC or TPS yielded a pyrophosphate and VII. Presumably the *o*-nitrobenzyl ester of tri- or tetra-substituted pyrophosphate was attacked by pyridine in a similar manner as in the case of the unsubstituted benzyl ester.[18] The triester IVb was synthesized from the mono cyanoethylester by treating with *o*-nitrobenzyl alcohol using TPS for 24 hr in pyridine and treatment with 80% acetic acid for 12 hr. The detritylated triester IVb was isolated by preparative TLC on silica gel in a yield of 57%. The diastereoisomer were separated in TLC and characterized by removing the protecting groups. The triester was stable to 80% acetic acid for at least

15) B. Amit, U. Zehavi, and A. Patchornik, *Israel J. Chem.*, **12**, 103 (1974).
16) M. Rubinstein, B. Amit, and A. Patchornik, *Tetrahedron Lett.*, **1975**, 1455.
17) E. Ohtsuka, M. Ubasawa, and M. Ikehara, *J. Am. Chem. Soc.*, **92**, 3445 (1970).
18) V.M. Clark and A.R. Todd, *J. Chem. Soc.*, **1950**, 2023.

45 hr at 30°. However, in anhydrous pyridine 40% of the *o*-nitrobenzyl group in IVb was cleaved after 120 hr. The pyridinium compound (VII) was detected in the reaction mixture. Although the cyanoethyl group of IVb could be removed selectively and the mono-*o*-nitrobenzyl ester of 2′-O-benzoyluridine 3′-phosphate may be stable in pyridine, the *o*-nitrobenzyl group on triesterified nucleotides would seem to be somewhat labile during prolonged condensation reactions.

2′-O-Benzoyluridine-3′-(trichloroethyl)phosphoranilidate (IVc)

Phosphormonoanilidates of protected nucleosides have been used as protecting groups of phosphate in ribooligonucleotide blocks and removal of these groups was easily performed by treating with isoamyl nitrite in pyridine–acetic acid.[4] For the synthesis of IVc introduction of anilino group to the diesterified phosphate was first attempted using TPS as the activation reagent. The result was not satisfactory. Even with a large excess (10 fold) of aniline and TPS (5 fold) the yield was only 20%. Using a 15 fold excess of triphenylphosphine-2,2′-dipyridyldisulfide[19] and aniline gave 60% of the anilidate as estimated by the amount of unchanged diester. The product (IVc) was isolated by preparative TLC on silica gel after removal of the monomethoxytrityl group. IVc was stable in anhydrous pyridine for at least 5 days and decomposed 1.5% in 80% acetic acid at 30° for 24 hr. Conversion of the anilidate to the phosphodiester was performed quantitatively by treatment with isoamyl nitrite in pyridine–acetic acid for 5 hr at 30°. It may be concluded that this trisubstituted mononucleotide can be used for the synthesis of dinucleotide blocks.

Synthesis of Dinucleotide Blocks using IVb and IVc

The mononucleotide IVb was allowed to react with 5′-O-monomethoxytrityl-N,2′-O-dibenzoylcytidine 3′-phosphate (VIII) using either DCC or TPS (Chart 3). The condensation

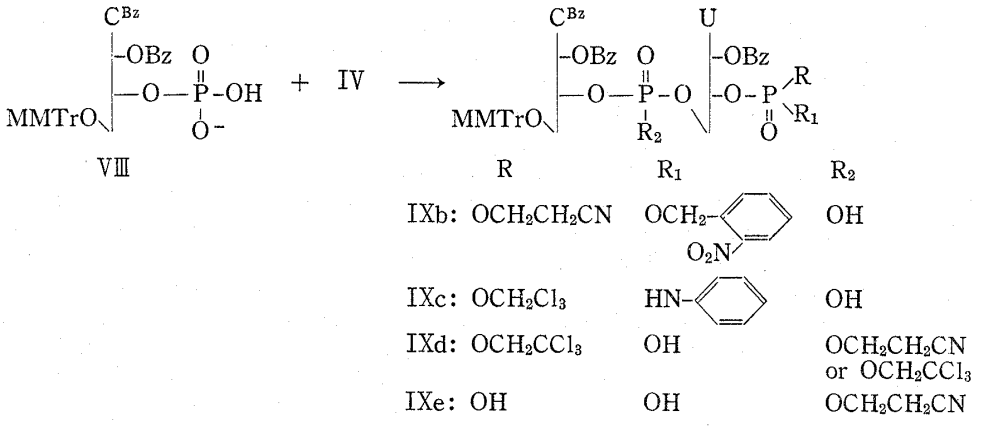

Chart 3

using DCC proceeded in 60% yield after 2 days as based on the amount of remaining 3′-phosphorylated compound (VIII) but 34% of the dinucleotide (IXb) lost the terminal *o*-nitrobenzyl group. In the TPS condensation reaction the *o*-nitrobenzyl ester of ca 50% of the product was cleaved after 18 hr reaction. The combined yield of the dimer was 47% as estimated from the unchanged monoester component (VIII). The product was characterized after removing the protecting groups, by hydrolysis with RNase A. Up and Cp were obtained in a ratio of 1 to 1.

The protected dinucleotide (IXc) was obtained from IVc by condensation with VIII and DCC. IXc was separated from the starting materials by preparative TLC and the yield was 67% as estimated from the ratio of IXc to VIII. The dinucleotide (IXc) may be useful if

19) T. Mukaiyama and M. Hashimoto, *J. Am. Chem. Soc.*, **94**, 8528 (1972).

the internucleotidic phosphate is esterified either with the trichloroethyl or cyanoethyl group and then the anilidate is removed with isoamyl nitrite to give a fully protected dinucleotide block IXd.

Conclusion

From above experiments it may be concluded that the triesterified nucleotide IVa could be used for 3′-end protection of oligonucleotide blocks with trichloroethylated internucleotide phosphates, since the removal of the cyanoethyl group of IVa was achieved selectively whereas removal of the trichloroethyl group resulted in N-debenzoylation. The trichloroethyl group would be removed only at the final stage prior to 2′-O-debenzoylation. The *o*-nitrobenzyl group seemed to be unsuitable as a protecting group for phosphate. Even a diesterified nucleotide such as the *o*-nitrobenzylester of uridine 5′-phosphate might be expected to loose the ester since activated pyrophosphates have trisubstituted phosphates. The mononucleotide IVc could serve as a 3′-terminal nucleotide in the synthesis of IXc, which could be converted to IXd by esterification of the internucleotide phosphate. Oligonucleotide blocks with a phosphodiester end such as IXd require vigorous activation compared with the activation required for phosphomonoesters. It would therefore seem to be desirable to obtain protected oligonucleotide having 3′-phosphomonoesters, such as IXe. The phosphorodianilidate of deoxyribonucleosides[20] was reported as a protection of terminal phosphates and seemed to be suitable for synthesis of fully protected oligonucleotide blocks with phosphomonoester termination. Introduction of phosphordianilidate to the 3′-position of protected ribodinucleotides is under investigation and will be reported shortly.

Experimental

Paper chromatography was performed by the descending technique using solvent systems: A, 2-propanol–ammonia–water (7 : 1 : 2, v/v); B, ethanol–1 M ammonium acetate, pH 7.5 (7 : 3, v/v). Paper electrophoresis was performed using 0.05 M triethylammonium bicarbonate, pH 7.5 at 900 V/40 cm. Other general methods[4b] and photolysis procedure[21] were as described previously.

2′-O-Benzoyluridine 3′-(Cyanoethyl-trichloroethyl)phosphate (IVa)——The pyridinium salt of 5′-O-monomethoxytrityl-2′-O-benzoyluridine-3′-phosphate (I) (1 mmol) was coevaporated with pyridine 5 times and then treated with trichloroethanol (0.68 ml, 7 mmol) in pyridine (6 ml) in the presence of DCC (2.06 g, 10 mmol). The solvent was evaporated to *ca.* 3 ml and the mixture was kept for 15 hr at room temperature. The extent of reaction was checked by TLC ($CHCl_3$–MeOH, 5 : 1) and paper electrophoresis of the detritylated mixture. Aqueous pyridine (50%, 20 ml) was added and DCC was removed with hexane (5 ml) 3 portions. The filtered solution was kept for 3 hr and extracted with $CHCl_3$ (20 ml) 3 portions. The organic layer was centrifuged to break the emulsion, washed with 50% pyridine and concentrated with pyridine. The product was precipitated with 1 : 1 ether–hexane from its solution in anhydrous pyridine. The yield of the diester was 78%. This diester (0.05 mmol) was rendered anhydrous by evaporation with pyridine 5 times and condensed with cyanoethanol (0.04 ml, 0.5 mmol) using TPS (61 mg, 0.2 mmol) in pyridine. The mixture was concentrated to a half the volume and kept for 2 days. TLC ($CHCl_3$–MeOH, 19 : 1) showed that 70% of the diester (*Rf*, 0.05) was converted to the triester (*Rf*, 0.54). Aqueous pyridine (50%, 10 ml) was added with cooling and after 4 hr the product was extracted with $CHCl_3$ (5 ml) 3 portions. The organic layer was washed with water (5 ml) 3 portions and pyridine was removed by evaporation with toluene. The residue was treated with 80% acetic acid for 5 hr at room temperature and the product was isolated by preparative TLC on silica gel by developing with 9 : 1 $CHCl_3$–MeOH. The *Rf* of IVa was 0.25.

The Selective Removal of the Cyanoethyl and/or Trichloroethyl Groups from IVa——IVa ($25A_{260}$) was dissolved in 30% aqueous pyridine (1 ml) adjusted to pH 9 with ammonium hydroxide and kept for 10 min at room temperature. Pyridinium Dowex 50 W×2 was added to remove ammonium ions and the removal of the cyanoethyl group was observed by application of the filtered solution to paper electrophoresis and TLC. *Rm* values are shown in Table 1. For the removal of the trichloroethyl group the decyanoethylated diester ($25A_{260}$) was dissolved in DMF (0.5 ml) and stirred with Zn–Cu (5 mg) under nitrogen at 50° for 2 days.

20) W.S. Zielinski and J. Smrt, *Collection. Czech. Chem. Commun.*, 39, 2483 (1974); M. Hashimoto and T. Mukaiyama, *Chem. Lett.*, 1973, 513.
21) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Nucleic Acids Res.*, 1, 1351 (1974).

The centrifuged solution was passed through a small column of pyridinium Dowex 50 W×2. The result is described in the text above. IVa was also treated by the same procedure. Treatment of IVa (2 mg) with Zn powder (13 mg) in 2:1 pyridine–acetic acid (0.5 mg) for 2 hr was stopped by filtration through pyridinium Dowex 50 W×2 resin.

5′-O-Monomethoxytrityl-2′-O-benzoyluridine 3′-(*o*-Nitrobenzyl)phosphate——The pyridinium salt of 5′-O-monomethoxytrityl-2′-O-benzoyluridine 3′-phosphate (1 mmol) was condensed with *o*-nitrobenzyl alcohol (1.53 g, 10 mmol) using DCC (2.06 g, 10 mmol) in pyridine (3 ml) for 2 days. The reaction mixture was worked up as described for the synthesis of IVa. The precipitated product was free from the starting 5′-O-monomethoxytrityl-2′-O-benzoyluridine 3′-phosphate (1 mmol) was condensed with *o*-nitrobenzyl alcohol (1.53 g, 10 mmol) using DCC (2.86 g, 10 mmol) in pyridine (3 ml) for 2 days. The reaction mixture material but contaminated with the 3′-N-phosphoryl dicyclohexylurea (22%). The yield of the diester was 72%.

2′-O-Benzoyluridine 3′-(Cyanoethyl, *o*-Nitrobenzyl)phosphate (IVb)——The pyridinium salt of 5′-O-monomethoxytrityl-2′-O-benzoyluridine 3′-phosphate (I) (0.73 mmol) was reacted with cyanoethanol (0.28 ml, 4 mmol) using DCC (618 mg) for 24 hr as described for the synthesis of IVa. For the synthesis of the triester the diester (0.6 mmol) was condensed with *o*-nitrobenzyl alcohol (968 mg, 6 mmol) in pyridine (2 ml) for 24 hr using TPS (902 mg, 3 mmol). The extent of the reaction was checked by TLC ($CHCl_3$–MeOH, 19:1). Aqueous pyridine (50%, 20 ml) was added with cooling and the triester was extracted with $CHCl_3$ (20 ml). The organic phase was washed with water (5 ml) 5 portions and the product was isolated by preparative TLC (4 sheets, 20×20 cm). The trityl positive bands was collected and eluted with MeOH–$CHCl_3$ (10:1). The filtered solution was concentrated and the residue was treated with 80% acetic acid (200 ml) for 12 hr at 30°. IVb was isolated by preparative TLC (4 plates, 20×20 cm). The overall yield of IVb was 48%.

Deprotection of IVb——IVb (60 A_{260}) was dissolved in dioxane (2 ml) and irradiated with UV light through a Pyrex filter.[21] 2′-O-Benzoyluridine 3′-cyanoethyl phosphate, thus obtained, ran as a single spot in paper electrophoresis (Table I). Decyanoethylation was effected by treating IIb (20 A_{260}) with 50% aqueous dioxane adjusted to pH 11 with ammonium hydroxide (2 ml) for 30 min at 30°. The diester was identified by paper electrophoresis and was converted to the monoester by irradiation with UV light as described above.

2′-O-Benzoyluridine 3′-(Trichloroethyl)phosphoranilidate (IVc)——5′-O-Monomethoxytrityl-2′-O-benzoyluridine 3′-trichloroethyl phosphate (0.76 mmol), which was synthesized as described in the synthesis for IVa, was rendered anhydrous by evaporation with pyridine and a mixture of 2,2′-dipyridyl disulfide (2.4 g, 11 mmol) and triphenylphosphine (3 g, 11 mmol) in anhydrous pyridine (5 ml) was added. After addition of aniline (1.1 ml, 11 mmol) half of the solvent was evaporated and the resulting solution was kept at room temperature for 60 hr. TLC ($CHCl_3$–MeOH, 19:1) showed 60% of the diester (*Rf*, 0) was converted to the trisubstituted product (*Rf*, 0.58). The concentrated mixture was evaporated with toluene and the residue was treated with 80% acetic acid (200 ml) for 12 hr at room temperature. The detritylated product IIc was separated on a column (2×65 cm) of silica gel. The column was first washed with $CHCl_3$ (3 ml) to remove the reagents and IVc was eluted with $CHCl_3$–MeOH (50:1). The product was precipitated with pentane from its pyridine solution. An aliquot (3 A_{260}) was treated with isoamyl nitrite (10 μl) in 1:1 pyridine–acetic acid for 5 hr at 30°. The diester was isolated by TLC ($CHCl_3$–MeOH, 9:1) (*Rf*, 0) and transfered to paper electrophoresis. A single spot with *Rm* 0.48 showed a UV spectrum characteristic of 2′-O-benzoyluridine.

The Dinucleotide, 5′-O-Monomethoxytrityl-(N, 2′-O-dibenzoylcytidylyl-(3′–5′)-2′-O-benzoyluridine 3′-(Cyanoethyl *o*-Nitrobenzyl)phosphate (IXb)——The mononucleotide (VIII) (0.04 mmol), the triester (IIb) (0.05 mmol) and pyridinium Dowex 50 W×2 (*ca.* 300 mg) were dried with pyridine and treated with DCC (33 mg, 0.16 mmol) in pyridine (1 ml) for 2 days at room temperature. Aqueous pyridine (50%, 10 ml) was added and DCC was removed by extraction with hexane. The filtered solution was evaporated and the product was precipitated with ether-hexane (1:1). An aliquot was treated with 80% acetic acid and then with methanolic ammonia. The *o*-nitrobenzyl group was removed with UV irradiation. CpUp (2 A_{260}) was hydrolyzed with RNase A as described previously and the products were analyzed by a nucleic acid analyzer to give a ratio 1.0:1.0 of Cp to Up.

The Dinucleotide, 5′-O-Monomethoxytrityl(N,2′-O-dibenzylcytidylyl-(3′–5′)-2′-O-benzoyluridine 3′-(Trichloroethyl)phosphoranilidate (IXc)——The mononucleotide (VIII) (0.14 mmol) and IIc (0.15 mmol) were treated with TPS (80 mg, 0.26 mmol) in pyridine (2 ml) for 24 hr at room temperature. The mixture was checked by TLC ($CHCl_3$–MeOH, 10:1) for the extent of the reaction. Aqueous pyridine (50%, 10 ml) was added and the mixture was kept for 1 hr. The nucleotides were extracted with $CHCl_3$ and precipitated by 1:1 ether–hexane from their solution in pyridine. An aliquot was treated with 80% acetic acid and then with methanolic ammonia. The mixture was applied to paper chromatography in solvent A. The yield estimated from the recovered mononucleotide Cp and the dimer CpUp2′(3′)(Cl_3Et) was 70%. The esterified dimer was digested with RNase A and the products were analyzed by a nucleic acid analyzer. The ratio of Cp to Up plus Up(2′)(Cl_3Et) was 1.0 to 1.0.

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