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A CONVENIENT SYNTHESIS OF DINUCLEOTIDES BY OXIDATION-REDUCTION CONDENSATION USING NUCLEOSIDE 5′-PHOSPHORODIANILIDATES

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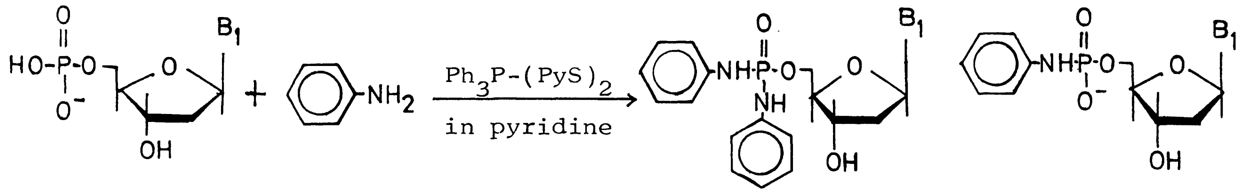
5′-Phosphorodianilidates of various N-protected nucleoside were prepared from the N-protected 5′-deoxyribomononucleotides (e.q., d-pA^{Bz}-OAc) and aniline with coupling reagents, triphenylphosphine and 2,2′-dipyridyl disulfide, in yields ranging from 60 to 65%. The phosphorodianilidates were condensed with suitably protected mononucleotides by the above mentioned oxidation-reduction condensation to afford the dinucleotide phosphorodianilidate, such as >d-pA^{Bz}pC^{An}, >d-pTpT, >d-pA^{Bz}pT in yields ranging from 50 to 75%. The phosphorodianilidates were successfully converted to the corresponding parent 5′-phosphate by treatment with isoamylnitrite.

It is well known that protected dinucleotides having 5′-phosphomonoester end groups (e.q., d-pA^{Bz}pC^{An} (I)) are important intermediates in the blockwise synthesis of deoxyribonucleotides of defined sequences. The preparation of dinucleotides such as type (I) was usually performed by coupling of 3′-hydroxyl group of 5′-protected nucleotide with 5′-phosphate group of 3′-protected nucleotides, followed by the selective removal of the 5′-phosphate protecting group. The β-cyanoethyl group[1] has been used most commonly so far for protection of 5′-phosphate of mononucleotides and, in addition, several useful protecting group[2-6] have been proposed recently for this purpose.

This communication describes a convenient and general method for the preparation of nucleoside 5′-phosphorodianilidates and for the synthesis of dinucleotides using these nucleoside 5′-phosphorodianilidates by the oxidation-reduction condensation.

This investigation was undertaken on the basis of the following facts; first, phosphorodianilidates were prepared easily from the 5′-nucleotides by one-step procedure with coupling reagents, triphenylphosphine (Ph_3P) and 2,2′-dipyridyl disulfide ($(PyS)_2$), which were very effective in the preparation of nucleotides[7] and phosphoramidates[8]. Secondly, the dianilidates (II) thus prepared are more stable in comparison with the corresponding phosphoromonoanilidate (III) under basic conditions. Concerning the removal of the anilino groups of 5′-phosphate, it was reported by Ohtsuka that amidates derived from the aromatic amines can be readily cleaved to give the parent 5′-phosphate group by treatment with isoamylnitrite under mild conditions^4.

In the first place, preparation of phosphorodianilidates was carried out in pyridine with Ph_3P-$(PyS)_2$. In a typical experiment, N^6-benzoyldeoxyadenosine 5′-phosphate (II) was obtained in 65% yield when N^6-benzoyldeoxyadenosine 5′-phosphate (1 mmol) and aniline (10 mmol) were treated with Ph_3P-$(PyS)_2$ (10 mmol) in anhydrous pyridine (10 ml) at room temperature for 2 days. The dianilidate was extracted with chloroform and purified with a thin layer chromatography on silica gel in tetrahydrofuran. This phosphorodianilidate was stable under basic conditions usually employed for the removal of the protecting groups of nucleotides. Deoxythymidine, deoxycytidine and deoxyguanosine phosphorodianilidates (II) can also be prepared in 60-65% yields along with the corresponding phosphoroanilidates (III) in 35-40% yields.



[I] [II] [III]

The dianilidates (II) can easily be isolated from the corresponding monoanilidates (III) with a thin layer chromatography on silica gel in tetrahydrofuran or ethyl acetate. These results are shown in Table I.

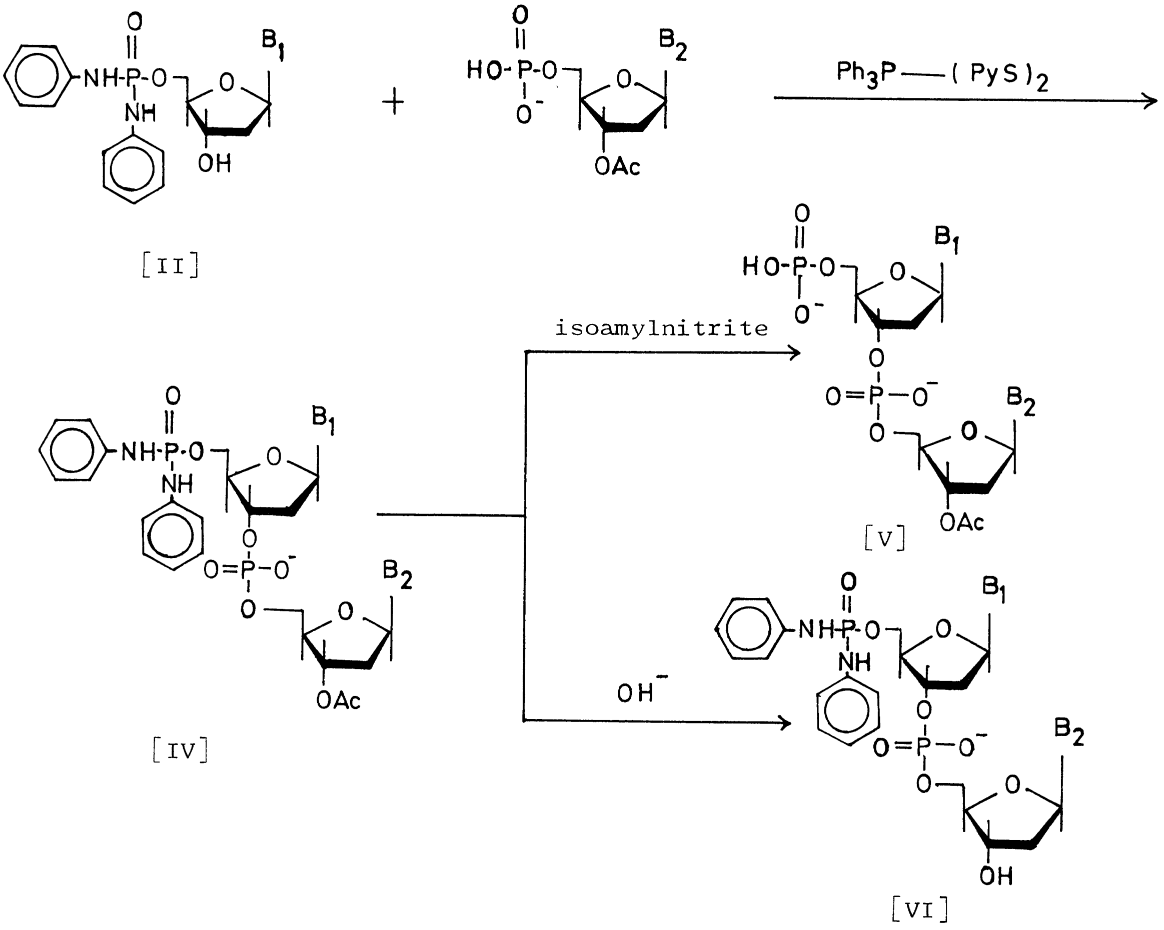
Table I. Experiments on the Synthesis of Nucleotide 5′-Phosphorodiamidate$^{a)}$ (II)

B_1	Amine	Reaction time (day)	Total yields of amidates II and III	Ratio of amidates		$\lambda_{max}^{pH 7}$
				Phosphoramidate(III)	Phosphorodiamidate(II)	
Thymine	Aniline	2	quant.	35	65	267,226
Thymine	Morpholine	5(hr)	"	82.5	17.5	267
		1	"	57	43	"
		3	"	17	64$^{b)}$	"
N-Benzoyl adenine	Aniline	2	"	35	65	280,230
N-Anisoyl cytosine	Aniline	2	"	41	59	302(285)
N-Benzoyl guanine	Aniline	2	"	40	60	290,260

a) 10 equiv. each of Ph_3P-$(PyS)_2$ and 10 equiv. amine were used based on parent nucleoside 5′-phosphate.

b) TppT was also produced in 17% yield.

Next, preparation of dinucleotides was tried by treating the phosphorodianilidates with various 5′-nucleotides. Condensation of the phosphorodianilidates (II) with suitably protected mononucleotides (e.q., d-pA^{Bz}-OAc) by Ph$_3$P-(PyS)$_2$ in pyridine proceeded smoothly to give the fully protected dinucleotides (IV) in the yields renging from 50 to 75% based on the phosphorodianilidate. For example, N^6-benzoyl deoxyadenosine 5′-phosphorodianilidate (II) (0.1 mmol) and N^6-benzoyl 3′-O-acetyl deoxyadenosine 5′-phosphate (d-pA^{Bz}-OAc, 0.15 mmol) were allowed to react with Ph$_3$P-(PyS)$_2$ (1 mmol) in pyridine (1 ml) for 3 days at room temperature. At this stage, paper electrophoresis showed four spots; that is, the desired nucleotide (70%), unreacted d-pA^{Bz}-OAc (0.06 mmol), II (20%) and a small amount of the other product which was not identified. The nucleotide (IV) was isolated by DEAE-cellulose column chromatography using a liner salt gradient of triethylammonium bicarbonate in 50% ethanol (1 ℓ. of 50% ethanol in the mixture chamber and the equal volume of 0.1M salt in the reservoir). The treatment of IV with alkali and with isoamylnitrite afforded VI and V quantitative yields respectively, according to the following scheme.



B$_1$ or B$_2$ = N-benzoyladenine, thymine, N-anisoyl cytosine, or N-benzoylguanine.

Similary various other dinucleotides d-pA^{Bz}pT, d-pA^{Bz}pC^{An}, d-pTpT, d-pTpA^{Bz}, d-pTpC^{An} were also prepared in good yields (Table II).

Table II. The Synthesis of Protected Dinucleotides Containing 5′-Phosphate Groups. Summary of Conditions and Yields of Condensation Reactions.

Dinucleotide	3′-OH containing component [b)]	5′-Phosphate containing component	Amount mmol	Ph_3P-$(PyS)_2$ mmol	Pyridine ml	Time day	Yield %[a)]
d-pTpT-OAc	p-dT	d-pT-OAc	0.1	0.5	0.8	2	70
d-pTpC^{An}-OAc	p-dT	d-pC^{An}-OAc	0.07	0.5	0.7	2	50
d-pTpA^{Bz}-OAc	p-dT	d-pA^{Bz}-OAc	0.07	0.5	0.7	2	51
d-pA^{Bz}pT-OAc	p-dA^{Bz}	d-pT-OAc	0.1	1	0.85	2	75
d-pA^{Bz}pA^{Bz}-OAc	p-dA^{Bz}	d-pA^{Bz}-OAc	0.1	1	0.9	2	70
d-pA^{Bz}pC^{An}-OAc	p-dA^{Bz}	d-pA^{An}-OAc	0.1	1	1	2	70

a) Yields were determined spectrophotometrically in water.

b) In each case, 0.05 mmol amount of component was used.

In conclusion, it is noted that various nucleoside 5′-phosphorodianilidates were prepared in good yields from 5′-nucleotides and aniline by oxidation-reduction condensation. Various protected dinucleotides of type; d-pA^{Bz}pC^{An} were synthesized in good yields by the reaction of nucleoside 5′-phosphates with 3′-hydroxyl nucleoside phosphorodianilidates.

The synthesis of oligonucleotides containing various kinds of base sequences and the development of other phosphorodianilidates are now under investigation.

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REFERENCES

1) G. Weimann and H. G. Khorana, J. Amer. Chem. Soc., 84, 419 (1962).
2) A. Franke, F. Eckstein, K. Scheit and F. Cramer, Chem. Ber., 101, 944 (1968).
3) A. F. Cook, M. J. Holman and A. L. Nussbaum, J. Amer. Chem. Soc., 91, 6479 (1969).
4) E. Ohtsuka, K. Murao, M. Ubasawa and M. Ikehara, ibid., 92, 3441 (1970).
5) K. L. Agarwal, A. Yamazaki and H. G. Khorana, ibid., 93, 2754 (1971).
6) S. A. Narang, O. S. Bhanot, J. Coodchild and J. Michniewic, Chem. Commun., 516 (1970).
7) T. Mukaiyama and M. Hashimoto, J. Amer. Chem. Soc., 94, 8528 (1972).
8) T. Mukaiyama and M. Hashimoto, Bull. Chem. Soc. Japan, 44, 2284 (1971).

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