REINVESTIGATION OF "ONE STEP" PREPARATIONS OF 5'-PROTECTED DINUCLEOSIDE MONOPHOSPHATE

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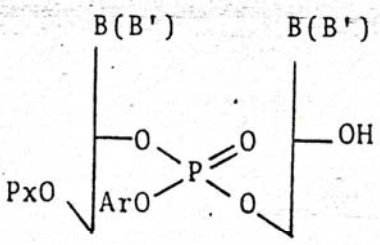
The recent reports[1,2,3] of a "one step"synthesis, combining both (i) introduction of a phosphate function (ii) and formation of an internucleotide linkage, have attracted our attention to explore the feasibility of synthesis of 5'-O-protected dideoynucleoside monophosphate (I) through such a method. Cashion and his co-workers[1] were the first to report such a synthesis in pyridine solution using an excess (3 equiv.) of thymidine or N^2-acylated deoxyguanosine to the 5'-protected component in presence of triethylamine (4 equiv.) and 1-methylimidazole (16 equiv.) to obtain d[DMTr TpT] and d[DMTr $A^{Bz}pG^{Ac}$] in 80-90% yield. Dobrynin et al.[3] have also reported a "one step" preparation using catalytic amounts of 4-dimethylaminopyridine in dry pyridine solution.

We have now reinvestigated these two reactions[1,3] in details. Following the reaction condition of Cashion and co-workers[1], we have prepared all sixteen dimers. The isolated yields in the form of precipitated powder are tabulated in table I. It should be emphasized that we have observed a considerable formation of 3'→3' linked dimer(3-14% of the total reaction product) beside the desired product. After a careful reinvestigation of the 4-dimethylaminopyridine mediated one step reaction[3], it can be safely concluded that a considerable excess of the latter reagent, at least 16 equiv., is necessary to drive the reaction to yield a optimum amount of the desired product (I) in all sixteen cases of dimer preparations. This conclusion is based on parallel sets of experiments with 0,4 and 16 equiv. of 4-dimethylaminopyridine with respect to 5'-protected component (1.5 equiv.) and thymidine or N-acylated deoxynucleoside (2.0 equiv.). In all these experimental conditions, we have also observed the formation of 3'→3' linked side products (3.2-9.0%). The 5'→5' and 3'→5' linked symmetrical products are also formed in smaller amounts which are separated during column chromatographic purification. The fact that 4-dimethylaminopyridine should be used in considerable excess to obtain a higher yield of the desired product might suggest the actual formation of an activated species like (2) at this concentration.

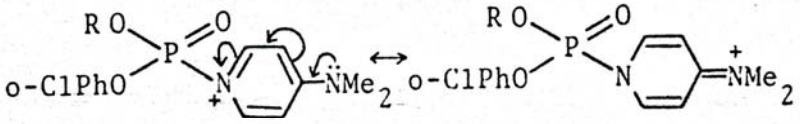
General method:

To a dry pyridine (6 ml) solution of 5'-pixyl thymidine or N-acyl deoxynucleoside[6] (1mmol), o-chlorophenylbis-trizolide in dry acetonitrile solution (6 ml, 0.25 mmol/ml) was added at 20°C. The reaction was complete within 30 min. To this stirring reaction mixture, thymidine or N-acylated deoxynucleoside (2mmol) and 4-dimethylaminopyridine (16mmol) were added simultaneously. The reaction was complete within 45 min. The reaction mixture was worked up following standard



(I), Ar= o-ClPh

Px= 9-phenylxanthen-9-yl



(2)

Table 1: "One pot" preparation of 5'-Pixyl dideoxynucleoside monophosphates (I)

Expt	Dimer[b,5,6]	% yield: 4-dimethylaminopyridine 0mmol 3'→5'[a]	4mmol 3'→5'[a,c]	16mmol 3'→5'[a,c]	3'→3'[a]	1-methylimidazole (Cashions's experimental condition[1]) 3'→5'[a,c]	3'→3'[a]	2-steps[4,5] MS-NT 3'→5'[a,c]	Solvent for chromatography[5] % EtOH-$CHCl_3$
1	d[Px-ApA-OH]	24.6	73.0	74.2	8.5	52.2	5.7	66.8	5.5
2	d[Px-ApG-OH]	-	34.8	61.2	3.2	66.7	7.8	68.4	6.0
3	d[Px-ApC-OH]	-	28.4	56.3	5.2	54.9	5.2	59.8	7.0
4	d[Px-ApT-OH]	-	57.0	68.2	5.4	57.5	10.9	58.5	6.0
5	d[Px-GpA-OH]	-	36.6	64.4	8.8	58.7	7.2	58.8	5.0
6	d[Px-GpG-OH]	-	41.0	48.0	3.3	47.0	3.0	63.4	8.0
7	d[Px-GpC-OH]	-	54.3	61.5	4.5	58.5	5.6	65.4	5.0
8	d[Px-GpT-OH]	34.5	58.2	66.2	4.5	71.5	5.3	61.4	6.0
9	d[Px-CpA-OH]	-	55.9	61.0	7.2	59.2	5.3	58.0	6.0
10	d[Px-CpG-OH]	-	65.6	73.4	5.2	70.3	4.7	72.2	8.0
11	d[Px-CpC-OH]	32.9	67.7	70.1	6.5	58.9	7.3	61.9	5.0
12	d[Px-CpT-OH]	-	40.1	58.4	7.7	70.0	7.7	66.1	6.5
13	d[Px-TpA-OH]	-	39.7	62.1	5.5	49.8	10.8	67.7	6.0
14	d[Px-TpG-OH]	-	39.8	59.5	4.0	65.2	4.1	65.8	7.0
15	d[Px-TpC-OH]	27.3	40.6	55.6	9.0	54.3	5.3	61.6	5.0
16	d[Px-TpT-OH]	25.8	41.6	62.0	8.0	72.0	14.0	53.6	6.0

[a] Isolated as powder from 1 mmol of the 5'-protected component.

[b] 9-Phenylxanthan-9-yl[6] at C-5' is abbreviated to Px(pixyl):6-N-(m-chlorobenzoyl)- 2'-deoxyadenosine, 2-N-(p-t-butylbenzoyl)-2'-deoxyguanosine, 4-N-benzoyl-2'-deoxycytidine are represented by A,G,C respectively. Abbreviations adopted here following the suggestion of Chattopadhyaya and Reese[4] MS-NT denotes 1-mesitylene sulphonyl-3-nitro-1,2,4-triazole.

[c] Dimers have been deprotected following a literature procedure[4,5]. They were pure on TLC[4,5] and were completely digested by snake venom and spleen phosphodiesterases.

procedure[4,5] to obtain a residue. A $CHCl_3$ solution of this residue (ca. 1 ml) was precipitated from a mixture of diethylether - petroleum ether (30-40°C) (1:1,v/v). The precipitate, thus obtained, was chromatographed through a column of silica gel (table I for solvent mixture for elution). The desired fractions were pooled and evaporated to obtain a glass which was dissolved in small volume of $CHCl_3$ (ca. 1 ml) and was precipitated from the same solvent mixture. Dried and weighed, % yield (Table 1).

References

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