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PHOSPHORYLATION OF NUCLEOSIDES USING MOLECULAR SIEVES AS ACID SCAVENGERS

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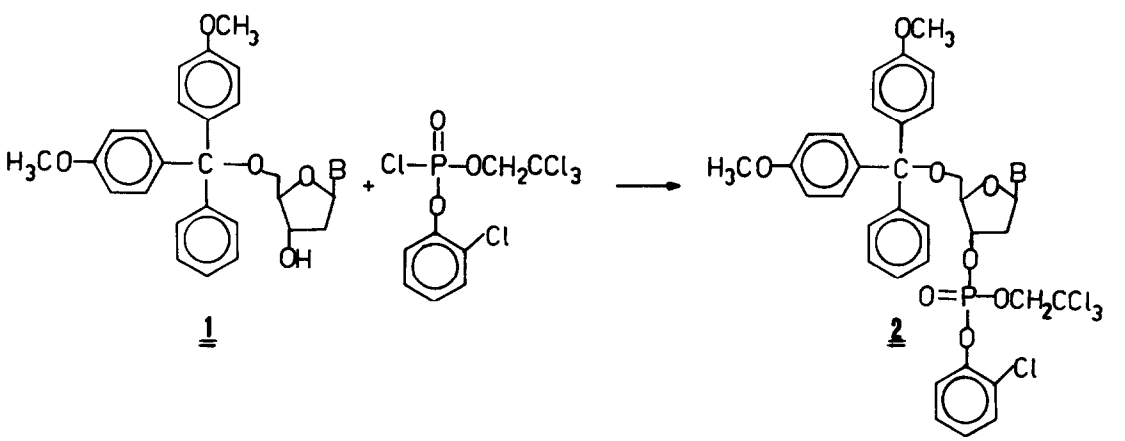
Summary : Molecular sieves (4Å) were used effectively to trap the generated hydrogen chloride during phosphorylation of protected nucleosides using phosphomonochloridates, thus preventing side reactions.

Phosphorylation of 3'-hydroxyl function of suitably protected deoxynucleosides by monofunctional phosphorylating reagents serves as a useful method for the preparation of phosphotriester intermediates.[1] One such phosphorylating reagent namely 2,2,2-trichloroethyl 2-chlorophenylphosphorochloridate was described by van Boom and his coworkers[2].

During the course of our studies on the synthesis of deoxyribooligonucleotides with defined sequences according to the triester approach we wanted to phosphorylate 5'-O-dimethoxytrityl N-acyldeoxyribonucleosides 1 at the 3'-hydroxyl function(see figure) using the conditions described by these workers for phosphorylation (i.e. for 1.3 mmole of 5'-O-dimethoxytrityl N-acyl deoxynucleoside, 1.8 mmole phosphomonochloridate and 2 mmole 1-methylimidazole in acetonitrile). However we observed considerable amounts of cleavage of the



acid sensitive dimethoxytrityl group. Consequently we observed some dimethoxytritanol and 5'-phosphorylated products in attempting to phosphorylate the 3'-hydroxyl of the protected nucleosides. To get rid of this problem we used

powdered molecular sieves of 4 Å (5g per mmole of protected nucleoside). We found this procedure very efficient for phosphorylation of the relatively sterically hindered 3'-position of protected nucleosides using even catalytic amounts of 1-methylimidazole with respect to phosphomonochloridate. The reaction is over in 10 minutes in the presence of molecular sieves. No detritylation and no phosphorylation at the heterocyclic ring were observed.

With carefully dried reagents and solvents detritylation could not be prevented. The action of molecular sieves therefore not only seems to be removing traces of moisture but to trap the hydrogenchloride generated during 3'-phosphorylation. The use of molecular sieves as a neutral acid scavenger has been recommended by another group for acylation of Cephamycins using acylchlorides[3].

Using this procedure we phosphorylated all the four 5'-dimethoxytrityl N-acyl deoxyribonucleosides[4] (1 a-e, see Figure). The 3'-phosphorylated products (2 a-e) were isolated as precipitated solids after short column chromatography over silica gel in excellent yields (see Table). The products were homogeneous by hplc and identified by ^{1}H-NMR, ^{31}P-NMR and U.V. spectroscopy and gave satisfactory elemental analyses.

Table. ^{31}P-NMR chemical shifts of 5'-O-dimethoxytrityl N-acyldeoxynucleoside phosphotriesters (2) and overall yields.

Compound	Yields(%)	^{31}P-Chemical Shift (ppm)
B= N-(Isobutyryl)guanine	87	-
B= N-(3,4-Dichlorobenzoyl)guanine[4]	83	s 9.245[a)]
B= N-(3,4-Dimethylbenzoyl)cytosine[4]	92	s 9.446
B= N-(Benzoyl)adenine	96	s 9.580
B= Thymine	92	s 9.178

a) Using 85% H_3PO_4 as external standard

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