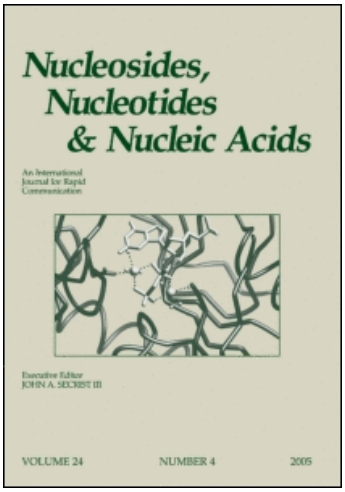

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The p-Phenylazophenyloxycarbonyl Protecting Group: Selective Deblocking and Oligonucleotide Synthesis Avoiding Acid Steps

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THE p-PHENYLAZOPHENYLOXYCARBONYL PROTECTING GROUP: SELECTIVE DEBLOCKING AND OLIGONUCLEOTIDE SYNTHESIS AVOIDING ACID STEPS

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SUMMARY: The p-phenylazophenyloxycarbonyl group is selectively introduced at nucleoside-5'-OH by reaction with the resp. chloroformate and deblocked e.g. by transesterification/β-elimination. With it we designed a reaction cycle for oligonucleotide support synthesis, that avoids acid deprotection.

A major concern in oligonucleotide synthesis has been the risk of depurination during the removal of substituted trityl groups from 5'-OH [1]. Although a high degree of selectivity is generally achieved, the demands of solid-phase polynucleotide synthesis would be better met by a reaction cycle, which avoids acid steps altogether. With this aim we have reinvestigated on the p-phenylazophenyloxycarbonyl- (= PAPoc-) group, which we previously recommended as affinity protecting group for diester preparations [2]. Here, we describe novel selective deprotection procedures and the use of this group in solid-phase oligonucleotide synthesis following the phosphoramidite route.

The PAPoc-group was introduced by treating (base-protected) deoxynucleosides (1 mmol) with 1.5 mmol PAP-chloroformate (obtained by phosgenation of phenylazophenol). The 5'-PAPoc-nucleosides I were obtained in ca. 70 % yield (after column chromatography) as yellow powders (λ_{max} = 320 nm) and characterized by elem. analysis, IR and FD-MS.

Several alternatives were found for deprotection, which was, in all cases, indicated by a bathochromic spectral shift (λ_{max} of phenolate = 396 nm): 1. alkali; 2. 4-dimethylami-

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nopyridine in THF (2.8 g in 40 ml + trace of H_2O, ca.10 sec. at room temp.); 3. a) β-cyanoethanol/$(C_2H_5)_3N/H_2O$ = 1:1:1 (1 min. at room temp.), then b) diazabicycloundecene/pyridine = 1:1 (1 min. at room temp.). Both conditions no. 2 and 3 are fast, mild and compatible with usual conditions of solid-phase phosphoramidite synthesis [3]. Since clogging of filters was occasionally observed with the near-saturated solution 2 we prefer the two-step deprotection (no. 3) for machine-aided preparations. That the latter proceeds via consecutive transesterification and β-elimination (Fig. 1) was shown e.g. by the characterization of a β-cyanoethylcarbonate intermediate.

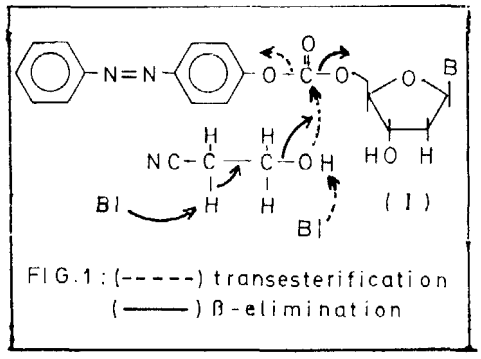

FIG.1 : (-----) transesterification
(——) ß-elimination

The compounds I were converted into the 3'-(methoxy-) N,N-dimethylaminophosphines II [3] and thus used for solid-phase oligonucleotide synthesis on silicagel supports [3], for which the following reaction cycle was developed:

1. deblocking as described above (no. 3) with intermediate CH_2Cl_2+pyridine washing; 2. condensation with II [3] + washing with CH_3CN + CH_2Cl_2; 3. capping (diethylchlorophosphine/N-methylimidazole/CH_2Cl_2 = 2:7:21) + washing with CH_2Cl_2 ; 4. oxidation [3] + washing with THF. Following this procedure, several oligonucleotides were synthesized; sequences and yields are given in Table 1. The purification, after demethylation and release from the support [3], could be done by standard C_{18}-HPLC [4], if a 5'-dimethoxytrityl-nucleoside-3'-(methoxy-)N,N-dimethylaminophosphine [3] was applied for the last condensation step. More recently, the compounds II were used throughout the synthesis, and the fully deblocked material directly subjected to gel electrophoresis.

Our results demonstrate the feasibility of using 5'-PAPoc-nucleosides I and their phosphoramidite derivatives II in oligonucleotide synthesis. From the selectivity of its

introduction and its chromatographic behavior, the PAPoc group is equivalent to trityl groups. Advantageous, however, is its facile removal under selective, near-neutral conditions. Examples of such deblocking reactions have been given above as no. 2 and 3 [5]; they should be applicable, as well, to other carbonate protecting groups, that have substituents with good leaving quality. An additional feature is the presence of a vis-chromophor, which allows direct optical reaction control. The results of Table 1 show, that the PAPoc-derivatives II are equally suitable for oligonucleotide synthesis as are tritylated monomers. Their use, however, permits to eliminate acid deprotection steps, an option, which should be of particular value in the preparation of longer oligo-deoxy- as well as -ribonucleotides.

TABLE 1 :

Oligonucleotide	Yield (%) total	average per cycle
dTA	86	--
dCA	91	--
dAA	93	--
dGA	95	--
$d(T)_9$	4	66
$d(A)_9$	26	85
dAGGTGA	3.3	51

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