THE 9-FLUORENYLMETHOXYCARBONYL-(FMOC) AND 2-NITROPHENYLSULFENYL-(NPS) GROUPS FOR THE PROTECTION OF THE BASE RESIDUES FOR THE CHEMICAL SYNTHESES OF DNA AND RNA FRAGMENTS

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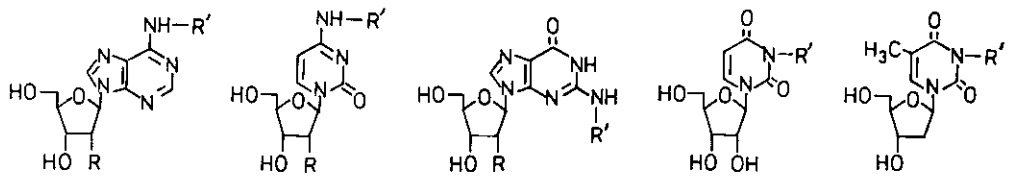
In the chemical synthesis of DNA and RNA fragments, it is desirable to protect the exocyclic amino functions of cytosine, adenine and guanine residues in view of their susceptibilities to attack by electrophiles such as phosphorylating agents. Khorana and his coworkers in their phosphodiester approach have introduced N-acyl groups to protect all three base residues and, subsequently, these acyl groups have also been used in the phosphotriester approach. The N-acyl groups on cytosine, adenine and guanine residues are relatively stable both under neutral and acidic conditions; however, their rates of removal, under an alkaline condition, are clearly dependent upon the nature of base residues. Thus, the removal of N-benzoyl groups are complete at room temperature with 5 M aq. NH_3 in dioxan (1:1;v/v) in 385, 1410 and 4350 minutes from the corresponding 2'-deoxyribofuranosyl derivatives of cytosine, adenine and guanine residues respectively. The above periods of deprotections seem to be too long, unless it is carried out at ca. 50°C, in view of the fact that the chemical synthesis of a fully protected 10-14 units long DNA sequence on the solid support takes only a day or so.

Thus, we wish to report on two alternative ways to protect the base residues of DNA and RNA components as a measure to reduce the side reactions during the oligonucleotide synthesis. They are as follows: (1) 9-fluorenylmethoxycarbonyl - (Fmoc) group as in (1) to (6): The Fmoc group could be completely deprotected, through a β-elimination pathway, from the corresponding derivatives of adenosine, guanosine, cytidine and their 2'-deoxysugar derivatives, with the help of aq, NH_3 (d 0.88) in pyridine solution (9:1 v/v) at 20°C within 30 min. (2) 2-nitrophenylsulfenyl-(NPS) group as in (7) to (14): The NPS group is removable with the help of triethylammonium thiocresolate (2-3 aq.) in dry CH_3CN at 20°C within 20 min.

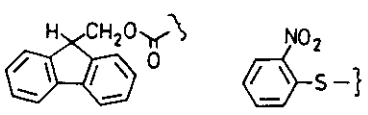
Both Fmoc and NPS protected derivatives, (1) to (14) gave crystalline compounds and were stable during the multi-step chemical synthesis. However, the advantages of the NPS group is that it may be used to protect both amino functions of cytosine, adenine and guanine residues as well as the imide function of uracil and 5-methyluracil residues of RNA and DNA respectively. It has emerged subsequently that the NPS group may be more conveniently and uniformly used over the Fmoc group, in terms of overall yields and facilities to isolate intermediates, in an exercise of a oligonucleotide synthesis.

It should, however, be added that the employment of either type of the protective group substantially reduces the length of time for deprotections and this facilitates the actual isolation procedures of oligonucleotides. Results would be presented to substantiate above observations.



(1) R = OH; R´= Fmoc (3) R = OH, R´= Fmoc (5) R = OH; R´= Fmoc
(2) R = H ; R´= Fmoc (4) R = H ; R´= Fmoc (6) R = H ; R´= Fmoc

(7) R = OH; R´= NPS (9) R = OH; R´= NPS (11) R = OH; R´= NPS (13) R´= NPS (14) R´= NPS
(8) R = H ; R´= NPS (10) R = H ; R´= NPS (12) R = H ; R´= NPS



Fmoc NPS