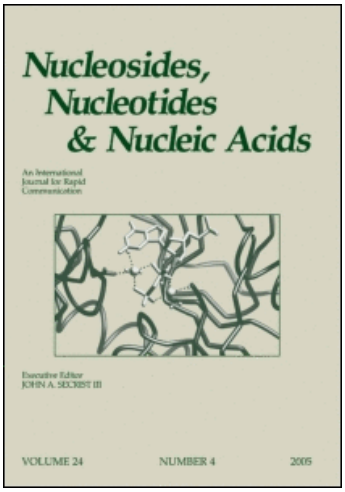

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New Results in Oligoribonucleotide Synthesis

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NEW RESULTS IN OLIGORIBONUCLEOTIDE SYNTHESIS

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Abstract. A new blocking group for 2'-OH protection of ribonucleosides was found in the p-cyanophenylethylsulfonyl (CPES) and the carbomethoxyethylsulfonyl (CMES) group. The former functionality is more stable than the p-nitrophenylethylsulfonyl (NPES) group, but can be cleaved by fluoride ion and DBU respectively.

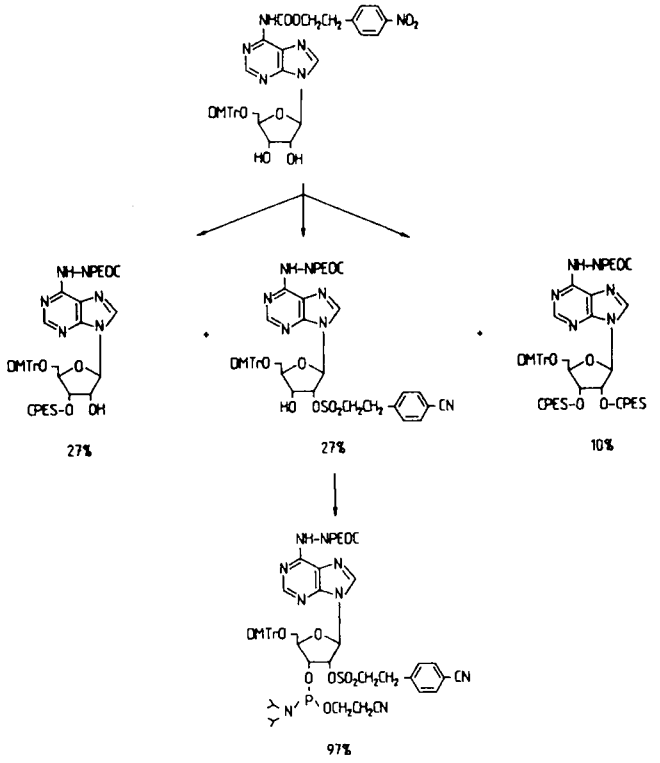
Nucleic acid chemistry has recently been developed almost to perfection in the field of oligodeoxyribonucleotide synthesis by the phosphoramidite approach[1,2], but a similar facile, rapid, and high-yield RNA-synthesis is far from general application due to principal structural problems. The major concern in the chemical synthesis of oligoribonucleotides is the presence of the additional 2'-hydroxyl group in the ribose moiety, which requires selective protection, may influence the formation of the internucleotide linkage by steric hindrance and affords especially a very distinct stability to guarantee clean and easy deprotection without harming the phosphodiester backbone at the end of the synthesis. The many trials and proposals to select the right combination of protecting groups for the various functionalities have in recent years only been of minor success and show

that we are dealing with a non-trivial matter. The so far best approach[3] recommends the use of the tert-butyldimethylsilyl or triisopropylsilyl group for the 2'-OH protection and allows both solution-[4] and solid-phase strategies[5] when used in conjunction with either the chlorophosphite[6] or phosphoramidite[7] coupling procedure.

Our own efforts favor in general ß-eliminating protecting groups of the type of substituted phenylethyl groups[8] including a broad range of stabilities suitable for almost all purposes. The p-nitrophenylethyl (NPE) and p-nitrophenylethoxycarbonyl (NPEOC) group respectively have shown the most universal applications[9], so that the introduction of the p-nitrophenylethylsulfonyl (BPES) group for the 2'-OH protection can be regarded as a logical extension of our general strategy to unify the blocking group variety.

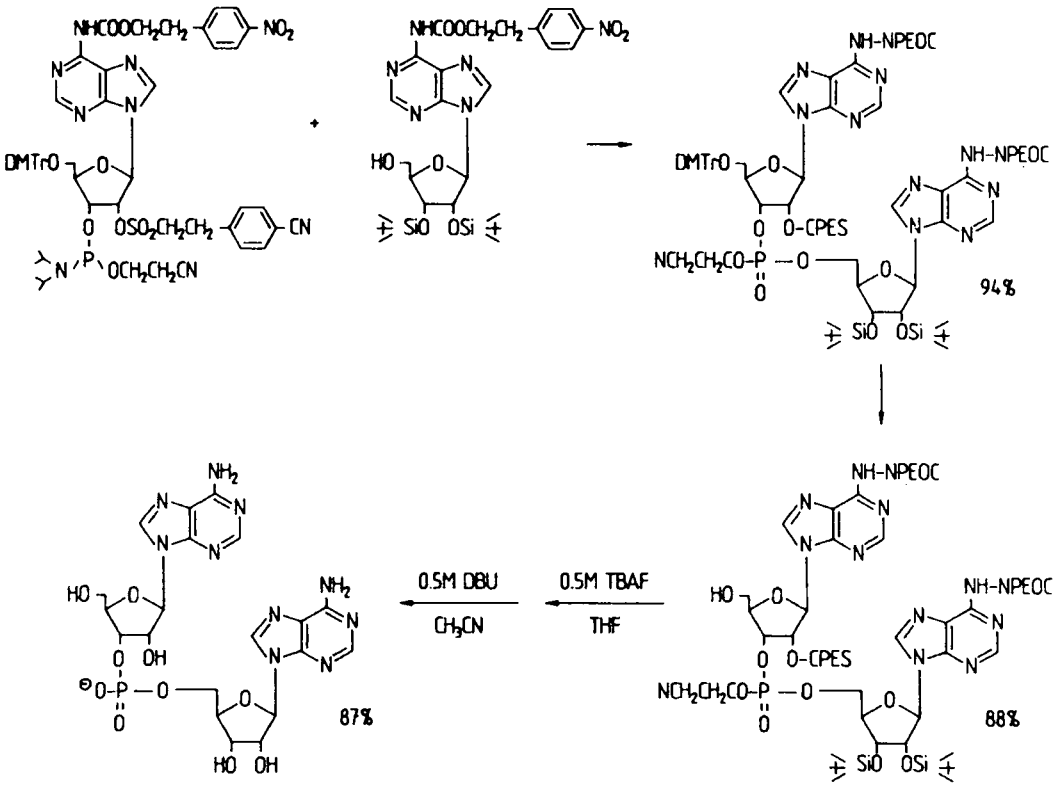
Detailed studies indicated that the NPES group can nicely be combined with the NPEOC group, whereas blocking of the phosphate function asks for the more labile ß-cyanoethyl- instead of the NPE group. The final deblocking sequence actually dictates the afforded chemical stabilities of the various protecting functions, which have to obey the order 1) phosphate, 2) 2'-OH group, and 3) aglycon in order to avoid side reactions including the cleavage of the internucleotide linkage or giving rise to anhydro nucleoside formation.

The search to improve the stability of the NPES group slightly led us to the development of the p-cyanophenylethylsulfonyl (CPES) group as a good alternative. The introduction of the CPES group into the 2'-position was achieved by direct sulfonylation of 5'-O-(dimethoxytrityl)-N^4-(p-nitrophenylethoxycarbonyl)-cytidine and 5'-O-(dimethoxytrityl)-N^6-(p-nitrophenylethoxycarbonyl)-adenosine respectively by p-cyanophenylethylsulfonyl chloride in NEt_3/CH_2Cl_2 to give a mixture of the corresponding 2'- and 3'-mono-CPES as well as the 2',3'-di-CPES derivatives.



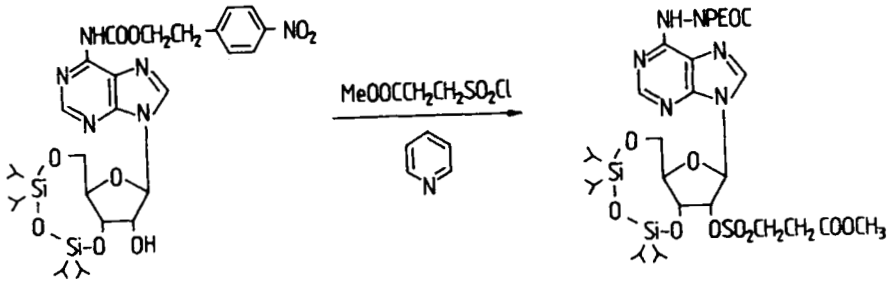
Chromatographical separation of the three compounds can be achieved but gives relatively low yields of the 2'-O-p-cyanophenylethylsulfonyl intermediates. Phosphitylations to the 3'-O-(ß-cyanoethyl)-phosphor-N-diisopropylamidites work very well and condensations in solution with a 5'-OH building block proceed also with high yields.

Studies with 3'-O-(p-cyanophenylethylsulfonyl)-5'-O-(dimethoxytrityl)-N^6-(p-nitrophenylethoxycarbonyl)-adenylyl-3'-[O^P-(ß-cyanoethyl)-5']-2',3'-di-O-(tert-butyldimethylsilyl)-N^6-(p-nitrophenylethoxycarbonyl)-adenosine revealed a subsequent deprotection sequence according to the above mentioned priorities and leading to a chromatographically pure A3'p5'A. The dimethoxy trityl group was removed first by acid followed by fluoride ion treatment in THF, which cleaves fast the ß-cyanoethyl group and then more slowly



the p-cyanophenylethylsulfonyl substituent, whereas the N^6-p-nitrophenylethoxycarbonyl residue remains almost untouched under these conditions. DBU in acetonitrile finally will achieve this cleavage by ß-elimination to form the free, unprotected dinucleosidemonophosphate. Analogous studies with the appropriately protected CpC, GpG, and UpU dimers are under investigation to prove the usefulness of the strategy in general.

The principle of ß-eliminating protecting groups can be extended further in various directions by activation of the ß-H-atoms through adjacent acceptor substituents. We introduced also the ß-carbomethoxyethylsulfonyl (CMES) group into N^6-p-nitrophenylethoxycarbonyl-3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-adenosine and found that removal of this new blocking group works straightforward with bases like DBU, DBN or tetramethylguanidine.



The CMES group seems to be more labile than the NPES and CPES group respectively and will therefore be recommended only for special purposes in nucleoside and nucleotide chemistry.

REFERENCES

1. S.L. Beaucage, M.H. Caruthers, Tetrahedron Lett. 1981, 1859.
2. L.J. McBride, M.H. Caruthers, Tetrahedron Lett. 1983, 245.
3. N. Usman, K.K. Ogilvie, M.Y. Jiang, R.J. Cedergreen, J.Am.Chem.Soc. 109, 7845 (1987).
4. K.K. Ogilvie, A.L. Schifman, C.L. Penney, Can.J.Chem. 57, 2230 (1979).
5. R.T. Pon, K.K. Ogilvie, Nucleoside & Nucleotide 3, 485 (1984).
6. K.K. Ogilvie, N. Theriault, K.L. Sadana, J.Am.Chem. Soc. 99, 7741 (1977).
7. N. Usman, R.T. Pon, K.K. Ogilvie, Tetrahedron Lett. 1985, 4567.
8. E. Uhlmann, W. Pfleiderer, Helv.Chim. Acta 64, 1688 (1981).
9. F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, Tetrahedron 50, 59 (1984).
10. W. Pfleiderer, H. Schirmeister, T. Reiner, M. Pfister, R. Charubala in "Biophosphates and their Analogues -

Synthesis, Structure, Metabolism and Activity", Ed. K.S. Bruzik, W.J. Stec, Elsevier Science Publishers 1987, 133.

11. M. Pfister, S. Farkas, R. Charubala, W. Pfleiderer, Nucleosides & Nucleotides, 1988,