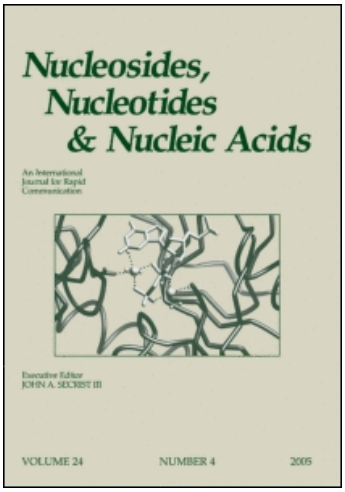


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Recent Progress in Oligoribonucleotide Synthesis

Magdalena Pfister[a]; Silke Farkas[a]; Ramamurthy Charubala[a]; Wolfgang Pfleiderer[a]
[a] Fakultät für Chemie, Universität Konstanz, Konstanz

To cite this Article Pfister, Magdalena , Farkas, Silke , Charubala, Ramamurthy and Pfleiderer, Wolfgang(1988) 'Recent Progress in Oligoribonucleotide Synthesis', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 595 — 600
To link to this Article: DOI: 10.1080/07328318808056292
URL: http://dx.doi.org/10.1080/07328318808056292

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 595-600 (1988)

RECENT PROGRESS IN OLIGORIBONUCLEOTIDE SYNTHESIS

Magdalena Pfister, Silke Farkas, Ramamurthy Charubala,
Wolfgang Pfleiderer

Fakultät für Chemie, Universität Konstanz,
Postfach 5560, D-7750 Konstanz

Abstract. The usefulness of the p-nitrophenylethylsulfonyl (NPES) group for 2'-OH protection in oligoribonucleotide synthesis is further investigated. The difficulties of uridine protection are discussed and the p-cyanophenylethyl (CPE) group introduced as a versatile new O^4-blocking group.

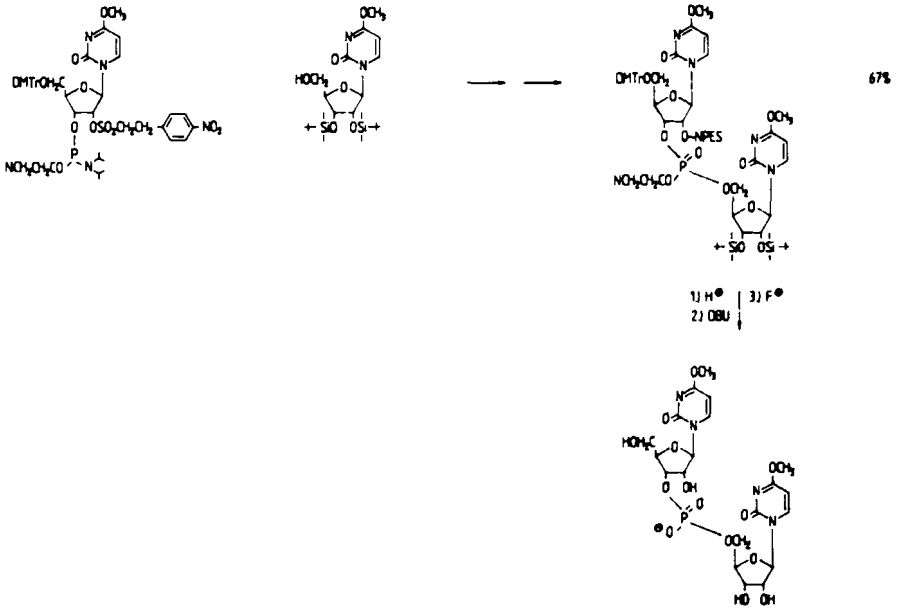
The most significant progress in nucleic acid chemistry in recent years is undoubtedly the development of the effective and convenient phosphoramidite approach[1,2] for the rapid synthesis of oligo- and poly-deoxyribonucleotides of defined sequence. Less successful, however, was so far the application of the analogous methodology in the oligoribonucleotide series due to the obvious problems concerned with the protection of the additional 2'-OH group. The correct choice of this protecting group, which should remain intact until the final deblocking steps, can be regarded as the crucial point of the whole strategy of a successful oligoribonucleotide synthesis in solution and especially in an automated process on a solid support. A number of protecting groups such as the tetrahydropyranyl-, tetrahydrofuranyl-, 4-methoxytetrahydropyranol-, 1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl, 3-methoxy-1,5-dimethoxycarbonyl-pent-3-yl-, benzyl, 2-nitrobenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl and tert.butyldimethylsilyl group have been applied and investigated, but so far no satisfactory solution of the com-

plex problem could be achieved despite the latter blocking group shows some promise[3].

Recently we introduced the p-nitrophenylethylsulfonyl (NPES)[4-6] group for 2'-OH protection due to the fact that sulfonates at the C-2 position of sugars are generally less reactive in nucleophilic displacement reactions[7], do not show acyl migration but can be removed in the case of the NPES group by a ß-elimination process. Detailed studies revealed that the use of the NPES groups in combination with the p-nitrophenylethoxycarbonyl (NPEOC) group for aglycon protection works very well, whereas phosphate protection has to be performed by the more labile ß-cyanoethyl group instead of the p-nitrophenylethyl (NPE) group[8]. The chemical stabilities of the various protected functions indicate that the deblocking sequence has to follow strictly the order 1) phosphate, 2) 2'-hydroxy group and 3) aglycon deprotection, otherwise side reactions occur, which involve the cleavage of the internucleotidic linkage or give rise to anhydronucleoside formation.

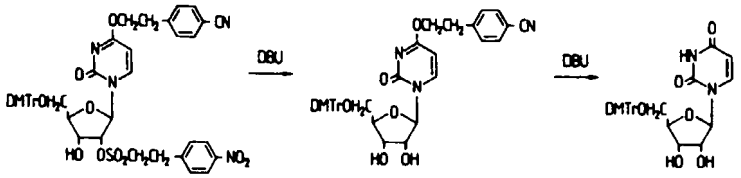
The fully blocked dimers 5'-O-dimethoxytrityl-N^6-p-nitrophenylethoxycarbonyl-2'-O-p-nitrophenylethylsulfonyladenylyl-[3'-(ß-cyanoethyl)-5']-N^6,N^6,2',3'-tetrabenzoyladenosine[4] and 5'-O-dimethoxytrityl-N^4-p-nitrophenylethoxycarbonyl-2'-O-p-nitrophenylethylsulfonyl-cytidylyl-[3'-(ß-cyanoethyl)-5']-2',3'-di-O-tert.butyldimethylsilyl-N^4-p-nitrophenylethoxycarbonylcytidine[5] respectively showed the expected clean deprotection on subsequent treatment by 1) H^+, 2) DBU, 3) TBAF and 4) DEAE-Sephadex work-up to give ApA and CpC in an isolated yield of 80 % and 79 % respectively.

More difficulties, however, have been encountered in the uridine series. The use of an unprotected agylcon moiety gives rise to anhydro nucleoside formation since DBU generates an anion which facilitates intramolecular attack of the O^2 at the C-2' center. O^4-Protection by the p-nitrophenylethyl group can also not be recommended since the deprotection rates of the NPES and NPE group are not so different that a unique reaction sequence is guaranteed.



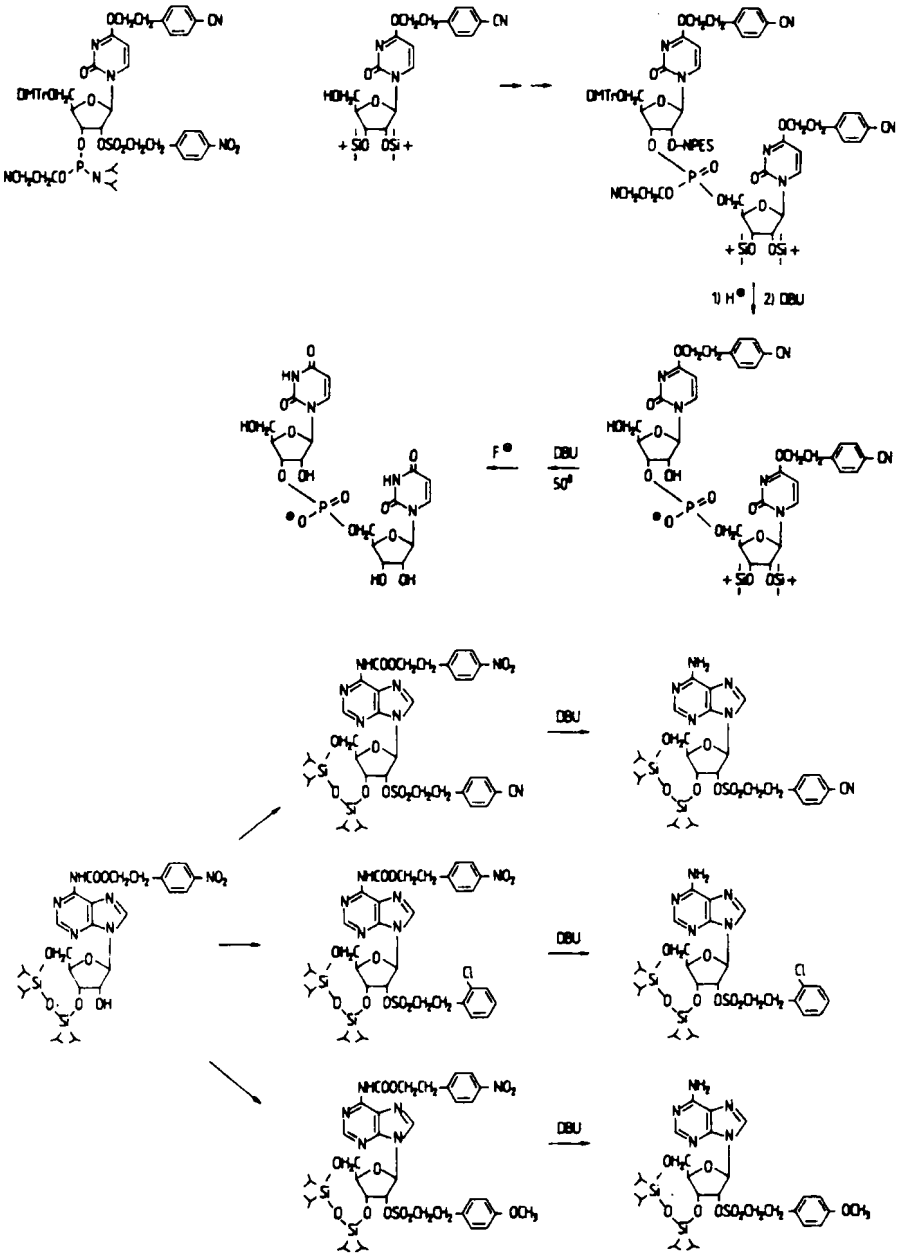
Model studies with the fully protected 5'-O-dimethoxytrityl-O^4-methyl-2'-O-p-nitrophenylethylsulfonyl-uridylyl-[3'-(β-cyanoethyl)-5']-2',3'-di-O-tert.butyldimethylsilyl-O^4-methyl-uridine, which was synthesized from the corresponding monomeric building blocks by the phosphoramidite approach, revealed a clean deblocking pattern and indicated this way the importance of a relatively stable O^4-protecting group if the NPSE group is in use.

In order to develop a new O^4-protecting group for uridine, which is more stable than the NPE group but will also be cleaved by a β-elimination process, we draw our attention to the p-cyanophenylethyl (CPE) group[9], in which the cyano function activates the β-hydrogen atoms much less than the nitro group. After synthesis of the O^4-p-cyanophenylethyl-5'-O-dimethoxytrityl-2'-O-p-nitrophenylethylsulfonyl-uridine we noticed that DBU treatment at room temp. removes only the NPES group, whereas the p-cyanophenylethoxy function stays intact. Cleavage by DBU takes place at elevated temp. of about 50°C and shows under these conditions no side reactions.



Thereafter the fully protected dimer O^4-p-cyanophenylethyl-5'-O-dimethoxytrityl-2'-O-p-nitrophenylethylsulfonyl-uridylyl-[3'-(β-cyanoethyl)-5']-O^4-p-cyanophenylethyl-2',3'-di-O-tert.butyldimethylsilyl-uridine has then been synthesized for further deblocking studies. Treatment first with acid and then with DBU at room temp. led to the partly protected phosphodiester intermediate, which was converted into UpU in high 80 % yield by DBU at 50°C and fluoride ion respectively.

Furthermore we also studied the stability of various substituted phenylethylsulfonyl groups towards the influence of DBU in the adenosine series. Introduction of the 4-cyanophenylethylsulfonyl, 2-chlorophenylethylsulfonyl and 4-methoxyphenylethylsulfonyl group into the N^6-p-nitrophenylethoxycarbonyl-3',5'-O-tetraisopropyldisiloxan-1,3-diyl-adenosine led to the fully protected analogues. DBU treatment at room temp. and 50°C respectively revealed that only the NPEOC group was eliminated, whereas the sulfonyl residues remained stable under these conditions. It was noticed, however, that treatment of the 2'-O-4-cyanophenylethylsulfonyl (CPES) derivative with fluoride ion does not only cleave the cyclic silyl blocking group but also the PCES group at a much faster rate than the relatively stable N^6-NPEOC protecting group. The 2-chloro- and the 4-methoxyphenylethylsulfonyl group respectively again did not show any tendency to cleavage under the influence of fluoride ions. The different reactivities of the β-eliminating blocking groups towards various bases will be subject of future investigations.



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. Cedergren, J.Am.Chem.Soc. 109, 7845 (1987).
4. 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.
5. M. Pfister, W. Pfleiderer, Nucleic Acids Res. Symp. Ser. 18, 165 (1987).
6. S. Farkas, W. Pfleiderer, Nucleic Acids Res. Symp. Ser. 18, 153 (1987).
7. C.H. Sapino, P.P. Brodfuehrer, S.P. Brundidge, C. Sapino, H.G. Howell, J.Org.Chem. 50, 3644 (1985).
8. F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, Tetrahedron 40, 59 (1984).
9. E. Uhlmann, W. Pfleiderer, Helv.Chim. Acta 64, 1688 (1981).