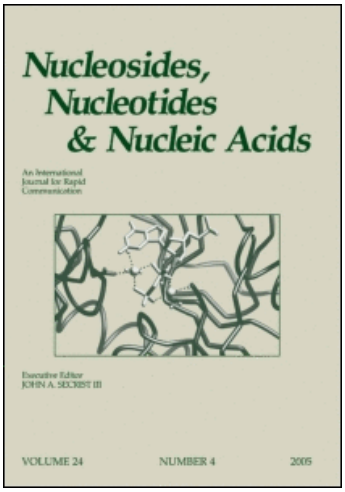

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Model Studies Towards the Automated Synthesis of tRNAs

W. Pfleiderer[a]; M. Pfister[a]; S. Farkas[a]; H. Schirmeister[a]; R. Charubala[a]; K. P. Stengele[a]; M. Mohr[a]; F. Bergmann[a]; S. Gokhale[a]
[a] Fakultät für Chemie, Universität Konstanz, Konstanz, Germany

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MODEL STUDIES TOWARDS THE AUTOMATED SYNTHESIS OF tRNAs

W. Pfleiderer, M. Pfister, S. Farkas, H. Schirmeister, R. Charubala, K.P. Stengele, M. Mohr, F. Bergmann and S. Gokhale

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

Abstract: The p-nitrophenylethylsulfonyl and methoxycarbonylethylsulfonyl group are alternative 2'-OH protecting groups in oligoribonucleotide synthesis. The corresponding, more reactive 3'-O-(ß-cyanoethyl, N,N-diethyl)phosphoramidites have to be applied in solid-phase synthesis to achieve high yields and short coupling times.

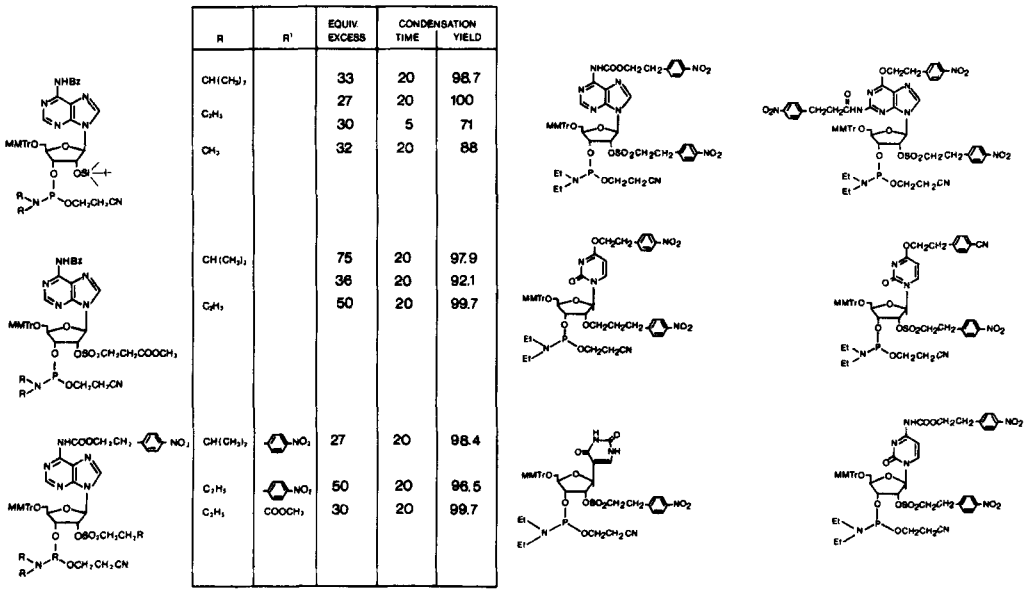
The development of a versatile automated solid-phase synthesis for oligoribonucleotides analogous to the phosphoramidite approach[1] in the oligo-2'-deoxyribonucleotide series is still far from perfection due to the unsolved problem of the proper choice of the various protecting groups. An even more sophisticated goal is the chemical synthesis of tRNAs incorporated also modified and supermodified bases into the oligonucleotide chain. So far little progress has recently been made on these lines by Ogilvie et al.[2], who have synthesized a 77-nucleotide-long RNA sequence with methionine-acceptance activity and by Téoule et al.[3], who have chosen a different blocking group combination to build a 35-mer constituting the 5'-end of the initiator tRNA from B. subtilis. In both cases the tert-butyldimethylsilyl group was chosen for 2'-OH protection giving rise to problems during the deblocking procedures[4]. C. Reese et al.[5,6] have pushed forward the phosphotriester approach to get the 3'-terminal heptatriacontamer (37-mer) of yeast alanine tRNA. Finally two recent reports on the solid-phase synthesis of oligori-

bonucleotides using acid-labile 2'-OH protecting groups and the H-phosphonate[7] and phosphoramidite approach[8] respectively show some other alternatives to solve the problems.

Since our general strategies are based on ß-eliminating protecting groups like the p-nitrophenylethyl (NPE) and the p-nitrophenylethoxycarbonyl (NPEOC) group[9] the development of the p-nitrophenylethylsulfonyl (NPES) and the methoxycarbonylethylsulfonyl (MCES) group for 2'-OH protection[10] was a consequent extension of the anticipated ß-elimination principle.

In the synthesis of the appropriate monomeric building blocks base protection by the NPE- and NPEOC blocking groups was achieved first[9]. The sugar moiety was then protected at the 5'-OH group by the monomethoxytrityl group and these derivatives applied for sulfonylation by p-nitrophenylethylsulfonyl chloride and methoxycarbonylethylsulfonyl chloride respectively giving rise to a mixture of the two isomeric 2'- and 3'-O-NPES derivatives as well as small amounts of the 2',3'-bis-O-NPES compounds. Their separation into the pure components by chromatographical means is a tedious and sometimes troublesome undertaking and leads usually to a yield of about 30 % of the 2'-O-NPES and 2'-O-MCES derivatives. In the final step phosphitylations at the 3'-OH position were achieved in the usual manner to form the corresponding 3'-O-(ß-cyanoethyl, N,N-diisopropyl)-phosphoramidites[11].

In preliminary studies with this type of phosphoramidites was, however, noticed that under the normal conditions of automated solid-phase syntheses only low yields were achieved in the coupling step. Prolongation of the reaction time and use of a large excess of the reagent improved to some extent the yields, but was still far from satisfaction. The decreased reactivity is attributed to the electronic influence of the electron-attracting sulfonyl groups and the steric bulkiness of the N,N-diisopropyl residue. Consequently, we then explored more reactive phosphoramidites, which are obviously represented by the N,N-diethyl derivatives.

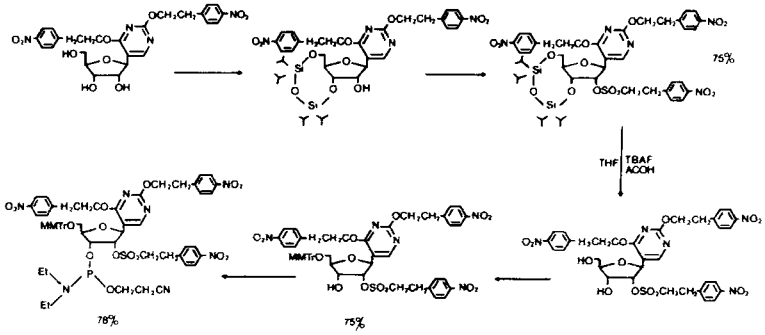


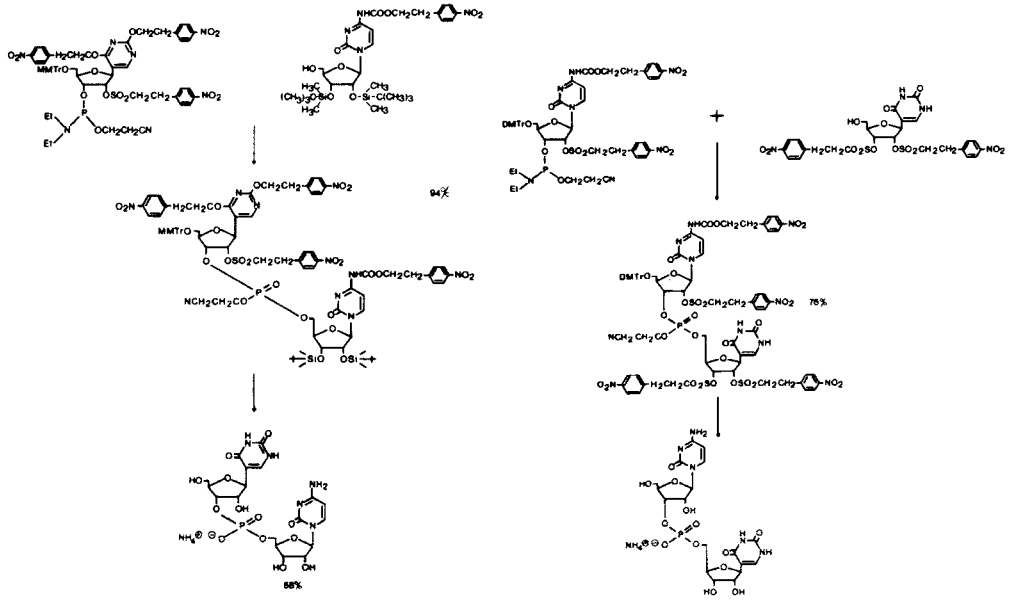
R	R'	EQUIV. EXCESS	CONDENSATION TIME	CONDENSATION YIELD
$CH(CH_3)_2$		33	20	98.7
C_2H_5		27	20	100
CH_2		30	5	71
		32	20	88
$CH(CH_3)_2$		75	20	97.9
		36	20	92.1
C_2H_5		50	20	99.7
$CH(CH_3)_2$	$-C_6H_4-NO_2$	27	20	98.4
C_2H_5	$-C_6H_4-NO_2$	50	20	96.5
C_2H_5	$COOCH_3$	30	20	99.7

Unfortunately their synthesis turned out to be quite difficult due to the high reactivity, which does not allow simple silica-gel chromatography or reprecipitation for purification. Chromatography on deactivated neutral aluminum oxide is so far the best isolation and purification method.

Comparative studies with various differently protected adenosine derivatives forming 10-mers on a newly developed solid-support material containing a new type of spacer[12] revealed that the coupling yields using 5'-O-monomethoxytrityl-2'-O-p-nitrophenylethylsulfonyl-3'-O-(β-cyanoethyl, N,N-diethyl)-ribonucleoside phosphoramidites can dramatically be increased applying a 30-50 equivalent excess and a reaction time of 20 min.. The use of the corresponding 2'-O-methoxycarbonylethylsulfonyl derivatives look even better and give almost the wanted quantitative coupling yields.

We have also been interested in the synthesis of a properly blocked pseudouridine derivative as a first example of a modified base common in most tRNAs. Pseudouridine and its O^2,O^4-di-p-nitrophenylethyl derivative respectively have first





been protected by Markiewicz's reagent in 3'- and 5'-position followed by sulfonylation at the 2'-OH group. Cleavage of the silyl group could be achieved by tetrabutylammonium fluoride in THF in presence of AcOH without harming the ß-eliminating protecting groups. Monomethoxytritylation in 5'-position and subsequent phosphitylation to the corresponding 3'-O-(ß-cyanoethyl, N,N-diethyl)-phosphoramidites proceeded under standard conditions in good yields.

Condensation reactions to various dimers in solution indicated that base protection in pseudouridine is in principle not necessary though blocking of the amide functions may have some advantage in causing higher solubilities in aprotic solvents.

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