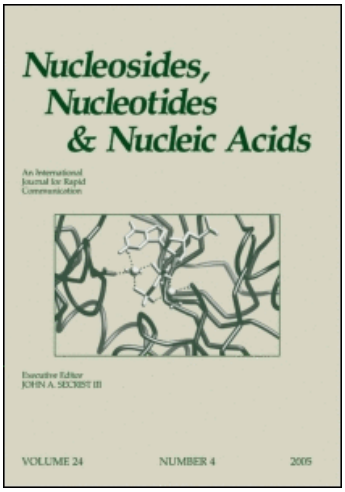


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

How to Synthesize A tRNA?

W. Pfleiderer[a]; K. P. Stengele[a]; F. Bergmann[a]; M. Resmini[a]; C. Henke[a]
[a] Fakultät für Chemie, Universität Konstanz, Konstanz

To cite this Article Pfleiderer, W. , Stengele, K. P. , Bergmann, F. , Resmini, M. and Henke, C.(1995) 'How to Synthesize A tRNA?', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 843 — 846
To link to this Article: DOI: 10.1080/15257779508012485
URL: http://dx.doi.org/10.1080/15257779508012485

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 843-846 (1995)

HOW TO SYNTHESIZE A tRNA ?

W.Pfleiderer*, K.P.Stengele, F.Bergmann, M.Resmini and C.Henke
Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-78434 Konstanz

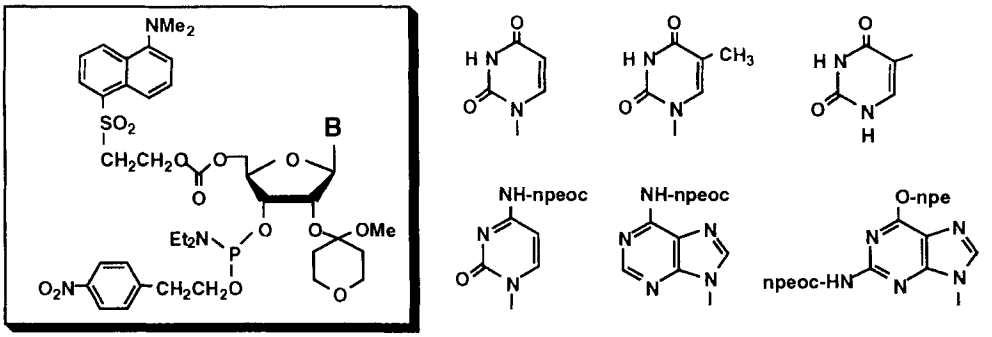
Abstract. New blocking group combinations have been investigated to achieve an automated synthesis of a tRNA and structural analogs on solid-support. The use of the 4-methoxytetrahydropyranyl group for 2'-OH-protection and the dansylethoxycarbonyl group for the 5'-OH position shows in the phosphoramidite approach good results. In the arabino series the 2-(4-nitrophenyl)ethoxycarbonyl group is a perfect 2'-OH blocking group which can be combined with the dimethoxytrityl residue in the usual manner to give high yields and pure materials.

The chemical synthesis of oligo- and poly**ribo**nucleotides can be regarded as a difficult challenge in synthetic organic chemistry and has, so far, not been solved in a satisfactory way during its 30 years of investigations. The dominance of 2'-deoxy-ribonucleotide sequences in biological research led, on one hand, to the development of the very successful phosphoramidite approach on solid-support materials which could, however, on the other hand, not been extended easily to the oligo-ribo series due to the presence of the additional 2'-OH function interfering with a simple blocking group strategy. C.B.Reese, the pioneer in oligoribonucleotide research, has made the most important contributions to this field due to his systematic studies regarding new types of 2'-OH protecting groups, quantitative kinetics for selective deblocking and the comparisons of the solution versus the solid-support syntheses.[1] The complexity of the protecting group strategy can best be seen from the fact that only two types of ribonucleoside-3'-phosphoramidites, namely the 5'-O-dimethoxytrityl-2'-O-tert-butyldimethylsilyl- [2] and the 5'-O-pixyl-2'-O-1-(2-fluorophenyl)-4-methoxypiperidin-4-yl-[3] combinations, respectively, are commercially available and have been applied with some success in the built-up of shorter oligoribonucleotide sequences.

Chemistry. We have shown recently[4] that the use of β-eliminating blocking groups for base, sugar and phosphate protection offers a series of advantages in the standard phosphoramidite approach concerning easy isolation and purification of the anticipated oligodeoxyribonucleotides. The 2-(4-nitrophenyl)ethyl-

(npe) and 2-(4-nitrophenyl)ethoxycarbonyl- (npeoc) group turned out to be of universal use due to their chemical stability towards hydrolyses with weak acids and bases but their highly selective removal by DBU in aprotic solvents in a β-elimination process. Based on these findings the 2-(4-nitrophenyl)ethylsulfonyl- (npes) group[5] was developed for 2'-OH protection overcoming the handicap of 2'-3' acyl migration during the synthesis of the monomeric ribonucleotide building blocks. Although this approach revealed some promising results in the synthesis of shorter oligoribonucleotides, it had to be given up due to difficulties in preparing the 2'-O-npes derivatives regioselectively in high yields and especially because of an useless side reaction taking place on DBU treatment of the uridine moiety in forming to some extent a 2,2'-anhydro bond.

We developed then a new blocking group combination using the 4-methoxytetrahydropyranyl- (mthp) residue as an acetal function for the 2'-OH group and the dansylethoxycarbonyl- (dnseoc) group for 5'-O-protection.[6] The latter blocking group was designed according to a high sensitivity towards dilute DBU,

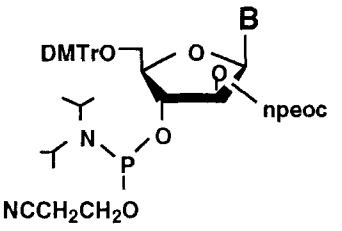


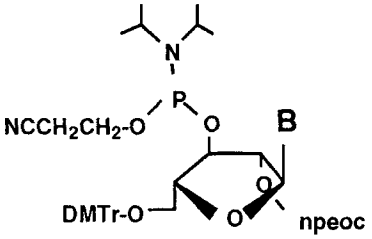
including the compatibility with the acid labile acetal function and the relatively stable npe and npeoc groups for base and phosphate protection and also due to its easy detection on cleavage by its color and fluorescence. The syntheses of the monomeric building blocks starting from uridine, ribothymidine, pseudouridine, cytidine, adenosine and guanosine proceeded well in the usual manner and led to the fully protected 3'-O-[2-(4-nitrophenyl)ethyl, N-diisopropyl and the more reactive N-diethyl)phosphoramidites. Automated syntheses of oligoribonucleotides in a DNA-synthesizer up to 20-mers worked very well and chain lengths of 40 units still gave satisfactory results. The npe / npeoc strategy allowed the cleavage of the β-eliminating blocking groups as long as the oligomer is still attached to the support, and after washing the oligoribonucleotide is then cleaved by

ammonia yielding the corresponding 2'-O-mthp-protected oligomers as ammonium salts in pure form without further purification. The fully deprotected oligoribonucleotide can then be freed from this stable storage form by treatment with 80% acetic acid if needed.

All efforts to synthesize the phage T5 tRNA ^{his}, which contains only pseudouridine and ribothymidine as minor components, showed clearly that the new method has its limitations at longer sequences. The crude material had to be purified in this case by HPLC to give the 2'-O-mthp-protected 78-mer in sufficient purity. We assume that the exchange of the npe-phosphate blocking group against the more stable 2-(4-cyanophenyl)ethyl group (cpe) will reduce some side reactions due to partial phosphate deprotection occurring during the chain elongation process.

The npe / npeoc strategy was furthermore applied to synthesize oligoarabinonucleotides and a structurally analogous tRNA. The arabinonucleosides were first protected by the Markiewicz reagent in 3'- and 5'-position followed by simultaneous protection of the 2'-OH and base amino groups by the npeoc function. Cleavage of the silyl blocking group was highly selective and the arabino configuration prevented the acyl migration at the sugar moiety. Dimethoxytritylation at the 5'-OH group followed by phosphitylation to the fully protected 3'-O-(β-cyanoethyl, N-diisopropyl)phosphoramidites[7] gave very stable and easily to handle monomeric building blocks. Automated syntheses of oligoarabinonucleotides of various chain lengths and up to the 78-meric structural analog of phage T5 tRNA ^{his} on CPG solid-phase supports proceeded extremely well in almost quantitative condensation yields. Also the two step deprotection (first treatment with acid for detritylation and second with DBU to achieve β-elimination) worked perfectly and led after final ammonia cleavage of the oligonucleotide from the support to very pure crude materials.





In a similar manner the fully protected α-arabinonucleoside-3'-O-(β-cyanoethyl, N-diisopropyl)phosphoramidites were prepared and applied with the

same success in oligo-α-arabinonucleotide chemistry. The 78-meric α-tANA analog of phage T5 $tRNA^{his}$ was obtained as a single peak in the capillary electrophoresis in the crude stage demonstrating the high efficiency of the method.

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