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Protecting Groups for the Synthesis of Ribonucleic Acids

Stefan Pitsch*

Abstract: Methods for the regioselective introduction of alkoxymethyl-groups into the 2'-O-position of 5'-O-dimethoxytritylated, nucleobase-protected ribonucleosides and for the N-alkyloxycarbonylation of adenine and guanine nucleosides were developed. These methods were used for the preparation of RNA-phosphoramidites carrying novel fluoride- and photolabile sugar and nucleobase protecting groups.

Keywords: Nucleic acids · Protecting groups · Phosphoramidites · RNA

1. Introduction

Deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) are linear macromolecules, each consisting of four structurally related nucleotide building blocks. The chemical synthesis of nucleic acids has been automated and is carried out on a solid-phase by stepwise addition of appropriately activated and protected nucleotide building blocks until the desired sequence has been obtained. Finally, all protecting groups required during the assembly-procedure are removed and the product is cleaved from the solid support (Scheme 1). Length and uniformity of the product are limited by the coupling and deprotection efficiencies.

The synthesis of DNA-oligonucleotides is probably the most evolved chemical process known so far as it has almost reached perfection in terms of efficiency and automation. More than $2 \cdot 10^6$ DNA-couplings/year are performed world-wide and DNA of defined sequence is available to everyone. In principle, the same methodology can be applied to the synthesis of structurally very similar RNA-oligonucleotides. Compared to DNA, however, each nucleotide unit within an RNA-strand contains an additional 2'-OH-group which is responsible for the relative instability of RNA under weakly basic conditions and which has to be protected during the assembly. These supplementary protecting groups have to be chosen carefully, both with respect to their deprotection efficiency and their influence on the coupling reaction.

From the large number of 2'-O-protecting groups investigated so far, the fluoride-labile *t*-butyldimethylsilyl (= *t*BDMS) group has found the widest application. However, several factors, including the relatively low coupling yields of typically ≤98% obtained with corresponding building blocks are not optimal, limiting the length of routinely synthesized RNA-sequences to about 40 nucleosides [1]. In order to overcome these limitations, we have investigated new photolabile and fluoride-labile protecting groups for the 2'-O-position of ribonucleosides.

A severe limitation in the chemical synthesis of oligonucleotide analogues is the need to remove the commonly used acyl-type nucleobase protecting groups with strong nucleophiles such as NH_3 or $MeNH_2$ (Scheme 1). A variety of naturally occurring modified nucleosides, such as dihydrouridine, and biologically relevant RNA-conjugates, such as aminoacylated RNA-sequences, are not stable under these deprotection conditions. In order to prepare such sensitive analogues, we have also developed new photolabile and fluoride-labile protecting groups for the nucleobase moieties.

*Correspondence: Dr. S. Pitsch
Laboratorium für Organische Chemie
ETH Zentrum
Universitätstr. 16
CH-8092 Zürich
Tel.: +41 1 632 44 81 / 29 69
Fax: +41 1 632 11 36
E-Mail: pitsch@org.chem.ethz.ch

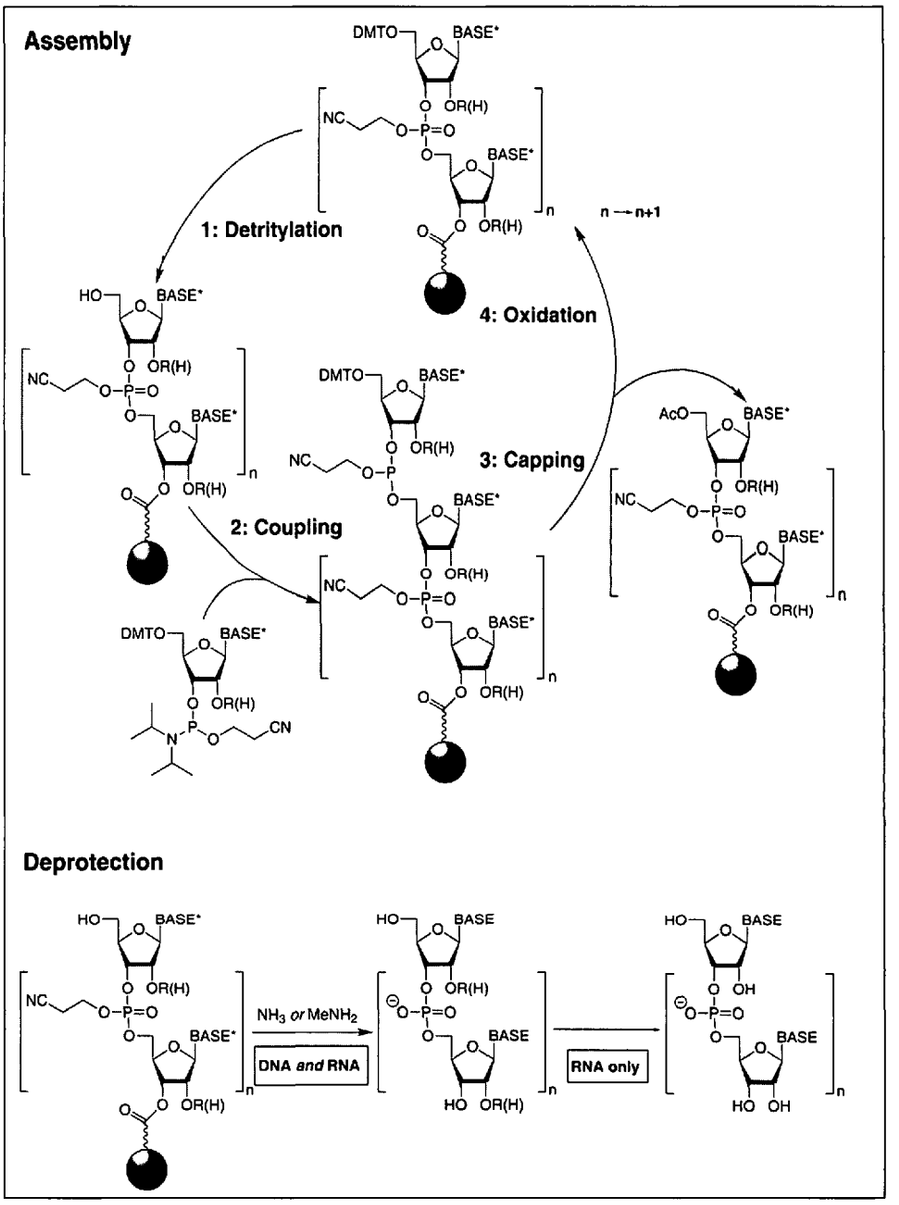
2. Protecting Groups for the Sugar-Moiety

When we started our RNA-related projects five years ago, we first investigated alternatives to the commonly used 2'-O-protecting groups of the silylether [1] or acetal-type [2]. The most attractive was the photolabile [2-(nitrobenzyl) oxy]methyl (= nbm) protecting group, introduced by Gough and coworkers [3], which leads to good coupling yields due to its minimal steric hindrance. However, the reported introduction of this group into ribonucleosides was not satisfactory for our purposes. We therefore developed a superior method for their introduction which later proved useful for the synthesis of a number of structurally related (formaldehyde acetal-derived) 2'-O-protecting groups [4]: Formation of a cyclic 2',3'-di-O-dibutylstannyl derivative of nucleobase-protected, 5'-O-dimethoxytritylated nucleosides with Bu_2SnCl_2/ $i Pr_2NEt$ in dichloroethane for 90 min at r.t., followed by addition of 1.1–1.5 equiv. of the corresponding chloromethyl ether ($ROCH_2Cl$), and heating for 15 min at 80° resulted in the exclusive formation of the two corresponding 3'-O- and 2'-O-protected nucleosides. With all nucleosides and chloromethyl ethers employed thus far, the 2'-O-alkylated product was formed as the main product (Scheme 2).

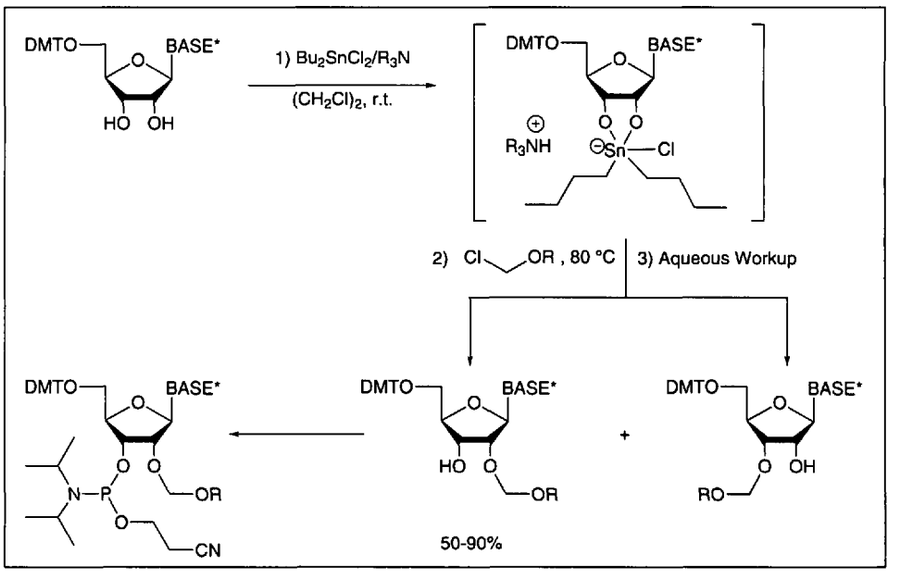
We subsequently prepared and investigated a number of related 2'-O-alkylated nucleoside phosphoramidites for the synthesis of RNA-sequences. About 3 years ago, we introduced novel 2'-O-[(triisopropylsilyl)oxy]methyl (=tom) pro-

CHIMIA 2001, 55, No. 4

Scheme 1. The solid-phase synthesis of oligonucleotides is accomplished by stepwise assembly of phosphoramidite building blocks. The product is then deprotected and cleaved from the support.



Scheme 2. General method for the introduction of alkoxymethyl-groups into protected ribonucleosides [4]. The 2'-O-alkylated products are finally transformed into phosphoramidite building blocks.



tected RNA-building blocks [5][6] (Fig. 1). The 2'-O-tom protecting group combines the advantages of the (sterically non demanding) nbm- and the (fluoride-labile) tBDMS-protecting group. Excellent coupling yields of typically >99.3% are obtained under DNA-coupling conditions (2 min coupling time). The deprotection of the resulting oligomers, first with $MeNH_2$ in $EtOH/H_2O$ and then with $Bu_4NF{\cdot}3H_2O$ in THF is quantitative, reliable, and occurs without concomitant destruction of the product. Employing 2'-O-tom protected phosphoramidites, we can meanwhile routinely prepare RNA-sequences up to 100mers.

For a variety of interesting applications, including structural and mechanistic investigations, we required building blocks with photolabile 2'-O-protecting groups which were completely orthogonal to the corresponding (fluoride-labile) 2'-O-tom-groups. For this purpose, we developed 2'-O-[(*R*)-1-(2-nitrophenyl) ethyloxy)methyl]- (= (*R*)-mnbm) protected phosphoramidites [5] (Fig. 2).

Such building blocks could be combined successfully with 2'-O-tom protected phosphoramidites. After deprotection of the resulting oligonucleotides with Bu_4NF, partially 2'-O-mnbm protected RNA-sequences were obtained. Such compounds are useful tools for the study of ribozyme cleaving reactions by preformation of inactive complexes with the ribozyme and the partially 2'-O-protected, 'caged' substrate; after photolysis efficient cleavage of the remaining protecting group occurs [5]. Using this orthogonal pair of 2'-O-protecting groups, we have also been able to develop an efficient, unambiguous method for the preparation of RNA-sequences by ligation of two or more fragments [7]. In combination with fluoride-labile nucleobase protecting groups, the 2'-O-tom and 2'-O-mnbm protecting groups are useful for a general synthesis of aminoacylated RNA-fragments [8].

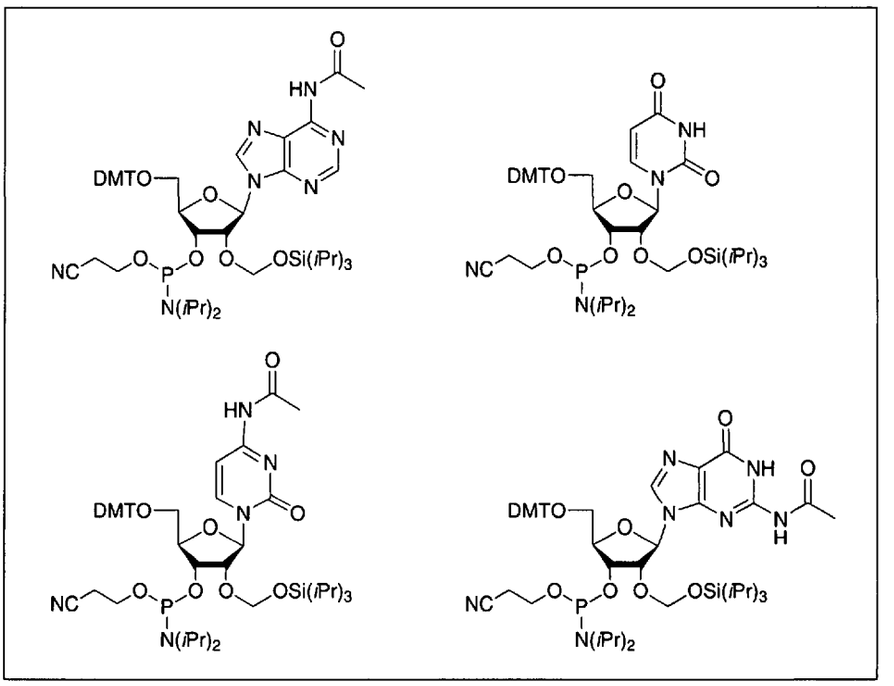


Fig. 1. 2'-O-tom-protected RNA-phosphoramidites for the routine synthesis of RNA-sequences [5][6].

3. Protecting Groups for the Nucleobase Moieties

In order to prevent side-reactions and to enhance solubility in organic solvents, the exocyclic NH_2-groups of adenine-, cytosine- and guanine-containing DNA and RNA phosphoramidite building blocks are usually protected by acylation. For example, our 2'-O-tom protected phosphoramidites carry acetyl nucleobase protecting groups that are removed with $MeNH_2$ (Fig. 1). In order to prepare labile RNA-derivatives, we investigated a variety of alternative nucleobase protecting groups, removable under milder, nonnucleophilic conditions.

The acylation of cytidine-derivatives is straightforward and can be carried out with almost any acylating agent. In contrast, acylation of the purine-nucleosides is more difficult due to their low nucleo-

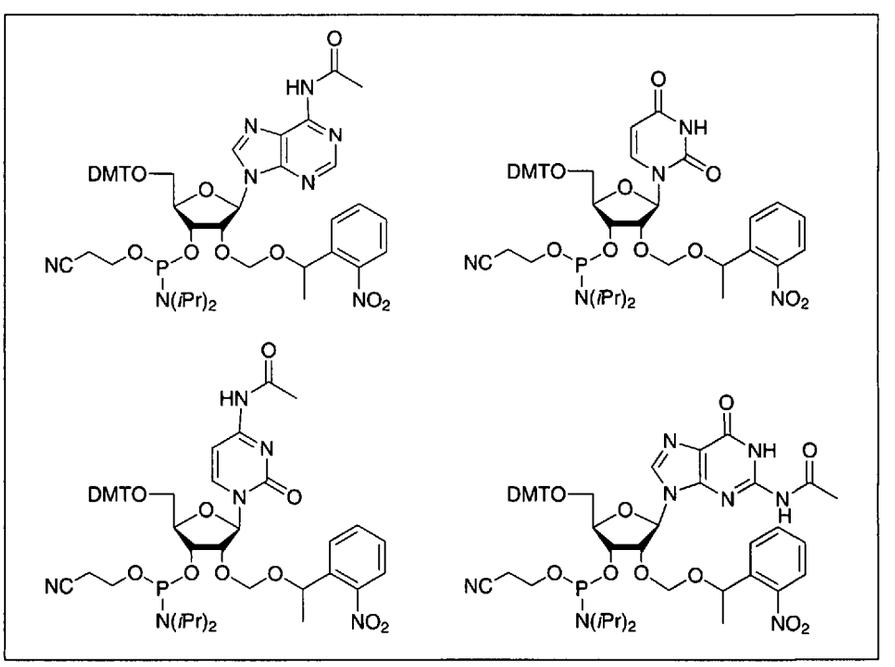
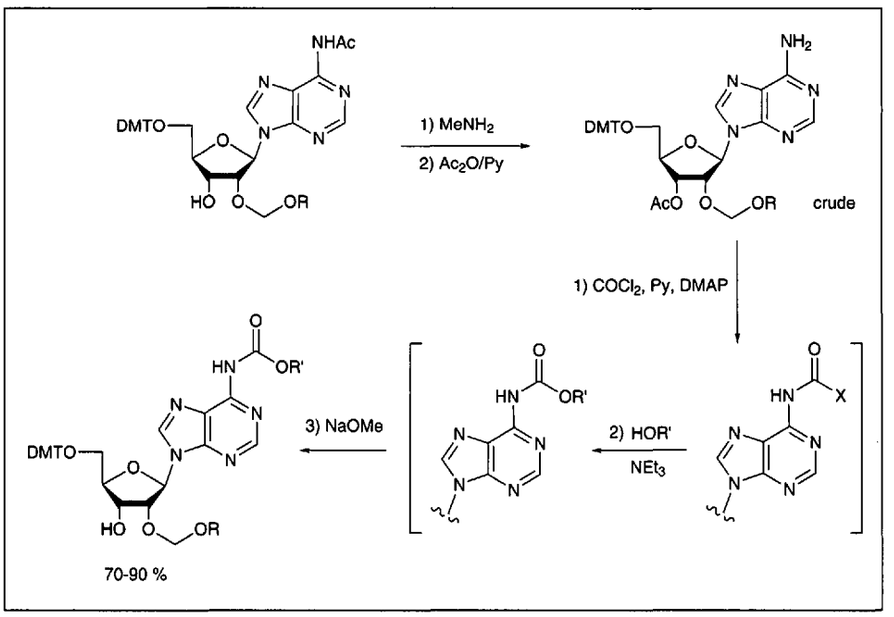


Fig. 2. 2'-O-mnbm-protected RNA-phosphoramidites for the introduction of photolabile 2'-O-protecting groups into RNA-sequences [5].



Scheme 3. General method for the introduction of N-carbamoyl-groups into protected purine nucleosides [9].

philicity. For the introduction of carbamoyl-type protecting groups into purine nucleosides, we developed a general reaction sequence, which is carried out without purification of the intermediates [8][9]: Treatment of 2'-O-protected, 5'-O-dimethoxytritylated purine nucleosides with Ac_2O/DMAP in pyridine led to quantitative acetylation of the 3'-O-position. After extractive workup, these intermediates were converted into the corresponding N-carbamoyl derivatives by subsequent treatment first with $COCl_2$/DMAP and then an appropriate alcohol (ROH) in the presence of NEt_3. After extraction, selective removal of the intermediate 3'-O-acetyl protecting groups was achieved with NaOH in THF/MeOH/H_2O. After extraction and purification, the protected purine nucleosides were obtained typically in yields >70% (Scheme 3).

In our first approach towards a general total synthesis of aminoacylated t-RNAs, we developed RNA-phosphoramidites carrying photolabile 2'-O-[2-(nitrobenzyl)oxy]methyl (= nbm) sugar and N-[2-(nitrobenzyl)oxy]carbonyl (= nboc) nucleobase protecting groups. These building blocks could be assembled to oligoribonucleotides, detached from the solid support and finally deprotected by photolysis under neutral and mild conditions (Fig. 3).

We soon realized that the purification and handling of aminoacylated RNA-sequences is very difficult due to their instability towards hydrolytic cleavage of the ester bond formed between the amino acid and the RNA [7][8]. We decided therefore to modify the original concept by preparing stabilized precursors that could be transformed into the target structures by a final photolytic step. For this purpose we prepared and evaluated a number of fluoride-labile nucleobase protecting groups. Finally, we chose the N-{[(2-triisopropylsilyloxy)benzyl]oxy}carbonyl (= toz) group which is both stable under all reaction conditions and very labile towards Bu_4NF deprotection. RNA phosphoramidites with 2'-O-tom sugar and N-toz base protecting groups were prepared (Fig. 4).

From these building blocks, oligoribonucleotides were assembled by automated synthesis under standard conditions. After the detachment from the solid support, the resulting fully protected sequences could be aminoacylated with amino-acid derivatives, carrying photolabile N-protecting groups. Upon removal of the fluoride-labile sugar and nucleobase protecting groups, partially photolabile-protected, stabilized precursors of aminoacylated RNA-sequences were obtained. Their photolysis under mild conditions resulted in an efficient formation of 3'(2')-O-aminoacylated RNA-sequences [8].

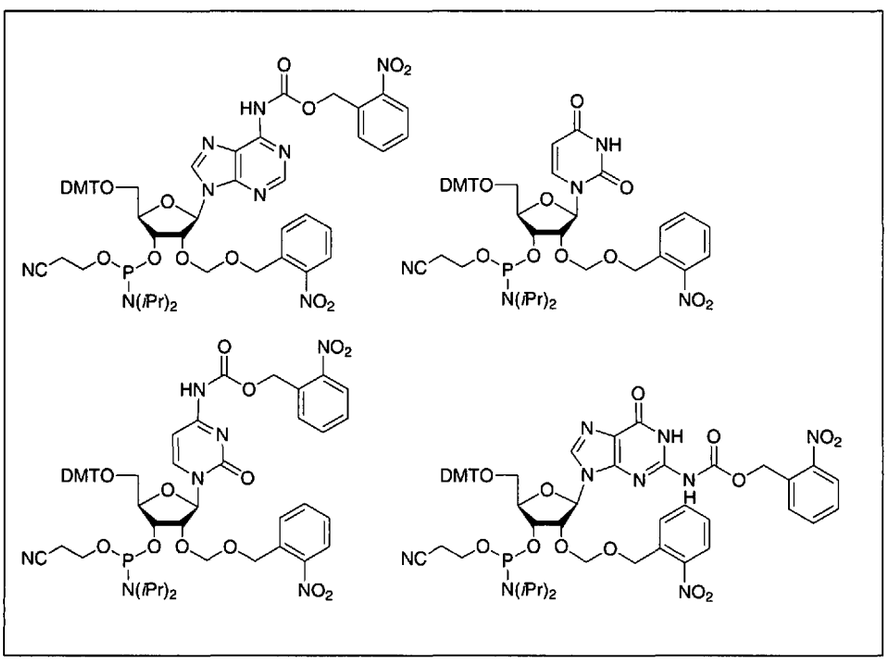


Fig. 3. 2'-O-nbm and N-nboc-protected RNA-phosphoramidites for the synthesis of base-labile RNA-analogues [9].

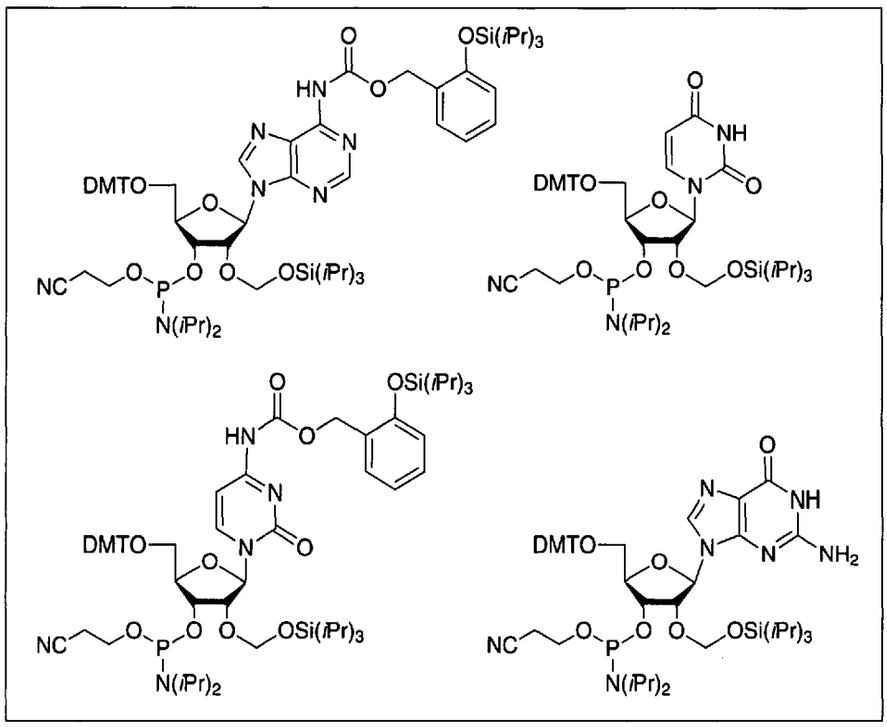


Fig. 4. 2'-O-tom and N-toz-protected RNA-phosphoramidites for the synthesis of amino-acylated RNA-sequences [8].

4. Conclusions and Outlook

The successful and unambiguous synthesis of nucleic acids requires reliable protecting groups which have to meet several stringent requirements. Their removal at the end of the synthesis must occur quantitatively without partial destruction of the product; they have to be orthogonal to each other and to be completely stable under all other reaction and deprotection conditions; they should not interfere with the coupling reactions; and they should be easy to introduce into monomers. To that end, we have created a set of reliable fluoride- and photolabile sugar- and nucleobase-protecting groups and developed synthetic methods for their introduction into ribonucleosides. Based on these building blocks we are now set to implement general strategies for the preparation of long RNA-sequences and aminoacylated t-RNAs. In sodoing, we hope to support biological research and contribute to the understanding of fundamental biological processes involving ribonucleic acids.

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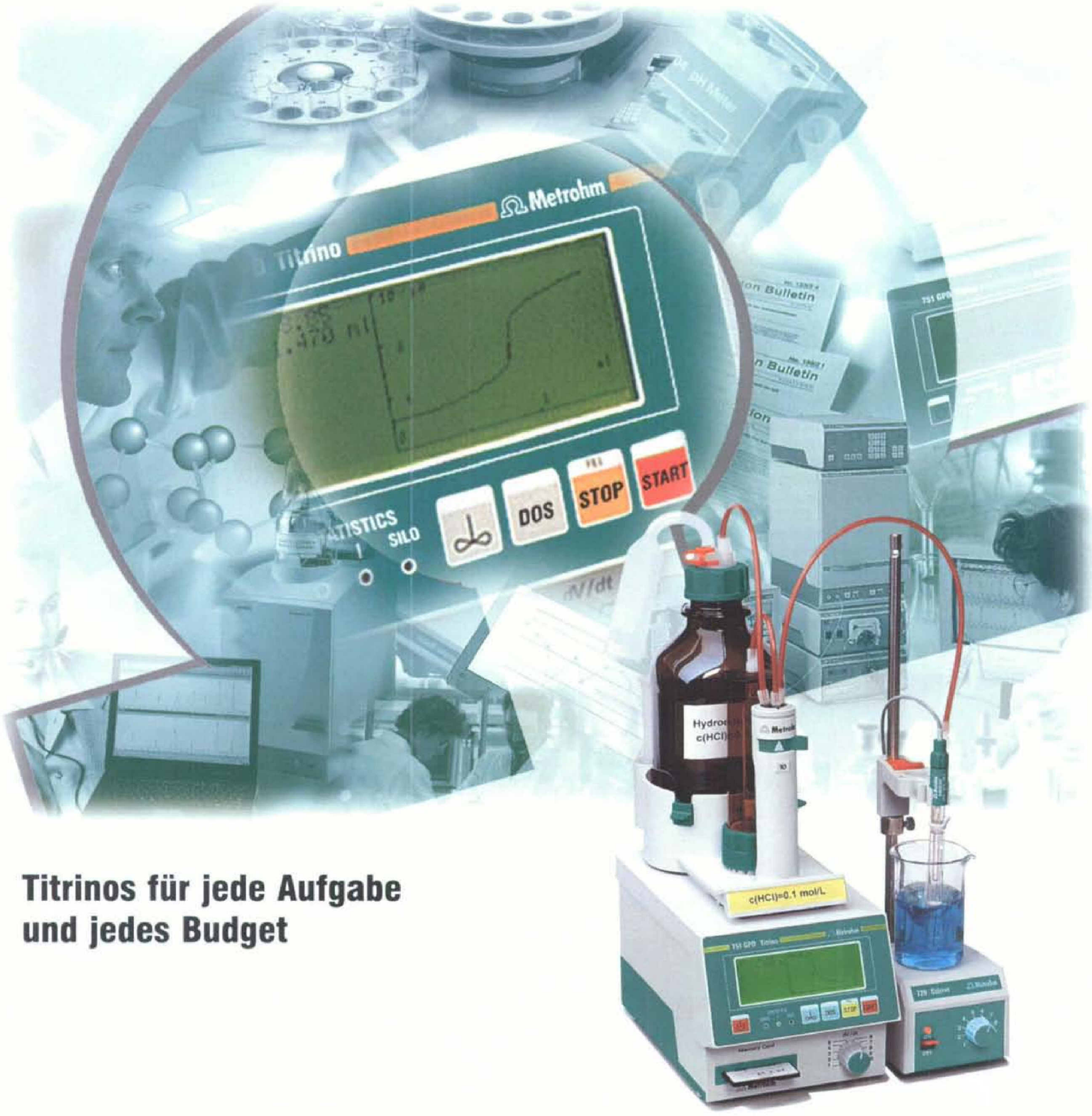

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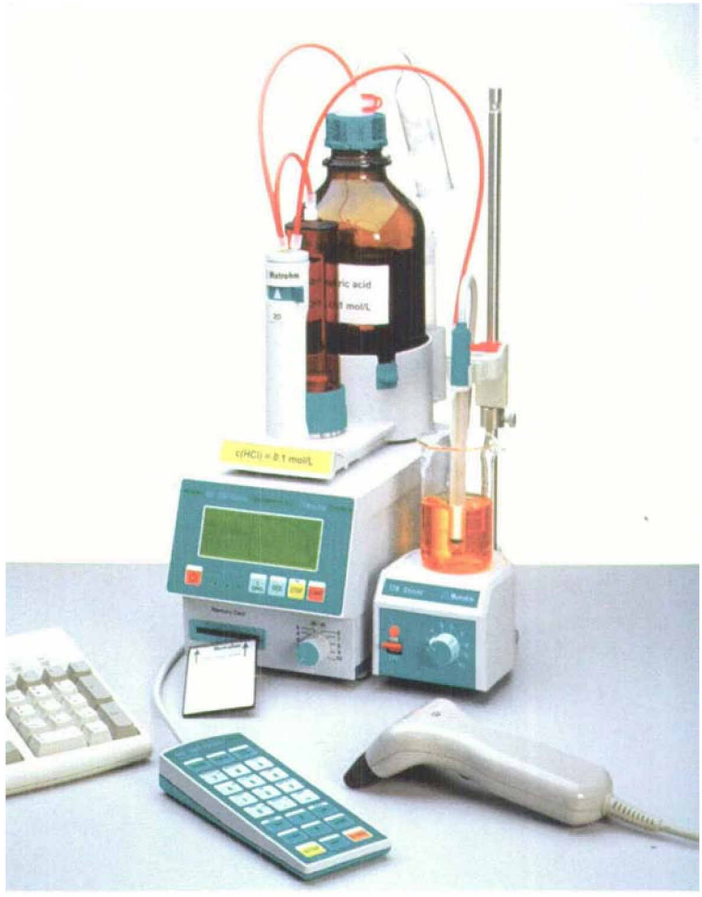

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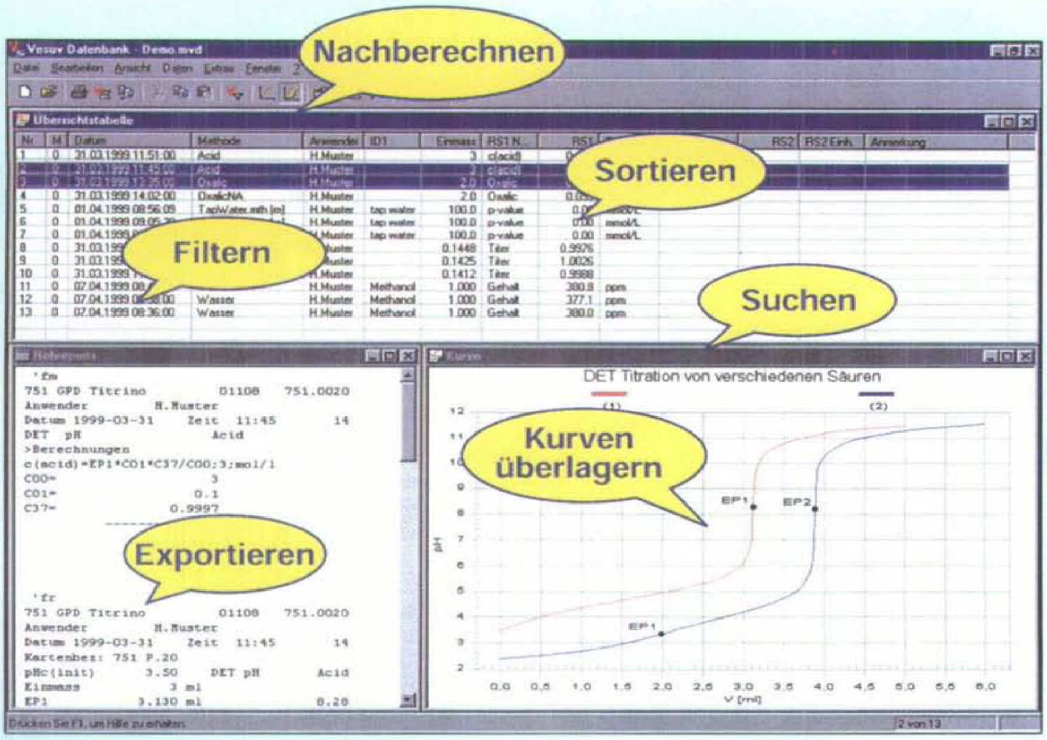

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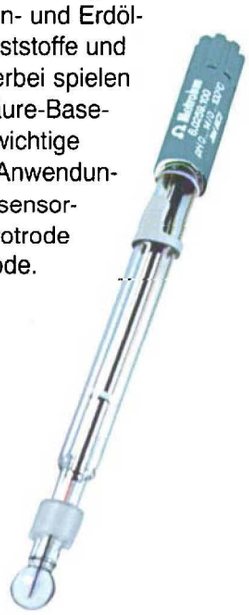

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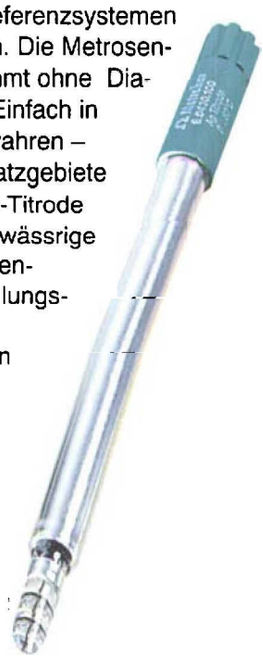

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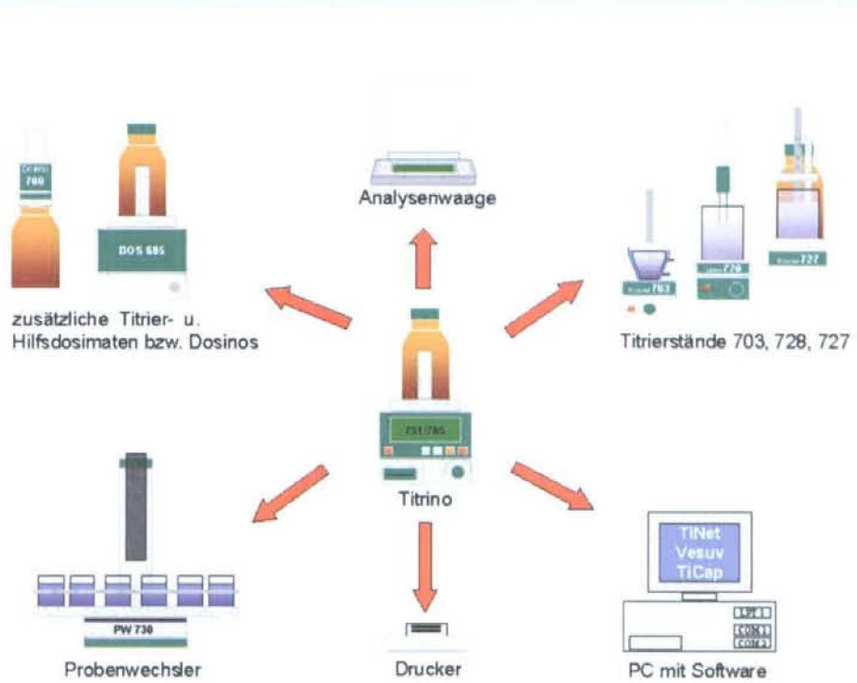

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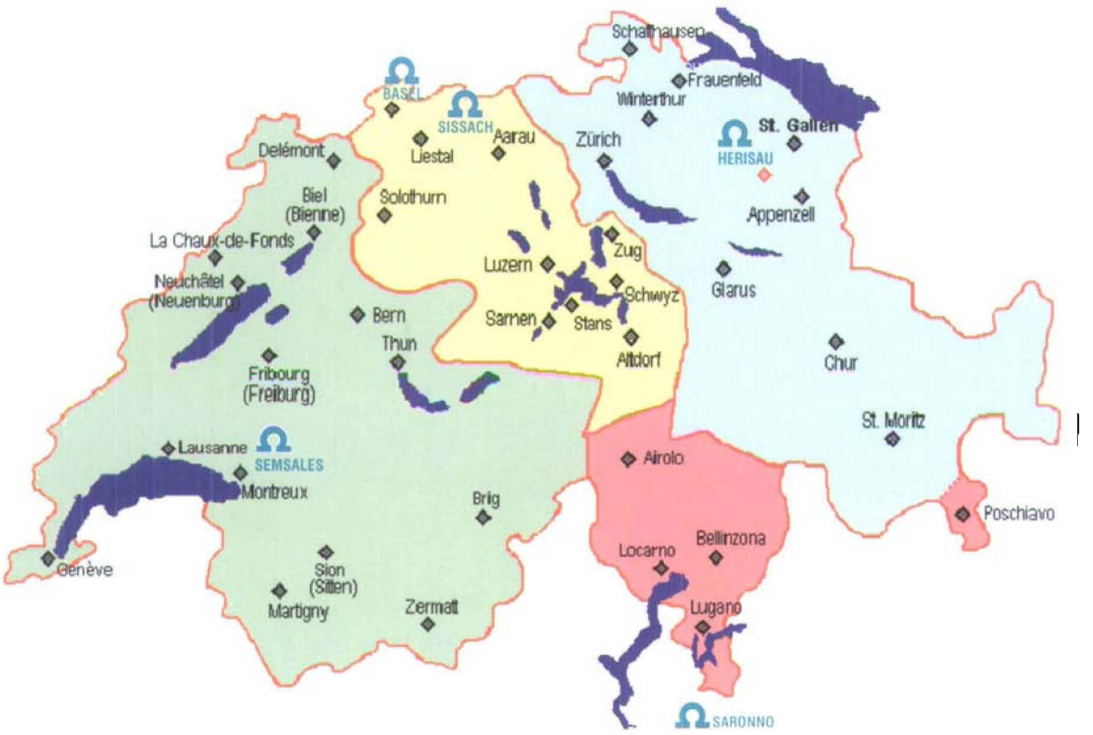

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Vertretung Westschweiz
Hubert Peyer
Au Village
1623 Semsales
Telefon 026 / 918 55 84
Telefax 026 / 918 55 84
h.peyer@metrohm.ch

Vertretung Nordwest- / Zentralschweiz und Clariant
Kurt Fankhauser
Bützenenweg 90
4450 Sissach
Telefon 061 / 971 78 10
Telefax 061 / 971 78 11
k.fankhauser@metrohm.ch

Vertretung Ostschweiz
Heinz Kellenberger
Oberdorfstrasse 68
9101 Herisau
Telefon 071 / 353 86 01
Telefax 071 / 353 89 05
kel@metrohm.ch

Vertretung Novartis und Hoffmann-La-Roche
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Käferholzstrasse 109
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Telefon 061 / 603 24 40
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Metrohm
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Metrohm AG CH-9101 Herisau
Schweiz
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