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QUARTERLY

This paper is dedicated to Professor David Shugar on the occassion of his 80th birthday and in admiration of his outstanding contributions to nucleic acid chemistry

Recent progress in oligonucleotide synthesis

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New blocking group combinations for the machine-aided oligoribonucleotide synthesis on solid phase material have been developed and tested regarding their general application. An acetal function for 2′-OH protection offers a series of advantages in the synthetic approach but special conditions have to be fullfilled in order to guarantee a selective cleavage of the temporary 5′-OH blocking group such as the dansylethoxycarbonyl or even the acid-labile dimethoxytrityl group in the chain elongation process. The final removal of the 2′-*O*-acetal function in the partially deblocked oligomer proceeds unexpectedly well under weak acidic conditions due to a supposed intramolecular acid catalysis.

The chemical synthesis of oligo-2′-deoxyribonucleotides has in recent years been developed almost to perfection based upon the very effective combination of phosphoramidite chemistry [1] with the solid-phase method [2, 3]. However, a machine-aided assembly of oligo**ri**bonucleotide chains has only partially been successful since the presence of a single extra hydroxy group at the 2′-position of a **ribo**nucleoside has given rise to many problems which so far could not been solved satisfactorily [4]. Only the *tert*-butyldimethylsilyl [5–13] and the 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp) group [14, 15] are in practical use so far, but the compatibility with the other blocking groups involved in the chain elongation process is not perfect and leads to side reactions which are recognized by the HPLC methods of analysis. The resumee from various investigations combining different protecting groups points towards the use of an acetal function for 2′-OH protection for several reasons but its chemical nature in regard to the temporary 5′-OH blocking group has to be tuned very specifically to be orthogonal in reactivity. New alternatives have been investigated with variable success showing that more attention has to be given this important field of nucleic acid chemistry.

RESULTS AND DISCUSSION

We have recently shown [16] 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 oligonucleotide. Especially, the 2-(4-nitrophenyl)ethyl (Npe) and

Abbreviations: CPG, controlled-pore glass; DBU, 1,8-diazabicyclo[5.4.0]-undec-7-one; DMTr, dimethoxytrityl group; Fpmp, 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl group; Npe, 2-(4-nitrophenyl)ethyl group; Npeoc, 2-(4-nitrophenyl)ethoxycarbonyl group.

the 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, and their highly selective removal by (1,8-diazabicyclo[5.4.0]-undec-7-ene) DBU in aprotic solvents in a β-elimination process [17]. The Npe/Npeoc strategy was then applied for permanent nucleo-base and phosphate protection whereas the sugar moiety was modified with the 2'-*O*-4-methoxytetrahydropyranyl group [18] and the newly developed, base-labile and fluorescing 5'-*O*-dansylethoxycarbonyl group [19, 20]. This new type of monomeric building block gave good results in the automated synthesis of shorter oligoribonucleotides up to a chain length of 20 and in special cases of 40 nucleotide units [21] (Scheme 1).

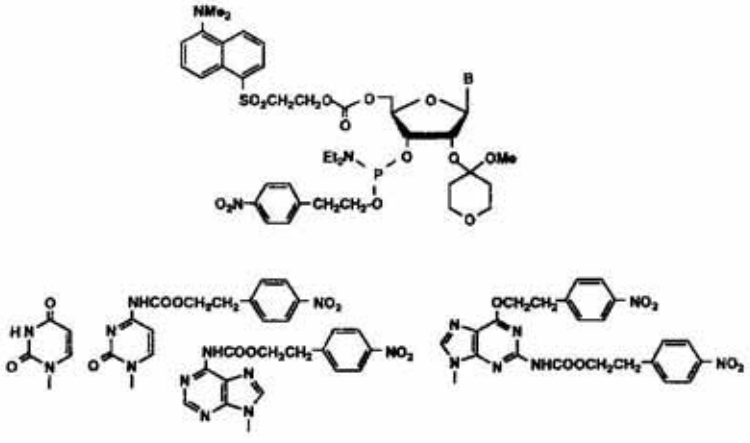
Attempts to synthesize the $tRNA^{His}$ of the phage T5 consisting of 78 nucleotides by machine-aided solid-phase assembly, however, were not successful and revealed the limitations of this method.

More recent studies have then been undertaken to look systematically for new acetal functions for 2'-OH protection which are compatible with the dimethoxytrityl (DMTr) in revealing high orthogonal reactivity of these blocking groups.

The principle of this approach has already been rationalized by Reese *et al.* [22, 23] in combining first the Mthp group with the DMTr and the pixyl group, respectively, but only with little success due to the close acid lability of the corresponding blocking groups. The introduction of the 2'-*O*-[1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl] [24, 25] and finally the 2'-*O*-Fpmp group [14,15] improved the results substantially due to a higher stability of the latter groups at lower pH afforded during 5'-detritylation and 5'-depixylation. On the other hand, Takaku and his group [26, 27] investigated the more simple 2'-*O*-1-(ethoxy)-ethyl, -1-(butoxy)ethyl, -1-(isopropoxy)ethyl and -1-(2-chloroethoxy)-ethyl acetals showing a structure-dependent acid stability over a broad pH range.

We extended the spectrum of acetal functions even further to 2'-*O*-1-(benzyloxy)ethyl and -1-(2-phenylethoxy)ethyl acetals and their *o*- and *p*-substituted derivatives as well as to other similar types such as the 1-[2-(*N*-phthalimido]ethoxy)ethyl, 1-(2-cyanoethoxy)ethyl and 1-(2-nitroethoxy)ethyl acetal, respectively (Table 1).

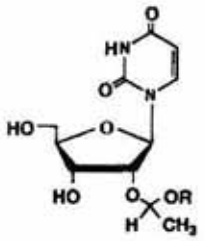
The acid stabilities of the corresponding uridine-2'-*O*-acetals have been comparatively studied by the determination of their half lifetimes at pH 2 which is considered as an appropriate pH range for fast cleavage of the dimethoxytrityl group during the chain elongation process. Table 1 indicates very nicely the structurally related stabilities ($t_{1/2}$) which range between 21 and 14 000 min. During these studies it was noticed that the electron-attracting highly acid-stable methylsulfonylethyl, phenylsulfonylethyl, cyanoethyl and nitroethyl acetals are prone to cleavage by DBU in a β-eliminating process which is unique to some extent and of synthetic value. From this list the 2-(4-nitrophenyl)ethyl group with a $t_{1/2}$ of 222 min was chosen as the most appropriate candidate since this acetal is stable enough to survive the detritylation steps during the chain elongation process in the synthetic cycle of the oligonucleotide synthesis but is also cleavable



Scheme 1

Table 1

Acid stabilities of uridine-2'-O-acetals at pH 2 (0.05 N HCl + MeOH 1:2, v/v).



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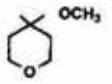	24
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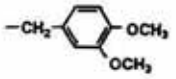	82
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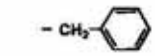
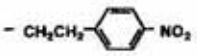
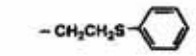
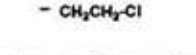
	175
	222
	230
	620

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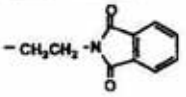
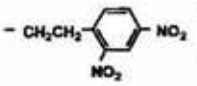
	840
	1 200
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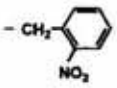
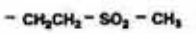
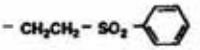
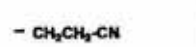
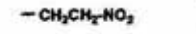
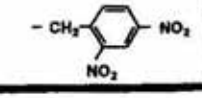
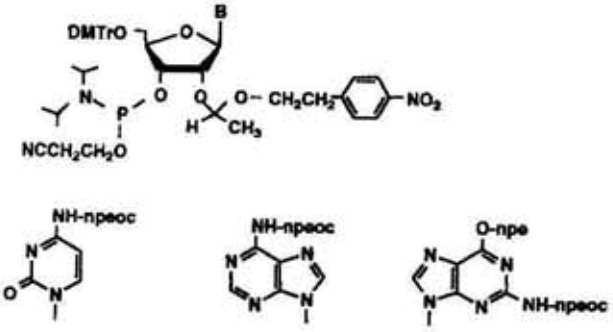
	2 240
	3 100
	3 100
	3 370
	4 000
	14 000

by prolonged treatment with acid without harming the glycosidic linkage.

Next the four phosphoramidite building blocks carrying the Npe and Npeoc protecting groups at the bases and the 5'-*O*-dimethoxytrityl as well as the 2'-*O*-1[2-(4-nitrophenyl)-ethoxy]-ethyl function at the sugar moiety were synthesized (Scheme 2).



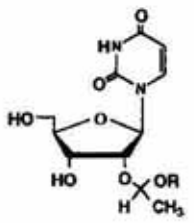
Scheme 2

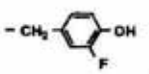
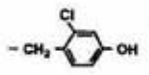
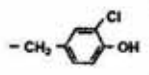
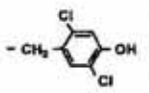
Automated solid-support syntheses of various oligoribonucleotides with the phosphoramidites proceeded well to chain lengths of 20- and moderately to 40-mers. However, it was also recognized during the final deblocking of the β-eliminating Npe and Npeoc base and β-cyanoethyl phosphate protecting groups by DBU that the Npe-ether acetal function takes also part to some extent in the cleavage process leading to some contamination of the oligomer.

In order to overcome these unexpected difficulties new types of acetals consisting of the so-called "protected protecting" groups have been developed to modify the stabilities of the acetal functions in an appropriate manner. It was found that the relatively acid-labile uridine-2′-*O*-1-(4-hydroxybenzyl)ethyl acetal and its various derivatives can be 5–6 times stabilized by acylation of the 4-hydroxy group converting an electron-donating into an electron-attracting group. The best compromise for our purpose seems to be the 3-fluoro-4-[2-(4-nitrophenyl)-ethoxycarbonyloxy]benzyl residue since the corresponding acetal shows in 80% AcOH a $t_{1/2}$ of 305 min whereas DBU treatment generates thereof the 3-fluoro-4-hydroxybenzyl acetal which under the same acidic conditions is cleaved much faster with $t_{1/2}$ of 48 min (Table 2).

A new set of the most common ribonucleoside 3′-*O*-phosphoramidites were synthesized as monomeric building blocks for the machine-aided assembly of oligoribonucleotides using a modified CPG-solid-phase material [16] (Scheme 3).

Table 2
Acid stabilities of uridine-2′-O-acetals in 80% AcOH at 20°C



R	$t_{1/2}$ (min)
	24
	48
	69
	96
	250
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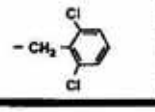
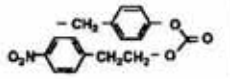
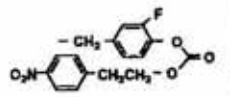
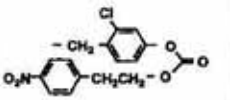
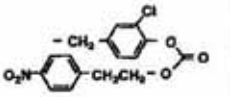
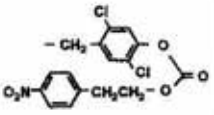
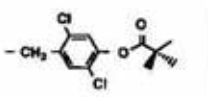
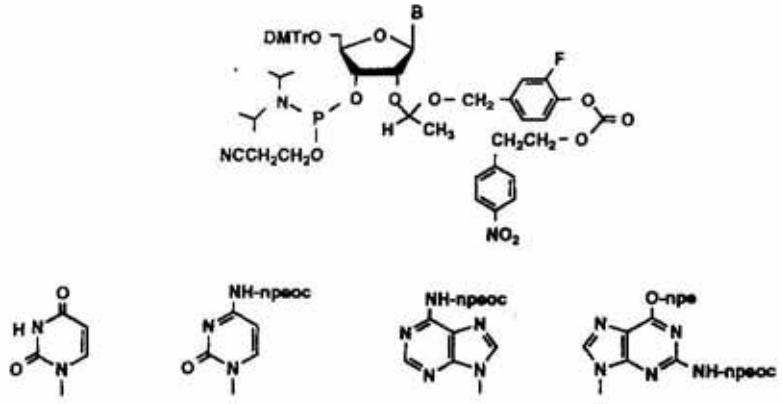
	1 130

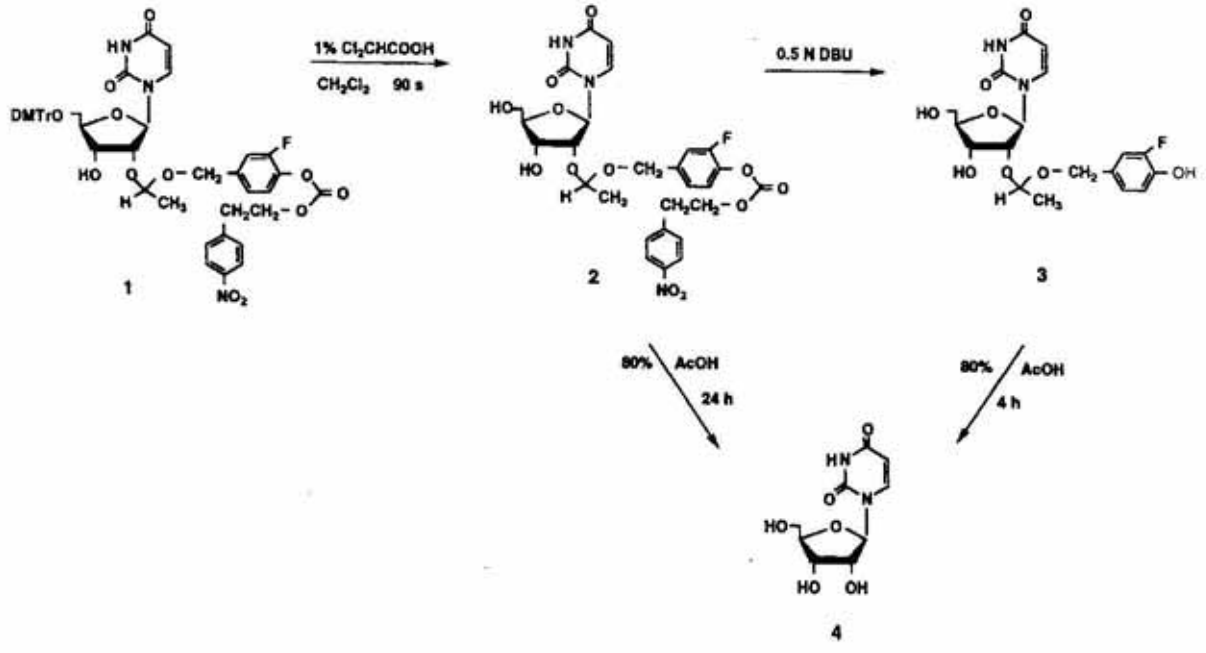
R	$t_{1/2}$ (min)
	104
	305
	272
	288
	1 300
	1 355



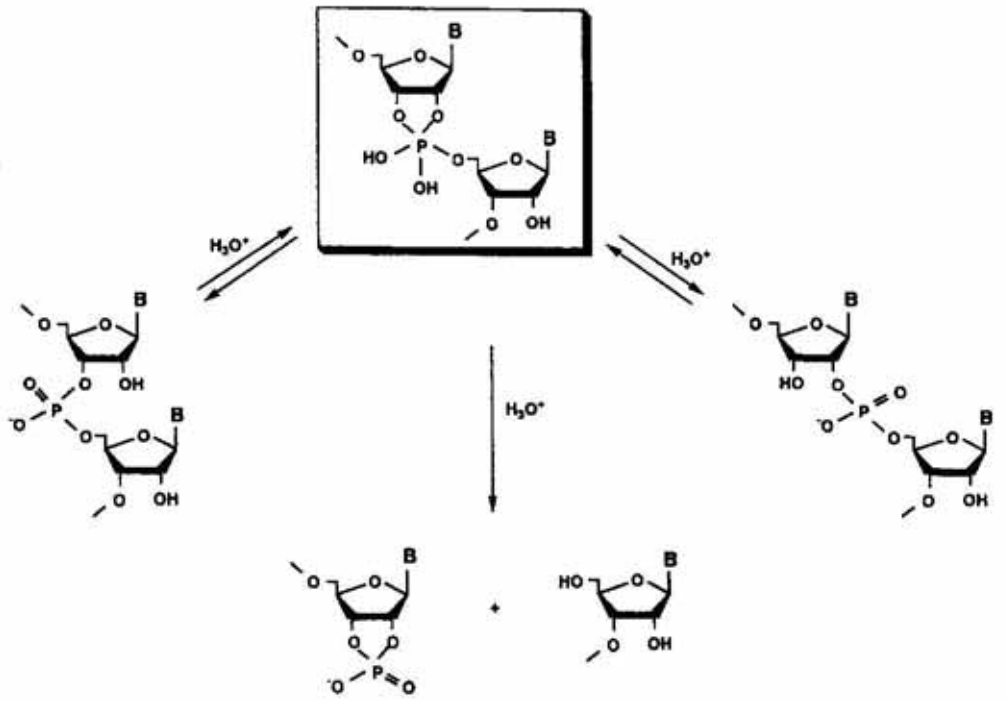
Scheme 3

In order to demonstrate the anticipated reactivity of the new protecting group, 5'-*O*-dimethoxytrityl-2'-*O*-1-{4-[2-(4-nitrophenyl)ethoxycarbonyloxy]-3-fluorobenzyloxy}ethyl-uridine (**1**) was treated with 1% of dichloro-acetic acid for 90 s removing selectively the dimethoxytrityl group to **2** without harming the relatively stable acetal function. Cleavage of the acetal group in **2** by treatment with 80% AcOH for 24 h afforded the quantitative conversion into uridine (**4**). On the other hand, removal first of the 2-[4-(nitrophenyl)ethoxycarbonyl group in **2** by 0.5 N DBU forming 2'-*O*-1-(3-fluoro-4-hydroxybenzyl-oxy)ethyluridine (**3**) and then treatment with 80% AcOH led to uridine (**4**) within 4 h (Scheme 4).

A series of homo- and mixed oligoribonucleotides have then been synthesized by the machine-aided approach to give excellent yields on applying a condensation time in each step of 20 min. Cleavage of the β-eliminating Npe, Npeoc and cyanoethyl groups by DBU followed by detritylation and then removal of the acetal protected oligomer from the solid-support material by NH_3 gave somewhat broad and structured peaks in HPLC due to the chirality of the 2'-*O*-acetal blocking group and therefore showed the presence of a diastereoisomeric mixture. During the final cleavage studies by acid treatment of the unprotected oligoribonucleotides it was noticed that the formerly applied 80% AcOH is much to strong for



Scheme 4

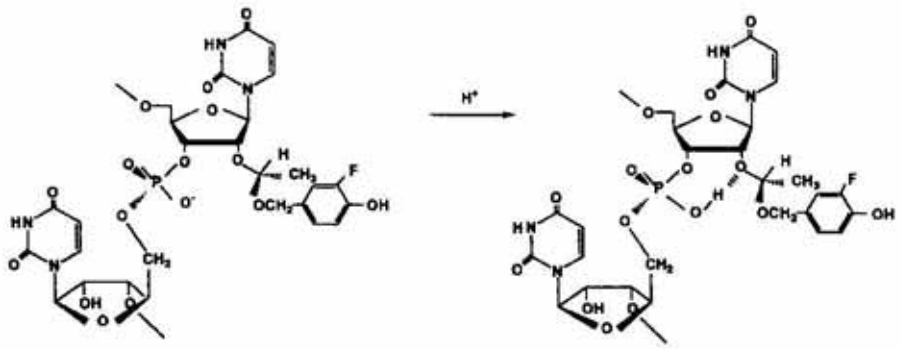


Scheme 5

the acetal deblocking since HPLC revealed impurities increasing with time. This was due to the fact that the internucleotidic linkage in the ribo series is prone at a pH < 3 to various side-reactions leading to a partial 2′-5′ isomerisation and scission of the phosphodiester bond *via* a pentavalent phosphorus intermediate [15] (Scheme 5).

If the AcOH concentration, however, was reduced drastically to 0.5% a very clean acetal deprotection took place at room temperature within 18 h indicating that most likely an intramolecular acid catalysis affords the anticipated favourable reaction conditions (Scheme 6).

Further systematic studies are under investigation to measure the suspected sequence dependence of the 2′-*O*-acetal cleavage in oligoribonucleotide chains.



Scheme 6

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