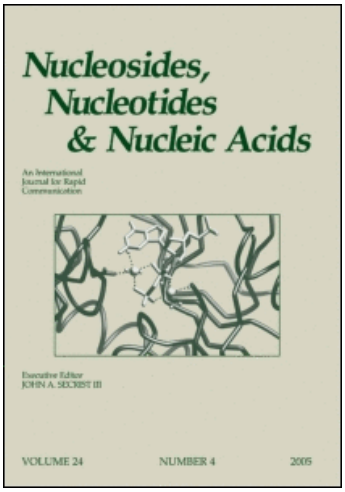

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EVALUATION OF THE USE OF THE t-BUTYLDIMETHYLSILYL GROUP FOR 2'-PROTECTION IN RNA-SYNTHESIS VIA THE H-PHOSPHONATE APPROACH.

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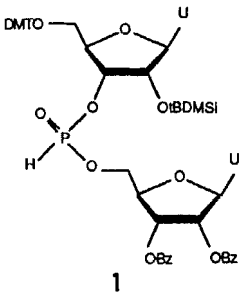
Abstract: *Two model compounds, **1** and **2**, have been studied to test the stability of the t-butyldimethylsilyl group towards anhydrous acid, aqueous ammonia and tetrabutylammonium fluoride in THF. Results of relevance to cleavage and migration of phosphodiesters during deprotection of synthetic RNA will be presented.*

We have recently developed a method for the synthesis of oligoribonucleotides[1] based on the use of 5'-O-(4,4'-dimethoxytrityl) 2'-O-t-butyldimethylsilyl-protected ribonucleoside 3'-H-phosphonates. This method is presently being optimized in order to achieve synthesis of longer (t-RNA size) oligomers in high yield. One part of this optimization is of course to get a consistently high coupling efficiency during the assembly of the RNA-chain by e.g. technical changes and/or the use of new condensing reagents. Another part is to optimize the deprotection conditions and/or the choice of protecting groups.

It has been shown, particularly by Ogilvie et. al.[2], that the tert.-butyldimethylsilyl (t-BDMSi) group can be successfully used for RNA-synthesis *via* the amidite approach. Also other protection[3] than the t-BDMSi group has been used in RNA-synthesis *via* H-phosphonates, but we still consider the t-BDMSi group as one of the most promising.

In order to optimize the deprotection scheme in our approach to RNA-synthesis, we felt that a quantitative study on the stability and deprotection of the t-BDMSi group was needed. The crucial points to investigate were: (i) whether the silyl groups are stable towards the repeated acid treatments used for removal of the temporary dimethoxytrityl group; (ii) if the t-BDMSi groups are lost during deprotection of the bases and if so how much subsequent cleavage of the RNA-chain will occur; (iii) whether any isomerisation of the phosphodiester linkage can be detected when the t-BDMSi groups are removed with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF).



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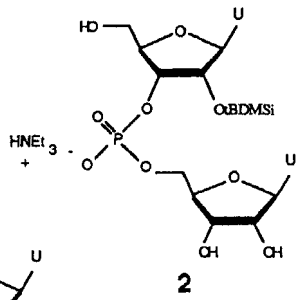

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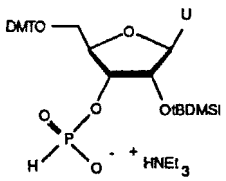

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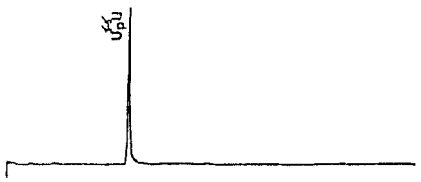

FIG. 1. H.p.l.c. chromatogram (Waters Resolve RP18, 20 min. linear gradient, 0.05M KH_2PO_4 – 25% MeCN (aq)) of product obtained after keeping **2** with TBAF·3H_2O in THF for 24h.

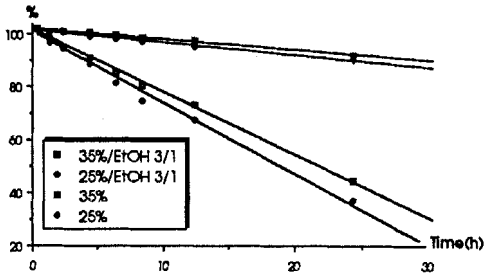

FIG. 2. Graph showing decrease of **2** (due to loss of the tBDMSi group) with time in various ammonia (aq) solutions at 55 °C.

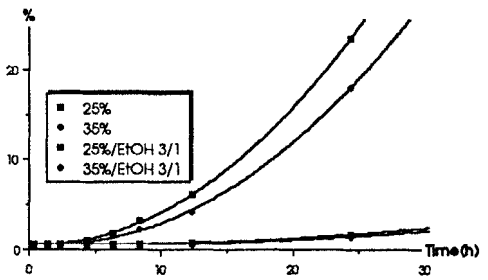

FIG. 3. Graph showing cleavage of the internucleotidic phosphodiester linkage as a function of time during various ammonia (aq) treatments, at 55 °C, of **2**.

FIG. 4. H.p.l.c. chromatogram (same system as in fig. 1) showing separation of all reference compounds.

Results.

It is of utmost importance that the fully protected building blocks e.g. **3** are not contaminated with the unwanted isomer i.e. the 3'-silyl 2'-H-phosphonate. The PCl_3/imidazole/Et_3N system[1] for phosphonylation of 5'-O-DMT-3'-O-t-BDMSi-U sometimes produced traces (< 0.5 %) of the unwanted isomer[4]. However, during the chromatographic purification[5], routinely used by us, such traces of contaminant are easily removed.

Treatment of the H-phosphonate diester **1**[6] with 2.5 % dichloroacetic acid in dichloromethane was followed by both ^{31}P n.m.r. and t.l.c.[7] It was found that, after the fast detritylation, no further changes occurred within 16 h (equal to 480 detritylation times in machine synthesis). The detritylated product was also isolated by preparative t.l.c. and its ^{1}H n.m.r. spectrum revealed that the silyl group was intact.

The t-BDMSi group was completely removed within 4h when **2**[8] was treated with tetrabutylammonium fluoride (TBAF·$3H_2O$ (Aldrich)) in THF. Furthermore, the fluoride treatment gave only the correct product and no isomerization of the phosphodiester linkage could be detected by h.p.l.c. (fig. 1) even after 24 h treatment with TBAF.

When the treatment of phosphodiester **2** with ammonia was followed by h.p.l.c. analysis it was found that the t-BDMSi group is not completely stable (fig. 2). When 25% aqueous ammonia was used at 55 °C, 73% of the silyl group remained intact after 8 h but only 35% after 24 h. This loss was also accompanied by cleavage of the phosphodiester linkage (fig. 3) (2.7% after 8 h and 23% after 24 h). If, however, a mixture of conc. ammonia and ethanol (3:1, v/v) is used a much milder treatment is obtained. The mildest conditions at 55 °C, 35% aqueous ammonia:EtOH 3:1, removed ca 10% of the silyl group and gave 0.9% cleavage after 24 h. However, no cleavage could be detected after 12h and only 4% of the silyl group was lost during this time. The h.p.l.c. system used for analysis during the ammonia treatments was the same as for the TBAF treatments (fig. 1). As can be seen in fig. 4 all relevant compounds separate well using this system.

Discussion and conclusions.

The t-BDMSi group is completely stable towards the detritylation conditions used and it can be easily removed by treatment with TBAF·$3H_2O$ in THF. Most important is that no migration of the phosphodiester linkage occurs, thus ensuring that the synthesized RNA-fragment will contain the desired 3'-5' linkage only.

However, the use of conventional conditions for base-deprotection will cause cleavage of phosphodiester linkages. If the proper conditions are used this should not be a problem for shorter oligomers, but for synthesis of longer fragments (t-RNA size) it is indeed serious if cleavage occurs since very little or no desired product may be obtained. This is to be expected if the most common base-protection (isobutyryl for G, benzoyl for A and C) is used, especially if the time needed for total deprotection of G is close to that for dG[9]. The solution to the problem of cleavage seems to be to use more labile protecting groups for the bases, as has recently been done in DNA-synthesis[9].

From the present study we conclude that it should be an advantage to use more labile base-protecting groups than those commonly employed[1,2], when applying t-BDMSi-protection for the 2'-OH. This, in combination with H-phosphonate chemistry, should give a safe synthesis of RNA-fragments containing only the correct 3'-5' phosphodiester linkages The yield of the desired oligomer should then depend mainly on the efficiency of the condensation step.

Acknowledgements.

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2. Usman N., Ogilvie K.K., Jiang M.-Y., Cedergren R.J., J. Am. Chem. Soc. 109,(1987),7845.
3. Tanaka T., Tamasukuri S., Ikehara M., Nucl. Acids Res., 15,(1987),7235.
4. T.l.c., silica gel , isopropanol/ammonia/water (8.5/0.5/1) ; R_f , 3'-H-phosphonate **3** = 0.8 ; R_f , 2'-H-phosphonate = 0.55.
5. Short column chromatography on silica gel, stepwise gradient from $CHCl_3/Et_3N$ (99.5/0.5) to $CHCl_3$/MeOH/Et_3N (90/9.5/0.5)
6. Synthesized by coupling of **3** with 2',3'-di-O-benzoyluridine using a procedure similar to Jäger A., Charubala R. and Pfleiderer W. (Nucl. Acids Res. Symp. Ser. No18,(1987),197).
7. After detritylation: ^{31}P n.m.r. ; 8.6 ppm, $^1J_{PH}$ = 757 Hz, $^3J_{PH}$ = 8.1 Hz.; T.l.c. ; $CHCl_3$/MeOH (9/1), R_f = 0.37.
8. Obtained by oxidation (with I_2 in pyridine/H_2O (98/2)) of **1** which was subsequent deprotected i.e. the resultant phosphodiester was first debenzoylated with conc. aq. ammonia /EtOH (3/1) and then detritylated with 80% HOAc. A final purification using preparative HPLC was applied in order to obtain analytically pure **2** .
9. Schulhof J.C., Molko D., Teoule R., Nucl. Acids Res., 15,(1987),397.