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Removal of *t*-butyldimethylsilyl protection in RNA-synthesis. Triethylamine trihydrofluoride (TEA, 3HF) is a more reliable alternative to tetrabutylammonium fluoride (TBAF)

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The *t*-butyldimethylsilyl (TBDMS) group is the most widely used 2′-OH protection in synthesis of oligoribonucleotides. The TBDMS-group became much used particularly after it was combined with phosphoroamidite methodology (1) and was also the choice when H-phosphonate based RNA-synthesis was introduced (2). Removal of TBDMS-protection is most commonly done with 1 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF). These conditions has proven efficient, but it has been reported that deprotection may be incomplete for longer RNA's such as a *t*-RNA (3). It has also been noted by a number of workers in the field (own experience and personal communication) that this can be the case even for shorter oligomers and that the results are not always reproducible. Moisture as a likely cause of such problems has recently been addressed by us (4) and others (5).

Lyophilising the TBAF from dioxane (~1 mmol in 5 ml) overnight (which leaves 2–2.5 eq. water ~4–5% w/w by KF titration) before preparing the reagent solutions has given us consistent results for oligomers around the size of 20-mers (standard conditions being 1 M TBAF in THF overnight). With all-2′-O-TBDMS-$(Up)_{20}U$ a small quantity of TBDMS-containing oligomer remains after 2 h but most of it is deprotected (Fig. 1a) and seems intact after 24 h (chromatogram not shown but is distinguishable from that in Fig. 1a only by the absence of the last eluting peak). With 3 additional equivalents of water present (i. e. about 5.5 eq. in total ~11% w/w) the deprotection is only about half-complete even after 24 h (Fig. 1b). In a recent report on desilylation of different dimers and an octamer containing 2′-O-TBDMS groups it was concluded that for efficient desilylation the TBAF solution should not contain more than 5% water (5). Drying of the TBAF solution with molecular sieves was recommended (our way of making sure that the reagent does not contain too much water is simply another alternative). The qualitative results of our study on deprotection of 21-mers with TBAF are in good agreement with their findings. Thus, after finding out about the work by Hogrefe *et al.* we thought that together with their results the picture is complete enough not to pursue this part of the study further, particularly having our results with TEA, 3HF in mind (see below).

Neat triethylamine trihydrofluoride was reported to be a more efficient desilylating agent than TBAF and successful in deprotecting a synthetic t-RNA (3). When we used TEA, 3HF directly from the bottle, our all-2′-O-TBDMS-$(UP)_{20}U$ was deprotected faster than with the standard TBAF solution. TEA, 3HF removes the silyl groups within 1h (Fig. 1c). Importantly the rate of desilylation with this reagent is also quite insensitive to the presence of moisture. A reagent with 1 M higher water concentration (from added extra water, 1.8% → total water content 2.2% w/w) still caused complete deprotection after 1 h (not shown) and this was true even with 3 M higher water content (total water content ~5.8% w/w, Fig. 1d). The TEA, 3HF reagent also does not seem to take up moisture particularly fast since bottles that were opened and reopened over more than a month only contained about 0.3–0.35 M water (~0.6–0.7% w/w, typical water content when newly opened was around 0.4% w/w). Deprotection when using TEA, 3HF is clearly not particularly sensitive to moisture and this should give a high

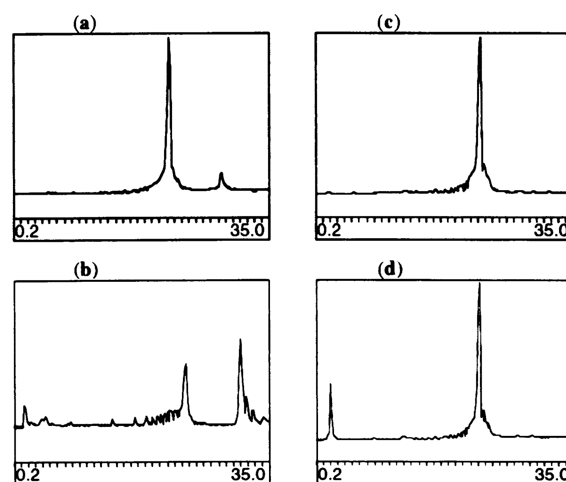

Figure 1. RP-HPLC analysis (Supelcosil LC-18, 3 µm, 4.6×150 mm, 1 ml/min and a linear gradient of 0–25% MeCN in 0.1 M triethylammonium acetate buffer (TEAA, pH ~6.5) during 60 min) of crude mixtures from treatment of all-2′-O-TBDMS-$(Up)_{20}U$ with; **(a)** 1 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) for 2 h; **(b)** 1 M TBAF in THF containing an additional 3 M water for 24 h; **(c)** neat triethylamine trihydrofluoride (TEA, 3HF) for 1 h; **(d)** neat TEA, 3HF containing an additional 3 M water for 1 h.

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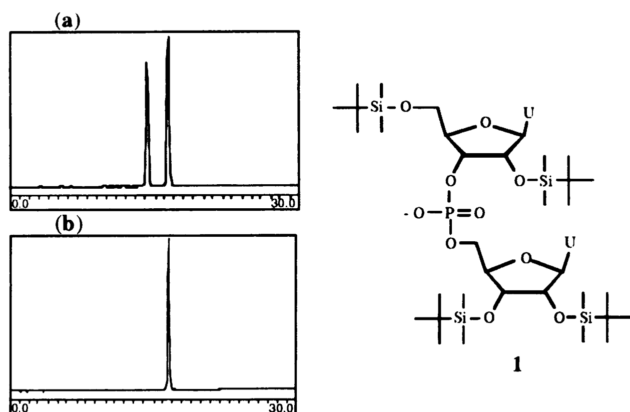

Figure 2. RP-HPLC analysis (as in Fig. 1 but with a gradient of 0−7.5% MeCN in 0.1 M TEAA (aq, pH ~7.4) during 24 min) of; (**a**) A mixture of 2′,5′-UpU (R_t = 14.2 min) and 3′,5′-UpU (R_t = 16.4 min); (**b**) The product after treatment of 1 with neat TEA, 3HF for 48 h.

reliability. The use of this reagent also offers a simpler workup procedure and faster deprotection. Longer oligomers may require longer deprotection time than for the 21-mers in this study and was also reported to be between 2 and 4 h for a 43-mer (3). We have turned to using this reagent rather than TBAF and to make certain that deprotection will be complete we are using longer deprotection times than should be necessary (4−8 h and often overnight for convenience) since this does not seem to cause any damage. Thus, $(Up)_{20}U$ as from Fig. 1c remains unchanged when kept in the reagent overnight (data not shown, but see also dimer below and ref. 3).

With TBAF/THF no significant migration of phosphodiester linkages takes place within 24 h (6) and a similar result for TEA, 3HF is likely. On the other hand, migration will always occur under neutral or acidic conditions, the question is only the extent of it. Since only the slightest amount of migration can be a serious problem we wished to make certain if it is neglectable even upon prolonged treatment with TEA, 3HF. We decided for a model study on 2′,5′-di-O-*t*-butyldimethylsilyluridine 3′-(2′,3′-di-O-*t*-butyldimethylsilyluridine 5′-phosphate) (**1**, produced from the corresponding H-phosphonate (8) with I_2 in aq. pyridine). **1** was kept in TEA, 3HF for up to 48 h, about an order of magnitude longer than required for deprotection of 20−40 mers and 3 times longer than the time used for a *t*-RNA (3). We can, however, not detect any 2′,5′-UpU when analysing the product from 48 h treatment of 1 with TEA, 3HF (Fig. 2).

The all-2′-O-TBDMS-$(Up)_{20}U$'s used above were made by automated solid support syntheses as reported (7) and cleaved from the support with 32% NH_3 (aq)-EtOH (3:1) for 14−16 h at room temperature. The oligomers (~0.5 µmol) were divided into 3 to 4 portions that where each dissolved in 1.0 M TBAF in THF (250 µl) or neat TEA, 3HF (100 µl), containing varying amounts of water (as stated above). Aliquots (60 µl of TBAF solution and 25 µl TEA, 3HF) were withdrawn at different times. The samples from TBAF treatment were passed through a cation exchange resin (SP-Sephadex G-25, 7.5×90 mm), lyophilised, redissolved in water, subsequently desalted using a Pharmacia NAP-25 column, lyophilised again and redissolved in 120 µl water of which 100 µl was injected when doing the HPLC analysis. The aliquots from TEA, 3HF treatment were diluted with 100 µl of water and analysed (injecting 100 out of 120 µl) by HPLC.

For analysis of migration was about 1 mg of 2′,5′-di-)-*t*-butyldimethylsilyluridine 3′-(2′,3′-di-O-*t*-butyldimethylsilyluridine 5′-phosphate) **1** dissolved in 300 µl TEA, 3HF. Aliquots of 20 µl were withdrawn at different times, diluted with 60 µl of water and extracted 5 times with 100 µl of ethyl acetate. The aqueous phase was lyophilised for 1 h, diluted with 100 µl of water and analysed by HPLC (injecting 90 µl). The reference solution containing a mixture of uridine 3′-(uridine 5′-phosphate) and uridine 2′-(uridine 5′-phosphate) was prepared by dissolving the commercially available dimers in TEA, 3HF. These solutions (also done separately for each dimer) were treated and analysed as for **1**.

Using 1 M TBAF in THF is an established method for removal of TBDMS-groups in synthesis of RNA-fragments. The reagent does however have the disadvantage of being quite sensitive to moisture. The water content can be kept down by lyophilising the reagent before use (or drying the solution with molecular sieves (5)) but the TBAF-reagent is still not as efficient as TEA, 3HF and also requires more tedious workup (as well as pre-preparation). Our results further confirms the finding of Gasparutto *et. al.* (3), that this reagent is more efficient than TBAF for TBDMS-deprotection of oligoribonucleotides, and we feel that the use of this reagent has not yet spread as much as it ought to. We have also found that the TEA, 3HF reagent has the additional advantage of being quite insensitive to moisture which should ensure a high reliability when using the reagent straight from the bottle. In addition we conclude that the reagent is quite safe with respect to migration of phosphodiester linkages since this event can not be detected in the product, 3′,5′-UpU, even after 48 h treatment of **1** with TEA, 3HF.

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