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Use of Silyl Ethers as Fluoride Scavengers in RNA Synthesis

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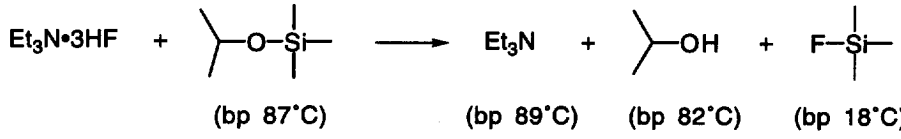
Abstract: Use of a fluoride ion scavenger significantly simplifies isolation of synthetic RNA.

Removal of the fluoride reagents used in desilylation of RNA prepared using the 2'-*O*-TBDMS protecting group is a critical step in isolation of synthetic RNA, and it can be especially troublesome as the scale of the synthesis is increased. We have developed a universal allyl linker for solid phase RNA synthesis that allows desilylation to be carried out while the RNA is attached to the support, so that the excess reagent is simply washed away.[1,2] Cleavage of the RNA from this linker relies on a Pd(0) mediated reaction. This cleavage works well, and we have found only trace amounts of Pd in the molecules we have prepared using the linker. Nevertheless, to avoid any possibility of Pd contamination, we have developed an alternative procedure that solves the fluoride problem by destroying the excess fluoride reagent once cleavage of the 2'-*O*-TBDMS groups is completed. In principle, any silyl ether or siloxane should function as such a fluoride scavenger. In conjunction with triethylamine tris(HF), and a volatile scavenger like 2-propyl trimethylsilyl ether,[3,4] volatile products are obtained that then can be removed on a rotary evaporator or speedvac.



We prefer to isolate synthetic RNA with the 5'-*O*-DMT group in place, and therefore use *N*-methylpyrrolidinone (NMP) with the TEA•3HF in the desilylation reaction.[5] The boiling point of NMP is too high for isolation of the RNA by concentration to dryness, but destruction of the excess TEA•3HF makes it possible to precipitate the RNA using diethyl ether at room temperature. The isolation procedure reported for the TEA•3HF/NMP reaction involves addition of 3 M NaOAc and precipitation with n-butanol cooled to -70°C, followed by an ethanol wash.[5] This procedure is somewhat inconvenient, and the ethanol wash to remove NaOAc can dissolve RNA oligomers to some extent. Ether precipitation after reaction with trimethylsilyl ether requires no NaOAc, and therefore no ethanol wash. Overall, this ether precipitation allows even faster isolation than does evaporation (when no NMP is used).

General procedure for deprotection, isolation, and purification of synthetic RNA fragments, which was used for the three molecules listed in the table and figure below:

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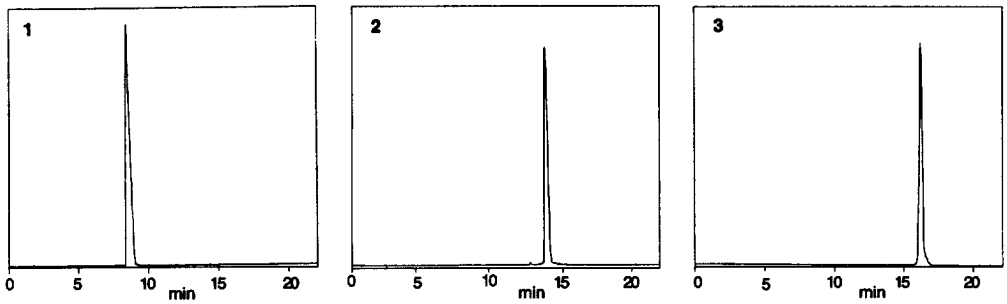
1. To support-bound RNA, add aqueous methylamine (40%; 10 mL), maintain at 65 °C for 10 min;
2. Filter, wash the residue with 50% aqueous ethanol;
3. Concentrate (SpeedVac) the filtrate to dryness;
4. Desilylate using NMP-TEA-TEA•3HF (6:3:4; 5 mL), maintain at 65 °C for 1.5 h;
5. Destroy fluoride reagent by addition of isopropyl trimethylsilyl ether (10 mL), shake gently for 10 min;
6. Precipitate the crude RNA by addition of diethyl ether (20 mL);
7. Isolate the crude RNA by centrifugation, wash with diethyl ether (2 x 10 mL);
8. Chromatograph, Nova-Pak C18 (19 x 300 mm) reversed phase column using a gradient of 2% to 40% acetonitrile:0.1 M triethylammonium acetate in 60 min at 10 mL/min, lyophilize appropriate fractions;
9. Detritylate using dilute acetic acid (5 mL, 0.1 to 0.5 M, pH 4) for 20 min, neutralize with solid ammonium bicarbonate;
10. Chromatograph on the above reversed phase column using a gradient of 2% to 10% acetonitrile:0.1 M ammonium or triethylammonium bicarbonate in 60 min at 10 mL/min, lyophilize appropriate fractions;
11. Convert the pure RNA fragment to the sodium form by ion exchange with Bio-Rad AG 50W-X2 (1x5 cm column).

Molecule (5'→ 3')	Scale (μmol)	Yield (OD_{260}, μmol)
1; CUA UUA UG	50	1480, 18
2; CUG GUC UG*A UGA GGC C; G* is [7,NH_2-$^{15}N_2$, 2-^{13}C]-G	30	720, 5
3; AAA GAG AGA AGU GAA CCA GAG AAA CAC ACG CG	20	520, 2



HPLC profiles of molecules **1**, **2**, and **3**, after isolation and purification as described above, on a DIONEX PA-100 (4x250 mm) column using a gradient of 0.02 M to 0.12 M $LiClO_4$ containing 10% acetonitrile in 20 min at 1.5 mL/min.

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3. Patel, K. M.; Baltisberger, R. J.; Virgil, I.; Woolsey, N. F. *J. Org. Chem* **1982,** *47*, 4250-4254.
4. A mixture of 2-propanol (77 mL, 1 mol), 1,1,1,3,3,3-hexamethyldisilazane (116 mL, 0.55 mol) and chlorotrimethylsilane (1 mL) was heated under reflux until evolution of ammonia ceased (*ca.* 4 h). Distillation of the reaction mixture gave 2-propyl trimethylsilyl ether as a colorless liquid (bp 87-89°C, 114.0 g, yield: 86%).
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