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1, 1, 3, 3-Tetraisopropyl-3-(2-(Triphenylmethoxy)ethoxy)disiloxane-1-yl Group, A Potential 5'-O-Protecting Group for Solid-Phase RNA Synthesis

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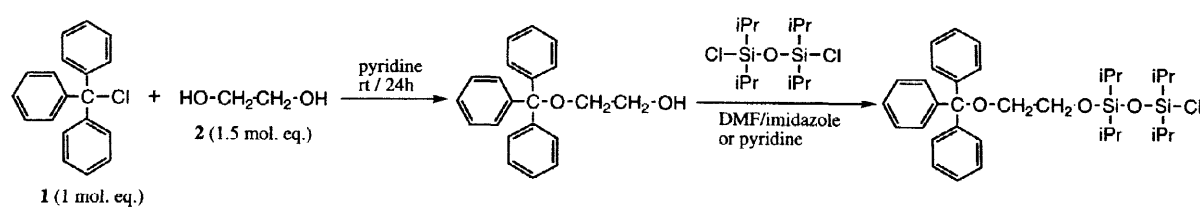
Abstract: 1, 1, 3, 3-Tetraisopropyl-3-(2-(triphenylmethoxy)ethoxy)disiloxane-1-yl (TES) group was designed for solid-phase RNA synthesis as a 5'-O-protecting group, which can be combined with the tetrahydropyranyl group, a 2'-hydroxyl protecting group. The TES group can be selectively introduced to the 5'-hydroxy group of nucleosides and can be easily removed by 0.1M tetrabutylammonium fluoride or 0.01M hydrochloric acid.

The protecting group strategy for the chemical synthesis of RNA oligomers is restricted by the 2'-hydroxyl groups of the ribose moieties. The *t*-butyldimethylsilyl (TBDMS) group is currently the most popular, commercially available protecting group for the 2'-hydroxyl protection in the phosphoramidite RNA-synthesis[1-3]. In spite of its successful use in RNA synthesis, the TBDMS-amidite system still has some problems: lower coupling efficiency compared with DNA synthesis using deoxyribonucleoside phosphoramidites, the disadvantageous cleavage of the 2'-O-TBDMS group under alkaline conditions, and a tedious deprotection procedure for removal of the TBDMS group[4-6].

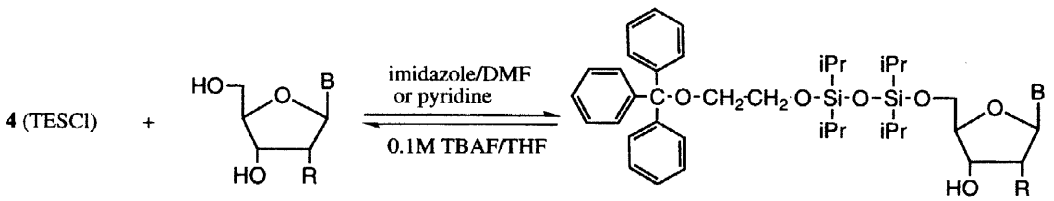
The acid-labile tetrahydropyranyl (THP) group is another option for protecting the 2'-OH group of nucleosides. It is easily prepared via 3', 5'-O-(tetraisopropyldisiloxane-1,3-diyl) derivatives and is removed under mild acidic conditions (0.01M hydrochloric acid)[7, 8]. However, this attractive protecting group is difficult to combine with the acid-labile dimethoxytrityl group (DMTr), which commonly protects 5'-hydroxyls. Even so, RNA synthesis up to 20mers using 2'-O-THP-5'-O-DMTr derivatives has been reported[9-13].

To use the THP group for RNA synthesis, we have designed the 1, 1, 3, 3-tetraisopropyl-3-(2-(triphenylmethoxy)ethoxy)disiloxane-1-yl (TES) group for a new 5'-hydroxyl protecting group. The TES group can be selectively introduced to the 5'-OH of nucleosides and is removed by the treatment of fluoride ion, without cleaving the THP group. In addition, the trityl moiety enables determination of the coupling yields during oligomer synthesis by the coloration of the deblocking solution under acidic conditions.



Scheme 1

PII: S0040-4039(98)00440-7



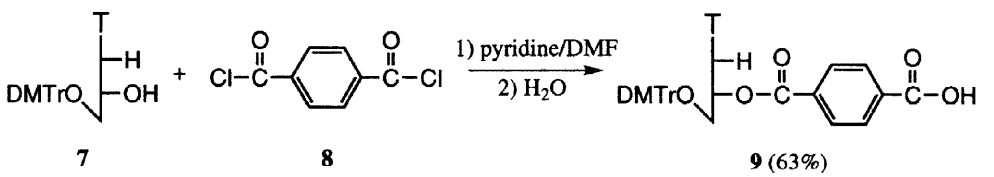
product	conditions	yield (%)
6a	DMF/imidazole	80
6a	pyridine	90
6b	DMF/imidazole	65

Scheme 2

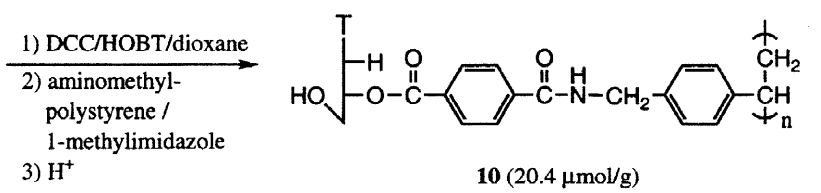
1-Chloro-1,1,3,3-tetraisopropyl-3-(2-(triphenylmethoxy)ethoxy)disiloxane (TESCl)(**4**) was prepared as shown in Scheme 1[14]. The resulting TESCl solution was used *in situ* to synthesize 5'-O-TES-thymidine (**6a**) and 5'-O-TES-3'-O-THP-uridine (**6b**) in 90% and 65% yields, respectively (Scheme 2)[15]. Cleavage of the TES group from **6a** and **6b** was achieved with 1) 0.1M tetrabutylammonium fluoride (TBAF) in THF at room temperature for 1 min, or 2) 0.01M hydrochloric acid at room temperature for 24 h. The TES group was stable in NH_3/MeOH solution at room temperature for 12 h.

Compounds **6a** and **6b** were phosphitylated with bis-(N,N-diisopropylamino)-2-cyanoethoxyphosphine using reported protocols[16]. Attempts to synthesize oligo-thymidilic acids using highly cross-linked polystyrene[17], however, were unsuccessful. Both the 2-cyanoethyl group on the phosphates and the succinate linkage of the 3'-terminal nucleoside connecting the aminomethylpolystyrene were cleaved during the deprotection step (0.1M TBAF/THF) of the 5'-TES group in the synthesis.

Thus, instead of the succinyl linker, we constructed a polymer-bound thymidine (**10**) using a terephthaloyl linker (Scheme 3), which should be stable under the conditions of TBAF/THF. No cleavage of the polymer-bound thymidine was observed upon treatment with a 0.1M TBAF/THF solution for 3 min. Using this polymer support, we have demonstrated the TpTpT and UpT synthesis with 3 min coupling and 3 min 5'-O-deprotection[18], though the 2-cyanoethyl cleavage in the syntheses remained. The average coupling yields of TpTpT and UpT syntheses were 97% and 95%, respectively. Each coupling yield was determined by the coloration (450 nm) of a perchloric acid-acetonitrile (3:1 v/v) solution added to the residue that remained after the evaporation of the TES-deblocking solution. After the synthesis, the resin bound oligomer was



7 **8** **9** (63%)



10 (20.4 μmol/g)

Scheme 3

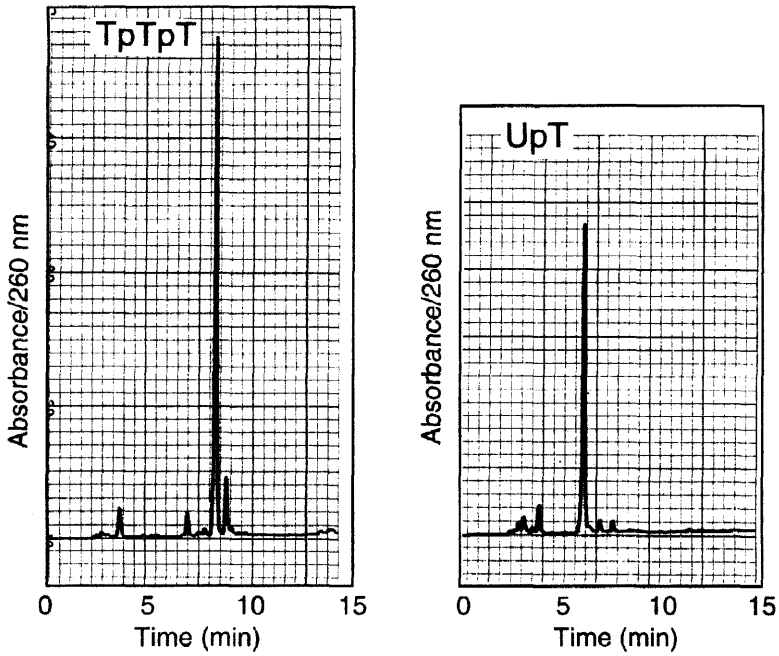


Fig. 1 HPLC profiles of crude TpTpT and UpT with a C-18 column (M&S pack C18, 4.6 mm ID x 15 cm). Eltution was performed with a liniear gradient of 5%-25% CH_3CN for 15 min in 0.1M triethylammonium acetate (pH 7.0).

heated with conc. NH_4OH for 6 h at 55℃[19]. The solution was then filtered and evaporated. In the case of UpT synthesis, the residue was treated with 0.01M hydrochloric acid at room temperature for 24 h to remove the THP group. The crude oligomer after deprotection was analyzed by C18-HPLC (Fig. 1). The main product can be identified by comparing its retention time with that of the authentic sample.

In conclusion, the combination of 5'-O-TES and 2'-O-THP groups for the solid phase RNA synthesis is proposed here; the stepwise coupling efficiency is over 95% within a coupling time of 3-5 min, the 5'-O-TES group is easily removed by a 0.1M TBAF/THF solution, and the release of the group can be spectrophotometrically monitored in an acidic conditions. The TES-THP protecting group combination has other possible merits. First, large scale RNA synthesis may be done by triphosphate liquid-phase synthesis. Second, RNA oligomers containing modified bases that are unstable under basic conditions may be synthesized by using the combination of 5'-O-TES, 2'-O-THP, acid-labile protecting group (such as an MMTr group) for exocyclic amines on the bases, and an acid-labile linkage to the resin[20].

Despite the specificity in deblocking, the 2-cyanoethyl group on the phosphates is susceptible to cleavage during the deprotection step of the 5'-O-TES group. The synthesis of pentaribonucleotides were unsuccessful using the TES-amidite system. Further experiments are planned to improve the protecting group of phosphate linkages instead of the 2-cyanoethyl group. The TES group may be more effective in an H-phosphonate method[21] than the phosphoramidite method.

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14. Compound **2** (0.36 ml; 6.5 mmol) was reacted with **1** (1.4 g; 5 mmol) in 20 ml of dry pyridine at room temperature for 24 h. After the purification by the silica gel column chromatography **3** (1.35 g; 90%) was crystalized from the dichloromethane - hexane solution. ^{1}H NMR (25℃, $CDCl_3$ - D_2O) δ = 7.46-7.28 (m, 18H, Ph-H), 3.76 (t, 2H, CH_2), 3.27 (t, 2H, TrOC<u>H</u>$_2$)

    Compound **3** (335 mg; 1.1 mmol) and imidazole (82 mg; 1.2 mmol) were added into a DMF (5 ml) or pyridine (8.4 ml) solution of dichloro 1,1,3,3-tetraisopropyldisilane (0.35 ml; 1.1 mmol) and stirred for 2 h at room temperature. The solution was directly used for the 5'-O-TES nucleosides.
15. ^{1}H NMR (25℃, $CDCl_3$) of **6a**; δ = 8.08-7.25 (m, 17H, NH, Ph-H, H-6), 6.24 (t, 1H, H-1'), 4.25 (m, 1H, H-3'), 4.01-3.91 (m, 3H, H-4', 5', 5"), 3.92 (t, 2H, CH_2-O-Si), 3.21 (t, 2H, TrOC<u>H</u>$_2$), 2.29 (m, 1H, H-2'), 2.06 (m, 1H,H-2"0, 1.88 (s, 3H, 5-CH_3), 1.08 (m, 28H isopropyl)

    ^{1}H NMR (25℃, $CDCl_3$) of **6b**; δ = 8.12-7.22 (m, 17H, NH, H-6, Ph-H), 5.95 (d, 1H, H-1'), 5.63 (m, 1H, H-5), 4.72 (m, 4H, THP-H), 4.24-3.89 (m, 7H, H-2', 4', 5', 5", CH_2-OSi, THP-H), 3.49 (m, 1H, THP-H), 3.19 (m, 1H, H-3'), 3.17 (m, 2H, TrOC<u>H</u>$_2$), 1.84 (m, 2H, THP-H), 1.53 (m, 4H, THP-H), 1.08 (m, 28H, isopropyl)
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18. The solid-phase oligonucleotide synthesis was performed using a DNA synthesizer (PE Applied Biosystems, 391). The deprotection process of the TES group during the synthesis is carried out by the following cycle (step 1 - step 4) twice; step 1; CH_3CN to column for 30 sec, step 2; reverse flush with argon for 30 sec, step 3; 0.1M TBAF/THF to the column for 30 sec, step 4; wait for 90 sec.
19. The polymer support linkage can not be cleaved completely with conc. NH_4OH for 1 h at room temperature.
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