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Triphenylmethanesulfenyl Group. A New Protecting Group for the Uracil Residue in Oligoribonucleotide Synthesis

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The selective protection of the N^3-imido group of uridine with triphenymethanesulfenyl group is described. This group could be introduced selectively onto the N^3-position of the uracil moiety of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine in a good yield and removed very easily by treatment with aqueous iodine.

It has been clearly shown by Reese that the protection of the uracil residue is necessary to prevent the side reactions in the chemical synthesis of oligoribonucleotides containing uridine units.[1] Recently, some workers have explored new protecting groups for the uracil residue in oligoribonucleotide synthesis.[2] These protecting groups were introduced onto the uracil residue via 2',3',5'-protected uridine intermediates. The direct protection of the uracil residue should be desired. However, only a few examples of the direct protection of the uracil residue can be found in the literature.[3] Further, most of new protecting groups are unstable under alkaline conditions.

In this paper, we wish to report the triphenylmethanesulfenyl (TPMS) group as a new protecting group for the uracil residue which can be introduced selectively and removed very easily by treatment with aqueous iodine. Recently, Chattopadhyaya[2g] reported the preparation of N^3-arenesulfenylated uridines, aiming to use the arenesulfenyl groups as the protecting groups of the uracil moiety.

First we examined the introduction of the TPMS group to the uracil residue. 2',3',5'-Tri-O-acetyluridine (1.23 g, 3 mmol) was treated with triphenylmethanesulfenyl chloride (TPMSCl) (1.86 g, 6 mmol) in the presence of triethylamine (0.78 ml, 6 mmol) in dry CH_2Cl_2 (10 ml) at room temperature for 8 h. After workup and chromatography of the reaction mixture, N^3-triphenylmethanesulfenyluridine derivative (2)[4] was obtained in 81% (1.56 g) yield. Introduction of the TPMS group onto the uracil residue would be expected to suppress the side reactions. Consequently, we examined the stabilities of uridine derivative (2) under a variety of deprotective conditions that would be used in oligoribonucleotide synthesis. These results are summarized in Table 1. Thus, it becomes clear from deprotective conditions (A, B, C, D, E, and F) in Table 1 that the TPMS group can be used in conjunction with other sugar and phosphate protecting groups in our approach to the oligoribonucleotide synthesis. In particular, nucleoside derivative 2 was treated with 0.1 M I_2 in THF-collidine-H_2O (44:3:3, v/v) at room

temperature for 10 min to afford the corresponding uridine.

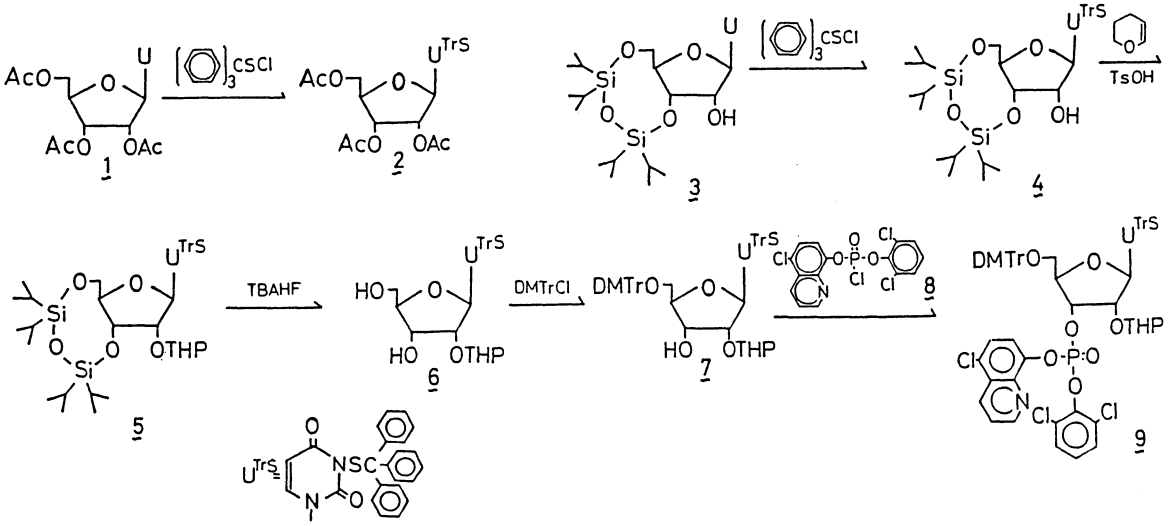
Next we found that the TPMS group can be introduced selectively onto the uracil residue of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine (3). Compound 3 (2.43 g, 5 mmol) was treated with TPMSCl (3.11 g, 10 mmol) in the presence of triethylamine (1.36 ml, 10 mmol) in CH_2Cl_2 at room temperature for 8 h. After the usual workup, silica gel chromatography using a mixture of CH_2Cl_2 and MeOH (98:2, v/v) as eluent afforded compound 4[5] (2.79 g, 75%). To a solution of N^3-triphenylmethanesulfenylated uridine(4) (3.04 g, 4.0 mmol) in dioxane (33 ml) were added p-toluenesulfonic acid monohydrate (800 mg, 4.28 mmol) and 2,3-dihydropyran (4.4 ml, 52 mmol). After being stirred for 2 h, the reaction mixture was neutralized with triethylamine, diluted with CH_2Cl_2 (80 ml) and washed with water. The CH_2Cl_2 solution was evaporated under reduced pressure and the residue was treated with 1 M tributylammonium hydrogen fluoride (TBAHF) at room temperature for 3 h to give, after workup and chromatography of the reaction mixture, 2'-substituted uridine 6[6] in 75% (1.86 g).[7]

Table 1. Stabilities of the uridine derivative under various conditions[a,b]

Compound	A	B	C	D	E	F	G
2	stable	stable	stable	stable	stable	stable	97%

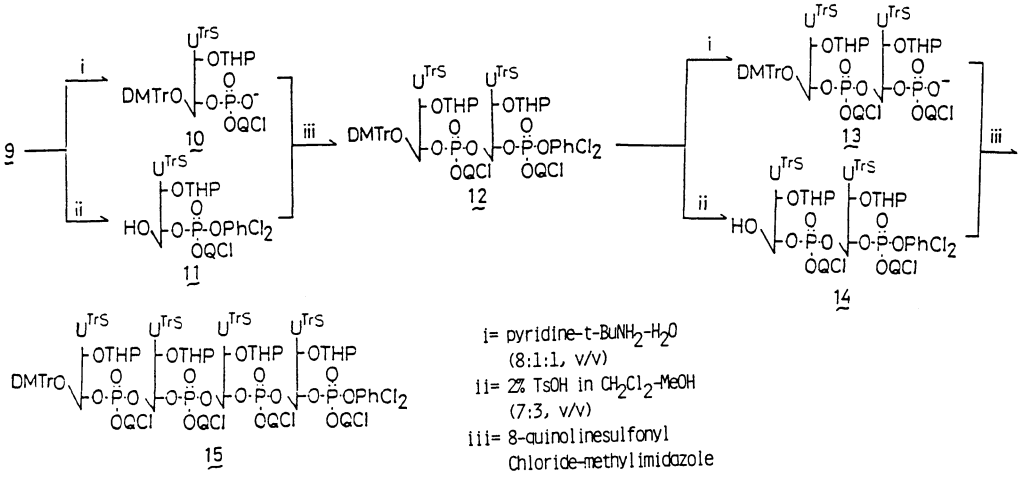
a) (A) 2% TsOH in CH_2Cl_2-MeOH (7:3, v/v), room temperature, 30 min; (B) 80% AcOH, room temperature, 2 h; (C) Et_3N-CH_3CN (1:1, v/v); room temperature, 1 d; (D) pyridine-t-$BuNH_2$-H_2O (8:1:1, v/v), 12 h; (E) 1 M TBAHF in THF, room temperature, 1 d; (F) concentrated NH_4OH-MeOH (9:1, v/v), room temperature, 3 h; (G) 0.1 M I_2 in THF-collidine-H_2O (44:3:3, v/v), room temperature, 10 min.

b) Aliquots of solution were taken after suitable interval time and analyzed by HPLC (Inertsil ODS).



To demonstrate the utility of the TPMS group, the tetramer, UUUUp was synthesized. Treatment of 6 with DMTrCl in dry pyridine gave the expected 5'-tritylated product 7 in a good yield. The fully protected mononucleotide unit (9)[8]

was obtained in 86% yield by treatment of the nucleoside derivative 7 with a phosphorylating agent, 2,6-dichlorophenyl 5-chloro-8-quinolyl phosphorochloridate (8) prepared simply from 2,6-dichlorophenyl phosphorodichloridate and 5-chloro-8-hydroxyquinoline in a one-flask reaction.[9] In this phosphorylation, no loss of the uracil protecting group was observed. According to our approach to the oligonucleotide synthesis,[9] the mononucleotide unit (9) (1.418 g, 1.12 mmol) was treated with pyridine-t-$BuNH_2$-H_2O (8:1:1, v/v) at room temperature for 3 h to give the corresponding phosphodiester (10) as triethylammoium salt which was used in the next coupling reaction without further purification. On the other hand, treatment of 9 (1.112 g, 0.88 mmol) with 2% TsOH in CH_2Cl_2-MeOH (7:3, v/v) at 0 °C for 10 min followed by washing with 5% $NaHCO_3$ solution gave the 5'-hydroxyl component (11) (0.718 g, 85%).[10] The condensation of 10 and 11 (0.718 g, 0.75 mmol) in the presence of 8-quinolinesulfonyl chloride (QsCl) (0.513 g, 2.25 mmol) and N-methylimidazole (MeIm) (357 ml, 4.5 mmol) in dry pyridine (4.5 ml) for 1.5 h. The fully protected dimer (12) was isolated in 89% (1.372 g) yield after separation by silica gel column chromatography. In a similar manner, the selective deprotection of the 2,6-dichlorophenyl and DMTr groups from 12 was performed. Thus, the dimer blocks (13 and 14) were obtained in good yields. A solution of both compounds 13 and 14 in dry pyridine was treated with QsCl and MeIm at room temperature for 1.5 h. The fully protected tetramer (15) was obtained by chromatography on a silica gel column in 85% yield.



Deprotection of the tetramer, UUUUp (10.2 mg, 5.0 umol) was performed as follows: 1) pyridine-t-$BuNH_2$-H_2O (8:1:1, v/v) at room temperature for 6 h to remove the 2,6-dichlorophenyl group; 2) zinc acetate in pyridine-H_2O (9:1, v/v) at room temperature for 2 days to remove the 5-chloro-8-quinolyl group; 3) 0.1 M I_2 in THF-collidine-H_2O (44:3:3, v/v) at room temperature for 2 h to remove the TPMS group; 4) 0.01 M HCl in dioxane-H_2O (1:1, v/v) at room temperature for 36 h to remove the THP and DMTr groups. The purification by TSKgel DEAE-2SW HPLC and reversed phase HPLC gave the pure tetramer UUUUp (5.2 OD). The tetramer was digested by spleen phosphodiesterase to give the corresponding Up.

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4) Mp 77-80 °C; UV λ max(MeOH) 263, 213 nm; ^{1}H-NMR ($CDCl_3$) δ 2.39 (t, 9H, CH_3), 4.47 (br s, 3H, H-4', H-5'), 5.32-5.48 (m, 2H, H-2', H-3'), 5.60 (d, 1H, $J_{5,6}$=8 Hz, H-5), 5.74 (d, 1H, $J_{1',2'}$=4 Hz, H-1'), 6.82-7.88 (m, 16H, H-6, ArH). Found: C,62.72;H,4.98;N, 4.11%. Calcd for $C_{34}H_{32}N_2O_9S.1/3\ CH_3OH$: C, 62.92, H, 5.12, N, 4.27%.

5) UV λ max(MeOH) 272, 216 nm; ^{1}H-NMR ($CDCl_3$) δ 1.05 (m, 28H, CH_3CSi), 3.80 (br s, 2H, H-5'), 3.95 (br s, 2H, H-3', H-4'), 4.25 (m, 2H, H-2', HO-2'), 5.10 (s, 1H, H-1'), 5.35 (d, 1H, $J_{5,6}$=8 Hz, H-5), 7.05-7.50 (m, 16H, H-6, ArH). Found: C, 65.45; H, 7.18; N, 3.54%. Calcd for $C_{40}H_{52}N_2O_7SSi_2.2/3CH_3C_6H_5$: C, 65.23; H, 7.03; N, 3.41%.

6) Mp 69-72 °C; UV λ max(MeOH) 271, 209 nm; ^{1}H-NMR ($CDCl_3$) δ 1.57 (m, 6H, C-methylene of THP), 3.25-4.35 (m, 9H, H-2', H-3', H-4', H-5', HO-3', HO-5', O-methylene of THP), 4.95 (m, 1H, acetal proton of THP), 5.40 (s, 1H, H-1'), 5.65 (d, 1H, $J_{5,6}$=8 Hz, H-5), 6.65-7.78 (m, 16H, H-6, ArH). Found: C, 64.01; H, 5.82; N, 4.44%. Calcd for $C_{33}H_{34}N_2O_7S$: C, 63.84; H, 5.69; N, 4.67%.

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8) UV λ max(MeOH) 276, 228 (sh), 209 nm; ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 6H, C-methylene of THP), 3.88 (s, 6H, CH_3O), 4.90 (m, 1H, acetal proton of THP), 5.60 (d, 1H, $J_{1',2'}$=4.1 Hz, H-1'), 5.78 (d, 1H, $J_{5,6}$=8 Hz, H-5), 6.70-7.72 (m, 35H, H-6, ArH), 8.65 (d, d, 1H, ArH), 8.95 (m, 1H, ArH). Found: C, 65,91; H, 4.95; N, 3.09%. Calcd for $C_{69}H_{59}N_3O_{12}SCl_3$: C, 65.74; H, 4.72; N, 3.33%.

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