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2′-(Trimethylsilyl)ethoxymethyl Protection of the 2′-Hydroxyl Group in Oligoribonucleotide Synthesis

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Abstract: 2′-*O*-[(Trimethylsilyl)ethoxymethyl]-5′-*O*-dimethoxytrityl uridine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (**1**) was synthesized from dimethoxytrityl uridine (**2**) in two steps. The amidite was then incorporated into a $(Up)_9U$ polymer. Removal of the (trimethylsilyl)ethoxymethyl (SEM) group was effected with $BF_3 \bullet OEt_2$ in 30 min.

In the synthesis of RNA it is critical that the 2′-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the same time, this group should also be readily removed when desired. To that end, the *t*-butyldimethylsilyl group has been efficacious.[1,2] However, long exposure times to TBAF are required to fully remove this blocking group from the 2′-hydroxyl. In addition, the bulky alkylsilyl group sterically hinders coupling, thereby requiring longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic ammonia.[2,3]

Thus, a new 2′-hydroxyl protecting group would be useful. The (trimethylsilyl)-ethoxymethyl ether (SEM) has appeal as a suitable substitute.[4] This protecting group is stable to base and all but the harshest acidic conditions. Therefore it is stable under all the conditions required for oligonucleotide synthesis. The SEM group can be readily introduced and the oxygen carbon bond, rather than an oxygen silicon bond, renders it unable to migrate. Finally, this protecting group can be readily removed with $BF_3 \bullet OEt_2$.

The SEM group was introduced using a modification of the Moffat procedure[5,6] (**Figure 1**). Treatment of 5′-*O*-dimethoxytrityl uridine (**2**) with dibutyltin oxide in the presence of TBAF gave the 2′,3′-*O*-dibutylstannylene derivative **3**. Addition of SEM-Cl provided a ~1:1 mixture of the 2′- **4** and 3′- **5** protected derivatives in 28% and 25% yield respectively. The two isomers were separated and the 2′-protected nucleoside was phosphitylated to give the amidite **1** in 73% yield.[7] The 3′-protected nucleoside was succinylated to give **6** and coupled to a polystyrene support with DCC to afford **7**. Using standard coupling conditions,[2] a uridine 10-mer, $(Up)_9U$, was synthesized with an average stepwise yield of 99%.

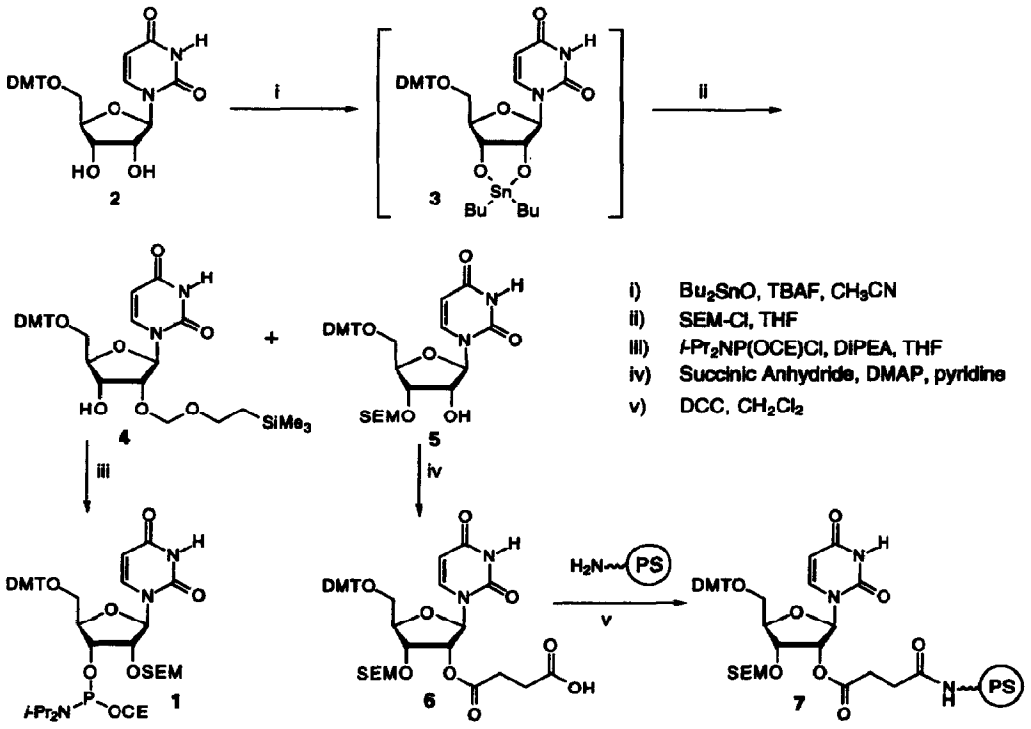

Figure 1. Synthesis of SEM-Protected Uridine Phosphoramidite and Solid Support

Before subjecting the oligonucleotide to deprotection conditions, the SEM deprotection was first tested at the nucleoside level (**Figure 2**). The 2′-protected nucleoside **4** was detritylated and peracetylated to provide the fully protected nucleoside **8** in 75% yield. Exposure of **8** to TBAF gave no reaction, however treatment with $BF_3 \cdot OEt_2$ for 15 min at RT provided an 88% yield of the deprotected nucleoside **9**. This model confirmed the feasibility of the removal of the SEM group from an oligonucleotide.

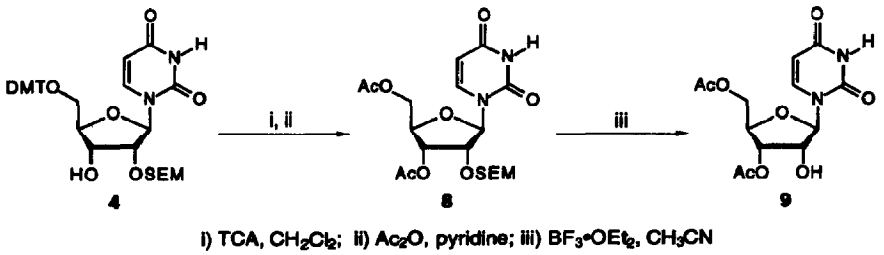

Figure 2. Deprotection of the SEM Group

To test an entire deprotection protocol, the uridine homopolymer was first treated with ammonium hydroxide/ethanol (3/1) at 65 °C for 4 h to cleave the oligonucleotide from the solid support and to simulate the removal of base protecting groups. The resulting SEM protected oligonucleotide was then dissolved in CH_3CN and exposed to 3 eq. of $BF_3 \bullet OEt_2$ per SEM group for 15-30 min to provide the fully deprotected 10-mer in 74% yield.

The $(Up)_9U$ sequence was then purified by ion-exchange HPLC (**Figure 3**) and subjected to nucleoside compositional analysis[8] to yield only uridine as expected.

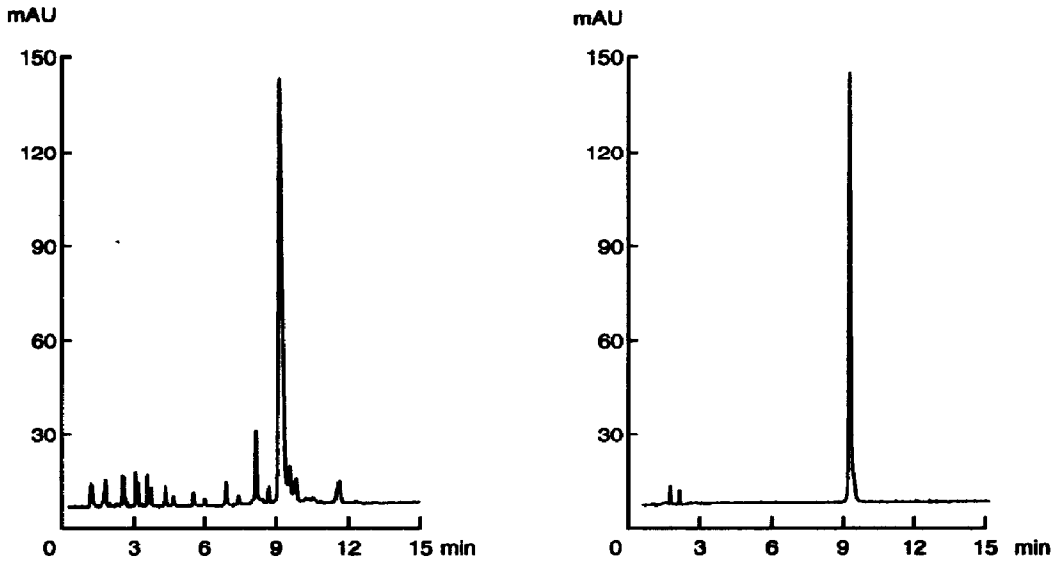

Figure 3. HPLC Analysis of Crude and Pure $(Up)_9U$

This report shows the potential of SEM protection for general RNA synthesis. The advantages imparted by using the SEM group include: ease of introduction, lack of migration, stability to RNA synthesis conditions and rapid and clean removal at the end of the synthesis. These criteria make 2'-hydroxyl SEM protection an attractive alternative to the TBDMS protecting group.

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7. To 5′-*O*-dimethoxytrityl uridine (2) (1.0 g, 1.83 mmol) in CH_3CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol) and TBAF (1.0 M, 2.38 mL). The mixture was stirred for 2 h at RT at which time (trimethylsilyl)ethoxymethyl chloride (SEM-Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred overnight, filtered and evaporated. Flash chromatography (30% hexanes in ethyl acetate) yielded 347 mg (28%) of the 2′-hydroxyl protected nucleoside 4 and 314 mg (25%) of the 3′-hydroxyl protected nucleoside 5. ^{1}H-NMR (^{1}H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to TMS. Solvent was $CDCl_3$ unless otherwise noted. 4: ^{1}H δ 7.91 (d, $J_{6,5}$ = 9.2, H6), 5.97 (d, $J_{1',2'}$ = 6.8, H1′), 5.25 (d, $J_{5,6}$ = 9.2, H5), 4.90 (d, $J_{A,B}$ = 4.5, OCH_2O), 4.85 (d, $J_{B,A}$ = 4.5, OCH_2O), 4.44 (dd, $J_{2',1'}$ = 6.8, $J_{2',3'}$ = 11.2, H2′), 4.28 (dd, $J_{3',2'}$ = 11.2, $J_{3',4'}$ = 4.3, H3′), 4.06 (dd, $J_{4',3'}$ = 4.3, $J_{4',5'}$ = 5.5, H4′), 3.79 (s, 2 x OMe), 3.55-3.75 (m, OCH_2CH_2), 3.50 (m, H5′,5″), 0.94 (m, $CH_2CH_2Si(Me)_3$). To 4 (400 mg, 0.59 mmol) in THF (2 mL) at 0 °C was added *N,N*-diisopropylethylamine (206 µL, 1.18 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (158 µL, 0.71 mmol) dropwise. The reaction mixture was stirred 5 min and then allowed to warm to RT and stirred for 30 min. The solution was again ice-cooled and quenched with dry methanol (1.5 mL). After 5 min the mixture was concentrated *in vacuo* and purified by flash chromatography (30% ethyl acetate in hexanes, 1% triethylamine) to yield 377 mg (73%) of the desired amidite 1. ^{31}P δ 151.923, 152.340.

8. The base compositional analysis of the oligomer was confirmed by digestion of the 10-mer and analysis by reverse phase HPLC. The oligomer was converted to the monomer nucleosides by incubation of 0.3 OD of oligomer with 10 units of P1 nuclease and 2 units of calf intestinal alkaline phosphotase (Boehringer-Mannheim) in 30 mM NaOAc, 1 mM $ZnSO_4$, at pH 5.2 overnight at 50 °C. The digested material was then injected directly onto a C18 column (Rainin, Dynamax, ODS 4 x 250 mm) and the nucleosides separated by an acetonitrile gradient buffered with 50 mM potassium phosphate, pH 7.0. The retention times were then compared with monomer standards.

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