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Efficient Large Scale Synthesis of 2′-*O*-Alkyl Pyrimidine Ribonucleosides

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Abstract:

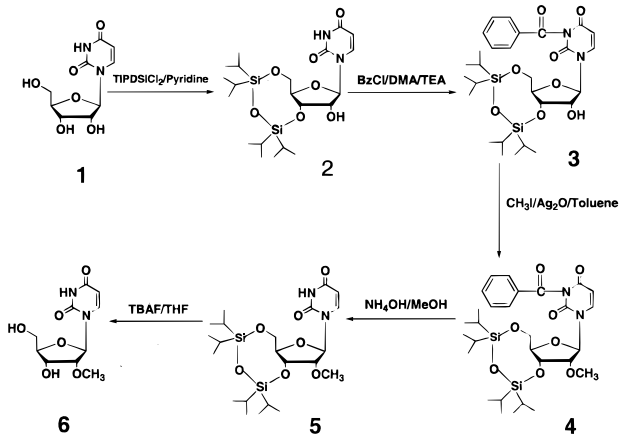
An efficient process to synthesize 2′-*O*-alkyl pyrimidine ribonucleosides in high yield has been described. The inexpensive method was used on a multikilogram-scale synthesis and optimized reaction conditions have been investigated.

Results and Discussion

The presence of 2′-*O*-alkyl ribonucleosides in oligonucleotides has been shown both in vitro and in vivo to provide a better stability and also strong binding capability to the complementary gene targets. Particularly 2′-*O*-methyl modified nucleosides have been widely used in oligonucleotides synthesis for the purpose of research and future therapeutic application.[1−3] Several 2′-*O*-methyl nucleoside modified oligonucleotides are at present in clinical trials including the antiangiogenic ribozyme molecule, RPI 4610.[4]

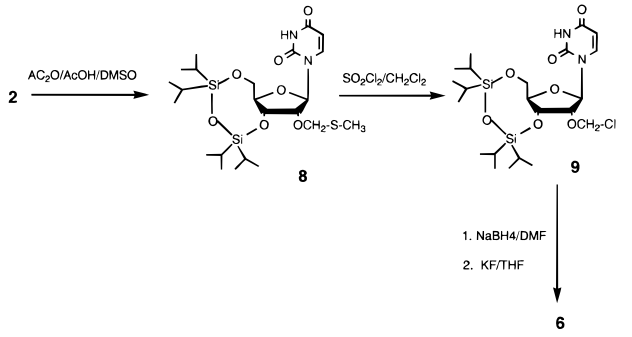
We require a large quantity of 2′-*O*-methyl uridine (10 kg) for phase II trials of GEM 231.[5] 2′-*O*-methyl uridine has been prepared by using following two methods (Schemes 1 and 2). Scheme 1 would involve N^3-protection by benzoyl or toluyl, and 3′,5′-*O*-protected with TIPDSi-Cl ribonucleosides have been subjected to alkylation of the ribose hydroxyl group using methyl iodide and silver oxide.[6−9] The overall yield after deportation of N3 and 3′,5′-*O*-TIPDSi ribonucleosides was 13%. The three purifications at intermediate level made the process labor-intensive.

Scheme 1



The second method also needed the 3′,5′-O-protection with TIPDSi−Cl followed by Pummerer rearrangement. The recovery of **6** after borohydride reduction and deportation of **9** was 18% (Scheme 2). These methods thereby increase

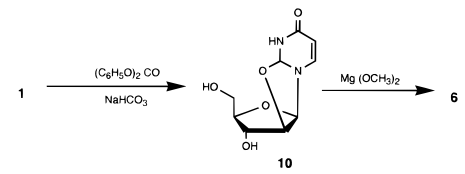
Scheme 2



the time and expense of synthesis while decreasing its efficiency and overall yield. The poor recovery, expensive reagents, and labor-intensive purification caused us to investigate high-yielding, inexpensive, and practical method amenable to a large-scale operation.

In this paper we report a simple, practical, and optimized method for the synthesis of **6** in large scale. The synthesis has been done in two steps via anhydronucleoside opening by a nucleophile (Scheme 3). This method provides efficient

Scheme 3



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synthesis of 2′-*O*-CH_3 substituted pyrimidine ribonucleosides. Synthesis of **10** has been carried out using diphenyl carbonate in DMF and with sodium bicarbonate in catalytic amount, which selectively gave the desired product in 90%. The product crystallized out from the reaction mixture during 12 h of stirring at room temperature. The crystallized material was 98% pure by HPLC. The second step was the ring opening of 2,2′-anhydrouridine using 4 equiv of magnesium methoxide, which afforded the desired product.

Magnesium methoxide is a weak base, which eliminates the possibility of forming the epoxide and consequently the formation of the arabinofuranosyl structure. Hence, the only possible reaction is the attack at C-2′ and the formation of a ribofuranosyl structure. 2,2′-Anhydrocytidine HCl was also reacted with magnesium methoxide to give the 2′-*O*-methyl cytidine in >76% recovery.

Conclusions

The method comprises the preparation of 2,2′-anhydrouridine and reaction with freshly prepared $Mg(OCH_3)_2$ in the corresponding alcohols to produce 2′-*O*-CH_3-substituted uridine and cytidine. Commercially available $Mg(OCH_3)_2$ was not successful (see Experimental Section). A further advantage of the present method is that the alkylation is essentially complete within 5 h.[10−12]

This improved method of alkylation through ring opening of anhydro ribonucleosides makes it possible to synthesize a broad range of 2′-*O*-substituted pyrimidine nucleosides via a divalent alkoxide acting as a nucleophile of the corresponding alcohol.

Experimental Section

All of the starting materials are commercially available from Aldrich (Madison, WI), except that 12% magnesium methoxide that was prepared freshly. HPLC analysis was performed on a Delta-Pak C18 Column (300 Å, 3.9 × 150 mm) by using a linear gradient of A (0.1 M ammonium acetate in H_2O) and B (80% CH_3CN in 20% buffer A) at a flow rate 1.0 mL/min with UV detection at 240 nm.

Preparation of 2,2′-Anhydrouridine (10). Diphenyl carbonate (1.928 kg, 9.0 mol) was added to the uridine (1.2 kg, 8.19 mol) in 4.9 L of DMF under inert atmosphere followed by sodium bicarbonate (20.7 g, 0.245 mol). The mixture was heated for 3 h at 80 °C. The disappearance of the starting material and the formation of a polar product was observed by in-process analysis with HPLC. The product slowly crystallized out from the reaction mixture. The mixture was stirred overnight at rt. The solid was filtered and washed with methanol. The product was 98% pure by RP-HPLC. The material was dried in a high-vacuum oven to its constant weight 1.69 kg (90% yield). The product was identified by comparison with an authentic sample purchased from Aldrich.

Preparation of 2′-*O*-Methyl Uridine (6). 2,2′-Anhydrouridine (**10**, 1.5 kg, 6.63 mol) was added to a freshly prepared solution of 12% magnesium methoxide (18.64 L, 25.88 mol) in methanol. The mixture was refluxed for 5 h. The commercially available magnesium methoxide did not work efficiently. The reaction was monitored by HPLC. The starting material was consumed in 5 h and the formation of a comparatively nonpolar peak observed by in-process analysis. The mixture was cooled to room temperature and then to 5 °C. The pH was adjusted to 7 by the addition of glacial acetic acid. The solvent was striped off to foam. The resultant foam was refluxed with ethyl alcohol (15 L) for 2 h to separate the desired product from inorganic impurities. The solid (magnesium salts) was filtered off and discarded. The filtrate was striped off to a solid mass to give compound **6**. The isolated yield of the compound **6** was 1.46 kg (92%). The product was >95% pure by HPLC. The yield after quantitation against uridine was 89%. ^{1}H NMR (300 MHz, D_2O) δ 7.85 (d, J = 8.09), 5.90 (d, J = 3.36), 5.82 (d, J = 7.93), 4.27 (m, 1H), 4.02 (m, 1H), 3.97 (m, 1H), 3.45 (s, 3H), 3.76−3.84 (m, 2H).

Preparation of 2′-*O*-Methyl Cytidine. 2′-*O*-Methyl cytidine was synthesized similarly by using 2,2′-anhydrocytidine HCl (3.1 g, 3.82 mmol) and freshly prepared solution of 10% magnesium methoxide (16.25 mL, 22.17 mmol) in methanol.

The mixture was refluxed to 5 h. The workup was similar to that described above, and the isolated yield after quantitation against cytidine was 752 mg (76%). The product was >93% pure by HPLC. ^{1}H NMR (300 MHz, D_2O) δ 7.82 (d, J = 7.48), 5.91 (d, J = 3.33), 6.01 (d, J = 7.33), 4.24 (m, 1H), 4.04 (m, 1H), 3.95 (m, 1H), 3.47 (s, 3H), 3.76−3.85 (m, 2H).

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(10) During the course of this work, a paper published by McGee, D.; Zhai, Y. *Nucleosides Nucleotides* **1996**, *15*, 1797−1803 reported selective alkylation of 5′-*O*-dimethoxytrityl anhydrouridine with magnesium or calcium alkoxide in DMF at 100 °C. Their attempts to react the unprotected anhydrouridine with $Mg(OCH_3)_2$ under the same conditions gave no product.

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