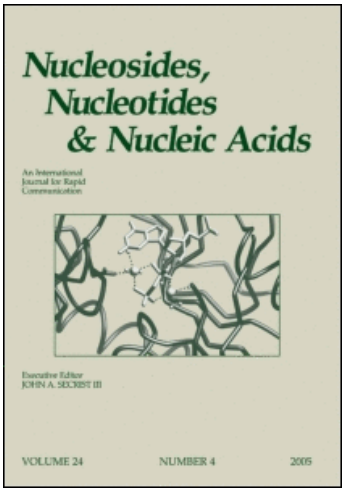

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Synthesis and Incorporation of 2'-O-Methyl-Pseudouridine into Oligonucleotides

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SYNTHESIS AND INCORPORATION OF 2'-0-METHYL-PSEUDOURIDINE INTO OLIGONUCLEOTIDES

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Abstract: A short multigram synthesis of 2'-O-methylpseudouridine and its phosphoramidite derivative is described which avoids the use of protecting groups on the nitrogens. A binding study of oligonucleotides containing this modification suggest an increased binding affinity to RNA when compared to oligonucleotides incorporating 2'-O-methyluridine.

2'-O-Methyluridine, pseudouridine (**1**, see Figure 1) and 2'-O-methylpseudouridine (**4**) are rare nucleosides found in tRNA, snRNA and rRNA. 2'-O-Methylribonucleosides have been tested widely in antisense and ribozyme oligonucleotides as RNA mimics which display greater binding affinity for RNA and some increase in nuclease resistance. The incorporation of the pseudouridine analog into oligonucleotides has only been reported once[1] and was found to increase the melting temperature against RNA complement about 1 °C per modification over 2'-O-methyluridine in the two sequences tested. Our goal was to make, with a minimum of time and effort, a larger amount of this product so that it could be tested in many sequences.

2'-O-methylpseudouridine was first reported[2] to be synthesized along side of 2'-O-methyluridine using a stannous chloride catalyzed diazomethane reaction with the ribonucleoside. A small amount of the correct isomer was separated by chromatography. The synthesis of the phosphoramidite derivative was later accomplished[1] by using a fully blocked intermediate (3' and 5' hydroxyl blocked with the Markiewicz disiloxane reagent and pivaloyloxymethyl groups on N-1 and N-3). This route required eight steps and seven chromatographic separations. The use of nitrogen protecting groups appeared unnecessary for oligomerization since the phosphoramidite of pseudouridine itself has been successfully incorporated into oligonucleotides without them.[3]

A shorter albeit less selective method to alkylate uridine which avoids nitrogen protection is through the dibutyltin oxide complex.[4] In an analogous manner, starting with 25 g of pseudouridine, the tin complex (**2**) was formed and isolated by crystallization in high yield. Treatment of **2** with methyl iodide in dimethylformamide afforded a mixture of the 2' and 3'-O-methyl isomers (**3** and **4**). After a short column to remove unreacted starting material, the mixture was reacted with dimethoxytriphenylmethyl chloride and the product isomers were separated chromatographically and their identities were confirmed by TOCSY and NOESY NMR.[5] A portion of the desired less polar 2' isomer (**6**) was carried on to give 5 g of its phosphoramidite (**7**). Although the overall non-optimized yield of this route was poor (7% vs 18% for the fully blocked route), there were four less steps and chromatographic separations.

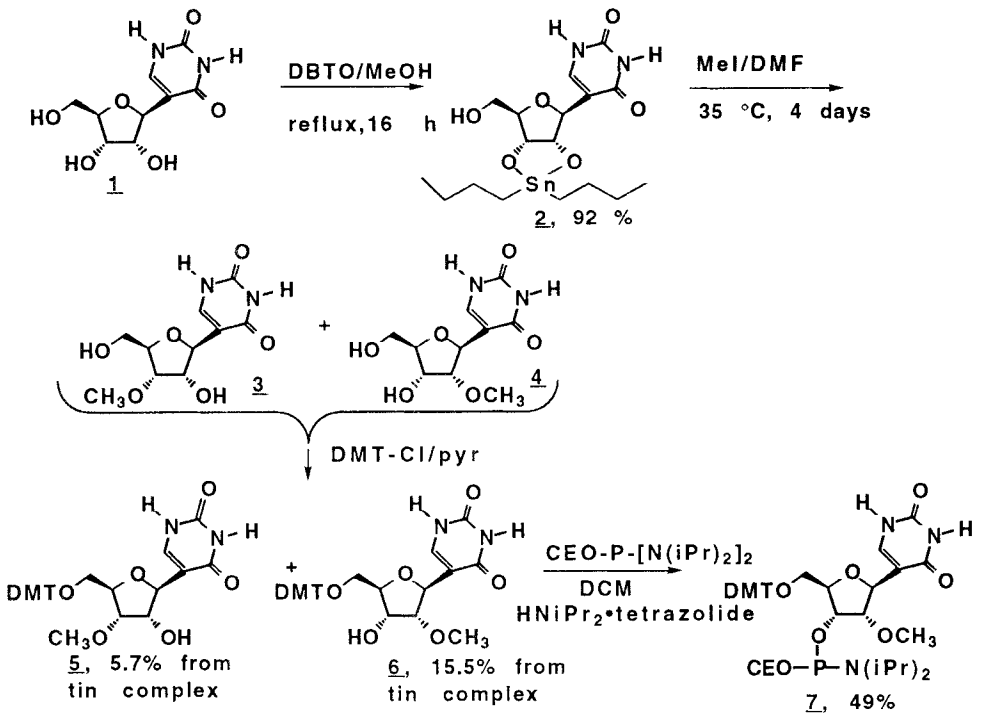

Figure 1

Table 1

Isis #	Sequence 5' to 3'	Tm-1	Tm-2	ΔTm/mod
13861	CTC GTA CCt TTC CGG TCC	65.0	64.5	0.53
13862	CTC GTA Ctt ttC CGG TCC	65.5	63.6	0.48
13863*	GCG ttt ttt ttt tGC G	61.7	52.3	0.93
13864	tCC AGG tGt CCG CAt C	68.0	63.1	1.22

A, C, G, T are deoxynucleosides, all diester linkage
Tm-1 vs RNA, t = 2'-O-Me-pseudoU
Tm-2 vs RNA, t = 2'-O-Me-U
ΔTm/mod = [(Tm-1) - (Tm-2)]/number of modifications

* This sequence was also tested against DNA to give a Tm of 41.3, ΔTm/mod of -0.06.

Four sequences were chosen for melting temperature studies to compare 2'-O-methylpseudouridine to 2'-O-methyluridine against RNA complement (see Table 1). The increase in melting temperature was dependent upon the number of incorporations and spacing, but clearly there was a positive effect which averaged 0.8 °C per modification over 2'-O-methyluridine which itself is about 0.7 °C per modification over thymidine. This result supports the reported value and invites the use of this material for biological screening as well as further structure activity relationships of related bases and 2' modifications.

EXPERIMENTAL

Oligonucleotide sequences incorporating 2'-O-methylpseudouridine were synthesized on an ABI 380B utilizing phosphoramidite chemistry with an increased coupling time. The pseudouridine phosphoramidite was used at a starting concentration of 0.1 M and 5-(ethylthio)-1H-tetrazole (American International Chemistry) was used as the activator at 1M. (Final concentrations of amidite and activator are 0.05 M and 0.5 M, respectively.) Deoxy phosphoramidites were from Perseptive BioSystems GmbH and were used at 0.2 M. The oligonucleotides were cleaved from the CPG support and the base protecting groups removed using concentrated ammonia: methylamine (13%) at 55 °C for 16 hours. The oligonucleotides were precipitated twice from ethanol with a final purification by size exclusion chromatography over Sephadex G25. Oligomer purity and identity checked by CE and electrospray MS.

REFERENCES

1. Pieles, U.; Beijer, B.; Bohmann, K.; Weston, S.; O'Loughlin, S.; Adam, V.; Sproat, B. *J. Chem. Soc. Perkins Trans. I* **1994**, 3423.
2. Robins, M.; Naik, S.; Lee, A. *Can. J. Org. Chem.* **1974**, *39*, 1891.
3. Hall, K.; McLaughlin, L. *Nucleic Acids Res.* **1992**, *20*, 1883.
4. Wagner, D.; Verheyden, J.; Moffat, J. *J. Org. Chem.*. **1974**, *39*, 24.
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