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Simplified Synthesis of 2'-O-Alkyl Ribopyrimidines

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Abstract: Direct 2'-hydroxyl alkylation (R= methyl, ethyl, propyl, pentyl or allyl) of 5'-dimethoxytrityl-N-(t-butylphenoxyacetyl or benzoyl)-cytidine 1 was achieved by reaction in the presence of silver I oxide and a catalytic amount of pyridine with excess alkyl halide. At 0°C alkylation of the 2'-hydroxyl was found to be highly selective; less than 10% di (2'3')-O-alkylated or 3'-O-alkylated side products were detected in most cases. Quantitative deamination of 2'-O-alkylated cytidines to 2'-O-alkylated uridines were performed by refluxing in 4M aq. LiOH solution. This method simplifies access to various 2'-O-alkylated ribopyrimidines using readily available starting materials in one or two steps.

The synthesis of 2'-O-alkyl ribonucleosides have been intensely investigated, largely in part due to increased importance of these monomers in antisense and antiviral oligonucleotide chemistry[1,2]. The advantages of permanent modifications at the 2'-hydroxyl include stabilization of RNA oligomers during synthesis and deprotection, increased nuclease resistance *in vitro* and enhancement of duplex stability upon hybridization to target sequences.[1] Notably 2'-O-methyl modifications in synthetic RNA oligomers have been shown useful for inhibition of *in vitro* mRNA processing,[2a] and in synthetic ribozymes have been shown to enhance catalytic activity.[2b] Thus, the need for improved methods of large scale synthesis of 2-'O-alkylated monomers has emerged. Specific alkylations and allylations of the 2' hydroxyl group have been reported using 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) (TIPS) protected ribonucleosides.[3-5] The TIPS group must be removed after alkylation for subsequent replacement with the 5'-O-dimethoxytrityl protecting group. Earlier methods of methylation using diazomethane on unprotected or minimally protected 2',3'-diol ribonucleosides[6] suffered from low yields and/or poor selectivity giving mixtures of 2'-O-Me; 3'-O-Me; N-Me products. Wagner et al.[7] have shown moderate selectivity in alkylations of the 2'-hydroxyl (2':3' ratio of 4:1) on unprotected cytidine or adenosine using 1 equivalents of sodium hydride and 1.5 - 2.0 equivalent of alkyl iodide. However, methylation products were difficult to separate at this stage requiring subsequent protection of the base exocyclic amine and the 5'-hydroxyl to facilitate purification. Furthermore alkylation of uridine was accomplished using the N^3-(2-cyanoethyl) protected 5'-O-DMT-uridine which is very base labile. Other methods of alkylation of partially protected uridines utilize several additional protection and deprotection steps and have resulted in low overall yields of desired product.[4d]

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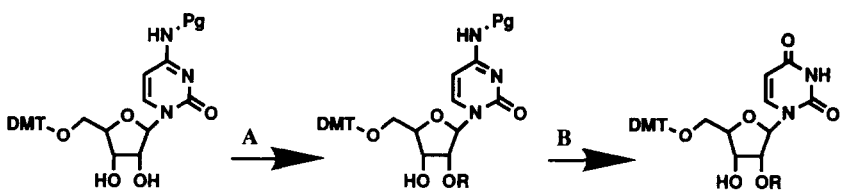
During our studies of hydroxyl alkylations of nucleosides we found that alkylation reactions on 5'-O-DMT-N-tBPA(or Bz)-cytidine occurred selectively at the 2'-hydroxyl without any observable N-alkylation (tBPA protected cytidines exhibit greater selectivity). In addition we found that refluxing the 2'-O-alkyl cytidine products in 4M lithium hydroxide converts these to 2'-O-alkyl uridines quantitatively in one step. Phosphitylation of all 2'-O-alkyl cytidine and uridine products could be performed immediately after purification to produce the corresponding phosphoramidites[8]. In this communication, we report that N-tBPA, 5'-O-DMT cytidine is an excellent starting material for the formation of several 2'-O-alkyl cytidine derivatives which can then be easily converted to the corresponding 2'-O-alkyl uridines with lithum hydroxide solution (Scheme 1.).



Scheme 1.

A). 25 eq. methyl iodide, 1.5 eq. Ag_2O, 0.2eq. pyridine, dry toluene, 0°C, 3hrs.
B). 4M lithium hydroxide, methanol: water (4:1), reflux, 4-16hrs.

General Alkylation Method

Addition of excess alkyl halide (25 eq.) in the presence of silver I oxide (1.5 eq.)[9] and trace amounts (0.1-0.2 eq.) of pyridine to a solution of 5'O-DMT-N^4-protected (benzoyl or t-butylphenoxyacetyl[10]) cytidine in dry toluene (5mL per gram of nucleoside) at 0°C gave the 2'-O-alkyl cytidine product (65-95%) and a small amount of the 2',3'-O,O-dialkylated species (3-5%). The reaction usually takes around 3 to 7hrs for methylations depending on the scale and reactivity of the alkylating agent (see Table 1.). Similar selectivity was found for alkylations with allyl, ethyl, propyl and pentyl; however for the higher alkyl chains, reaction times were much slower and the temperature was allowed to rise to room temperature. Reactions were considered complete when less than 10% starting material remained. The reaction slurry was filtered through a celite plug (twice for larger scales) and evaporated. The residue was then redissolved in ethyl acetate and extracted with 5% sodium iodide in 1M citrate buffer (pH 6.0). The organic layer was then dried over sodium sulfate, filtered and evaporated. Normal phase HPLC of the crude product showed the presence of 3-5% dialkylated product[11] (confirmed by isolation, ^1H NMR and mass spectrometry) and a trace (1-2%) remaining starting material; both can be easily separated by silica gel chromatography (using Merck Silica Si-60 and solvents methylene chloride and 1% water containing ethylacetate). The condition used for a large scale purification[13] (725.0 g of crude material) was Solvent A (a mixture of 78% methylenechloride

in ethylacetate containing 1% water) and Solvent B (ethylacetate containing 1% water); the material was eluted initially with 100% Solvent A for 30 min and then with a linear gradient of a mixture of Solvent A and Solvent B reaching 20% Solvent B in 30 min using the Biotage kilo prep 1000 HPLC system.

Table 1. Alkylation Results

Pg	Scale (g)	Alkyl Iodide	Equiv. Base	Equiv. Ag_2O	Temp. °C	Rxn Time	%products (2', diMe, Sm[a])
Bz	1	methyl	0.2	1.5	0-23	5h	50,16,24
tBPA	1	methyl	0.1	2.5	0-5	7 h	93,1,3
tBPA	5	methyl	0.2	2.5	0-5	3 h	91,3,4
tBPA	50	methyl	0.1	1.5	0-5	9 h	92,1,5
tBPA	132	methyl	0.1	2.5	0-5	17 h	83,5,2
tBPA	1	allyl	0.2	2.5	0-5	4h	74,2,9
tBPA	1	ethyl	0.2	2.5	0-10	10h	98,0,1

[a] Sm = Starting material (N-Bz-5'-O-DMT-cytidine or N-tBPA-5'-O-DMT-cytidine)

With the success of the 2'-O-alkyl cytidine synthesis, our next goal was to convert these 5'-O-DMT, 2'-O-alkyl cytidines directly to the corresponding 5'-O-DMT uridine compounds. It is well established that cytidine derivatives can be deaminated to uridine using either acidic or basic deamination conditions.[12] Attempted bisulfite catalyzed deamination conditions[12a] were found to be unsuitable due to significant loss of the 5'-O-DMT from the nucleoside because of the acidic nature of this reagent and prolonged refluxing. We found that refluxing N-tBPA (or Bz), 5'-O-DMT, 2'-O-Me cytidine in 4M lithium hydroxide in water:methanol (4:1) gave consistent yields (50-70%) of the uridine product in 4-16hrs. Loss of the trityl group was also minimized (<3%) with this process. Base protecting groups were removed rapidly (within 30 minutes for Bz and <15 minutes for tBPA) followed by slow deamination. Upon analysis of the crude product, a small amount (5-10%) 3'-O-alkyl products were discovered; probably due to intramolecular transfer of the alkyl group (longer chains showed less of this rearrangement). However, it is worth noting that no significant rearrangement or loss of the allyl group was observed with the 2'-O-allyl cytidine derivatives under these conditions. Following purification by chromatography (MeOH:CH_2Cl_2), these 2'O-alkyl uridines could be phosphitylated directly using standard methods.[8]

Table 2. Deamination Results

2b, R=	Rxn (g)	Time	%2'prod.	Ratio 2':3'
methyl	1	4hrs	60	10:1
methyl	30	6hrs	74	16:1
methyl	80	8hrs	60	16:1
allyl	0.6	10hrs	59	20:1
ethyl	1.1	16hrs	53	7:1
propyl	0.7	24 hrs	59	24:1
pentyl	0.8	2days	55	100:1

General Deamination Method

To 1mmole 5'-O-DMT N^4-tBPA (or Bz) 2'-O-alkyl cytidine was added 2 mL methanol and 8mL 4M aq. LiOH. The suspension was then attached to a reflux condenser and refluxed for 6 to 10hrs. Upon completion the reaction was cooled, neutralized with ammonium chloride to pH 7-8, diluted with 100mL of ethyl acetate and the layers extracted. The organic layer was dried over sodium sulfate, filtered and evaporated. Crude products were then purified by silica gel chromatography (0-1% MeOH / CH_2Cl_2).

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