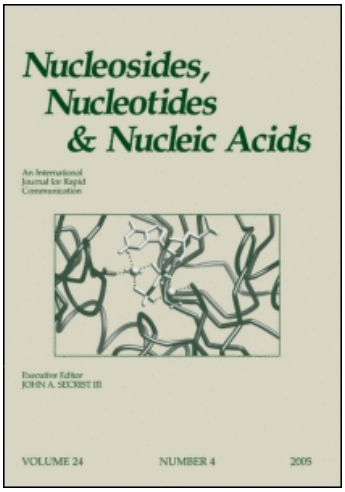

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Commercial-Scale Synthesis of Protected 2′-Deoxycytidine and Cytidine Nucleosides

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ABSTRACT

Transformation of 2′-deoxyuridine and uridine analogs to protected 2′-deoxycytidine and cytidine analogs has been investigated by two different methods. First, traditional triazolation protocol and second p-nitrophenoxylation method. Our studies conclude that the triazolation method is better and suitable for commercial scale–up.

Key Words: Cytidine; Deoxycytidine; Triazolation; 4-Nitrophenoxylation.

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INTRODUCTION

With the progress of several antisense oligonucleotide drugs through the clinical trials and consequent requirement of large quantities of these products, an increased demand for larger quantity of protected nucleoside building blocks was inevitable.[1,2] Protected 2′-deoxycytidine and cytidine analogs, which are also required in larger quantities, are generally prepared from: (a) 2′-deoxycytidine (dC) or cytidine (C); (b) 2′-deoxyuridine (dU) or uridine (U); (c) thymidine.

The second option is more attractive for reasons of economy and easy access of starting materials. Several methods have been reported describing conversion of dU/U derivatives to the corresponding dC/C derivatives. We have investigated two approaches, first through triazolation and secondly via 4-nitrophenoxylation of dU/U derivatives, with special emphasis on their usefulness for commercial scale production.

CHEMISTRY AND DISCUSSION

Protected dC and C building blocks, which are required for the synthesis of deoxyribo- and ribo-oligonucleotides respectively are synthesized either from dC or C[3] or alternatively, from dU or U. The latter option is more attractive for reasons of cost and accessibility of raw materials. For example, we estimate that the cost of protected dC **8** synthesized from dU is lower by 15–20% when compared to that synthesized from dC.

Therefore, we investigated the synthesis of dC and C building blocks, starting from dU and U. Many methods are available for the conversion of 4-oxopyrimidine nucleosides to the corresponding 4-aminopyrimidine nucleosides.[4] Among these methods, the 4-triazol-1-yl-pyrimidine nucleoside mediated procedure had been successfully employed[5] for the conversion of 5′-O-DMT protected thymidine to the corresponding 4-amino analog. As a result, we opted for this method for the proposed conversion of other 4-oxopyrimidine nucleosides **1** (See Sch. 1) to the 4-aminopyrimidine nucleosides **8**.

Several variants of this method are available in the literature for the synthesis of 4-triazol-1-yl pyrimidine nucleosides **3**. Literature method describes addition of the suitably protected 4-oxo-pyrimidine nucleoside to a solution of phosphoryl-tris-triazolide [formed in situ by the reaction of phosphorous oxychloride and 1,2,4-(1*H*)-triazole in the presence of triethylamine] in acetonitrile. The product is isolated after quenching (triethylamine + water mixture) and partitioning between an organic solvent and saturated sodium bicarbonate solution.[6] We designate this as Method A.

In another variant, designated as Method B, the protected 4-oxopyrimidine nucleoside to be triazolated is mixed with 1,2,4-triazole and triethylamine and to this cooled mixture is added phosphorous oxychloride. The 4-(1,2,4-(1*H*) -triazolyl product **4** or **6** is isolated as above.[7] We decided to try both variants for the synthesis of the required 4-amino-pyrimidine nucleosides **8**.

Thus, 5′-O-(4,4′-Dimethoxytrityl) -deoxyuridine **1a** was treated with acetic anhydride in pyridine to yield **2a** or trimethyl chlorosilane in acetonitrile containing triethylamine to provide **5a**. All attempts to triazolate **2a** by either method A or

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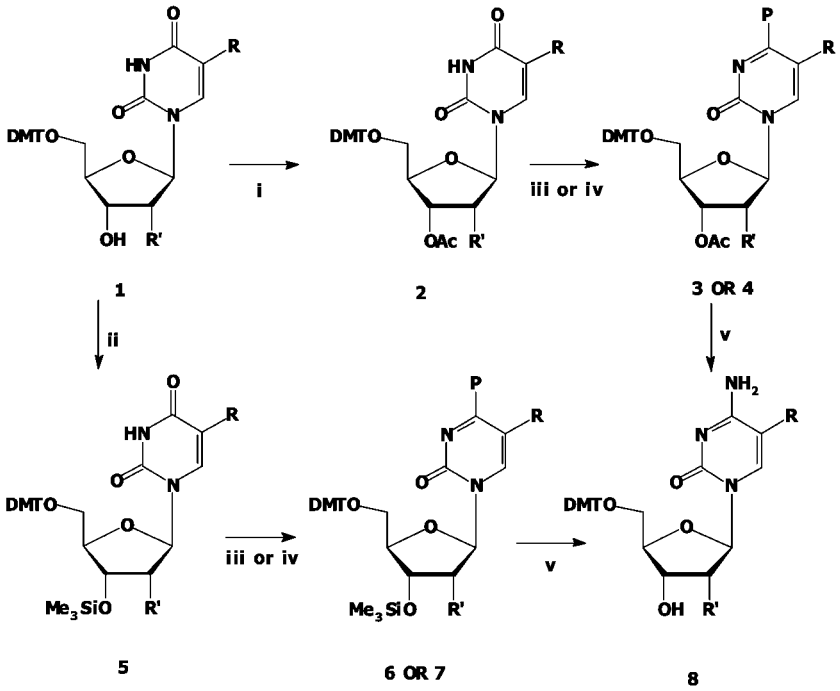

a: R = R'= H; b: R= CH_3, R'= $OCH_2CH_2OCH_3$; c: R= H, R'= OCH_3
For **3, P** = (1(H)-1,2,4-Triazol)-1-yl
For **4, P** = 4-Nitrophenoxy

Reaction Conditions:
i = Acetic anhydride / Pyridine / 0-5^{0}C; **ii** = TMSCl / AcCN / TEA/ 0-5^{0}C
iii = Cpd in AcCN ,1,2,4-Triazole / Triethylamine then $POCl_3$ / -15 to 0^{0}C
iv = $POCl_3$ / 4-Nitrophenol / N-Me-pyrrolidine ; **v** = Aq. NH3 / dioxan / RT

Scheme 1.

method B failed in our hands. Furthermore, treatment of the product of this reaction after workup with 1,4-dioxane and aqueous ammonia did not yield 5′-(4,4′-dimethoxytrityl)-deoxycytidine **8a**. We rationalized this outcome as due possibly to the instability of the 4-(1,2,4-(1*H*)-triazolyl derivative **3a** or **6a.** Interestingly, examples of triazolation mediated conversion of protected dU to the corresponding dC derivative were unavailable in the literature. Recently, such instability of the triazolyl derivative has also been observed by another group.[8]

As an alternative approach, we attempted the conversion by the procedure described by Miah et al.[7] While Miah et al. used the unprotected nucleoside substrates for their transformations, we used either **2a** or **5a** as the starting materials. This change required the work up of the product differently. Treatment of a mixture of **2a** and *N*-methylpyrrolidine or triethylamine in acetonitrile with phosphorous oxychloride and 4-nitrophenol followed by work up of the concentrated reaction mixture by partitioning between ethyl acetate and sodium bicarbonate solution gave

the 4-nitrophenoxy derivative **4a**. Similarly, treatment of **5a** with phosphorous oxychloride and 4-nitrophenol in the presence of *N*-methyl pyrrolidine (or *N*-methyl-morpholine) followed by work up of the concentrated reaction mixture by partitioning between ethyl acetate and sodium bicarbonate solution afforded the 4-nitrophenoxy derivative **7a**. Compound **4a** or **7a** was not purified but treated with a mixture of aqueous ammonia and 1,4-dioxane to obtain **8a** in 57% yield. This product was contaminated with 4-nitrophenol and required purification by silica gel chromatography. On small-scale (<10 g), this procedure looked promising. However, further scale-up to pilot–plant level (5 Kg) and removal of traces of 4-nitrophenol by chromatography proved time consuming and expensive. The overall yield of **8a** from **1a** was only 30–40% on pilot scale. Furthermore, ammonolysis of **7a** required 60 h for completion resulting in higher plant occupation cost.

Reverting to triazolation, we focused our attention on the following aspects that could improve the yield of 4-triaolyl derivative **3a**: (a) use of anhydrous solvents and reagents and inert conditions during reaction; (b) ensuring efficient stirring of the reaction mixture and temperature control; (c) ensuring efficient heat dissipation during the addition of phosphorous oxychloride. Measures (b) and (c) were proved essential by the reaction calorimetry study of the triazolation step which indicated that the addition of phosphorous oxychloride to the protected nucleoside **5** was prone to form ''hot spots''.

After many attempts, successful triazolation of **1a** was accomplished employing method B. The key to success was avoiding the isolation of triazolyl intermediate **3a**. The crude product was subjected to ammonolysis under usual conditions to provide, after work up, **8a** which was purified by column chromatography. An overall yield of 70% was obtained for the crystalline **8a** on a 10 kg scale.

Similarly, **1b** was protected on 3′-end as acetate **2b** and the product triazolated by method A in good yields (70%). Unlike in case of the dU derivatives, here, triazolation proceeded without any problems. For the conversion of **2b** to **8b** via method B on a larger scale, ''transient'' silyl protection offered the advantage of reducing two process steps. After ammonolysis and chromatographic purification, a 86% yield of 5′-O-(4,4′-dimethoxytrityl)-2′-O-methoxyethyl-5-methylcytidine **8b** was achieved. This transformation is now routinely performed on 10–20 kg scales. We have also utilized the triazolation process to convert 5′-O-(4,4′-dimethoxytrityl)-2′-O-methyl-uridine **1c** to 5′-O-(4,4′-dimethoxytrityl)-2′-O-methyl cytidine **8c** in overall yields of 66% on kilo scale.

In summary, we have shown that while both the 4-(1,2,4-1H-triazolyl)- and 4-(4-nitrophenoxy)-derivatives are useful intermediates for conversion of 4-oxopyrimidine nucleosides to the corresponding 4-aminopyrimidine nucleosides on smaller scales, on commercial scale conversions, the former process offered cleaner product in higher yields and at lower cost.

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