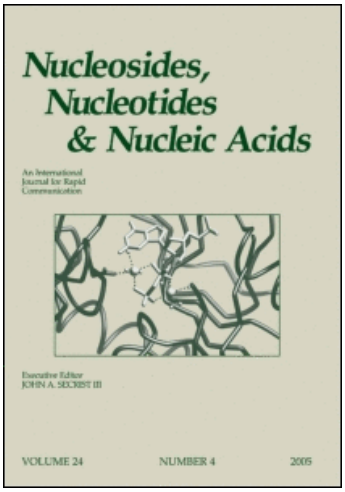

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EVALUATION OF LACTAM PROTECTION FOR SYNTHESIS OF 2′-O-ALKYLATED URIDINES

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□ *Two different classes of protection for the uridine lactam function have been evaluated. These are benzoyl protections and different acetal functions. In particular the triisopropylsiloxymethyl protection is a most promising lactam protecting group for use in synthesis of 2′-O-alkyl-uridines.*

Keywords Base protection; nucleoside; uridine; lactam protection

INTRODUCTION

Modification of oligonucleotides literature with 2′-O-alkyl groups is in many cases highly beneficial with respect to the thermodynamic stability of duplexes formed with target RNA as well as for resistance toward nucleases.[1] Direct alkylation of the 2′-hydroxyl of unprotected uridine has earlier been accomplished, e.g., with dibutyltin oxide and alkylhalides.[2] This generally gives poor yields and low selectivity between the 2′-OH and the 3′-OH. Different 2′O-alkyluridines have also been obtained by reacting 2,2′-anhydro-1-β-*D*-arabinofuranosyluracil with, e.g., metal alkoxides.[3] This excellent method is however limited in that preparation of metal alkoxides is not always readily done.

Direct alkylation of unprotected uridine with strong bases (such as NaH) generally gives problems with alkylation of the lactam function. There are a number of reports on alkylation of base protected uridine but as we felt that no reported approaches are ideal we started to evaluate a number of protecting groups for the lactam function that we thought could be of potential use in 2′-O-alkylations of uridine. The main categories in the study presented here are acetal derivatives and acyl groups.

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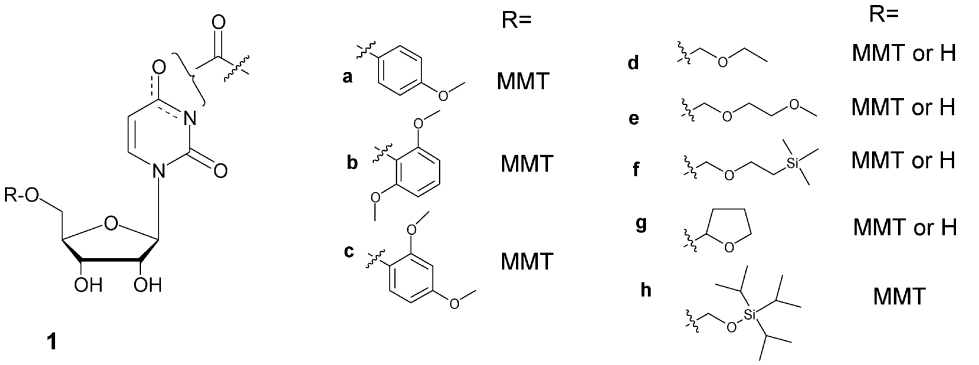


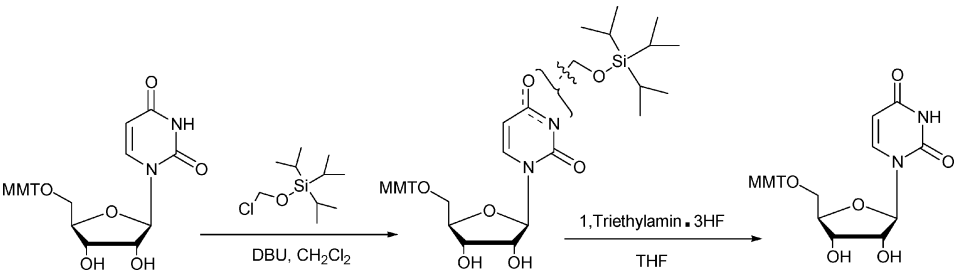
FIGURE 1 Synthesized lactam protected uridine derivatives.

RESULTS AND DISCUSSION

Several protected uridines were synthesized (Figure 1) and 5′-O- 4-monomethoxytrityl (MMT) protection was chosen in most cases to get higher yield and better solubility during protection and further alkylation studies. The aim is to find a derivative that is readily introduced and stable enough during the strongly basic alkylation conditions and yet readily removable either directly after the alkylation or at a later stage of oligonucleotide synthesis. The aroylated derivatives **1a–c** were made essentially according to the procedure published for **1a**.[4]

However, the benzoylderivatives were abandoned as TLC experiments indicated that these were in general not resistant enough to the strongly basic alkylation conditions (0.025 M potassium tert-butoxide in tetrahydrofuran (THF)). The alternative approach investigated was to protect the lactam of uridine with an acetal function. Five different, **1d–e** derivatives were made by alkylation with the corresponding alkoxymethyl chlorides (tetrahydrofuranyl chloride was prepared and used directly in situ[5]) in the presence of either 1,8-Diaza-7-bicyclo[5.4.0]undecene (DBU) or diisopropylethylamine in THF or dichloromethane. Although **1g** (R = H) could be deprotected by 80% HOAc over night, this group was abandoned since it only was obtained in low yield as an isomeric mixture. The acetals in **1d** and **e** (R = H) were not cleaved using up to 10% trifluoroacetic acid (TFA) in dichloromethane and was therefore also not studied further. The (2-trimethylsilylethoxy)methyl group (SEM) of **1f** was not cleaved upon treatment neither with 0.38 M tetrabutylammonium fluoride in dichloromethane nor with neat triethylamine trihydrofluoride even after overnight treatment at room temperature.

The triisopropylsiloxymethyl (TOM) group[6] did however display more promising properties. **1h**[7] was produced from MMT-U by alkylation with TOM-Cl (1 eq.) in the presence of DBU (1 eq.) in dichloromethane (Scheme 1). Interestingly, it appears that the main product is the O-4-triisopropylsiloxy methyluridine derivative as suggested by ^{15}N-^{1}H and



SCHEME 1 Introduction and removal of the TOM group from 5′O-MMT-uridine.

^{13}C-^{1}H NMR correlation spectroscopy (HMBC) experiments. The TOM group was readily removed from **1h** by a few drops of triethylamine trihydrofluoride in 1 ml dich-loromethane in less then 2 hours. When submitting **1h** to condition for 2′-O-alkylation we found that this protection is kept largely intact. Potassium tert-butoxide treatment (0.025 M in THF) of **1h** did not cause any detectable cleavage of the TOM-group (as monitored by TLC).

Protection of the uridine lactam function with TOM appears to be most promising for use in synthesis of 2′-O-alkyluridine derivatives. We are presently implementing the use of this group for synthesis of alkyluridine derivatives and the initial results show that it can indeed be used successfully for this purpose, eg., **1h** has been alkylated with methyl bromoacetate.

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7. NMR-data for **1h**: ^{1}H-NMR ($CDCl_3$): 1.1 (d, 18H), 1.19 (m, 3H), 3.46 (dd, 1H), 3.50 (dd, 1H), 3.82 (s, 3H), 4.25 (m, 1H), 4.32 (m, 1H), 4.41 (dd, 1H), 4.70 (d, 1H), 5.47 (d, 1H), 5.61 (s, 2H), 5.84 (d, 1H), 6.84 (d, 2H), 7.26-7.41 (m, 12H), 7.88 (d, 1H); ^{13}C-NMR ($CDCl_3$): 12.1, 17.8, 55.2, 62.3, 64.9, 70.3, 75.9, 84.0, 87.3, 91.1, 101.9, 113.3, 127.3, 128.1, 128.4, 130.5, 134.6, 138.5, 143.7, 143.9, 151.5, 158.8, 162.1.