

The acetal levulinyl ester (ALE) group for the 2'-hydroxyl protection of ribonucleosides and the synthesis of oligoribonucleotides

Jeremy G. Lackey¹ and Masad J. Damha^{1,*}

¹Department of Chemistry, McGill University, Montreal, QC, Canada H3A 2K6

ABSTRACT

A novel 2'-*O*-ALE (Acetal Levulinyl Ester) uridine phosphoramidite derivative has been efficiently prepared and used for the solid-phase synthesis of a chimeric oligonucleotide strand, 5'-rU₁₁-dT-3'. The average coupling yield of the phosphoramidite was comparable to one obtained with a 2'-TBDMS rU phosphoramidite reagent. Upon completion of the RNA chain assembly, the cyanoethyl phosphate protecting groups were removed using a solution of triethylamine in acetonitrile (2:3 v/v). The 2'-*O*-ALE protecting groups were cleaved under hydrazinolysis conditions. Finally, the RNA oligonucleotide was released from the Q-CPG support when treated with 1 molar TBAF in THF. When the TBAF step was carried out prior to hydrazinolysis, an oligonucleotide with its intact 2'-*O*-ALE groups was obtained.

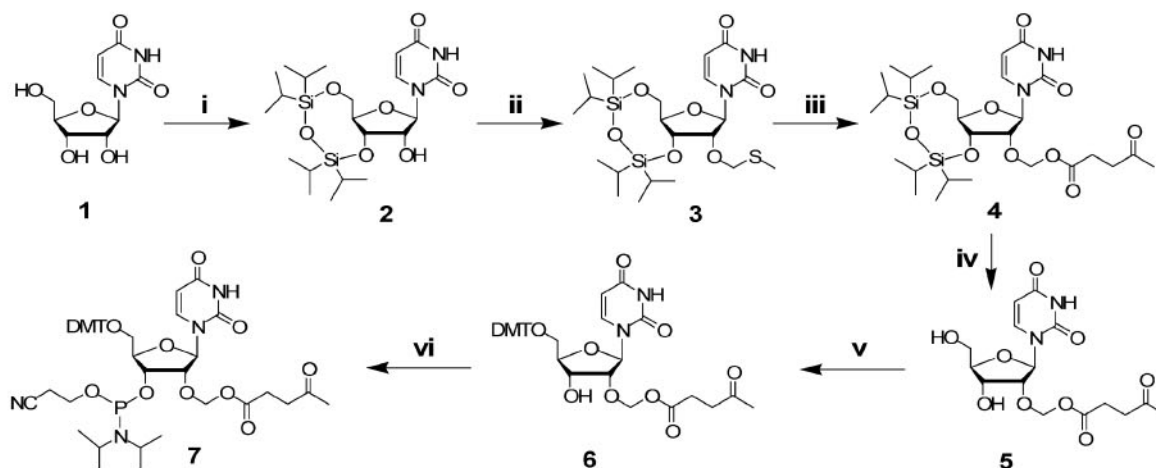
INTRODUCTION

RNA interference (RNAi) therapeutics represent a fundamentally new way to treat human diseases.¹ However, achieving targeted tissue and cellular delivery, stabilization *in vivo*, and cost effective large scale synthesis of RNA are significant bottlenecks in the development of RNAi technology. Recent advances in RNA synthesis have been summarized recently in a review by Beaucage.² There is

also a need to develop synthetic strategies that permits *both* the growth and deprotection of RNA chains that remain attached to a solid polymer support or to a glass or chip surface. Towards achieving these goals, we recently reported the use of the 2'-*O*-levulinyl (Lv) for the 2'-hydroxyl protection of ribonucleoside phosphoramidites.³ The 2'-*O*-Lv group could be removed on-column and the fully deprotected RNA could be subsequently released from a Q-CPG support when treated with 1 M TBAF in THF. In addition, we showed that an oligouridylic acid could be released from the solid support with its 2'-*O*-Lv esters intact. These 2'-*O*-Lv RNAs showed greater stability in fetal bovine serum compared to the fully deblocked 21-mer. However, synthesis of the phosphoramidite monomer building blocks was low yielding due to difficult separation of 2' and 3'-Lv phosphoramidite regioisomers. We have solved this problem while retaining the advantages of the Lv group through the design of a novel acetal levulinyl ester (ALE) protecting group for the 2'-hydroxyl of ribonucleosides. Here we present the synthesis of the 2'-*O*-ALE rU phosphoramidite and its use in the synthesis of a 12-mer chimeric strand (5'-rU₁₁-dT-3').

RESULTS AND DISCUSSION

The 2'-*O*-ALE rU phosphoramidite **7** was synthesized according to scheme 1. Thus, uridine (**1**) was reacted with 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane in pyridine according to Markiewicz³ to give **2** in quantitative yield.



Scheme 1. Synthesis of 5'-DMT-2'-ALE-3'-phosphoramidite-G monomer. i) TIPDSiCl₂, pyr. ii) DMSO, AcOH, Ac₂O iii) a) SO₂Cl₂, CH₂Cl₂ b) NaLv, 15-C-5, CH₂Cl₂ iv) NEt₃-3HF, THF v) DMTCl, pyr. vi) (i-Pr₂N)PClOCE, Hunig's base, THF.

This material was then dissolved in DMSO and further treated with acetic acid and acetic anhydride forming methylthioacetal intermediate **3** (90%).⁴ It was then freeze-dried in benzene and dissolved in dry dichloromethane where it was treated with 1M sulfuryl chloride for one hour. The solvent was evaporated and the resulting rU (2'-O-CH₂Cl) intermediate was redissolved in dry dichloromethane. Sodium levulinate was then added followed by 15-Crown-5 ether to afford **4** (87%).⁵ Compound **4** was then dissolved in dry THF and treated with neat triethylamine trihydrofluoride for 2 hours. The



Figure 1. 24% PAGE of a crude 5'-UUUUUUUUUUUdT-3' oligomers synthesized with 2'-ALE and 2'-TBDMS chemistry.

solvent was then evaporated, and 2'-O-ALE rU (**5**) was precipitated from ether in quantitative yield. As expected, there was no detectable 2'↔3'-isomerization of the ALE protecting group as assessed by ¹H NMR. The recovered 2'-O-ALE rU (**5**) was then 5'-O-tritylated (DMT-Cl/pyridine; 90%) and 3'-O-phosphitylated (90%) under standard conditions to yield **7**. This phosphoramidite was activated with 4,5-dicyanoimidazole (DCI), and a coupling time of only 1 min was set for the preparation of a 12-mer chimeric oligonucleotide (5'-rU₁₁-dT-3') starting from Q-linked dT-CPG.⁷ The average coupling yield of phosphoramidite **7** was >98% and this was comparable to the average coupling yields of standard TBDMS RNA phosphoramidites under similar conditions.

Once the synthesis of the 2'-O-ALE oligomer was completed, the phosphate cyanoethyl groups were removed using 2:3 NEt₃/MeCN for 60 min. The 2'-O-ALE groups were then cleaved with 0.5 M hydrazine hydrate in 3:2 pyridine/acetic acid, r.t., overnight. After a washing step, the oligomer was released from the Q-CPG solid support when treated with 1 M TBAF, r.t., overnight.⁷ Its mass was confirmed by MALDI-TOF (calc. 3610, found 3611 [M + H]). Despite their different coupling time (1 versus 10

min), both silyl and ALE phosphoramidite monomers afforded crude oligomers of excellent purity (figure 1).

CONCLUSION

We have shown that 5'-UUUUUUUUUUUdT-3' could be synthesized from a phosphoramidite synthon **7** in excellent yields. The synthetic strategy developed here allows for the "on column" deprotection of oligoribonucleotide. A similar strategy using all four 2'-O-ALE phosphoramidite synthons should allow the assembly and deprotection of RNA on glass or chip surfaces. Furthermore oligomers released with their 2'-O-ALE groups intact should exhibit an increased stability to nucleases and improved cellular uptake as a consequence of the lipophilic character of their 2'-O-protected sugar moieties. Moreover, each 2'-O-ALE protecting group should be cleaved by intracellular carboxyesterases to generate the native siRNAs inside cells and potentially lead to novel siRNAi prodrugs. These experiments are in progress.

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*Corresponding Author. E-mail: masad.damha@mcgill.ca