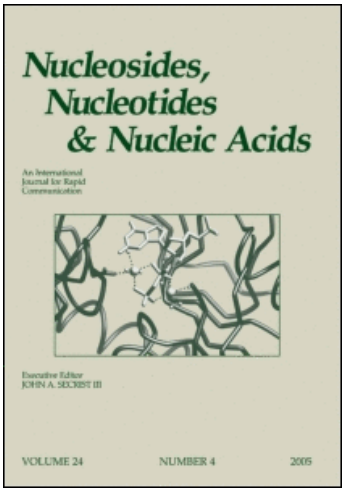

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Chemical Incorporation of 1-Methyladenosine, Minor tRNA Component, into Oligonucleotides

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ABSTRACT

The synthesis of suitably protected 1-methyladenosine derivatives has been developed and its successful chemical incorporation into oligonucleotides was achieved.

Key Words: 1-Methyladenosine; Minor tRNA component; Chemical synthesis; Oligonucleotides.

Modified nucleosides offer an overwhelming variety of chemistry and potential biological functions. More than hundred modified nucleosides have been discovered in nature, most of them occur in tRNAs. The contribution of these modifications to the functional chemistry, structure and biological activity of the RNAs largely

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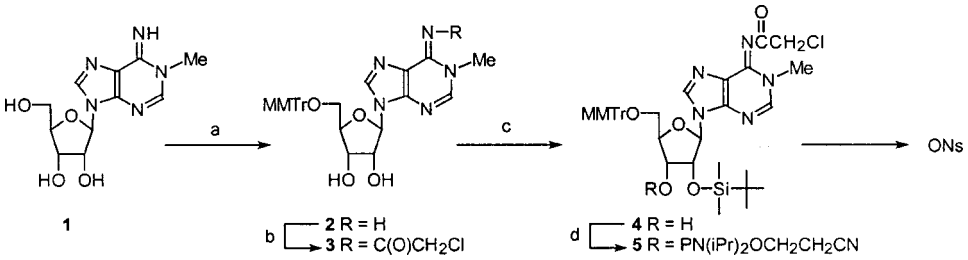
remains to be discovered. Approximately 25% of all tRNAs have 1-methyladenosine (m^1A) at position 58 in the T loop.

Here we present our results on the preparation of m^1A building block and its successful incorporation into oligonucleotides (ONs). Previously oligoribonucleotides containing m^1A (**1**) were prepared using T4 RNA ligase[1]. To the best of our knowledge before our recent publication[2] no attempts have been made for chemical incorporation of m^1A into ONs.



a. MMTrCl/Py; b. $(ClCH_2CO)_2O$/Py; c. $tBuMe_2SiCl$; d. $iPr_2NPCl(OCH_2CH_2CN)$.

Under alkaline conditions, m^1A (**1**) rearranges to N^6-methyladenosine (m^6A).[3] m^1A is difficult to handle due to its polar character. We have examined the stability of **1** under different conditions of deblocking which are used in ON synthesis. In 25% aq. ammonia the half-time of Dimroth rearrangement of **1** to m^6A was 36 h at 20°C. However, **1** was stable at least for 3 days in 2 M NH_3 in MeOH and for 2 days at 20°C in 1M Bu_4NF in THF.

Tritylation of the hydroiodide salt of **1**[3] gave **2** in 68% yield. The benzoyl and acetyl groups for base protection of **2** were found to be too stable towards NH_3 in MeOH.[2] Therefore, more labile chloroacetyl group was chosen. Compound **3** was prepared in 80% yield using chloroacetic anhydride in pyridine with subsequent cleavage of *O*-chloroacetyl groups with NH_3 in MeOH (0°C, 30 min). After silylation 2′-isomer **4** was converted to the phosphoramidite **5** suitable for DNA and RNA synthesis. Deblocking of base-labile groups should be done in anhydrous conditions (2M NH_3in MeOH 60 h, 20°C). Mass spectrometry together with enzymatic hydrolysis were used to prove ONs structure. The modification destabilizes a DNA duplex but slightly improves the stability of a hairpin when incorporated into the loop.

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