This article was downloaded by:

On: 26 January 2011

Access details: Access Details: Free Access

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-

41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713597286

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

E. V. Efimtseva^a; S. N. Mikhailov^a; J. Rozenski^b; R. Busson^b; A. Van Aerschot^b; P. Herdewijn^b
^a Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia ^b Rega Institute Katholieke Universiteit Leuven, Leuven, Belgium

Online publication date: 09 August 2003

To cite this Article Efimtseva, E. V. , Mikhailov, S. N. , Rozenski, J. , Busson, R. , Van Aerschot, A. and Herdewijn, P.(2003) 'Chemical Incorporation of 1-Methyladenosine, Minor tRNA Component, into Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1113-1115

To link to this Article: DOI: 10.1081/NCN-120022749 URL: http://dx.doi.org/10.1081/NCN-120022749

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

www.dekker.com

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS Vol. 22, Nos. 5-8, pp. 1113-1115, 2003

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

E. V. Efimtseva,^{1,*} S. N. Mikhailov,¹ J. Rozenski,² R. Busson,² A. Van Aerschot,² and P. Herdewijn²

¹Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia ²Rega Institute Katholieke Universiteit Leuven, Leuven, Belgium

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

1113

DOI: 10.1081/NCN-120022749 1525-7770 (Print); 1532-2335 (Online) Copyright © 2003 by Marcel Dekker, Inc.



^{*}Correspondence: Ekaterina V. Efimtseva, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, 119991 Moscow, Russia; Fax: +7 095 135 1405; E-mail: smikh@imb.ac.ru.

Downloaded At: 11:11 26 January 2011

1114 Efimtseva et al.

remains to be discovered. Approximately 25% of all tRNAs have 1-methyladenosine (m¹A) at position 58 in the T loop.

Here we present our results on the preparation of m¹A building block and its successful incorporation into oligonucleotides (ONs). Previously oligoribonucleotides containing m¹A (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 m¹A into ONs.

a. MMTrCl/Py; b. (ClCH₂CO)₂O/Py; c. tBuMe₂SiCl; d. iPr₂NPCl(OCH₂CH₂CN).

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 NH₃ in MeOH and for 2 days at 20°C in 1M Bu₄NF 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 NH₃ 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 NH₃ 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 NH₃in 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.

Authors thank KUL Research Council and RFBR for financial support.

REFERENCES

1. Helm, M.; Giege, R.; Florentz, C. A Watson-Crick base-pair-disrupting methyl group (m¹A9) is sufficient for cloverleaf folding of human mitochondrial tRNA^{Lys}. Biochemistry **1999**, *38*, 13,338–13,346.

Nucleic Acids Res. 2002, 30, 1124-1131.

Downloaded At: 11:11 26 January 2011

- 2. Mikhailov, S.N.; Rozenski, J.; Efimtseva, E.V.; Busson, R.; Van Aerschot, A.; Herdewijn, P. Chemical incorporation 1-methyladenosine into oligonucleotides.
- 3. Jones, J.W.; Robins, R.K. Purine nucleosides. III. Methylation studies of certain naturally occurring purine nucleosides. J. Am. Chem. Soc. 1963, 85, 193-201.