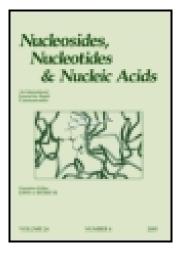
This article was downloaded by: [Carnegie Mellon University] On: 21 January 2015, At: 08:18 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides and Nucleotides

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/lncn19

Synthesis of 6-Aza- & 6-Methylpyrimidine Ribonucleoside Phosphoramidites and Their Incorporation in Hammerhead Ribozymes

Leonid Beigelman^a, Alexander Karpeisky^a & Nassim Usman^a ^a Department of Chemistry and Biochemistry Ribozyme Pharmaceuticals Inc., 2950 Wilderness Place, Boulder, CO, 80301, USA Published online: 16 Feb 2007.

To cite this article: Leonid Beigelman, Alexander Karpeisky & Nassim Usman (1995) Synthesis of 6-Aza- & 6-Methyl-pyrimidine Ribonucleoside Phosphoramidites and Their Incorporation in Hammerhead Ribozymes, Nucleosides and Nucleotides, 14:3-5, 895-899

To link to this article: http://dx.doi.org/10.1080/15257779508012497

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

SYNTHESIS OF 6-AZA- & 6-METHYL-PYRIMIDINE RIBONUCLEOSIDE PHOSPHORAMIDITES AND THEIR INCORPORATION IN HAMMERHEAD RIBOZYMES

Leonid Beigelman, Alexander Karpeisky & Nassim Usman*

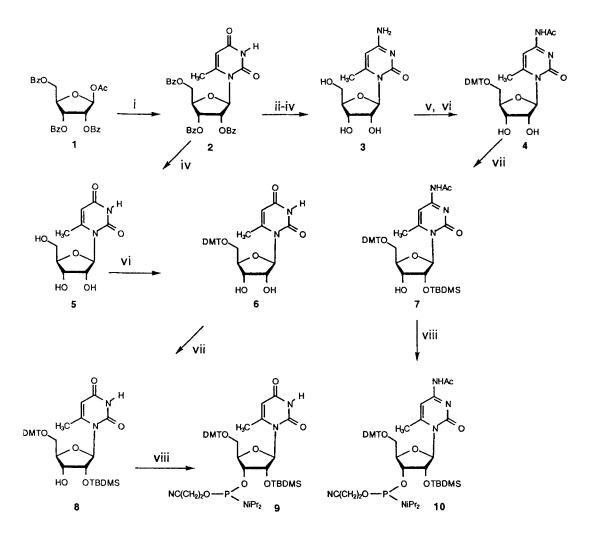
Department of Chemistry and Biochemistry Ribozyme Pharmaceuticals Inc., 2950 Wilderness Place, Boulder, CO 80301, USA

Abstract: The synthesis of phosphoramidites of 6-modified pyrimidine ribonucleosides and their incorporation into hammerhead ribozymes and influence on nuclease stability and catalytic activity is described.

As a part of our studies on the structure-activity relationships and molecular mechanism of action of hammerhead ribozymes, we were interested in the effect of the incorporation of pyrimidine nucleotides modified at the 6-position in a hammerhead ribozyme. These heterocyclic modifications alter the *syn-anti* conformation around the glycosidic bond¹⁻³ and may affect Watson-Crick base pairing at specific positions. They may also provide nuclease resistance since the 6 position is often required for base-specific nuclease binding. We describe the synthesis of 6-methyl- and 6-aza-pyrimidine ribonucleoside phosphoramidites **9**, **10** and **16**, **17** and their incorporation into a 36-mer hammerhead ribozyme by solid phase RNA synthesis. The resulting modified ribozymes were tested for their catalytic activity and nuclease stability in human serum.

Vorbrüggen glycosylation of 6-methyluracil⁴ at 0 °C in the presence of trimethylsilyl trifluoromethane sulfonate gave the nucleoside derivative 2 (Figure 1) in 75% yield. The latter was debenzoylated to give 6-methyl uridine (5). Subsequent standard dimethoxy-tritylation, *t*-butyldimethylsilylation and phosphitylation yielded uridine amidite 9. Protected 6-methyluridine 2 was converted into the corresponding cytidine derivative 3 using a triazolide intermediate.⁵ 6-Methylcytidine (3) was N⁴-acetylated using the "transient protection" procedure⁶ and, without separation, dimethoxytritylated to give compound 4 in 74% yield. Standard *t*-butyldimethylsilylation and phosphitylation led to the cytidine phosphoramidite 10.

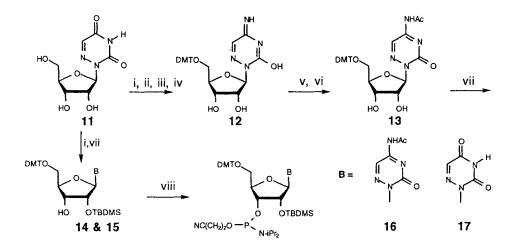
6-Aza-uridine phosphoramidite 17 was synthesized from 6-aza-uridine (11) using the standard steps of dimethoxytritylation, *t*-butyldimethylsilylation and phosphitylation



Reagents and Conditions: *i*) 6-Me-Ura^{TMS}, CF₃SO₃SiMe₃, 0 °C; *ii*) 1,2,4-triazole, POCl₃; *iii*) NH₄OH/dioxane; *iv*) 2M NaOH/Pyr/MeOH; *v*) Me₃Si-Cl/Pyr, then Ac₂O; *vi*) DMT-Cl/Pyr; *vii*) TBDMS-Cl/AgNO₃/Pyr/THF; *viii*) 2-Cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

FIGURE 1

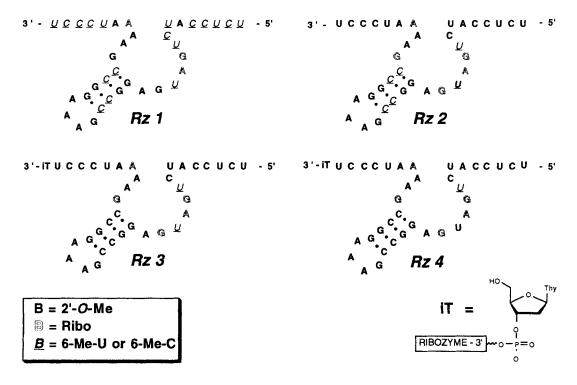
Synthesis of 6-Methyl-Uridine & Cytidine Phosphoramidites



Reagents and Conditions: *i)* DMT-Cl/Pyr; *ii)* Ac₂O/Pyr; *iii)* 1,2,4-triazole, POCl₃, Et₃N; *iv)* NH₄OH/dioxane; *v)* Me₃Si-Cl/Pyr; *vi)* Ac₂O/Pyr; *vii)* TBDMS-Cl, AgNO₃, Pyr/THF; *viii)* 2-Cyanoethyl-N,N-diisopropyl-chlorophosphoramidite, DIPEA/CH₂Cl₂.

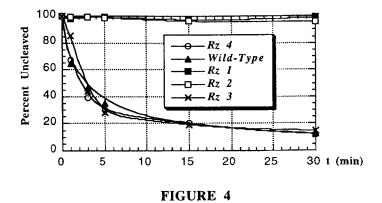
FIGURE 2







Hammerhead Ribozymes Containing 6-Methyl-Uridines & Cytidines



Cleavage Activity of Ribozymes Containing 6-Me-Uridines & Cytidines

(Figure 2). To obtain the 6-aza-cytidine amidite, 6-aza-uridine (11) was dimethoxytritylated and acetylated (without intermediate isolation) to give 5'-O-dimethoxytrityl-2',3'-di-Oacetyl-6-aza-uridine in 75% yield. Amination of this compound through the corresponding triazolide intermediate⁵ led to 6-aza-cytidine 12 in 50% yield. The latter was N⁴acetylated via the "transient protection" procedure⁶ to give 5'-O-DMT-N⁴-acetyl-6-azacytidine 13. The diol 13 was then silylated and phosphitylated to give 6-aza-cytidine phosphoramidite 17. The structures of all compounds synthesized were confirmed by NMR spectroscopy.

6-Methyluridine and 6-methylcytidine were incorporated into the hammerhead ribozymes shown in Figure 3. Figure 4 shows a time course of ribozyme cleavage of a 17-mer RNA substrate sequence 5'- AGG GAU UCA UGG AGA -3'.

Total substitution of all C's and U's (Rz I) resulted in complete loss of catalytic activity. The ribozyme with 6-Me-C substituted Stem II and 6-Me-U modifications at the U4 and U7 positions of the catalytic core (Rz 2) also had no cleavage activity. This data indicates that 6-Me-pyrimidine nucleosides, that exist preferably in the *syn*- conformation, most probably affect duplex formation and thus inactivate the ribozyme. However, ribozymes modified only at U4 or at both U4 and U7 in the catalytic core (Rz 3 and Rz 4) still have almost wild-type cleavage activity.

We compared the stability of Rz 4 (U4 = 6-Me-U; U7 = 2'-O-Me-U) to a control Rz (U4 = ribo U; U7 = 2'-O-Me-U, not shown) in human serum. The control Rz was instantaneously cleaved providing degradation products corresponding to cleavage at position U4. In contrast Rz 4 remained intact after a 24 h incubation (approximate half-life ~40 h), providing an improvement in stability of more than 3 orders of magnitude.

REFERENCES

- 1. Schweizer, M.P.; Banta, E.B.; Witkowski, J.T.; Robins, R.K. J. Amer. Chem. Soc. 1973, 95, 3370-3378.
- 2. Saenger, W.; Suck, D.; Knappenberg, M.; Dirkx, J. Biopolymers 1979, 18, 2015-2036.
- 3. George, A.L.; Hruska, F.E.; Ogilvie, K.K.; Holy, A. Can. J. Chem 1978, 56, 1170-1176.
- 4. Niedballa,U.; Vorbrüggen,H. J. Org. Chem. 1974, 39, 3660-3663.
- 5. Sung, W.L. J. Org. Chem. 1982, 47, 3623-3628.
- 6. Kierzek, R. Nucleosides & Nucleotides 1985, 4, 641-649.