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DNAzymes and ribozymes carrying 2'-*C*-methyl nucleotides

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ABSTRACT

DNAzymes and Ribozymes find applications as inhibitors of gene expression and in detection systems such as biosensors, among others.

In particular, we are interested in the properties of hammerhead ribozymes carrying 2'-*C*-methylnucleotides and 10-23 DNAzymes containing (2'*R*) or (2'*S*)- 2'-*C*-methyl 2'-deoxynucleotides.

In this work a new synthesis of 2'-*C*-Methyluridine phosphoramidite is presented. Special emphasis is dedicated to the improvement of the protection of the tertiary 2'-hydroxyl group. Comparison to previous protecting strategies as well as the stability under oligonucleotide synthesis conditions is discussed.

2'-*C*-methyl-2'-deoxynucleosides show differential preferred conformations depending on the configuration of the 2'-carbon. The influence of these modifications on the activity of 10-23 DNAzymes is also assessed.

INTRODUCTION

The development of synthetic oligonucleotides with non conventional activities, like ribozymes, DNAzymes, ligand aptamers and more recently siRNA, have found applications in different fields, as therapeutics, biosensing and molecular biology. The inherent susceptibility of these molecules to the attack of nucleases, limited its field of application. A way to overcome this restriction is by the use of chemical modifications, which should confer increased stability for their biological uses, and also appropriate characteristics such as adequate hybridization and cellular uptake. Another important feature to be considered is related to structure; in this sense sugar puckering of modified nucleosides can play an important role, facilitating helix formation by nucleoside conformation pre-organization, like in the case of locked nucleic acids (LNA).

2′-*C*-methylribonucleosides are attractive candidates to be used in modified RNA structures, because they gather two previously mentioned characteristics: increased nuclease resistance, when incorporated into RNA sequences[1]; and can adopt C-3′*endo* (RNA-like) conformation on the sugar moiety and *anti* orientation of the base. Other important feature of this kind of nucleosides is that they keep the 2′-hydroxyl. This functional group is essential for maintaining RNA folding when hydrophilic interactions and hydrogen bonding are involved, like in positions 4, 5, 6, 7, 8 and 12 of the hammerhead ribozyme catalytic core.

In order to introduce 2'-*C*-methyl nucleosides in an oligonucleotide sequence, proper phosphoramidite building blocks must be synthesized, which requires adequate protection of the 2'-hydroxyl. We have used in the past[2], tetrahydropyranyl (THP) protection of the tertiary alcohol, unfortunately this group is not stable enough to completely survive acid deprotection conditions of 5'-dimethoxytrityl (DMT), during oligonucleotide solid phase synthesis.

Taking into account these antecedents we decided to explore the preparation of 2'-*C*-methyl phosphoramidite by alkaline degradation of fructose, using an improved lactone reduction procedure and a more acid-stable protecting group at 2'-position.

We are also interested in the properties of the corresponding deoxy analogs, namely 2'-*C*-methyl-2'-deoxynucleosides. These nucleosides show differential preferred sugar conformations depending on the absolute configuration of 2'-Carbon. (2'*S*)-2'-C-methyl-2'-deoxynucleosides mainly adopt the C-3' *endo* conformation (RNA like) while those with 2'*R* configuration adopt the C-2'-*endo* conformation (DNA like). Our final goal is to make use of the availability of both nucleosides to explore the conformational requirements of the nucleotides present in the catalytic core of the 10-23 DNAzyme selected by Joyce.

RESULTS AND DISCUSSION

In an attempt to improve 2'-*C*-methyluridine preparation, the 2'-modified sugar moiety was first prepared, starting from fructose and using an alkaline degradation that afforded the intermediate 2-*C*-methylribo-1,4-lactone (**1**, Figure 1).

Following the procedure depicted in Fig. 1 the corresponding uridine nucleoside **9** was prepared. For the preparation of the corresponding phosphoramidite, 3' and 5' were acetylated in the presence of the tertiary hydroxyl

2'. This alternative was preferred, because traditional Marckiewicz protection of positions 3' and 5', generates a bulk impediment that makes difficult the access to the 2'-tertiary position. In this way 3',5'-di-*O*-acetyl-2'-*C*-methyluridine (**10**, Figure 2) was prepared from **9** in 90 % yield.

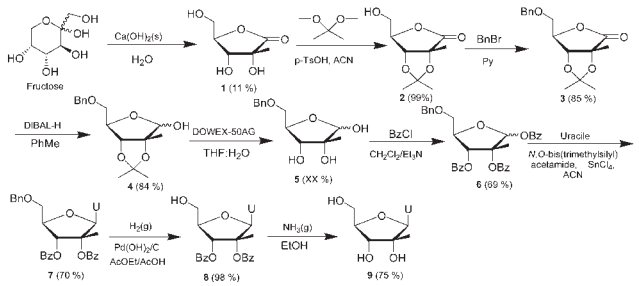

Fig. 1

Then, different protective groups, orthogonal to 4,4'-dimethoxytrityl 5'-protection, were tested. Classical *ter*-butyl-dimethylsilyl (TBDM) protection was assayed, but negative results were obtained using either chloride or triflate. Then, (triisopropylsilyl)oxymethyl chloride (TOM-Cl), which is known to have less steric hindrance was tested. Different bases were used under several reaction conditions (different solvents and temperatures) with negative results. Finally, 2-chloroethylvinyl ether was tested as protecting group, what was successfully achieved, obtaining product **11** (Figure 2) in 74 % yield. The next step consisted in the removal of the acetyls under basic conditions to give 2'-*O*-(1-(2-choroethoxy)ethyl)-2'-*C*-methyluridine (**12**, Figure 2) in 83 % yield.

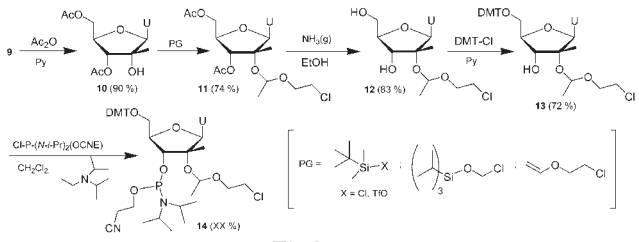

Fig.2

The acid stability of the protection on the 2'-position, compared to the previously reported group (THP), was also assessed.

Regarding the corresponding deoxy analogs, the synthesis and the hybridization properties of oligonucleotides carrying 2'-*C*-methyl-2'-deoxynucleosides have been previously explored by us. This work intends to study the influence on DNAzyme activity of this modification. 2'*R* (2'-methyl up) and 2'S (2'-methyl down) epimers adopt differential sugar conformations, 2'*R* is locked in the C-2'-*endo* conformation (DNA like) while 2'*S* is locked in the C-3' *endo* conformation (RNA like). Therefore, the substitution of these building blocks in structures such as DNAzymes may be useful in the study of conformational requirements.

The synthesis of the modified uridine phosphoramidites has been carried out according to the scheme shown in Fig. 3.

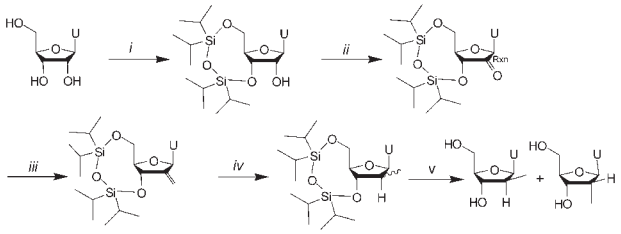

Fig.3

i) TIPDSCl, Py ii) CrO_3, Py, Ac_2O, CH_2Cl_2; iii) Ph_3PCH_3Br, BuLi, THF; iv) H_2(g) 55psi, EtOAc, Pd/C 10% v) 1)chromatographic separation 2) TBAF, py : MeOH : H_2O

In particular, the introduction of the (2'*R*)-methyl-2'-deoxyuridine building bolck in position 8 of the catalytic core of the 10-23 DNAzyme selected by Joyce produced a complete lost of activity, while the corresponding 2'-methyl up diasteromer maintained the catalytic action of the wild type DNAzyme as shown in Table 1.

$MgCl_2$ (mM)	K (m^{-1}) DNAz WT	K (m^{-1}) DNAz 8UP
5	1.01 +/- 0,15	1.37 +/- 0,18
10	2.33 +/- 0,43	1.10 +/- 0,18

Table 1

CONCLUSION

In this work we show a new synthesis of 2'-*C*-methyluridine phosphoramidite. The protection of the tertiary 2'-hydroxyl group was studied and the 2-choroethoxy ethyl group was selected as the best candidate. This protective group stands the acid conditions used during DMTr deprotection while can be still easily removed at the end of the oligonucleotide synthesis.

In addition, (2'R) and (2'S)-2'-*C*-methyl-2'-deoxyuridine were also synthesized and introduced in a DNAzyme sequence, showing differential behavior when the catalytic activity was measured.

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