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2'-O-Hydroxyalkoxymethylribonucleosides and their Incorporation into Oligoribonucleotides

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2'-O-HYDROXYALKOXYMETHYLRIبونUCLEOSIDES AND THEIR INCORPORATION INTO OLIGORIBONUCLEOTIDES

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□ *A simple and efficient method for the preparation of pyrimidine 2'-O-hydroxyethoxymethylribonucleosides and 2'-O-hydroxypropoxymethylribonucleosides has been developed. These modified nucleosides were incorporated into oligoribonucleotides, which were shown to form stable RNA/RNA duplexes. The effect of 2'-O-modification in the antisense and sense strands of small interference RNA was evaluated in multi-drug resistant NIH 3T3 cells.*

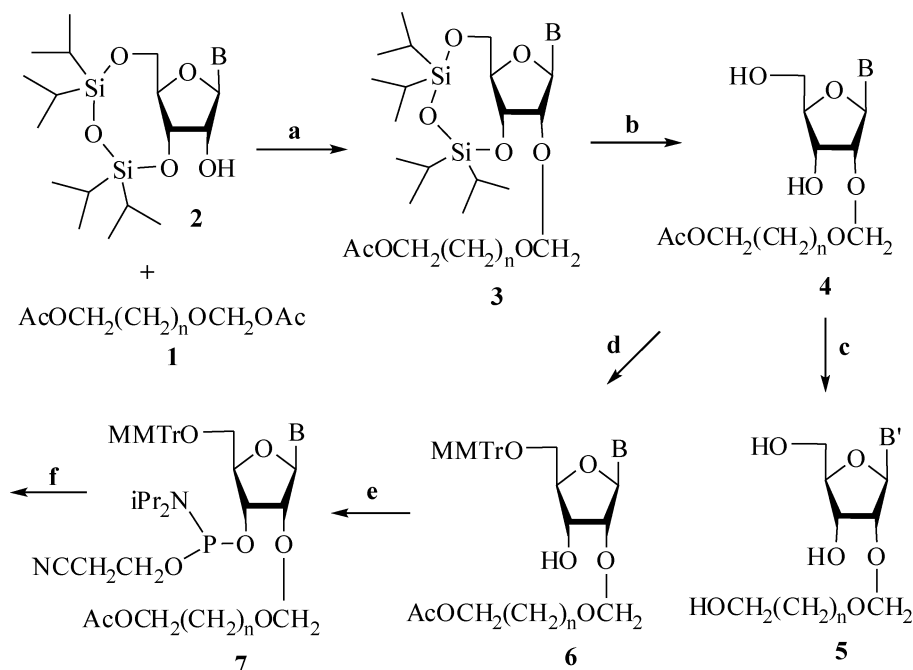
Keywords Oligoribonucleotides; ONs; RNA/RNA duplexes

Modified oligonucleotides (ONs) are widely used in molecular biology and medicinal chemistry. Antisense agents are valuable tools to inhibit the expression of a target gene in a sequence specific manner, and may be used for functional genomics, target validation and therapeutic purposes.^[1] Research in this field increased in impact by the discovery of RNA interference.^[2,3]

Numerous attempts have been made to improve the properties of natural ONs. It is believed that the 2'-carbohydrate modifications are the most universal and promising.^[4–6] A typical strategy for the preparation of such ONs is the synthesis of modified nucleoside followed by conversion to the corresponding phosphoramidite suitable for automated ON

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SCHEME 1 B = Ura, Cyt^{Bz}; B' = Ura, Cyt; n = 1,2. a) SnCl₄/ClCH₂CH₂Cl, -12°C; b). Bu₄NF/THF; c) NH₃/MeOH; d) MMTrCl/Py; e) ClP(NiPr₂)(OCH₂CH₂CN); f) oligoribonucleotide synthesis.

synthesis. Most of these modifications were achieved via alkylation reactions of partially protected ribonucleosides. The heterocyclic bases should be protected in order to avoid their alkylation. For each nucleoside the specific blocking groups are used,^[7] which in most cases are not compatible with standard automated ON synthesis. Very recently for the preparation of 2'-O-modified nucleosides we have developed another type of chemistry based on *O*-glycosylation reactions, which have several important advantages, such as increased yields and simplification.^[8,9] A simple and effective method for the preparation of 2'-O-(β-D-ribofuranosyl)nucleosides starting from readily available 3',5'-O-blocked *N*-acylribonucleosides and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose preactivated with tin tetrachloride in 1,2-dichloroethane at 0°C, has been recently developed.^[10–12]

RESULTS AND DISCUSSION

Here we present our recent results on the preparation of 2'-O-hydroxyalkoxymethylribonucleosides and their incorporation into oligoribonucleotides.^[13] This scheme is nearly the same as for the preparation of disaccharide nucleosides and their incorporation into ONs.^[14] The starting compounds 1 (n = 1, 2)^[15] were easily prepared from

1,3-dioxolane and 1,3-dioxalane, respectively, and were condensed with 3',5'-O-blocked ribonucleosides **2** (B = Ura, Cyt^{Bz}) in the presence of tin tetrachloride in 1,2-dichloroethane at -12°C for 20 minutes (Figure 1). The yields (85–87%) of products **3** were even higher than in the case of preparation of above mentioned disaccharide nucleosides.^[10] The silyl group was cleaved to yield partially protected **4** and subsequent deblocking with ammonia in methanol gave free 2'-O-substituted nucleosides **5** in high overall yield. Nucleosides **4** were converted using standard procedures to the corresponding monomethoxytrityl derivatives **6** and their phosphoramidites **7**. With these phosphoramidites, several oligoribonucleotides with one or more modifications were assembled. It was shown that modified ONs form stable RNA/RNA duplexes, with about 0.5°C destabilization per modification.

In addition several siRNAs comprising one or more of the new modifications have been prepared and are currently under evaluation. The effect of 2'-O-modification in the antisense and sense strands of small interfering RNA targeting the MDR1 gene was evaluated in multi-drug resistant NIH 3T3 cells using previously described techniques.^[16] Standard siRNA or siRNAs with 2'-O-hydroxyalkoxymethylribonucleosides in the sense strand effectively inhibited P-glycoprotein expression, whereas modifications in the antisense strand were not tolerated.

CONCLUSIONS

The general method for the preparation of 2'-O- β -D-ribofuranosyl nucleosides was found to be applicable for the synthesis of pyrimidine 2'-O-hydroxyalkoxymethylribonucleosides. The 2'-O-substituent was found to be stable during oligonucleotide synthesis. Additional work on the preparation of other 2'-O-functionalized nucleosides and oligonucleotides bearing amino groups is in progress and will be published shortly.

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