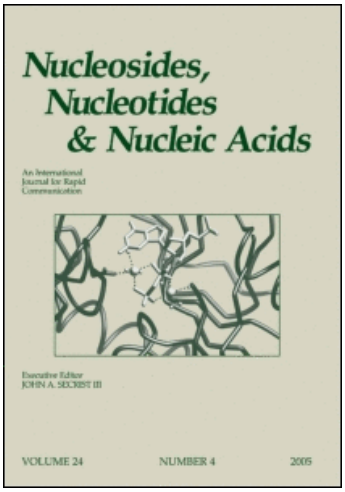

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Rnai Activity of Sirnas Modified with 2'-Aminoalkyl-Substituted Fluorinated Nucleobases

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RNAi ACTIVITY OF siRNAs MODIFIED WITH 2′-AMINOALKYL-SUBSTITUTED FLUORINATED NUCLEOBASES

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□ *We recently reported that a 1′-deoxy-1′-(4,6-difluoro-1H-benzimidazol-1-yl)-2′-(β-aminoethyl)-β-D-ribofuranose nucleoside appears to be a universal nucleoside which does not differentiate between the four natural nucleosides A, C, G, and U in duplexes. Moreover, ribozymes modified with this nucleoside analog showed a better or at least equal catalytic activity relative to Watson-Crick mismatches.[1] Due to these data, we investigated the ability of this compound to tolerate Watson-Crick mismatches in order to avoid HIV escape mutations in RNA interference. The influence of this nucleoside analog on siRNA efficiency was analyzed with a proven siRNA targeting GFP.*

Keywords RNAi; siRNA; universal nucleosides; 2′-modification

INTRODUCTION

According to literature it is well established that due to the high mutation rate of HIV-1 the virus can escape from RNAi-mediated inhibition.[2,3] However, due to the behavior of 1′-deoxy-1′-(4,6-difluoro-1H-benzimidazol-1-yl)-2′-(β-aminoethyl)-β-D-ribofuranose as a universal nucleoside we investigated, if an insertion of this compound into the antisense strand of a siRNA could keep the efficiency of RNAi mechanism by silencing the corresponding mRNA at a high level. Therefore we synthesized the phosphoroamidite of this compound and incorporated it into siRNA 21-mers. For all RNAi-assays we have chosen a siRNA which is targeted to

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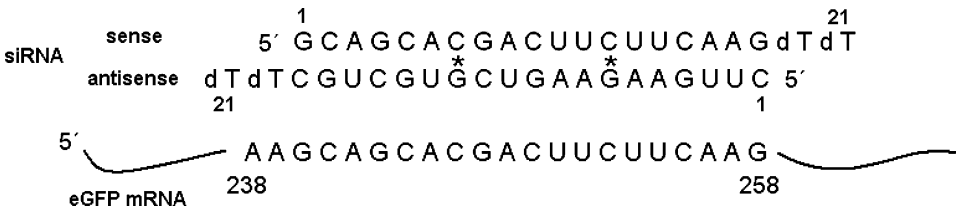

FIGURE 1 Schematical illustration of used siRNA construct and its target sequence of eGFP mRNA. 2′-(β-aminoethyl)-4,6-difluorobenzimidazole substituted nucleoside was inserted at positions 7 and 13 in the antisense strand. As a control, Watson-Crick mismatches were inserted at the same positions.

nucleotides 238–258 of eGFP mRNA.[4] The eGFP served as target gene, which was stably expressed in HeLa cells. The siRNA knock-down efficiency was measured by real time TaqMan-PCR,[5] thereby expression of eGFP was normalized to huGAPDH expression. Down regulation on eGFP protein level was confirmed via FACS analysis. To monitor transfection efficacy, the uptake of Cy5-labelled siRNA was measured by flow cytometry.

RESULTS AND DISCUSSION

Given that some Watson-Crick mismatch base pairs were tolerated in RNA interference,[6] we first investigated a Watson-Crick mismatch that

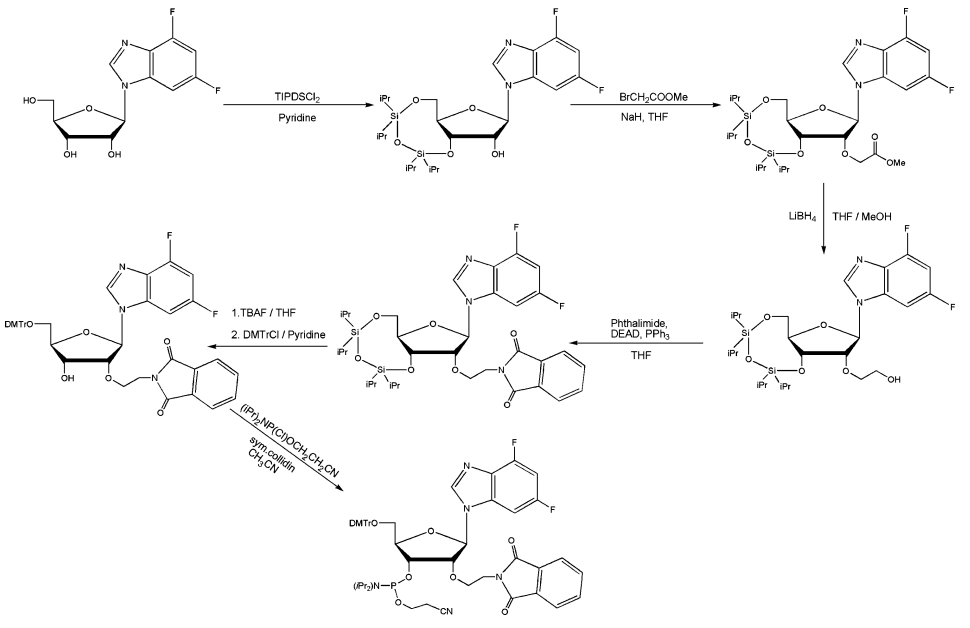

FIGURE 2 Synthetical pathway for the synthesis of 1′-deoxy-1′-(4,6-difluoro-1H-benzimidazol-1-yl)-2′-(β-aminoethyl)-β-D-ribofuranose.

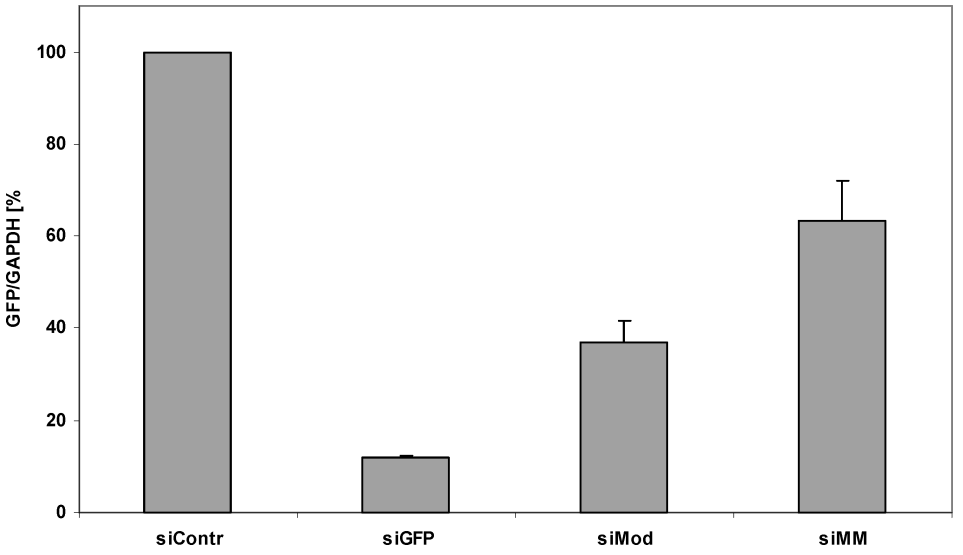

FIGURE 3 Analysis of the mRNA downregulation of modified siRNAs via TaqMan-PCR. HeLa-GFP cells were transfected with the 2′-(β-aminoethyl)-4,6-difluorobenzimidazole modified siRNA (siMod) and the mismatch control (siMM). As a negative control a nonsilencing siRNA (siContr) and as a positive control the unmodified siRNA (siGFP) were used. The determiniation of the GFP/GAPDH ratio of the transfected cells occurred 24 h after transfection via quantitative TaqMan-PCR. Percentage GFP-mRNA expression is illustrated relating to the control. Error bars represent the standard deviation of average values.

leads to a significant loss in siRNA activity. Thus, point mutations were inserted at positions 5 to 8 in the antisense-strand of the siRNA targeting GFP (Figure 1). The point mutation at position 7 (G→C) was 6-fold less active in mRNA silencing compared to the unmodified control (data not shown). We tested if an insertion of 1′-deoxy-1′-(4,6-difluoro-1H-benzimidazol-1-yl)-2′-(β-aminoethyl)-β-D-ribofuranose on this position into the antisense strand of the siRNA could enhance its efficiency compared to the siRNA containing the Watson-Crick mismatch.

The synthesis of the protected phosphoroamidite of 1′-deoxy-1′-(4,6-difluoro-1H-benzimidazol-1-yl)-2′-(β-aminoethyl)-β-D-ribofuranose, shown in Figure 2, is described in detail elsewhere by Kloepffer and Engels.[1]

The siRNA containing the universal nucleoside at pos. 7 in the antisense strand was analyzed for silencing activity by TaqMan-PCR and compared to the mismatch siRNA (Figure 3).

The modified siRNA showed a 71% higher silencing activity than the siRNA containing the C:C mismatch at the same position. However, another siRNA antisense strand with incorporated universal nucleoside at position 13 showed a significant decrease of silencing activity (data not shown).

These data suggest that an incorporation of 2′-(β-aminoethyl)-4,6-difluorobenzimidazole substituted nucleoside may increase the silencing activity in appearance of possible Watson-Crick mismatches.

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