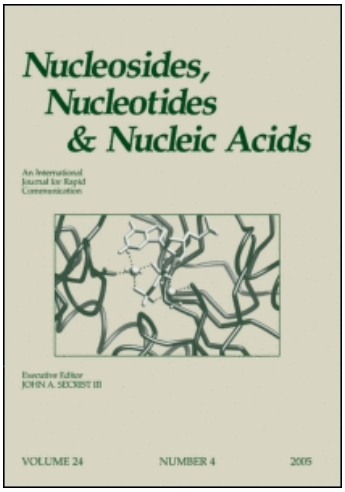

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SYNTHESIS OF 2'-C-DIFLUOROMETHYL SUBSTITUTED NUCLEOSIDE ANALOGS AS RIBONUCLEOSIDE REPLACEMENTS IN HAMMERHEAD RIBOZYMES

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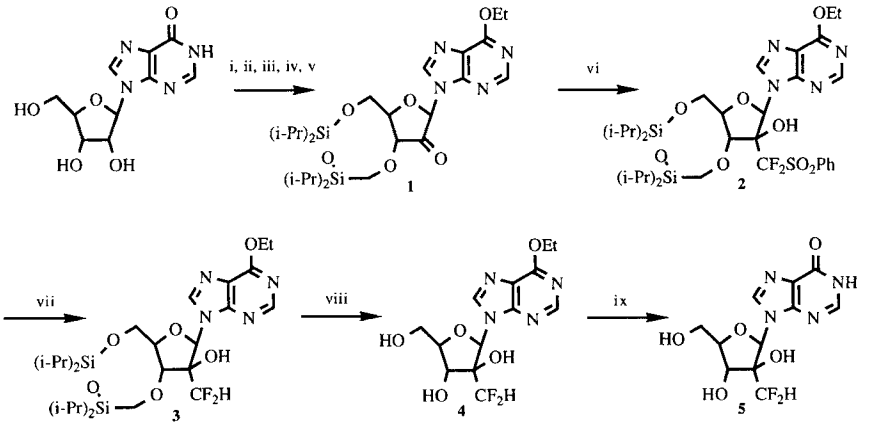
ABSTRACT: 2'-Difluoromethyl modified nucleoside analogs **4** and **9** have been prepared and converted into phosphoramidites for the incorporation into hammerhead ribozymes.

INTRODUCTION

Systematic replacement of the ribonucleosides in the catalytic core of the hammerhead ribozyme with 2'-deoxy nucleosides or 2'-O-allylribonucleosides allowed the identification of G^5, A^6, G^8, $A^{15.1}$ as those positions, where the presence of the 2'-hydroxyl groups is essential for cleavage activity[1, 2]. The role of these essential 2'-OH groups is not exactly known. They are probably involved in important hydrogen bond interactions, stabilizing the tertiary structure of the hammerhead and/or in interaction with the Mg^{2+} bound water molecules. At single nucleoside positions of the catalytic core, 2'-hydroxyl replacements other than 2'-O-allyl or 2'-H have been found possible without complete loss of the catalytic activity. At positions G^5 and G^8 2'-amino substitution gives only a 15-fold reduction of cleavage activity[3]. Similarly, the 2'-fluoro substitution at one of the A^6, A^9 or $A^{15.1}$, A^{14}, A^{13} adenosines leads to only a small decrease in catalytic activity in the presence of Mn^{2+} ions[3]. In order to find non-ribonucleoside replacements which could be accepted at all residual positions of the 2'-O-alkyl- or 2'-deoxynucleoside substituted hammerhead ribozyme, we have synthesised new purine nucleoside analogs which contain a 2'-CF_2H group in the 2'-position. The difluoromethyl group is non-nucleophilic, but isosteric and isopolar with the hydroxyl group and has both H-bond donor and acceptor abilities. This residue has already been used successfully in the carbohydrate and nucleoside fields as a replacement for a variety of hydroxyl functions[4-6].



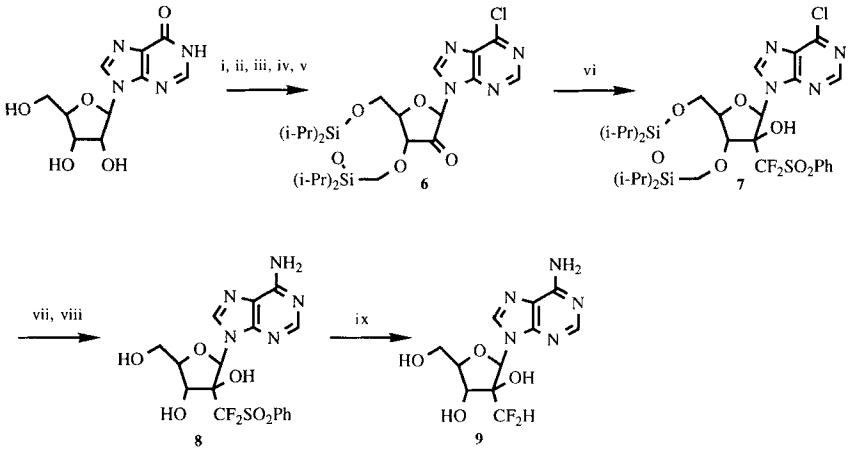
i: Ac_2O, pyridine; ii: $SOCl_2$-DMF, $CHCl_3$; iii: NaOEt, EtOH; iv: $TIPDSiCl_2$, pyridine;
v: Dess-Martin periodinane, CH_2Cl_2; vi: $PhSO_2CF_2H$-LDA, THF-HMPA; vii: Na-Hg, Na_2HPO_4, CH_3OH;
viii: TBAF, THF; ix: adenosine deaminase

Scheme 1

RESULTS

Compounds **1** resp. **6** were prepared by standard methods from inosine. Oxidation of the 2'-hydroxyl was achieved in both cases by the Dess-Martin periodinane. **1** and **6** were then alkylated with phenyldifluoromethylsulfone-LDA following the procedure of McCarthy et al.[7], to give compounds **2** and **7**. Removal of the activating phenylsulfonyl group was first tried with SmI_2 in THF-HMPA[8]. In the case of **2** the desired compound **3** was isolated. The 6-chloro derivative **7** though was over-reduced to give the nebularine difluoromethyl analog. Therefore, and because SmI_2 is difficult to handle, we looked for an alternative reducing agent. Sodium amalgam in phosphate-buffered methanol has been described as an efficient reagent for reductive desulfonylation[9]. Treatment of **2** resp. **7** with Na-Hg resulted in clean cleavage of the phenylsulfonyl moiety. **3** was then desilylated to give compound **4** which was in turn converted to the inosine analog **5** by adenosine deaminase in phosphate buffer[10] (Scheme 1).

The sodium amalgam reduction in methanol of **7** afforded the 6 -O-methyl derivative through attack on the aglycon. Therefore we had to convert the 6-chloropurine derivative **7** first to the adenosine analog **8** by treatment with methanolic ammonia followed by NH_4F in methanol. Subsequent desulfonylation with sodium amalgam gave the desired 2'-C-difluoromethyl adenosine **9** (Scheme 2).



i: Ac_2O, pyridine; ii: $SOCl_2$-DMF, $CHCl_3$; iii: K_2CO_3, MeOH; iv: $TIPDSiCl_2$, imidazole, DMF; v:Dess-Martin periodinane, CH_2Cl_2;vi: $PhSO_2CF_2H$-LDA, THF-HMPA; vii: NH_3-CH_3OH; viii: NH_4F-CH_3OH; ix: Na-Hg, Na_2HPO_4, CH_3OH

Scheme 2

Incorporation into Oligonucleotides

Compound **4** served us as a model nucleoside for coupling experiments with the novel sugar modification on a DNA-RNA synthesizer. Dimethoxytritylation followed by phosphitylation and acetylation afforded the 2'-O-protected phosphoramidite. This phosphoramidite was incorporated at the 3'-end of a T hexamer to give $T_6\underline{X}$ and in the middle position of a T$\underline{X}$T trimer under routine coupling conditions. With 5-(2-nitrophenyl)-1-H-tetrazole as activator the coupling yields for the analog were >98%.

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