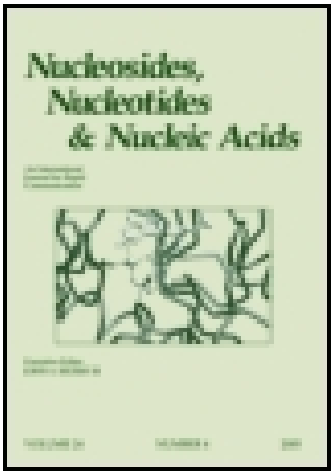

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Synthesis and NMR Spectra of Some New Carbohydrate Modified Uridine Phosphoramidites

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SYNTHESIS AND NMR SPECTRA OF SOME NEW CARBOHYDRATE MODIFIED URIDINE PHOSPHORAMIDITES

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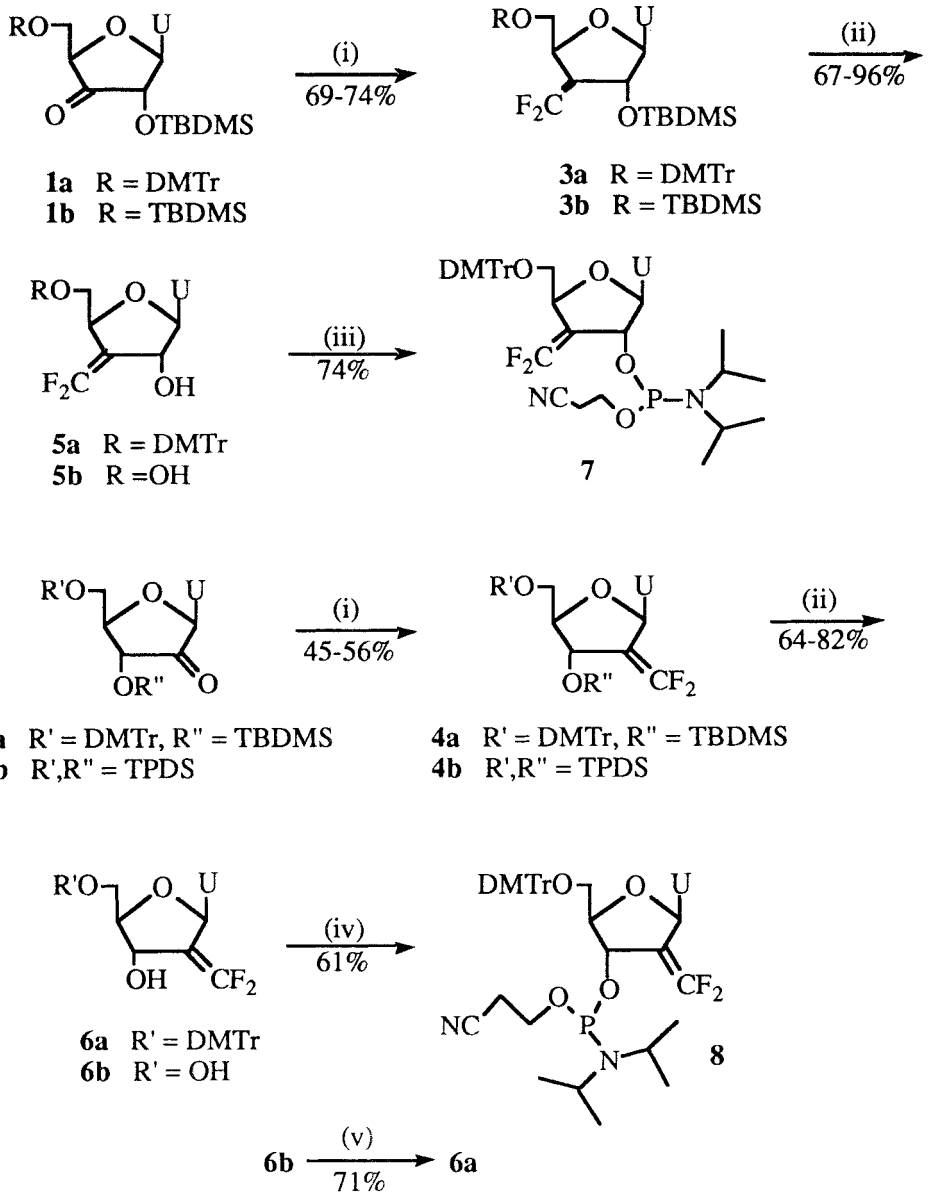
ABSTRACT: The one step reaction of 2'- and 3'-keto derivatives of uridine with bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide and zinc gives the corresponding 2'- and 3'-difluoromethylene nucleosides in good yield. Desilylation and phosphitylation of the resultant 2'- or 3'-hydroxyls provides the target 2'- and 3'-phosphoramidites **7** and **8** for use in oligonucleotide synthesis[1].

As part of our antisense programme we wished to investigate the properties of 3'-5' or 2'-5' linked oligonucleotides having the 2'- and 3'- positions modified with a difluoromethylene group. It was expected that such oligonucleotides would possess increased stability against nucleases owing to conformational restrictions imposed by the difluoromethylene groups on the pucker of riboses[2]. For the synthesis of the target oligonucleotides we elected to employ the phosphoramidite approach[3], which entailed prior preparation of the appropriate 2'- and 3'-difluoromethyleneuridine phosphoramidites **7** and **8** (Scheme 1).

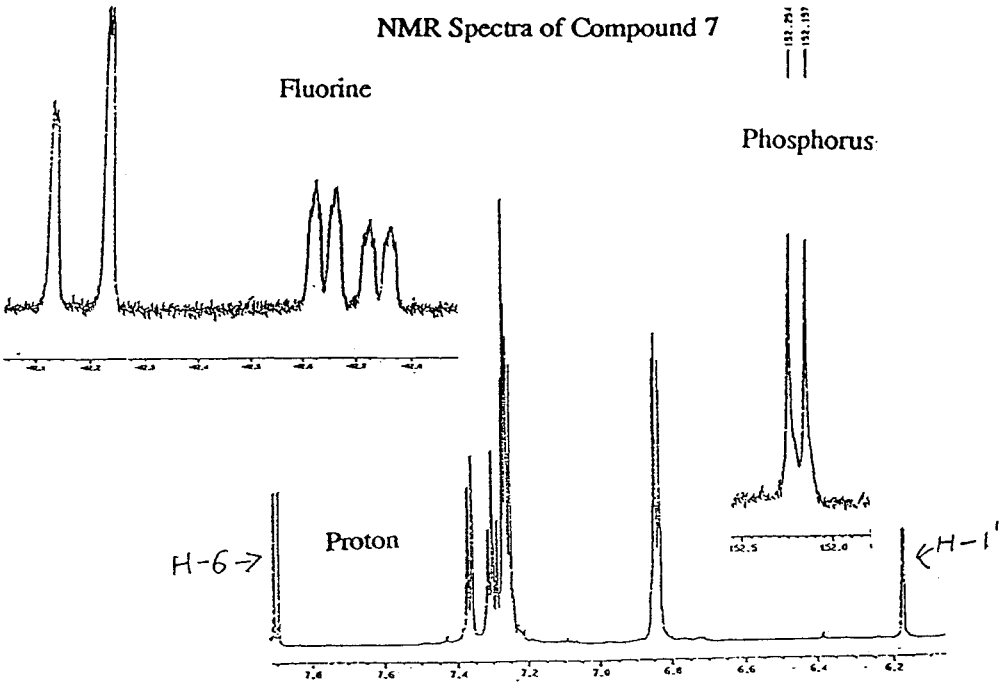
A new and efficient method was required for the introduction of nucleosidic 2'- and 3'-difluoromethylene functionality. It was found that treatment of suitably protected 2'- and 3'-ketonucleosides **1a, 1b, 2a,** and **2b** with zinc and bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide (**9**), afforded the difluoromethylene derivatives **3a, 3b, 4a, 4b** in 55-74%, respectively. Compounds **3b** and **4a** were also obtained in good yields when the Wittig reaction of **1b** and **2a** with **9**, was carried out in a sonic bath at a considerably lower temperature.

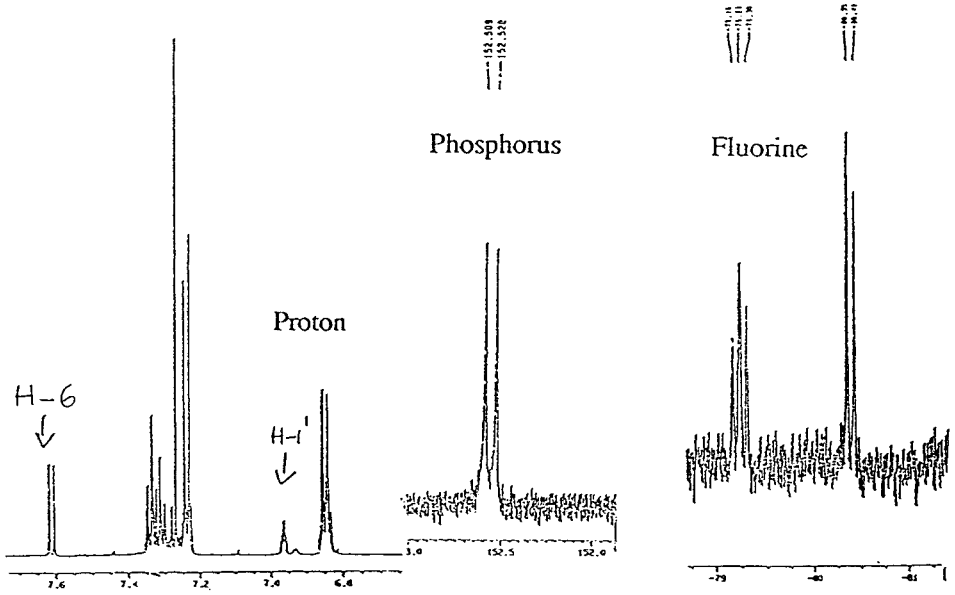
Compounds **3a, 3b, 4a** and **4b** were deprotected with tetrabutylammonium fluoride in tetrahydrofuran (THF) or ammonium fluoride in methanol to give 3'-deoxy-3'-difluoromethylene-5'-O-dimethoxytrityluridine (**5a**), 3'-deoxy-3'-difluoromethyleneuridine (**5b**), 2'-deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-



(i) $[(Me_2N)_3PCF_2Br]Br$, Zn, THF; (ii) TBAF, THF or NH_4F, MeOH; (iii) Diisopropylammonium tetrazolide, $NCCH_2CH_2OP(N^iPr_2)_2$, CH_2Cl_2; (iv) DMTrCl, pyridine.

Scheme 1





uridine (**6a**) and 2'-deoxy-2'-difluoromethyleneuridine (**6b**), respectively, in 70-96% yield. Compounds **5a** and **6a** were phosphitylated with 2-cyanoethyl-bis-diisopropylaminophosphine in the presence of diisopropylammonium tetrazolide to give the target phosphoramidites **7** and **8**.

NMR. Introduction of the difluoromethylene group resulted in considerable downfield shift of neighbouring protons and a characteristic fluorine pattern of two doublets between -80 and -90 ppm, unique to isolated, unsaturated difluoromethylene groups.

The NMR spectra of compounds **7** and **8** are quite unusual. Generally two phosphoramidite isomers can be seen by routine NMR techniques, even at a low field strength, but both the proton and phosphorus spectra for compounds **7** and **8**, measured at 250 MHz, were unresolved. The phosphorus spectra appeared as a singlet and the proton showed only one set of peaks where multiplets would be expected (e.g. H-1', H-6 seen as clean doublets). There was a slight splitting of the two fluorine doublets, which could be explained by either long range phosphorus coupling or the presence of two isomers.

The latter was confirmed by spectra recorded at a higher field (600 MHz, $CDCl_3$) (shown on the previous page), which did indeed show two very close phosphorus peaks for both compounds. The fluorine spectra at the higher field were both resolved into multiple doublets, although this was far greater for the 3'-difluoromethylene analogue, where one of the doublets was split completely into four separate peaks. Surprisingly at high field there was still no splitting of the proton spectra and hence both the anomeric and H-6 protons appeared as clean doublets (singlet in the case of H-1' for **8**).

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