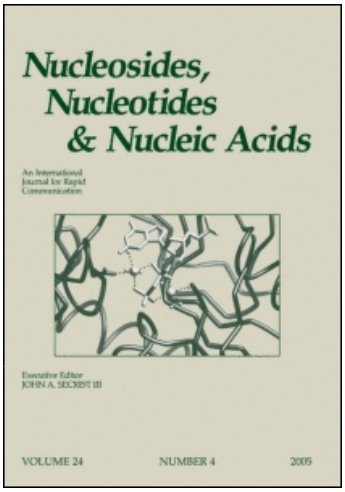

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NEW METHOD FOR THE PREPARATION OF 3′- AND 2′-*O*-PHOSPHORAMIDITES OF 2′- AND 3′-DIFLUOROMETHYLURIDINE DERIVATIVES

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ABSTRACT

Hydrogenation of 2′-deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**1**) and 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**7**), gave the corresponding 2′- and 3′-difluoromethyluridine derivatives **2a** and **8a**. Detritylation of compounds **2a**, **2b** and **8a**, **8b** resulted in the formation of 1-(2-deoxy-2-*C*-difluoromethyl-β-*D*-*arabino*-pentofuranosyl)uracil (**3a**) and 1-(3-deoxy-3-*C*-difluoromethyl-β-*D*-*xylo*-pento furanosyl)- uracil (**9a**) as well as corresponding minor isomers **3b** and **9b**. Compounds **3a** and **3b** were also obtained from 2′-deoxy-2′-difluoromethylene-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**4**). Finally, phosphitylation of **2a** and **8a** provided the title 2′- and 3′-*O*-phosphoramidites **6** and **10**.

INTRODUCTION

Various structural modifications to the carbohydrate moiety have been found to markedly enhance key properties of antisense oligonucleotides[1,2]. In particular, the 2′- and 3′-*O*-alkyl sugar modifications had been studied extensively but there was considerably less work on 2′- and 3′-*C*-alkyl

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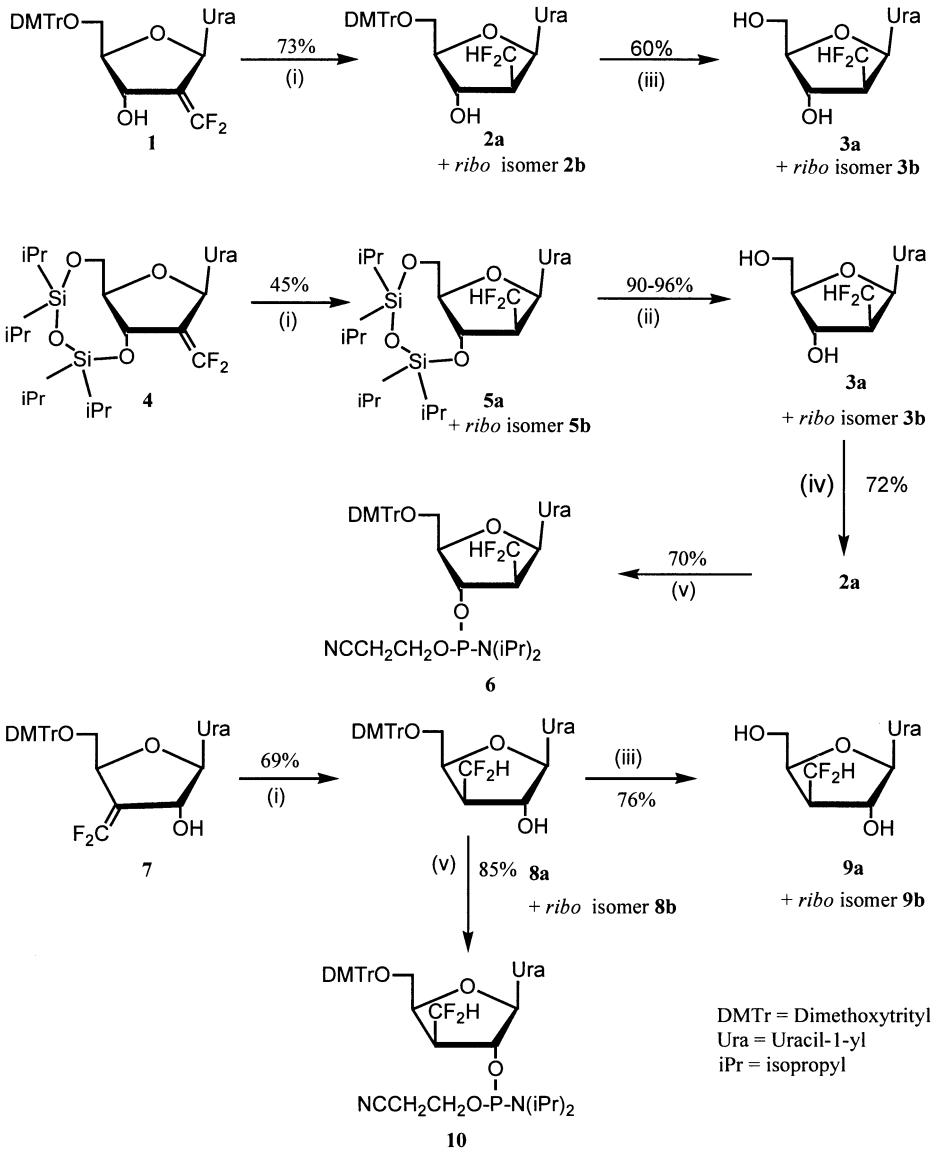
modifications. Although 2′-*C*-alkyl modified oligodeoxynucleotides have shown reduced binding affinity for complementary DNA and RNA, they exhibited increased stability against nucleases with dramatic increases being seen with 2′-*C*-methyl, methoxymethyl and phenyl groups[3]. Owing to the shortcomings of existing synthetic methods, however, oligonucleotides modified with electronegative 2′-trifluoromethyl and 2′-fluoromethyl substituents were investigated to only a limited extent while 2′- or 3′-difluoromethyl modifications were not investigated at all[3,4]. The difluoromethyl group has been proposed as an isosteric and isopolar replacement of a hydroxyl group[5–7]. The electronegativity of this group is likely to cause the sugar ring to adopt the 3′-endo conformation which is the preferred conformation of RNA in DNA/RNA hybrids[8]. Unlike CF_3 substituents, the CF_2H groups can act as a hydrogen bond donor, which may be related to the enhanced biological activity of the CF_2H compound over its CF_3 counterpart[9]. Thus, ''RNA like'' hydrogen bonding could result in oligonucleotides with higher binding affinity for target RNA[10]. Fluorine has also been identified as improving lipophilicity and hence may aid the distribution of subsequent modified oligonucleotides within an organism[5]. Until recently, the synthesis of 2′-deoxy-2′-difluoromethyl nucleosides remained unreported[11–13]. Dunkel and Reither[14] described the preparation of 2′-*C*-α-difluoromethylarauridine via the addition of difluoromethylphenylsulfone to a suitably protected 2′-oxouridine derivative[15]. Deoxygenation of the unreactive, tertiary 2′-hydroxyl function resulting from this addition, was, however, not carried out presumably due to problems with its derivatisation. Similar difficulties were encountered by us during the attempted synthesis of 3′-difluoromethylene-3′-deoxythymidine using the same approach[16]. Some 3′-deoxy-3′-*C*-difluoromethyl nucleosides were obtained by the treatment of corresponding 2′,5′-di-*O*-protected-3′-*C*-formyl nucleosides with (diethylamino)sulphur trifluoride (DAST)[17] but the syntheses of the required 3′-*C*-formyl nucleosides are low yielding and some crucial steps lack stereoselectivity[18–21]. In view of this, it was of interest to investigate the synthesis of 2′- and 3′-*C*-difluoromethyl substituted nucleoside precursors and subsequently examine the properties of oligonucleotides modified with 2′- and 3′-*C*-difluoromethyl groups. It was thought that the catalytic hydrogenation of the 2′- and 3′-difluoromethylene substituted nucleosides such as 2′-deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine and 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine reported by us previously[22,23], would provide a viable route towards 2′- and 3′-*C*-difluoromethyl substituted nucleosides which in turn could be phosphitylated to furnish the corresponding phosphoramidites, **6** and **10**, required for the automated synthesis of the target, modified oligonucleotides.

RESULTS AND DISCUSSION

2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**1**) and 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**7**), required as starting materials for the hydrogenation step, were prepared from the corresponding 2′- and 3′-oxonucleosides following the procedure reported by us earlier[22]. Compounds **1** and **7** were hydrogenated in the presence of palladium on activated carbon (10% Pd) to give the corresponding 2′-deoxy-2′-difluoromethyluridine derivatives **2a** and **2b** as well as 3′-deoxy-3′-difluoromethyluridine derivatives **8a** and **8b**, respectively. In each case the reaction showed high regioselectivity for the S isomers, **2a** and **8a**, with the R isomers, **2b** and **8b**, being formed only in a small amount. This was thought to be a result of attack by the catalyst on the least hindered α-face. Neither pair of diastereoisomers could be resolved at this stage but both the major isomers, **2a** and **8a**, were obtained pure by column chromatography on silicagel in 73% and 69% yield, respectively (**Scheme**). Removal of the dimethoxytrityl group was carried out only on the crude mixtures of **2a** and **2b** as well as **8a** and **8b** using 80% aqueous acetic acid. Thus, detritylation of the crudecompound **2a** contaminated with **2b** afforded 1-(2-deoxy-2-*C*-difluoromethyl-β-D-*arabino*-pentofuranosyl)uracil [(2′S)-2′-deoxy-2′-*C*-difluoromethyluridine] (**3a**) and the corresponding 2′R isomer, 1-(2-deoxy-2-*C*-difluoromethyl-β-D-*ribo*-pentofuranosyl)uracil [(2′R)-2′-deoxy-2′-*C*-difluoromethyluridine] (**3b**), in the ratio of six to one. Similarly, detritylation of **8a** contaminated with **8b** gave 1-(3-deoxy-3-*C*-difluoromethyl-β-*D*-*xylo*-pentofuranosyl)uracil [(3′S)-3′-deoxy-3′-*C*-difluoromethyluridine] (**9a**) and the corresponding 3′R isomer, 1-(3-deoxy-3-*C*-difluoromethyl-β-D-*ribo*-pentofuranosyl)-uracil [(3′R)-3′-deoxy-3′-*C*-difluoromethyluridine] (**9b**), in the ratio of eight to one. Each pair of diastereoisomers could be resolved at this stage by preparative HPLC. The isolated isomers were fully characterised by their MS and NMR spectra. ^{1}H-NMR of **3a**, **3b**, **9a** and **9b** showed a characteristic triplet of doublets at around 6 ppm corresponding to the CF_2H protons. ^{19}F-NMR was consistent with the presence of two diastereotopic fluorine atoms. Thus, **3a** displayed a doublet of doublets at -117 ppm and a doublet of doublets at -121 ppm whereas compounds **3b**, **9a** and **9b** showed two doublets of doublets of doublets each, between -112 and -123 ppm. These findings are in agreement with available literature data[19,24]. Configurations at the 2′-carbon and 3′-carbon for compounds **3a**, **3b**, **9a** and **9b** were assigned by 2D NOESY studies which is discussed in detail in the experimental section.

Compounds **3a** and **3b** were also obtained via a different route starting from 2′-deoxy-2′-difluoromethylene-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**4**). 2′-Deoxy-2′-difluoromethylene-3′,5′-*O*-(tetraisopropyl-



Scheme. Synthesis of 3′- and 2′-*O*-Phosphoramidites of 2′- and 3′-Difluoromethyluridine (i) 10% palladium on activated carbon, EtOH; (ii) 80% triethylamine trihydrofluoride, THF; (iii) 80% aqueous acetic acid; (iv) dimethoxytrityl chloride, pyridine; (v) diisopropyl-ammoniumtetrazolide, $NCCH_2CH_2OP(N^iPr_2)_2$, CH_2Cl_2.

disiloxane-1,3-diyl)uridine (**4**) was synthesised as described earlier[22] and was subsequently hydrogenated in an atmosphere of hydrogen over 10% palladium on charcoal to give (2′S)-2′-difluoromethyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**5a**) and the corresponding 2′R isomer **5b**. Compounds **5a** and **5b** were readily separated by column

chromatography on silica gel. The hydrogenation was rather low yielding, 45% overall yield, and took seventy two hours to complete but resulted in a more favourable, 3:2, ratio of 2′S/2′R isomers. It was of particular importance in view of the hydrogenation of compound **1** leading to the mixture of 2′S/2′R isomers in the ratio of 6:1 and paved the way for future preparation of the target phosphoramidite **6** having the 2′-difluoromethyl group in the R configuration. The observed stereoselectivity was found to be similar to that observed by Cicero and co-workers during the hydrogenation of 2′-deoxy-2′-methylene-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine[25]. Subsequent desilylation of compounds **5a** and **5b** with triethylamine trihydrofluoride[26] gave 1-(2-deoxy-2-*C*-difluoromethyl-β-*D-arabino*-pentofuranosyl)uracil (**3a**) and 1-(2-deoxy-2-*C*-difluoromethyl-β-*D-ribo*-pentofuranosyl)uracil (**3b**), respectively, in nearly quantitative yields. 1-(2-Deoxy-2-*C*-difluoromethyl-β-*D-arabino*-pentofuranosyl)uracil (**3a**) was tritylated with dimethoxytrityl chloride in pyridine to give 1-(2-deoxy-2-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D-arabino*-pentofuranosyl)uracil (**2a**) (**Scheme**) thus providing an alternative route for the preparation of compound **2a** required for the synthesis of the target phosphoramidite **6**.

The methodology for the synthesis of 2′-deoxy-2′-difluoromethyl nucleosides and 3′-deoxy-3′-difluoromethyl nucleosides described in this report combines four relatively simple and high yielding steps of oxidation, difluoromethylenation, desilylation and catalytic hydrogenation. This route is considerably simpler and more efficient than the methods suggested so far for similar compounds and would appear to be the method of choice for a large scale preparation of such derivatives.

Compounds **2a** and **8a** were phosphitylated with 2-cyanoethyl-bis-diisopropylamino-phosphine in the presence of diisopropylammonium tetrazolide[27] to give the corresponding phosphoramidites **6** and **10** in 70 and 85% respectively. The compounds were characterised by means of their MS and NMR. The ^{31}P-NMR confirmed the formation of two pairs of diastereoisomers. Each compound showed two singlets of roughly equal intensity between -150 and -153 ppm. The new phosphoramidite monomers are currently being used for the preparation of 2′- and 3′-*C*-difluoromethylated oligonucleotides having (3′–5′) or (2′–5′)-internucleotide linkages and the results will be published elsewhere[11].

EXPERIMENTAL

Melting points were determined on a Reichert micro hot stage apparatus and are uncorrected. UV spectra were measured in 95% ethanol with a Pye-Unicam SP-8-150 UV-vis spectrophotometer. ^{1}H and ^{19}F-NMR spectra, were recorded at 250 MHz using a Bruker WH-250 spectrometer with TMS or $CFCl_3$ as internal standards. The protons of 2′-OH, 3′-OH, 5′-OH, and

NHCO were exchangeable with D_2O. ^{13}C-NMR spectra with ^{1}H decoupling were recorded at 100 MHz using a Bruker AMX400. Unless otherwise indicated, DMSO-d_6 was used as the solvent. Phase sensitive NOESY was run at 400 MHz on a Bruker AMX-400 using the Bruker software package with D1 = 1.47 and D8 = 0.5 sec. ^{31}P-NMR spectra were recorded at 400 MHz on a Bruker AMX-400 spectrometer with 85% aqueous H_3PO_4 as an external standard. Mass spectra were obtained on a VG ZAB-SE spectrometer with FAB ionisation. Accurate masses were determined with MNOBA + Na as the matrix. HPTLC was run on Merck Kieselgel 60F_{254} analytical plates in the following systems: A CH_2Cl_2/EtOAc (4:1), B CH_2Cl_2/EtOH (19:1), C $CHCl_3$/MeOH (9:1), D Hexane/Acetone (6:4), E CH_2Cl_2/EtOAc (1:1), Merck Kieselgel 60H was used for short column chromatography.

Reverse phase HPLC was performed using a Waters chromatography system with a variable wavelength detector set at 254 nm and 280 nm. Columns Apex WP ODS (250 × 10 mm id, 7 μ, 300 Å) used for analytical and preparative scales were supplied by Jones Chromatography. The mobile phases were A 0.05 M aqueous $[Et_3NH]^+$ $[CH_3COO]^-$ and B MeCN. Solvent removal was performed in vacuo at 30–40°C. Other solvents used in reactions were purchased anhydrous from Aldrich. Solvents for chromatography were BDH GPR grade reagents.

2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**1**), 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**7**) and 2′-deoxy-2′-difluoromethylene-3′,5′-*O*-(tetraisopropyl-disiloxane-1,3-diyl)uridine (**4**) were prepared as described earlier (22). Palladium on activated carbon (10% Pd) was purchased from Aldrich. Triethylamine trihydrofluoride was purchased from Fluka.

Hydrogenation of 2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine(1) and 3′-Deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine (7) (General Procedure). To a solution of 2′-deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**1**) or 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**7**) (0.58 g, 1 mmol) in dry ethanol (80 mL) 10% palladium on activated carbon (0.58 g for **1** and 0.29 g for **7**) was added. The apparatus was evacuated and flushed with hydrogen three times. Each solution was then stirred at rt under an atmosphere of hydrogen for 7 h (**1**) or 18 h (**7**). The apparatus was evacuated and flushed with argon three times, the catalyst was filtered off (glass microfibre filter) and the filtrate was concentrated in vacuo. Each colourless residue was dissolved in dry dichloromethane (3 mL) and chromatographed on silicagel eluting with CH_2Cl_2/EtOH (96:4) for **2a** or CH_2Cl_2/EtOH (97:3) for **8a**.

1-(2-Deoxy-2-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*arabino*-pentofuranosyl)-uracil [(2′S)-2′-deoxy-2′-*C*-difluoromethyl-5′-*O*-dimethoxytrityluri-

dine] (2a). Yield: 0.42 g (73%); Rf 0.56 (B) 0.33 (D); colourless glass; ^{1}H-NMR δ 3.16 (m, 1H, H-2′), 3.27 (m, 2H, H-5′, H-5″), 3.74 (s, 6H, OCH_3), 3.86 (m, 1H, H-4′), 4.44 (m, 1H, H-3′), 5.27 (d, 1H, H-5, J = 8.17 Hz), 5.73 (m, 1H, 3′-OH), 6.08 (t of d, 1H, CF_2H, $J_{HF} = 50.5$ Hz, $J_{HH} = 4.72$ Hz), 6.27 (d, 1H, H-1′, J = 7.50 Hz), 6.85–7.31 (m, 13H, trityl), 7.72 (d, 1H, H-6, J = 8.17 Hz), 11.36 (s, 1H, NH); ^{19}F-NMR δ-132.3 (dd, 2F, $J_{HgemF} = 49.6$ Hz, $J_{FF} = 74.9$ Hz); observed FAB MS 579.1960, $[C_{31}H_{30}F_2N_2O_7\text{-}H]^-$ requires 579.1943.

1-(3-Deoxy-3-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*xylo*-pentofuranosyl)uracil [(3′S)-3′-deoxy-3′-*C*-difluoromethyl-5′-*O*-dimethoxytrityluridine] (8a). Yield: 0.403 g (69.4%); R_f 0.52 (B), 0.29 (D); white solid, mp 105–107 °C; ^{1}H-NMR δ 2.92 (m, 1H, H-3′), 3.24 (m, 2H, H-5′, H-5″), 3.71 (s, 6H, OCH_3), 4.30 (m, 1H, H-2′), 4.45 (m, 1H, H-4′), 5.52 (d, 1H, H-5, J = 8.10 Hz), 5.74 (d, 1H, H-1′, J = 3.40 Hz), 5.86 (d, 1H, 2′-OH, J = 5.57 Hz), 6.14 (t of d, 1H, CF_2H, $J_{HF} = 61.5$ Hz, $J_{HH} = 5.86$ Hz), 6.85–7.42 (m, 13H, trityl), 7.55 (d, 1H, H-6, J = 8.10 Hz), 11.34 (s, 1H, NH); ^{19}F-NMR δ −112.80 1F d ($J_{FF} = 267$ Hz) of d ($J_{HgemF} = 46.4$ Hz) of d ($J_{H3'F} = 13.56$ Hz), −118.17 1F d ($J_{FF} = 267$ Hz) of d ($J_{HgemF} = 55.4$ Hz) of d ($J_{H3'F} = 16.4$ Hz); observed ES MS 603.1940, $[C_{31}H_{30}F_2N_2O_7 + Na]^+$ requires 603.1919.

Detritylation of Crude 1-(2-Deoxy-2-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*arabino*-pentofuranosyl)uracil (2a/2b) or 1-(3-Deoxy-3-*C*-di- fluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*xylo*-pentofuranosyl)uracil (8a/8b) (General Procedure). Crude 1-(2-deoxy-2-*C*-difluoromethyl-5-*O*-dim- ethoxytrityl-β-*D*-*arabino*-pentofuranosyl)uracil (**2a**/**2b**) or 1-(3-deoxy-3-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*xylo*-pentofuranosyl)uracil (**8a**/**8b**) (0.58 g, 1 mmol) was dissolved in 80% aqueous acetic acid (10 mL) and the solution was stirred at rt for 30 min. The solvent was removed in vacuo, each residue was coevaporated with toluene (2 × 20 mL) and partitioned between $CHCl_3/H_2O$ (1:4, 50 mL). Each aqueous layer was extracted with chloroform (4 × 20 mL) and concentrated in vacuo. Each colourless residue was dissolved in methanol (10 mL), silicagel (0.5 g) was added, and the suspension was concentrated in vacuo. Each residue was treated with $CHCl_3$/MeOH (97.5:2.5) (5 mL) and the slurry was chromatographed on silicagel eluting with $CHCl_3$/MeOH (92.5:7.5) to give isomeric mixtures **3a**/**3b** and **9a**/**9b** as colourless froths. Each froth was dissolved in water (2 mL) and freeze dried to give compounds **3a**/**3b** and **9a**/**9b** as white powders. Analysis of 3a/3b by reverse phase HPLC (gradient elution; 5% B–60% B over 26 min) showed the main product at retention time of 7.7 min and a minor component at 8.35 min. Preparative HPLC followed by freeze drying resulted in compounds **3a** and the isomeric **3b**. Both the compounds were found to be > 98% pure by analytical reverse-phase HPLC. Analysis of

9a/**9b** by reverse phase HPLC (gradient elution; 5% B–60% B over 26 min) showed the main product at retention time of 7.62 min and a minor component at 8.23 min. Preparative HPLC followed by freeze drying resulted in compounds **9a** and the isomeric **9b**. Both the compounds were found to be $>98\%$ pure by analytical reverse-phase HPLC.

1-(2-Deoxy-2-*C*-difluoromethyl-β-*D*-*arabino*-pentofuranosyl)uracil [(2′S)-2′-deoxy-2′-*C*-difluoromethyluridine] (3a). Yield: 0.14 g (51.4%); Rf 0.14 (C); white solid, mp 172–176 °C; ^{1}H-NMR δ 3.02 (m, 1H, H-2′), 3.63 (m, 3H, H-4′, H-5′, H-5″), 4. 33 (t, 1H, H-3′ J = 7.69 Hz), 4.95 (bs, 1H, 5′-OH), 5.61 (d, 1H, H-6, J = 8.12 Hz), 5.83 (d, 1H, 3′-OH, J = 7.25 Hz), 6.02 (t of d, 1H, CF_2H, J_{HF} = 45.6 Hz, J_{HH} = 4.47 Hz), 6.21 (d, 1H, H-1′, J = 7.75 Hz), 7.84 (d, 1H, H-6, J = 8.12 Hz), 11.41 (bs, 1H, NH); ^{13}C NMR δ 51.60 (t, $J_{C\text{-}F}$ = 19 Hz, C-2′), 57.96 (C-5′), 66.31 (C-3′), 81.04 (C-1′), 83.84 (C-4′), 100.44 (C-5), 114.54 (t, $J_{C\text{-}F}$ = 240 Hz, CF_2H), 140.18 (C-6), 149.31 (C-2), 162.13 (C-4); ^{19}F-NMR δ −117.17 (1F, d (J_{FF} = 292Hz) of d (J_{HgemF} = 54.6 Hz), −121.46 (1F, d (J_{FF} = 292 Hz) of d (J_{HgemF} = 53.53 Hz); UV λ_{max}259 nm ε_{max} 6468, λ_{min} 229 nm ε_{min} 1564; Observed FAB MS 279.0770, $[C_{10}H_{12}F_2N_2O_5 + H]^+$ requires 279.0793.

1-(2-Deoxy-2-*C*-difluoromethyl-β-*D*-*ribo*-pentofuranosyl)uracil [(2′R)-2′-deoxy-2′-*C*-difluoromethyluridine] (3b). Yield 0.024 g (8.6%); Rf 0.16 (C); colourless foam; ^{1}H-NMR δ 2.89 (m, 1H, H-2′), 3.59 (m, 2H, H-5′, H-5″), 3.85 (m, 1H, H-4′), 4.36 (d, 1H, H-3′, J = 4.48 Hz), 5.12 (bs, 1H, 5′-OH), 5.69 (d, 1H, H-5, J = 8.13 Hz), 5.79 (bs, 1H, 3′-OH), 6.33 (d, 1H, H-1′, J = 8.53 Hz), 6.19 (t of d, 1H, CF_2H, J_{HF} = 55.4 Hz, J_{HH} = 6.80 Hz), 7.85 (d, 1H, H-6, J = 8.13 Hz), 11.32 (bs, 1H, NH); ^{19}F-NMR δ −114.58 (1F, d (J_{FF} = 295 Hz) of d (J_{HgemF} = 54.1 Hz) of d ($J_{H2'F}$ = 10.0 Hz), −123.51 (1F, d (J_{FF} = 295 Hz) of d (J_{HgemF} = 55.9 Hz) of d ($J_{H2'F}$ = 14.3 Hz); UV λ_{max} 260 nm ε_{max} 7426, λ_{min} 230 nm ε_{min} 1098; Observed FAB MS 279.0702, $[C_{10}H_{12}F_2N_2O_5+H]^+$requires 279.0793.

1-(3-Deoxy-3-*C*-difluoromethyl-β-*D*-*xylo*-pentofuranosyl)uracil [(3′S)-3′-deoxy-3′-*C*-difluoromethyluridine] (9a). Yield: 0.211 g (76%); R_f 0.16 (C); colourless glass; ^{1}H-NMR δ 2.87 (m, 1H, H-3′), 3.57 (m, 2H, H-5′, H-5″), 4.30 (m, 2H, H-2′, H-4′), 5.32 (bs, 1H, 5′-OH), 5.71 (m, 3H, H-1′, 2′-OH, H-5), 6.31 (t of d, 1H, CF_2H, J_{HF} = 49.08 Hz, J_{HH} = 6.88 Hz), 7.84 (d, 1H, H-6, J = 8.16 Hz), 11.34 (bs, 1H, NH); ^{13}C NMR δ 45.66 (C-2′), 49.38 (t, $J_{C\text{-}F}$ = 19.5 Hz, C-3′), 60.58 (C-5′), 76.90 (C-4′), 87.96 (C-1′), 102.40 (C-5), 116.96 (t, $J_{C\text{-}F}$ = 251 Hz, CF_2H), 140.69 (C-6), 150.92 (C-2), 163.03 (C-4); ^{19}F-NMR δ −112.08 1F d (J_{FF} = 294 Hz) of d (J_{HgemF} = 55.55 Hz) of d ($J_{H3'F}$ = 11.57 Hz), −116.75 1F d (J_{FF} = 294 Hz) of d (J_{HgemF} = 56.3 Hz) of d ($J_{H3'F}$ = 15.08 Hz); UV λ_{max} 260 nm ε_{max} 8778 λ_{min} 229 nm ε_{min} 3436; Observed ES MS 279.0802, $[C_{10}H_{12}F_2N_2O_5 + H]^+$ requires 279.0793.

1-(3-Deoxy-3-*C*-difluoromethyl-β-*D*-ribo-pentofuranosyl)uracil [(3′R)-3′-deoxy-3′-*C*-difluoromethyluridine] (9b). Yield: 0.025 g (9%); R_f 0.20 (C); colourless glass; ^{1}H-NMR δ 2.73 (m, 1H, H-3′), 3.52 (m, 1H, H-5′), 3.75 (m, 1H, H-5″), 4.34 (m, 3H, H-2′, H-4′, 5′-OH), 5.61 (d, 1H, J = 8.24 Hz, H-5) 5.68 (bs, 2H, 3′-OH, H-1′), 6.20 (t, J_{HF} = 56.1 Hz of d J_{HH} = 5.28 Hz, 1H, CF_2H), 7.98 (d, 1H, H-6, J = 8.24 Hz), 11.30 (bs, 1H, NH); ^{19}F-NMR − 116.03 1F d (J_{FF} = 290 Hz) of d (J_{HgemF} = 55.6 Hz) of d ($J_{H3'F}$ = 10.06 Hz), − 122.70 1F d (J_{FF} = 290 Hz) of d (J_{HgemF} = 57.1 Hz) of d ($J_{H3'F}$ = 18.7 Hz); UV λ_{max} 262 nm ε_{max} 8885 λ_{min} 230 nm ε_{min} 2020; Observed FAB MS 301.0600, $[C_{10}H_{12}F_2N_2O_5+Na]^+$ requires 301.0612. The stereochemistry of the products was confirmed by a series of 2D NOESY experiments.

3a – 2D NOESY showed a cross peak between H-2′ and H-4′ and at the same time the lack of a cross peak between H-2′ and H-5′. There was also a cross peak between H-6 and CF_2H. This is only possible if the H-2′ proton is on the α face.

3b – 2D NOESY showed a cross peak between H-2′ and H-5 and at the same time the lack of a cross peak between H-2′ and H-4′. This is only possible if the H-2′ proton is on the β face.

9a – 2D NOESY showed a cross peak between between H-5′ and CF_2H and at the same time the lack of a cross peak between H-3′ and H-5′. There was also a cross peak between H-6 and CF_2H. This is only possible if the H-3′ proton is on the α face.

9b – 2D NOESY showed a cross peak between H-4′ and CF_2H and at the same time the lack of a cross peak between H-5′ and CF_2H. There was also a cross peak between H-3′ and H-6. This is only possible if the H-3′ proton is on the β face.

Hydrogenation of 2′-Deoxy-2′-difluoromethylene-3′, 5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (4). To a solution of 2′-deoxy-2′-difluoro- methylene-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**4**) (0.22 g, 0.42 mmol) in dry ethanol (35 mL), 10% palladium on activated carbon (0.10 g) was added. The apparatus was evacuated and flushed with hydrogen three times. The solution was then stirred at rt under an atmosphere of hydrogen for 72 h. The apparatus was evacuated and flushed with argon three times, the catalyst was filtered off (glass microfibre filter) and the filtrate was concentrated in vacuo. The colourless residue was dissolved in dry dichloromethane (3 mL) and chromatographed on silicagel eluting with CH_2Cl_2/EtOAc (4:1).

1-[2-Deoxy-2-*C*-difluoromethyl-β-*D*-*arabino*-pentofuranosyl-3,5-*O*-(tetraisopropyl-disiloxane-1,3-diyl)]uracil [(2′S)-2′-deoxy-2′-*C*-difluoromethyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine] (5a). Yield: 0.06 g (27%); R_f 0.55 (A); colourless foam; ^{1}H-NMR δ 0.83–1.13 (m, 24H, iPr), 3.29 (m, 1H,

H-2′), 3.50 (m, 4H, iPr), 3.29 (m, 1H, H-2′), 3.84 (m, 1H, H-4′), 3.93–4.10 (m, 2H, H-5′, H-5″), 4.65 (m, 1H, H-3′), 5.60 (d, 1H, H-5, J = 7.92 Hz), 5.97 (t of d, 1H, CF_2H, $J_{HF} = 54.32$ Hz, $J_{HH} = 3.22$ Hz), 6.21 (d, 1H, H-1′, J = 7.82 Hz), 7.52 (d, 1H, H-6, J = 7.92 Hz), 11.41 (s, 1H, NH); ^{19}F-NMR δ −117.10 (1F, d of m), −122.10 (1F, d of m); Observed ES MS 543.2124, $[C_{22}H_{38}F_2N_2O_6Si_2+Na]^+$ requires 543.2134.

1-[2-Deoxy-2-*C*-difluoromethyl-β-*D*-*ribo*-pentofuranosyl-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)]uracil [(2′R)-2′-deoxy-2′-*C*-difluoromethyl-3′,5′-*O*-(tetraiso-propyldisiloxane-1,3-diyl) uridine] (5b). Yield: 0.04 g (18%); R_f 0.43 (A); colourless foam; ^{1}H-NMR δ 0.81–1.12 (m, 24H, iPr), 3.31 (m, 1H, H-2′), 3.68 (m, 4H, iPr), 3.77–4.11 (m, 3H, H-4′, H-5′, H-5″), 4.76 (m, 1H, H-3′), 5.66 (d, 1H, H-5, J = 8.10Hz), 6.07 (d, 1H, H-1′, J = 5.43 Hz), 6.26 (t of d, 1H, CF_2H, $J_{HF} = 54.60$ Hz, $J_{HH} = 4.13$ Hz), 7.65 (d, 1H, H-6, J = 8.10 Hz), 11.38 (s, 1H, NH); ^{19}F-NMR δ −117.85 (1F, d ($J_{FF} = 306$ Hz) of d ($J_{HgemF} = 57.75$ Hz) of d ($J_{H2'F} = 11.0$ Hz) , −123.56 (1F d ($J_{FF} = 307$ Hz) of d ($J_{HgemF} = 58.88$ Hz) of d ($J_{H2'F} = 22.6$ Hz); Observed ES MS 543.2120, $[C_{22}H_{38}F_2N_2O_6Si_2+Na]^+$ requires 543.2134.

Desilylation of 1-[2-Deoxy-2-*C*-difluoromethyl-β-*D*-*arabino*-pento furano- syl-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)]uracil (5a) and 1-[2-Deoxy-2-*C*-difluoromethyl-β-*D*-*ribo*-pentofuranosyl-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)]uracil (5b). Compounds **5a** (0.06 g,0.11 mmol) and **5b** (0.04 g, 0.08 mmol) were dissolved in THF (3 mL) for **5a** and 2 mL for **5b**. Triethylamine trihydrofluoride 98% (0.2 mL, 0.2 g, 12.3 mmol) (**5a**) or (0.13 mL, 0.13 g, 8.2 mmol) (**5b**) was added and each solution was stirred at rt for 2 h. The solvent was removed in vacuo and the organic layer was washed with 5% aqueous sodium bicarbonate (10 mL), water (10 mL), brine (10 mL), dried over anhydrous sodium sulphate and concentrated in vacuo to give compounds **3a** and **3b**.

1-(2-Deoxy-2-*C*-difluoromethyl-β-*D*-*arabino*-pentofuranosyl)uracil (3a). Yield: 0.031 g (96%).

1-(2-Deoxy-2-*C*-difluoromethyl-β-*D*-*ribo*-pentofuranosyl)uracil (3b). Yield: 0.019 g (90%). The spectroscopic and analytical data were consistent with those quoted above.

Dimethoxytritylation of 1-(2-Deoxy-2-*C*-difluoromethyl-β-*D*-*arabino*-pentofuranosyl)uracil (3a). Compound **3a** (0.03 g, 0.1 mmol) and dimethoxytrityl chloride (0.041 g, 0.12 mmol) were dissolved in dry pyridine (1.5 mL) and the solution was stirred at 50 °C for 4 h and then at rt for 18 h. The solvent was removed in vacuo and the residue was partitioned between $CHCl_3$/sat. aq. $NaHCO_3$ (2:1, 20 mL), the organic layer was washed with

H_2O (2×5 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (2 mL) and chromatographed on silicagel, eluting with CH_2Cl_2/EtOH (24:1), to give compound **2a** as a colourless glass.

1-(2-Deoxy-2-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*arabino*-pentofuranosyl)uracil (2a).

Yield: 0.045 g (72%). The spectroscopic and analytical data were consistent with those quoted above.

Phosphitylation of 1-[2-Deoxy-2-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*arabino*-pentofuranosyl]uracil (2a) and 1-(3-Deoxy-3-*C*-difluoromethyl-5-*O*-dimethoxytrityl-β-*D*-*xylo*-pentofuranosyl]uracil (8a) (General Procedure). Compounds **2a** or **8a** (0.58 g, 1 mmol) and bis-diisopro- pylammonium tetrazolide (0.180 g, 1.05 mmol) were dissolved in dry dichloromethane (10 mL) and the solution was stirred at rt under argon. 2-Cyanoethoxy-bis-diisopropylaminophosphine (0.37 g, 0.4 mL, 1.2 mmol) in dry dichloromethane (2.5 mL) was added and the mixture was stirred at rt under argon for 8 h. The reaction was quenched by the addition of dry dichloromethane (40 mL). The organic layer was washed with 5% aqueous sodium bicarbonate (10 mL), water (10 mL), brine (10 mL), dried over anhydrous sodium sulphate and concentrated in vacuo to give compounds **6** and **10**.

1-{2-Deoxy-2-*C*-difluoromethyl-5-*O*-dimethoxytrityl-3-*O*-[(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite]-β-*D*-*arabino*-pentofuranosyl}uracil {(2′S)-2′-deoxy-2′-*C*-difluoromethyl-5′-*O*-dimethoxytrityluridine-3′-*O*-[(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite]}(6). Yield: 0.55 g (70%); R_f 0.49, 0.36 two diasteroisomers (A); colourless glass; ^{1}H-NMR δ 1.05–1.14 (m, 14H, iPr), 2.59 (m, 1H, H-2′), 2.72 (t, 2H, J = 5.50 Hz, OCH_2CH_2CN), 3.45 (m, 2H, iPr), 3.51 (m, 2H, H-5′, H-5″), 3. 73 (s, 6H, OCH_3), 4.04 (m, 3H, OCH_2CH_2CN, H-4′), 4.67 (m, 1H, H-3′), 5.31 (d, 1H, H-5, J = 9.13 Hz), 6.10 t (J_{HF} = 56 Hz) of m, 1H, CF_2H), 6.30 (m, 1H, H-1′), 6.87–7.42 (m, 13H, trityl), 7.65 (2d, unresolved, H-6), 11.39 (bs, 1H, NH); ^{19}F-NMR δ −117.9 (d of m, 1F, CF_2H), −122.2 (d of m, 1F, CF_2H); ^{31}P-NMR δ −150.18 (s), −150.64 (s); Observed FAB MS 779.3050, [$C_{40}H_{47}F_2N_4O_8P$-H] requires 779.3021.

1-{3-Deoxy-3-*C*-difluoromethyl-5-*O*-dimethoxytrityl-2-*O*-[(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite]-β-*D*-*xylo*-pentofuranosyl}uracil {(3′S)-3′-deoxy-3′-*C*-difluoromethyl-5′-*O*-dimethoxytrityluridine-2′-*O*-[(2-cyanoethyl-*N*,*N*-diisopropyl)-phosphoramidite]}(10). Yield: 0.764 g(98%); R_f 0.44 (A); white froth, mp indef; ^{1}H-NMR δ (1.21, m, 14H, iPr), 2.88 (t, 2H, J = 6.82 Hz, OCH_2CH_2CN), 2.99 (m, 1H, H-3′), 3.48(m, 4H, H-5′, H-5″, iPr), 3.71 (s, 6H, OCH_3), 4.04 (m, 3H, OCH_2CH_2CN, H-4′), 4.51 (m, 1H, H-2′), 5.59 (d, 1H, H-5, J = 8.11 Hz), 5.89 (d, 1H, H-1′, J = 5.62 Hz), 6.18 t

($J_{HF} = 48.5$ Hz) of m, 1H, CF_2H), 6.82–7.45 (m, 13H, trityl), 7.55 (d, 1H, H-6, J = 8.11 Hz), 11.34 (s, 1H, NH); ^{19}F-NMR δ −112.58 - (−114.86) (m, 1F, CF_2H) −117.11 - (−118.99) (m, 1F, CF_2H); ^{31}P-NMR δ −152.502 (s), −152.409 (s); Observed FAB MS 779.3063, [$C_{40}H_{47}F_2N_4O_8P$-H] requires 779.3021.

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12. After completion of this manuscript, a paper appeared, ref. 13, in which the synthesis of compounds **3a** and **3b** as well as **9a** and **9b** was described using different methods than those presented in this manuscript. Thus, an ''inseparable'' mixture of compounds **3a** and **3b** was obtained starting from 2′-deoxy-2′-

difluoromethylene-3′, 5′-*O*-(tetraisopropyldisiloxa-ne-1,3-diyl)uridine (**4**)(22). The crucial hydrogenation step, however, was performed on the deprotected 2′-deoxy-2′-difluoromethyleneuridine. Similarly, an "inseparable" mixture of compounds **9a** and **9b** was obtained starting from 3′-deoxy-3′-difluoromethylene-2′, 5′-*O*-bis-t-butyldimethylsilyl uridine (22). Again, the hydrogenation step was performed on the deprotected 3′-deoxy-3′-difluoromethyleneuridine. Eventually, compound **3a** and its α-anomer were obtained via a multistep elaboration of a carbohydrate precursor and its subsequent condensation with silylated uracil. Comparison of the spectroscopic data is difficult since the NMR spectra were recorded in different solvents.

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