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New Method for the Preparation of 3'- and 2'-Phosphoramidites of 2'- and 3'-Difluoromethyleneuridine

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Abstract: The one step reaction of 2'- and 3'-keto derivatives of uridine **2a**, **2b**, **7a** and **7b** with bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide (**13**) and zinc gives the corresponding 2'- and 3'-difluoromethylene nucleosides **3a**, **3b**, **8a** and **8b** in good yield. Removal of the silyl groups affords difluoromethylenated uridines **4a**, **4b**, **9a** and **9b**. Phosphitylation of **4a** and **9a** provides the target 2'- and 3'-phosphoramidites **5** and **10** for use in oligonucleotide synthesis.

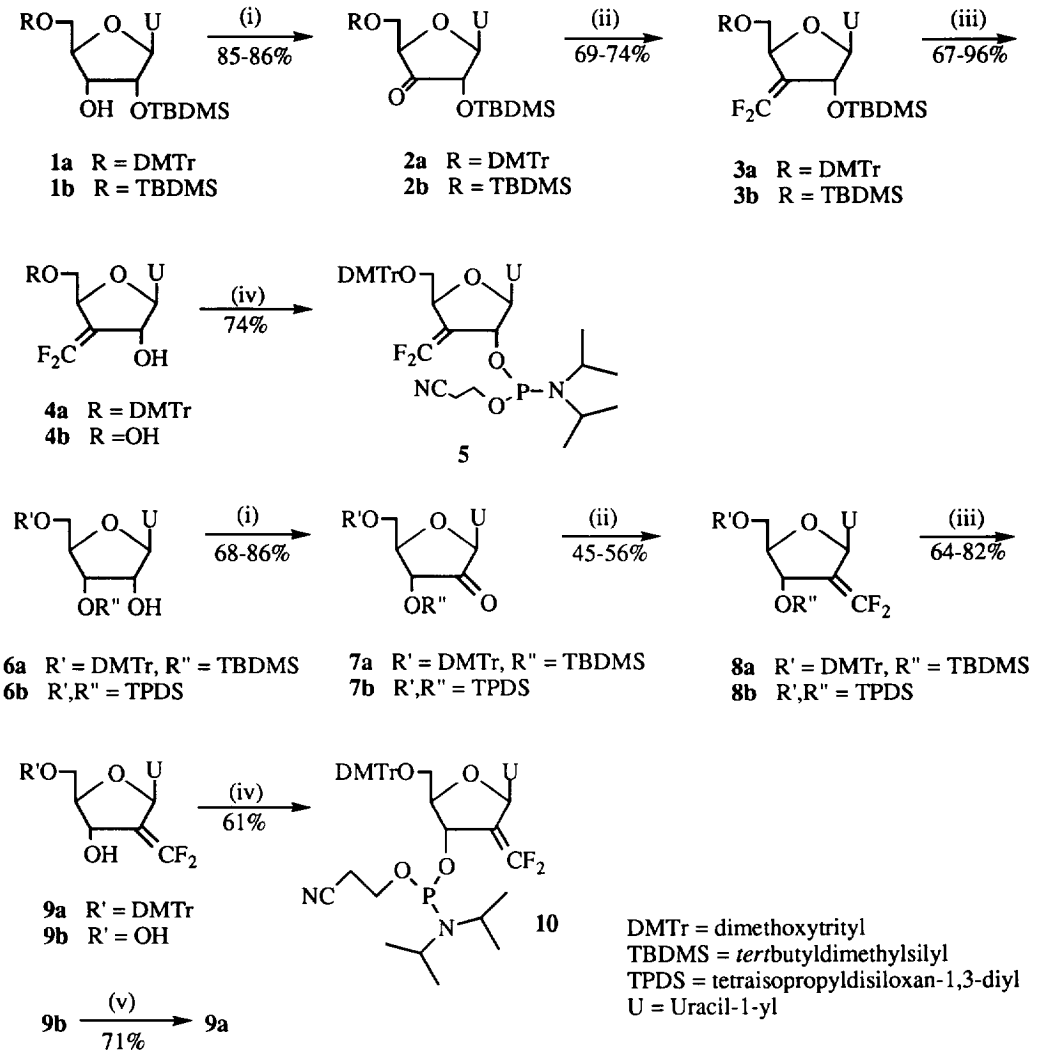
INTRODUCTION

Antisense oligonucleotides have been identified as selective inhibitors of point mutated oncogenes whose expression is linked with a variety of cancers[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[2,3]. Automated solid phase synthesis of oligonucleotides is based on the use of trivalent phosphorus intermediates such as phosphoramidites[4] or H-phosphonates[5]. For the synthesis of the target oligonucleotides we elected to use the phosphoramidite approach, which entailed preparation of the appropriate 2'- and 3'-difluoromethylenephosphoramidites of types **5** and **10**. In this report we present the synthesis of monomers in the uridine series.

RESULTS AND DISCUSSION

Phosphoramidites **5** and **10** were prepared according to the synthetic route outlined in Scheme 1. The crucial step in the synthesis is difluoromethylenation of 2'- or 3'-ketonucleosides. Currently no syntheses of 3'-difluoromethylene nucleosides have been reported. Synthetic strategies employed to prepare difluoromethylene derivatives in the 2'- series are based on the addition of difluoromethylphenylsulfone to a carbonyl group[6], Wadsworth-Emmons olefinations with difluoromethyldiphenylphosphine oxide[7,8] or Wittig type olefination with chlorodifluoroacetate/triphenylphosphine[3]. The three step difluoromethylphenylsulfone approach appeared theoretically superior to other methods due to the compatibility with the starting unstable ketonucleosides. Our attempts at this method in the synthesis of 2',3'-dideoxy-3'-difluoromethylenethymidine foundered, however, owing to problems with mesylation of the unreactive tertiary 3'-hydroxyl group produced from addition to the carbonyl[9].

We turned our attention to the Wittig olefination with the reagent generated *in situ* from dibromodifluoromethane (CF_2Br_2) and hexamethylphosphoroustriamide (HMPT). This methodology was



(i) Pyridinium dichromate, molecular sieves 3Å, CH_2Cl_2 or Dess Martin periodinane; (ii) $[(Me_2N)_3PCF_2Br]Br$, Zn, THF; (iii) TBAF, THF or NH_4F, MeOH; (iv) Diisopropylammonium tetrazolide, $NCCH_2CH_2OP(N^iPr_2)_2$, CH_2Cl_2; (v) DMTrCl, pyridine.

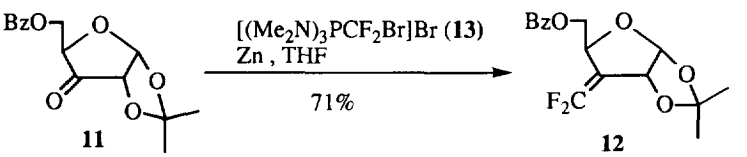
Scheme 1: Synthesis of 2'- and 3'-Difluoromethylene Uridine Derivatives

applied effectively to the preparation of various difluoromethylenated carbohydrate derivatives[10], but was reported to be unsuccessful in the nucleoside series[6]. In view of this conflicting evidence, as well as the shortcomings of methods used previously, we decided to reinvestigate applicability of the CF_2Br_2/HMPT methodology in the nucleoside series.

The suitably protected 2'- and 3'-ketonucleosides were required as starting materials in the synthesis. Oxidation of 5'-*O*-dimethoxytrityl-2'-*O*-*tert*-butyldimethylsilyluridine (**1a**)[11], 2',5'-bis-*O*-*tert*-butyldimethylsilyluridine (**1b**)[12] and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldimethylsilyluridine (**6a**)[11] with pyridinium

dichromate in dichloromethane[13] gave the corresponding 2'- and 3'-keto derivatives (**2a, 2b** and **7a**) in 68-86 % yield after column chromatography. In the case of 3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **6b**[14] it proved more expedient to use the Dess-Martin reagent[15]. The expected 2'-keto derivative **7b** was obtained in 86 % yield and did not require purification by column chromatography.

Treatment of the keto derivatives **2a** and **2b** with zinc and bromodifluoromethyl[tris(dimethylamino)]-phosphonium bromide (**13**), generated *in situ* from five equivalents of dibromodifluoromethane and HMPT[10,16], gave the expected difluoromethylene derivatives **3a** and **3b** in 37 and 68 % respectively, but yields were much lower and variable when the scale of syntheses was increased. It was therefore decided to investigate the reaction on a model carbohydrate derivative (**11**) (**Scheme 2**) in order to optimise conditions. It was found that if, instead of being made *in situ*, the quaternary phosphonium salt **13** was prepared before the reaction, much more reproducible and higher yielding results were obtained.



Scheme 2: Difluoromethylenation of the Model Carbohydrate Derivative

Reaction of 1,2-*O*-isopropylidene-5-*O*-benzoyl–α–D-furanosulose (**11**)[17] with 2 equivalents of the salt and zinc gave the expected difluoromethylene derivative **12** in 41 % yield, but when the amount of salt was increased to 3 equivalents, the isolated yield was 71 %. In contrast to a previous report[10], formation of side products deriving from partially hydrated salt were not observed.

Similar experiments in the nucleoside series also showed the yields depend strongly on the amount of salt **13** taken. Five equivalents were found to be sufficient to difluoromethylenate keto derivatives **2a, 2b** and **7b** giving **3a, 3b** and **8b** in 69, 71 and 55 % yields, respectively, after column chromatography. It is thought that the larger excess of the salt necessary in these reactions is due to the reversible formation of adducts with the pyrimidine base. 5'-*O*-Dimethoxytrityl-2'-oxo-3'-*O*-*tert*-butyldimethylsilyluridine (**7a**) seemed to be less reactive, as ten equivalents of the salt were required, with the expected 2'-deoxy-2'-difluoromethylene derivative **8a** isolated in 56 % yield. In this way, gram quantities of the difluoromethylenated nucleosides could be prepared.

Compounds **3b** and **8a** were also obtained in good yields when the Wittig reaction of **2b** and **7a** with the salt **13** was carried out in a sonic bath at considerably lower temperature. We are investigating these methods further, since they offer great potential for mild difluoromethylenation of more labile 2'- and 3'-keto nucleosides in the purine series as well as 2'-deoxy-3'-keto nucleosides and the results will be published elsewhere[9].

The structures of **3a, 3b, 8a, 8b** and **12** were unequivocally assigned by ^{1}H and ^{19}F NMR, mass spectroscopy as well as elemental analysis[18]. A particular characteristic of these compounds is the ^{19}F NMR spectrum which shows two doublets between -80 and -90 ppm; unique to isolated, unsaturated difluoromethylene groups[6,19]. Chemical shifts for individual sugar protons were assigned using homonuclear spin-spin decoupling methods. Introduction of the difluoromethylene group resulted in considerable downfield shift of neighbouring protons. The H-4' protons in the 3'-difluoromethylene derivatives **3a** and **3b** appeared at 5 ppm; whereas the shifts in the keto derivatives **2a** and **2b** were only 4.5 ppm. This effect was even more pronounced in the 2'- series, as the H-1' protons of **7a** and **7b** appeared at 5.5 ppm, whilst the H-1' protons of difluoromethylene derivatives **8a** and **8b** were around 7 ppm.

The *tert*-butyldimethylsilyl or tetraisopropyldisiloxanyl protecting groups were removed from **3a**, **3b**, **8a** and **8b** with tetra-*n*-butylammonium fluoride in tetrahydrofuran[20] or ammonium fluoride in methanol[21] to give 3'-deoxy-3'-difluoromethylene-5'-*O*-dimethoxytrityluridine (**4a**), 3'-deoxy-3'-difluoromethyleneuridine (**4b**), 2'-deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityluridine (**9a**) and 2'-deoxy-2'-difluoromethyleneuridine (**9b**), respectively, in 64-96 % yield. The use of ammonium fluoride proved more convenient since it simplified purification of the products and yields were generally higher.

2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityluridine (**9a**) was also prepared in 71 % yield by reaction of 2'-deoxy-2'-difluoromethyleneuridine (**9b**) with dimethoxytrityl chloride in pyridine[18].

Compounds **4a** and **9a** were phosphitylated with 2-cyanoethyl-bis-diisopropylaminophosphine in the presence of diisopropylammonium tetrazolide[22] to give the corresponding phosphoramidites **5** and **10** in 74 and 61 % yield, respectively. The compounds were characterised by means of their ^{1}H, ^{19}F and ^{31}P NMR as well as IR, MS and UV spectroscopy. Currently, the new phosphoramidite monomers[18] are being used for the preparation of 2'- and 3'-difluoromethylenated oligonucleotides, having 2'-5' and 3'-5' internucleotide linkages, and the results will be published at a later date[23].

EXPERIMENTAL SECTION

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. Unless otherwise indicated, DMSO-d_6 was used as the solvent. ^{31}P NMR spectra were recorded at 600 MHz on a Bruker AMX-600 spectrometer with 85 % aqueous H_3PO_4 as an external standard. In cases where analytical data are given for hydrates, the presence of water was confirmed by ^{1}H NMR. The protons of 2'-OH, 3'-OH, 5'-OH, and NHCO were exchangeable with D_2O. Mass spectra were obtained on a VG ZAB-SE spectrometer with FAB ionisation. Accurate masses were determined with MNOBA+Na as the matrix. IR spectra (KBr discs) were determined on a Perkin Elmer 1720 FT IR spectrometer. HPTLC was run on Merck Kieselgel $60F_{254}$ analytical plates in the following systems: A $CHCl_3$/MeOH (9:1), B CH_2Cl_2/EtOH (19:1), C CH_2Cl_2/EtOAc (9:1), D CH_2Cl_2/Et_3N/EtOAc (79.8:0.2:20), E Hexane/Acetone (6:4), F Hexane/EtOAc (7:3), G CH_2Cl_2/EtOAc (7:3). Merck Kieselgel 60H was used for short column chromatography.

Solvent removal was performed *in vacuo* at 30-40 °C. Tetrahydrofuran (THF) was distilled from potassium/benzophenone immediately prior to use. Other solvents used in reactions were purchased anhydrous from Aldrich. Solvents for chromatography were BDH GPR grade reagents. Uridine was purchased from Sigma. 5'-*O*-Dimethoxytrityl-2'-*O*-*tert*-butyldimethylsilyluridine (**1a**), 2',5'-bis-*O*-*tert*-butyldimethylsilyl-uridine (**1b**), 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldimethylsilyluridine (**6a**) and 3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)uridine (**6b**) were made according to the procedures described by Hakimelahi *et al*[11], Samano and Robins[12] and Markiewicz[14]. The 12-I-5 triacetoxyperiodinane (the Dess-Martin Periodinane) was obtained as recommended by Dess and Martin[15] whereas 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-furanosulose (**11**) according to Serra *et al*[17]. Bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide (**13**) was prepared essentially as described by Houlton *et al* [10] and Riesel *et al*[16]. Zinc was activated according to Hu *et al* [24].

Oxidation of protected nucleosides **1a**, **1b** *and* **6a** *(General Procedure)*

A solution of 5'-*O*-dimethoxytrityl-2'-*O*-*tert*-butyldimethylsilyluridine (**1a**)[11], 2',5'-bis-*O*-*tert*-butyldimethylsilyluridine (**1b**)[12] or 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldimethylsilyluridine (**6a**)[11] (10 mmol) in CH_2Cl_2 (50 mL) was added with a syringe, under argon, to a stirred suspension of pyridinium dichromate (7.50 g, 20 mmol) and powdered 3Å molecular sieves (6.55 g) in CH_2Cl_2 (75 mL). Each mixture was stirred at r.t. for 18 h and then concentrated and applied onto a column of silica gel. The columns were eluted with

CH_2Cl_2/EtOAc (9:1) to give the products **2a, 2b** and **7a** as colourless foams.

5'-O-Dimethoxytrityl-3'-oxo-2'-O-tert-butyldimethylsilyluridine (**2a**). Yield: 5.60 g (86 %); Rf 0.37 (B), 0.56 (E), 0.23 (F); mp 105-107 °C; δ_H 0.01 (s, 3H, CH_3), 0.06 (s, 3H, CH_3), 0.83 (s, 9H, t-Bu), 3.06 (dd, 1H, H-5', *J*=1.89 Hz, *J*=8.37 Hz), 3.46 (m, 1H, H-5''), 3.74 (s, 6H, OMe), 4.56 (d, 1H, H-4', *J*=4.06 Hz), 4.68 (d, 1H, H-2', *J*=7.71 Hz), 5.73 (d, 1H, H-5, *J*=8.11 Hz), 6.17 (d, 1H, H-1', *J*=7.68 Hz), 6.87-7.36 (m, 13H, trityl), 7.83 (d, 1H, H-6, *J*=8.11 Hz), 11.56 (s, 1H, NH); IR C=O 1782 cm^{-1}; UV λ_{max} 235 nm, ε 22490, λ_{min} 224 nm, ε 20605; Observed FAB MS 681.2603, $C_{36}H_{42}N_2O_8SiNa$ requires 681.2608; Found: C 64.38, H 6.61, N 4.05 %, $C_{36}H_{42}N_2O_8Si.0.75H_2O$ requires C 64.31, H 6.52, N 4.16 %.

2',5'-Bis-O-tert-butyldimethylsilyl-3'-oxouridine (**2b**). Yield: 4.0 g (85 %); Rf 0.48 (F); IR C=O 1769 cm^{-1}. The spectroscopic data were in agreement with those quoted in the literature[12].

5'-O-Dimethoxytrityl-2'-oxo-3'-O-tert-butyldimethylsilyluridine (**7a**). Yield: 4.45 g (68 %); Rf 0.32 (B), 0.44 (E), 0.13 (F); mp 110-112 °C; δ_H -0.11 (s, 3H, CH_3), 0.02 (s, 3H, CH_3), 0.74 (s, 9H, t-Bu), 3.10 (m, 1H, H-5'), 3.26 (m, 1H, H-5''), 3.73 (s, 6H, OMe), 4.04 (m, 1H, H-4'), 4.80 (d, 1H, H-3', *J*=9.11 Hz), 5.53 (s, 1H, H-1'), 5.74 (d, 1H, H-5, *J*=7.68 Hz), 7.38-6.84 (m, 13H, trityl), 7.42 (d, 1H, H-6, *J*=6.95 Hz), 11.67 (s, 1H, NH); IR C=O 1779 cm^{-1}; UV λ_{max} 234 nm, ε 22692, λ_{min} 224 nm, ε 20667; Observed FAB MS 681.2605, $C_{36}H_{42}N_2O_8SiNa$ requires 681.2608; Found: C 65.52, H 6.48, N 4.27 %, $C_{36}H_{42}N_2O_8Si$ requires C 65.63 H 6.43 N 4.25 %.

*Oxidation of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine (**6b**) with the Dess-Martin reagent*

3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)uridine (**6b**)[14] (3.37 g, 7 mmol) in dry CH_2Cl_2 (25 mL) was added with a syringe, under argon, to a solution of the Dess-Martin reagent (6.0 g, 14 mmol) in dry CH_2Cl_2 (60 mL) at 0-5 °C. The resulting mixture was stirred at 0-5 °C for 20 min and then at r.t. for 18 h. The reaction was quenched with diethyl ether (210 mL), poured onto an ice cold, saturated aqueous solution of $NaHCO_3$ (140 mL) containing $Na_2S_2O_3.5H_2O$ (17.5 g) and stirred for 5 min. The organic layer was washed with sat. aq. $NaHCO_3$ (2x40 mL), water (50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give compound **7b** as a colourless solid.

2'-Oxo-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine (**7b**). Yield: 2.92 g (86 %); Rf 0.28 (C) 3 developments, 0.51 (E); IR C=O 1787 cm^{-1}. The spectroscopic data were in agreement with those quoted in the literature[12].

Difluoromethylenation of 1,2-O-isopropylidene-5-O-benzoyl-α-D-furanosulose (**11**)

1,2-*O*-Isopropylidene-5-*O*-benzoyl-α-D-furanosulose (**11**)[17] (0.29 g, 1 mmol), bromodifluoromethyl-[tris(dimethylamino)]phosphonium bromide (**13**) [10,16] (1.1 g, 3 mmol) and powdered activated, zinc[24] (0.32 g, 5 mmol) were suspended in THF (10 mL) and the mixture was stirred magnetically under reflux for 40 min. The solid was filtered off on a glass microfibre filter and the filtrate was concentrated *in vacuo*. The oily, yellowish, residue was partitioned between $CHCl_3$/sat. aq. $NaHCO_3$ (4:1, 50 mL). The aqueous layer was extracted with $CHCl_3$ (2x10 mL) and the combined chloroform extracts were washed with water (2x10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The oily residue was dissolved in CH_2Cl_2 (2 mL) and applied onto a column of silica gel. The column was eluted with CH_2Cl_2 to give product **12** as a colourless oil.

5-O-Benzoyl-3-deoxy-3-difluoromethylene-1,2-isopropylidene-α-D-furanose (**12**). Yield: 0.31 g (71 %); Rf 0.57 (E); δ_H ($CDCl_3$) 1.43 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 4.36-4.63 (m, 2H, H-5), 5.23 (m, 1H, H-4), 5.32 (m, 1H, H-2), 6.00 (d, 1H, H-1, J=4.03 Hz), 7.42-8.03 (m, 5H, benzoyl); δ_F ($CDCl_3$) -81.70 (d, 1F, $J_{F\text{-}F}$=38.6 Hz), -83.01 (d, 1F, $J_{F\text{-}F}$=38.4 Hz); IR C=CF_2 1771 cm^{-1}; UV λ_{max} 230 nm, ε 11512, λ_{min} 213 nm, ε 4083; Observed EI MS 327.1040, $C_{16}H_{16}F_2O_5$ requires 327.1044; Found: C 58.66, H 5.03 %, $C_{16}H_{16}F_2O_5$ requires C 58.90, H 4.94 %.
When compound **11** reacted with 2 equivalents of bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide and 4 equivalents of zinc, under similar conditions, the yield was 41 %.

Difluoromethylenation of protected ketonucleosides **2a**, **2b**, **7a** *and* **7b** *(General Procedure)*

5'-*O*-Dimethoxytrityl-3'-oxo-2'-*O*-*tert*-butyldimethylsilyluridine (**2a**), 2',5'-bis-*O*-*tert*-butyldimethylsilyl-3'-oxouridine (**2b**), 5'-*O*-dimethoxytrityl-2'-oxo-3'-*O*-*tert*-butyldimethylsilyluridine (**7a**) or 2'-oxo-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**7b**) (1 mmol), bromodifluoromethyl[tris(dimethylamino)]-phosphonium bromide (**13**) (1.85 g, 5 mmol for **2a**, **2b** and **7b** or 3.70 g, 10 mmol for **7a**), powdered activated zinc (0.48 g, 7.5 mmol for **2a**, **2b** and **7b** or 0.96 g, 15 mmol for **7a**) were suspended in THF (20 mL) and the mixture was stirred magnetically under reflux for 25 min (**2a**, **2b**, **7b**) or 35 min (**7a**).

The solid was filtered off on a glass microfibre filter and the filtrate was concentrated *in vacuo*. Each brownish residue was partitioned between $CHCl_3$/sat. aq. $NaHCO_3$ (3:1, 80 mL). The aqueous layer was extracted with $CHCl_3$ (2x10 mL) and the combined chloroform extracts were washed with water (2x15 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Each oily residue was dissolved in CH_2Cl_2 (3 mL) and applied onto a column of silica gel. The column was eluted with CH_2Cl_2/EtOH (98.5:1.5) (**3a**, **3b**, **8b**) or with CH_2Cl_2/Et_3N/EtOAc (95.75:0.25:4) (**8a**) to give the products as pale yellow solids (**3a**, **3b**, **8a**) or a colourless glass (**8b**).

3'-Deoxy-3'-difluoromethylene-5'-O-dimethoxytrityl-2'-O-tert-butyldimethylsilyluridine (**3a**). Yield: 0.48 g (69 %); Rf 0.38 (B), 0.58 (E), 0.28 (F); mp 85-88 °C; δ_H -0.04 (s, 3H, CH_3), 0.04 (s, 3H, CH_3), 0.82 (s, 9H, t-Bu), 3.18-3.33 (m, 2H, H-5', H-5''), 3.74 (s, 6H, OMe), 5.00 (m, 1H, H-4'), 5.13 (m, 1H, H-2'), 5.50 (d, 1H, H-5, J=8.01 Hz), 5.85 (d, 1H, H-1', J=5.19 Hz), 6.87-7.37 (m, 13H, trityl), 7.76 (d, 1H, H-6, J=8.08 Hz), 11.50 (s, 1H, NH); δ_F -83.06 (d, 1F, $J_{F\text{-}F}$=47.8 Hz), -86.66 (d, 1F, $J_{F\text{-}F}$=47.5 Hz); IR C=CF_2 1772 cm^{-1}; UV λ_{max} 234 nm, ε 11850, λ_{min} 224 nm, ε 10495; Observed FAB MS 715.2622, $C_{37}H_{42}F_2N_2O_7NaSi$ requires 715.2627; Found: C 62.41, H 5.96, N 4.03 %, $C_{37}H_{42}F_2N_2O_7$ Si.H_2O requires C 62.52, H 6.24, N 3.94 %.

2',5'-Bis-O-tert-butyldimethylsilyl-3'-deoxy-3'-difluoromethyleneuridine (**3b**). Yield: 0.358 g (71 %); Rf 0.56 (F); mp indef.; δ_H -0.04 (s, 6H, CH_3), 0.04 (s, 6H, CH_3), 0.83 (s, 9H, t-Bu) 0.88 (s, 9H, t-Bu), 3.82 (m, 2H, H-5', H-5''), 4.92 (m, 2H, H-2' H-4'), 5.68 (d, 1H, H-5, J=8.07 Hz), 5.84 (d, 1H, H-1', J=5.13 Hz), 7.73 (d, 1H, H-6, J=8.09 Hz), 11.45 (bs, 1H, NH); δ_F -83.05 (d, 1F, $J_{F\text{-}F}$=47.8 Hz), -86.66 (d, 1F, $J_{F\text{-}F}$=47.5 Hz); IR C=CF_2 1780 cm^{-1}; UV λ_{max} 260 nm, ε 10221, λ_{min} 223, nm, ε 2710; Observed FAB MS 527.2180, $C_{22}H_{38}F_2N_2O_5Si_2Na$ requires 527.2185; Found: C 52.31, H 7.63, N 5.51 %, $C_{22}H_{38}F_2N_2O_5Si_2$ requires C 52.35, H 7.59, N 5.55 %.

2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-3'-O-tert-butyldimethylsilyluridine (**8a**). Yield: 0.390 g (56 %); Rf 0.38 (B), 0.58 (E), 0.28 (F); mp 87-91 °C; δ_H ($CDCl_3$) -0.13 (s, 3H, CH_3), 0.06 (s, 3H, CH_3), 0.81 (s, 9H, t-Bu), 3.34-3.50 (m, 2H, H-5', H-5''), 3.80 (s, 6H, OMe), 4.05 (m, 1H, H-4'), 5.20 (m, 1H, H-3'), 5.31 (d, 1H, H-5, J=7.83 Hz), 6.81-7.33 (m, 14H, trityl, H-1'), 7.61 (d, 1H, H-6, J=8.14 Hz); δ_F -81.05 (d, 1F, $J_{F\text{-}F}$=33.2 Hz), -81.51 (d, 1F, $J_{F\text{-}F}$=33.4 Hz); IR C=CF_2 1769 cm^{-1}; UV λ_{max} 234 nm, ε 26040, λ_{min} 223 nm, ε 24371; Observed FAB MS 715.2623, $C_{37}H_{42}F_2N_2O_7SiNa$ requires 715.2627; Found:

C 64.20, H 5.82, N 3.87 %, $C_{37}H_{42}F_2N_2O_7Si$ requires C 64.14, H 6.11, N 4.04 %.

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine (**8b**)[18]. Yield: 0.284 g (55 %); Rf 0.28 (B), 0.60 (E); δ_H 0.94-1.10 (m, 24H, iPr), 3.87 (m, 4H, iPr), 3.95 (m, 2H, H-5', H-5''), 3.99 (m, 1H, H-4'), 5.34 (m, 1H, H-3'), 5.67 (d, 1H, H-5, *J*=8.05 Hz), 6.66 (s, 1H, H-1'), 7.72 (d, 1H, H-6, *J*=8.05 Hz), 11.48 (bs, 1H, NH); δ_F -80.98 (d, 1F, J_{F-F}=40.3 Hz), -84.85 (d, 1F, J_{F-F}=39.2 Hz); IR C=CF_2 1774 cm^{-1}; UV λ_{max} 257 nm, ε 6182, λ_{min} 230 nm, ε 2748; Observed FAB MS 541.1970, $C_{22}H_{36}F_2N_2O_6Si_2Na$ requires 541.1978.

Difluoromethylenation of protected ketonucleosides **2b** *and* **7a** *in a sonic bath (General Procedure)*

2',5'-Bis-*O*-*tert*-butyldimethylsilyl-3'-oxouridine (**2b**) or 5'-*O*-dimethoxytrityl-2'-oxo-3'-*O*-*tert*-butyldimethylsilyluridine (**7a**) (1 mmol), bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide (**13**) (1.85 g, 5 mmol for **2b** or 3.70 g, 10 mmol for **7a**), powdered activated zinc (0.48 g, 7.5 mmol for **2b** or 0.96 g, 15 mmol for **7a**) were suspended in dry THF (20 mL) and the mixture was sonicated in a sonic bath (Camlab Transsonic T460/H) at 25-40 °C for 2 hours. The solid was filtered off on a glass microfibre filter and the filtrate was concentrated *in vacuo*. Each yellowish residue was worked up and purified as described above to give the products **3b** and **8a** as pale yellow solids, identical to those obtained by difluoromethylenation under reflux.

2',5'-Bis-O-tert-butyldimethylsilyl-3'-deoxy-3'-difluoromethyleneuridine (**3b**). Yield: 0.358 g (74 %).

2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-3'-O-tert-butyldimethylsilyluridine (**8a**). Yield: 0.390 g (45 %).

Desilylation of protected difluoromethylene nucleosides **3a**, **3b**, **8a** *and* **8b** *with ammonium fluoride in methanol (General Procedure)*

3'-Deoxy-3'-difluoromethylene-5'-*O*-dimethoxytrityl-2'-*O*-*tert*-butyldimethylsilyluridine (**3a**), 2',5'-bis-*O*-*tert*-butyldimethylsilyl-3'-deoxy-3'-difluoromethyleneuridine (**3b**), 2'-deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldimethylsilyluridine (**8a**) or 2'-deoxy-2'-difluoromethylene-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**8b**) (1 mmol) were dissolved in dry methanol (10 mL for **3a**, **8a** or 20 mL for **3b**, **8b**) and 0.5 M ammonium fluoride in methanol (10 mL for **3a**, 20 mL for **3b** and **8b** or 15 mL for **8a**) was added with a syringe under argon. Each solution was heated under reflux for 55 min (**3a**, **8b**), 90 min (**3b**) or 70 min (**8a**). In the case of **4a** and **9a** the solvent was removed *in vacuo*, and the residue was partitioned between $CHCl_3/H_2O$ (4:1, 50 mL). The organic layer was washed with H_2O (2x10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude products **4a** and **9a** as colourless solids. The crude products were chromatographically homogeneous and could be used in the next step without further purification. Analytical samples were prepared after each colourless solid was dissolved in CH_2Cl_2 (3 mL) and applied onto a column of silica gel. The columns were eluted with CH_2Cl_2/EtOH (97:3) to give products **4a** and **9a**, respectively, as colourless froths. In the case of **4b** and **9b** silica gel (0.5 g) was added and the suspension was concentrated *in vacuo*. Each residue was treated with $CHCl_3$/MeOH (97:3) (5 mL) and the resulting slurry was applied onto a column of silica gel. The columns were eluted with $CHCl_3$/MeOH (91:9) to give **4b** and **9b**, respectively, as colourless solids. Each solid was dissolved in water (2 mL) and the solution was freeze-dried to give compounds **4b** and **9b** as fluffy powders.

3'-Deoxy-3'-difluoromethylene-5'-O-dimethoxytrityluridine (**4a**). Yield: 0.554 g (96 %); Rf 0.26 (B), 0.33 (G); mp 91-96 °C; δ_H 3.27 (m, 2H, H-5', H-5''), 3.74 (s, 6H, OMe), 4.96 (m, 2H, H-2', H-4'), 5.38 (d, 1H, H-5, *J*=8.04 Hz), 5.85 (d, 1H, H-1', *J*=4.43 Hz), 6.15 (d, 1H, 2'-OH, *J*=6.30 Hz), 6.87-7.34 (m,

13H, trityl), 7.70 (d, 1H, H-6, J=8.01 Hz), 11.43 (s, 1H, NH); δ_F -83.91 (d, 1F, $J_{F\text{-}F}$=47.8 Hz), -86.43 (d, 1F, $J_{F\text{-}F}$=47.1 Hz); UV λ_{max} 234 nm, ε 20289, λ_{min} 223 nm, ε 18607; Observed FAB MS 601.1762, $C_{31}H_{28}F_2N_2O_7SiNa$ requires 601.1766; Found: C 62.92, H 5.00, N 4.64 %, $C_{31}H_{28}F_2N_2O_7.0.75H_2O$ requires C 62.89, H 5.07, N 4.73 %.

3'-Deoxy-3'-difluoromethyleneuridine (**4b**). Yield: 0.215g (78 %); Rf 0.17 (A); mp indef.; δ_H 3.59 (m, 2H, H-5', H-5''), 4.80 (m, 2H, H-2', H-4'), 5.13 (d, 1H, 2'-OH, J=4.53 Hz), 5.68 (d, 1H, H-5, J=8.06 Hz), 5.82 (d, 1H, H-1', J=4.43 Hz), 6.09 (m, 1H, 5'-OH), 7.82 (d, 1H, H-6, J=8.09 Hz), 11.37 (bs, 1H, NH); δ_F -84.26 (d, 1F, $J_{F\text{-}F}$=49.2 Hz), -87.45 (d, 1F, $J_{F\text{-}F}$=48.8 Hz); UV λ_{max} 260 nm, ε 10665, λ_{min} 230 nm, ε 2956; Observed EI MS 277.0632, $C_{10}H_{10}F_2N_2O_5$ requires 277.0636; Found: C 43.17, H 3.73, N 9.87 %, $C_{10}H_{10}F_2N_2O_5$ requires C 43.49, H 3.65, N 10.14 %.

2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityluridine (**9a**). Yield: 0.473g (82 %); Rf 0.32 (B), 0.43 (G); mp 92-94 °C; δ_H 3.25 (m, 2H, H-5', H-5''), 3.73 (s, 6H, OMe), 3.95 (m, 1H , H-4'), 4.83 (m, 1H, H-3'), 5.52 (1H, H-5, J=8.03 Hz), 5.88 (d, 1H, 3'-OH J=7.10 Hz), 6.74 (s, 1H, H-1'), 6.80-7.35 (m, 13H , trityl), 7.63 (d, 1H, H-6, J=8.07 Hz), 11.46 (s, 1H, NH); δ_F -81.86 (d, 1F, $J_{F\text{-}F}$=38.6 Hz), -84.58 (d, 1F, $J_{F\text{-}F}$=38.4 Hz); UV λ_{max} 234 nm, ε 22127, λ_{min} 223 nm, ε 19301; Observed FAB MS 601.1762, $C_{31}H_{28}F_2N_2O_7Na$ requires 601.1766; Found: C 63.97, H 5.11, N 4.94 %, $C_{31}H_{28}F_2N_2O_7.0.25H_2O$ requires C 63.86, H 4.93, N 4.80 %.

2'-Deoxy-2'-difluoromethyleneuridine (**9b**)[18]. Yield: 0.190 g (69 %); Rf 0.14 (A); mp indef.; δ_H 3.61 (m, 2H, H-5', H-5''), 3.80 (m, 1H, H-4'), 4.82 (m, 1H, H-3'), 4.99 (d, 1H, J=4.51 Hz, 5'-OH), 5.68 (d, 1H, H-6, J=8.08 Hz), 5.84 (d, 1H, 3'-OH, J=6.94 Hz), 6.73 (s, 1H, H-1'), 7.67 (d, 1H, H-5, J=8.09 Hz), 11.41 (bs, 1H, NH); δ_F -82.83 (d, 1F, $J_{F\text{-}F}$=40.2 Hz), -84.36 (d, 1F, $J_{F\text{-}F}$=40.5 Hz); UV λ_{max} 257 nm, ε 9457, λ_{min} 228 nm, ε 2574; Observed EI MS 277.0632, $C_{10}H_{10}F_2N_2O_5$ requires 277.0636; Found: C 41.73, H 3.89, N 9.81 %, $C_{10}H_{10}F_2N_2O_5.0.75H_2O$ requires C 41.45, H 4.00, N 9.67 %.

Desilylation of protected difluoromethylene nucleosides **3a**, **3b** *and* **8a** *with tetrabutylammonium fluoride in THF (General Procedure)*

3'-Deoxy-3'-difluoromethylene-5'-*O*-dimethoxytrityl-2'-*O*-*tert*-butyldimethylsilyluridine (**3a**), 2'-deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldimethylsilyluridine (**8a**) or 2',5'-bis-*O*-*tert*-butyldimethylsilyl-3'-deoxy-3'-difluoromethyleneuridine (**3b**) (1 mmol) were dissolved in THF (33 mL) and 0.5 M tetrabutylammonium fluoride (2.4 mL, 1.2 mmol for **3a** and **8a** or 4.8 mL, 2.4 mmol for **3b**) was added with a syringe, under argon. Each solution was stirred at r.t. for 35 min (**3a**), 15 min (**3b**) or 20 min (**8a**). The solvent was removed *in vacuo*, and in the case of **4a** and **9a** the residue was partitioned between $CHCl_3/H_2O$ (4:1, 50 mL) and the organic layer was washed with H_2O (2x10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude products **4a** and **9a** as colourless glasses. Each colourless glass was dissolved in CH_2Cl_2 (3 mL) and applied onto a column of silica gel. The columns were eluted with CH_2Cl_2/EtOH (97:3) to give **4a** and **9a** as colourless froths. In the case of **4b** silica gel (0.5 g) and MeOH (20 mL) were added and the suspension was concentrated *in vacuo* and the residue was applied onto a column of silica gel as described above for the same compound obtained *via* desilylation with ammonium fluoride.
The products were identical to those obtained by desilylation with ammonium fluoride.

3'-Deoxy-3'-difluoromethylene-5'-O-dimethoxytrityluridine (**4a**). Yield: 0.404 g (70 %).

3'-Deoxy-3'-difluoromethyleneuridine (**4b**). Yield: 0.185 g (67 %).

2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityluridine (**9a**). Yield: 0.37 g (64 %).

Dimethoxytritylation of 2'-deoxy-2'-difluoromethyleneuridine (**9b**)[18]

2'-Deoxy-2'-difluoromethyleneuridine (**9b**) (0.276 g, 1 mmol) and dimethoxytrityl chloride (0.41 g, 1.2 mmol) were dissolved in dry pyridine (15 mL) and the solution was stirred at 50 °C for 4 h and then at r.t. for 18 h. The solvent was removed *in vacuo* and the residue was partitioned between $CHCl_3$/sat. aq. $NaHCO_3$ (2:1, 60 mL), the organic layer was washed with H_2O (2x10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (3 mL) and applied onto a column of silica gel. The column was eluted with CH_2Cl_2/EtOH (97:3) to give compound **9a** as a colourless froth.

2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityluridine (**9a**). Yield: 0.411 g (71 %). The product was identical to that obtained by desilylation of **8a**.

Phosphitylation of nucleosides **4a** *and* **9a**

3'-Deoxy-3'-difluoromethylene-5'-*O*-dimethoxytrityluridine (**4a**) or 2'-deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityluridine (**9a**) (0.578 g, 1 mmol) and diisopropylammonium tetrazolide (0.180 g, 1 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and 2-cyanoethoxy-bis-diisopropylaminophosphine (0.37 g, 0.4 mL, 1.2 mmol) in dry CH_2Cl_2 (2 mL) was added with a syringe under argon. Each mixture was stirred at r.t. for 8 h and then the reaction was quenched by addition of CH_2Cl_2/Et_3N (99.75:0.25) (50 mL). The solutions were washed with sat. aq. $NaHCO_3$ (2x10 mL), water (2x10 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Each oily residue was dissolved in CH_2Cl_2/Et_3N (99.75:0.25) (3 mL) and applied onto a silica gel column packed in CH_2Cl_2/Et_3N (99.75:0.25). The column was eluted with CH_2Cl_2/Et_3N/EtOAc (95.75:0.25:4) to give the products **5** and **10** as colourless froths.

3'-Deoxy-3'-difluoromethylene-5'-O-dimethoxytrityluridine-2'-O-[(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite] (**5**). Yield: 0.576 g (74 %); Rf 0.31 (D); mp indef.; δ_H 1.07-1.24 (m, 12H, iPr), 2.71 (t, 2H, $NCCH_2$), 3.28(m, 2H, H-5', H-5''), 3.57 (m, 4H, $POCH_2$, iPr), 3.73 (s, 6H, OMe), 5.03 (m, 1H, H-4'), 5.19 (d, 1H, H-5, *J*=8.16 Hz), 5.99 (d, 1H, H-1', *J*=4.35 Hz), 6.85-7.37 (m, 13H, trityl), 7.75 (d, 1H, H-6, *J*=8.07 Hz), 11.45 (s, 1H, NH); δ_F -82.85 (d, 1F, $J_{F\text{-}F}$=43.5 Hz), -83.89 (m, 1F); δ_P (600 MHz; $CDCl_3$) 152.53 (s), 152.59 (s); UV λ_{max} 234 nm, ε 21540, λ_{min} 226 nm, ε 20474; Observed FAB MS 801.2847, $C_{40}H_{45}F_2N_4O_8PNa$ requires 801.2841.

2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityluridine-3'-O-[(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite] (**10**). Yield: 0.475 g (61 %); Rf 0.42 (D); mp indef.; δ_H ($CDCl_3$) 1.07-1.29 (m, 12H, iPr), 2.24 (t, 2H, CH_2CN), 3.55 (m, 6H, H-5', H-5'', $POCH_2$, iPr), 3.79 (s, 6H, OMe), 4.26 (m, 1H, H-4'), 5.19 (d, 1H, H-5, *J*=8.16 Hz), 5.32 (d, 1H, H-3', *J*=7.60 Hz), 6.80-7.35 (m, 14H, trityl, H-1'), 7.61 (d, 1H, H-6, *J*=8.11 Hz); δ_F ($CDCl_3$) -79.30 (m, 1F), -80.48 (d, 1F, $J_{F\text{-}F}$=28.3Hz); δ_P (600 MHz; $CDCl_3$) 152.16 (s), 152.25 (s); UV λ_{max} 234 nm, ε 22822, λ_{min} 230 nm, ε 21670; Observed FAB MS 801.2845, $C_{40}H_{45}F_2N_4O_8PNa$ requires 801.2841.

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