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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. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Antisense oligonucleotides have been identified as selective inhibitors of point mutated oncogenes whose expression is linked with a variety of cancers¹. 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 phosphoramidites⁴ or H-phosphonates⁵. 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 group⁶, Wadsworth-Emmons olefinations with difluoromethyldiphenylphosphine oxide^{7,8} or Wittig type olefination with chlorodifluoroacetate/triphenylphosphine³. 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 carbonyl⁹.

We turned our attention to the Wittig olefination with the reagent generated in situ from dibromodifluoromethane (CF₂Br₂) and hexamethylphosphoroustriamide (HMPT). This methodology was



(i) Pyridinium dichromate, molecular sieves 3\AA , 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 derivatives¹⁰, but was reported to be unsuccessful in the nucleoside series⁶. In view of this conflicting evidence, as well as the shortcomings of methods used previously, we decided to reinvestigate applicability of the CF₂Br₂/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 dichloromethane¹³ 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 reagent¹⁵. 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 report¹⁰, 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 elsewhere⁹.

The structures of **3a**, **3b**, **8a**, **8b** and **12** were unequivocally assigned by ¹H and ¹⁹F NMR, mass spectroscopy as well as elemental analysis¹⁸. A particular characteristic of these compounds is the ¹⁹F 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 tetrahydrofuran²⁰ or ammonium fluoride in methanol²¹ 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 pyridine¹⁸.

Compounds 4a and 9a were phosphitylated with 2-cyanoethyl-bis-diisopropylaminophosphine in the presence of diisopropylammonium tetrazolide²² to give the corresponding phosphoramidites 5 and 10 in 74 and 61 % yield, respectively. The compounds were characterised by means of their ¹H, ¹⁹F and ³¹P NMR as well as IR, MS and UV spectroscopy. Currently, the new phosphoramidite monomers¹⁸ 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 date²³.

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. ¹H and ¹⁹F NMR spectra were recorded at 250 MHz using a Bruker WH-250 spectrometer with TMS or CFCl₃ as internal standards. Unless otherwise indicated, DMSO-d₆ was used as the solvent. ³¹P NMR spectra were recorded at 600 MHz on a Bruker AMX-600 spectrometer with 85 % aqueous H₃PO₄ as an external standard. In cases where analytical data are given for hydrates, the presence of water was confirmed by ¹H NMR. The protons of 2'-OH, 3'-OH, 5'-OH, and NHCO were exchangeable with D₂O. 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 60F₂₅₄ analytical plates in the following systems: A CHCl₃/MeOH (9:1), B CH₂Cl₂/EtOH (19:1), C CH₂Cl₂/EtOAc (9:1), D CH₂Cl₂/Et₃N/EtOAc (79.8:0.2:20), E Hexane/Acetone (6:4), F Hexane/EtOAc (7:3), G CH₂Cl₂/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-butyldimethylsilyluridine (**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 Robins¹² and Markiewicz¹⁴. The 12-I-5 triacetoxyperiodinane (the Dess-Martin Periodinane) was obtained as recommended by Dess and Martin¹⁵ 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-tertbutyldimethylsilyluridine $(1b)^{12}$ or 5'-O-dimethoxytrityl-3'-O-tert-butyldimethylsilyluridine $(6a)^{11}$ (10 mmol) in CH₂Cl₂ (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 CH₂Cl₂ (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 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; $\delta_{\rm H}$ 0.01 (s, 3H, CH₃), 0.06 (s, 3H, CH₃), 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, C₃₆H₄₂N₂O₈SiNa requires 681.2608; Found: C 64.38, H 6.61, N 4.05 %, C₃₆H₄₂N₂O₈Si.0.75H₂O 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 literature¹².

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; $\delta_{\rm H}$ -0.11 (s, 3H, CH₃), 0.02 (s, 3H, CH₃), 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, C₃₆H₄₂N₂O₈SiNa requires 681.2608; Found: C 65.52, H 6.48, N 4.27 %, C₃₆H₄₂N₂O₈Si 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 CH₂Cl₂ (25 mL) was added with a syringe, under argon, to a solution of the Dess-Martin reagent (6.0 g, 14 mmol) in dry CH₂Cl₂ (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 NaHCO₃ (140 mL) containing Na₂S₂O₃.5H₂O (17.5 g) and stirred for 5 min. The organic layer was washed with sat. aq. NaHCO₃ (2x40 mL), water (50 mL) and brine (50 mL), dried (Na₂SO₄) 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 literature¹².

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, zinc²⁴ (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 CHCl₃/sat. aq. NaHCO₃ (4:1, 50 mL). The aqueous layer was extracted with CHCl₃ (2x10 mL) and the combined chloroform extracts were washed with water (2x10 mL) and brine (10 mL), dried (Na₂SO₄) and concentrated *in vacuo*. The oily residue was dissolved in CH₂Cl₂ (2 mL) and applied onto a column of silica gel. The column was eluted with CH₂Cl₂ 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); $\delta_{\rm H}$ (CDCl₃) 1.43 (s, 3H, CH₃), 1.54 (s, 3H, CH₃), 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); $\delta_{\rm F}$ (CDCl₃) -81.70 (d, 1F, J_{F-F}=38.6 Hz), -83.01 (d, 1F, J_{F-F}=38.4 Hz); IR C=CF₂ 1771 cm⁻¹; UV λ_{max} 230 nm, ε 11512, λ_{min} 213 nm, ε 4083; Observed EI MS 327.1040, C₁₆H₁₆F₂O₅ requires 327.1044; Found: C 58.66, H 5.03 %, C₁₆H₁₆F₂O₅ 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 CHCl₃/sat. aq. NaHCO₃ (3:1, 80 mL). The aqueous layer was extracted with CHCl₃ (2x10 mL) and the combined chloroform extracts were washed with water (2x15 mL) and brine (20 mL), dried (Na₂SO₄) and concentrated *in vacuo*. Each oily residue was dissolved in CH₂Cl₂(3 mL) and applied onto a column of silica gel. The column was eluted with CH₂Cl₂/EtOH (98.5:1.5) (3a, 3b, 8b) or with CH₂Cl₂/Et₃N/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; $\delta_{\rm H}$ -0.04 (s, 3H, CH₃), 0.04 (s, 3H, CH₃), 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); $\delta_{\rm F}$ -83.06 (d, 1F, *J*_{F-F}=47.8 Hz), -86.66 (d, 1F, *J*_{F-F}=47.5 Hz); IR C=CF₂ 1772 cm⁻¹; UV λ_{max} 234 nm, ε 11850, λ_{min} 224 nm, ε 10495; Observed FAB MS 715.2622, C₃₇H₄₂F₂N₂O₇NaSi requires 715.2627; Found: C 62.41, H 5.96, N 4.03 %, C₃₇H₄₂F₂N₂O₇Si.H₂O 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.; $\delta_{\rm H}$ -0.04 (s, 6H, CH₃), 0.04 (s, 6H, CH₃), 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); $\delta_{\rm F}$ -83.05 (d, 1F, *J*_{F-F}=47.8 Hz), -86.66 (d, 1F, *J*_{F-F}=47.5 Hz); IR C=CF₂ 1780 cm⁻¹; UV $\lambda_{\rm max}$ 260 nm, ε 10221, $\lambda_{\rm min}$ 223, nm, ε 2710; Observed FAB MS 527.2180, C₂₂H₃₈F₂N₂O₅Si₂Na requires 527.2185; Found: C 52.31, H 7.63, N 5.51 %, C₂₂H₃₈F₂N₂O₅Si₂ 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; $\delta_{\rm H}$ (CDCl₃) -0.13 (s, 3H, CH₃), 0.06 (s, 3H, CH₃), 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); $\delta_{\rm F}$ -81.05 (d, 1F, $J_{\rm F-F}$ =33.2 Hz), -81.51 (d, 1F, $J_{\rm F-F}$ =33.4 Hz); IR C=CF₂ 1769 cm⁻¹; UV $\lambda_{\rm max}$ 234 nm, ϵ 26040, $\lambda_{\rm min}$ 223 nm, ϵ 24371; Observed FAB MS 715.2623, C₃₇H₄₂F₂N₂O₇SiNa requires 715.2627; Found:

C 64.20, H 5.82, N 3.87 %, C₃₇H₄₂F₂N₂O₇Si 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); $\delta_{\rm H}$ 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); $\delta_{\rm F}$ -80.98 (d, 1F, J_{F-F}=40.3 Hz), -84.85 (d, 1F, J_{F-F}=39.2 Hz); IR C=CF₂ 1774 cm⁻¹; UV $\lambda_{\rm max}$ 257 nm, ϵ 6182, $\lambda_{\rm min}$ 230 nm, ϵ 2748; Observed FAB MS 541.1970, C₂₂H₃₆F₂N₂O₆Si₂Na 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*-tertbutyldimethylsilyluridine (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'-Odimethoxytrityl-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 CHCl₃/H₂O (4:1, 50 mL). The organic layer was washed with H₂O (2x10 mL) and brine (10 mL), dried (Na₂SO₄) 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 CH2Cl2 (3 mL) and applied onto a column of silica gel. The columns were eluted with CH2Cl2/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 CHCl3/MeOH (97:3) (5 mL) and the resulting slurry was applied onto a column of silica gel. The columns were eluted with CHCl3/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; $\delta_{\rm H}$ 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-F} =47.8 Hz), -86.43 (d, 1F, J_{F-F} =47.1 Hz); UV λ_{max} 234 nm, ϵ 20289, λ_{min} 223 nm, ϵ 18607; Observed FAB MS 601.1762, C₃₁H₂₈F₂N₂O₇SiNa requires 601.1766; Found: C 62.92, H 5.00, N 4.64 %, C₃₁H₂₈F₂N₂O₇.0.75H₂O 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-F}=49.2 Hz), -87.45 (d, 1F, J_{F-F}=48.8 Hz); UV λ_{max} 260 nm, ε 10665, λ_{min} 230 nm, ε 2956; Observed EI MS 277.0632, C₁₀H₁₀F₂N₂O₅ requires 277.0636; Found: C 43.17, H 3.73, N 9.87 %, C₁₀H₁₀F₂N₂O₅ 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; $\delta_{\rm H}$ 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); $\delta_{\rm F}$ -81.86 (d, 1F, $J_{\rm F-F}$ =38.6 Hz), -84.58 (d, 1F, $J_{\rm F-F}$ =38.4 Hz); UV $\lambda_{\rm max}$ 234 nm, ϵ 22127, $\lambda_{\rm min}$ 223 nm, ϵ 19301; Observed FAB MS 601.1762, C₃₁H₂₈F₂N₂O₇Na requires 601.1766; Found: C 63.97, H 5.11, N 4.94 %, C₃₁H₂₈F₂N₂O₇.0.25H₂O 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-F}=40.2 Hz), -84.36 (d, 1F, J_{F-F}=40.5 Hz); UV λ_{max} 257 nm, ε 9457, λ_{min} 228 nm, ε 2574; Observed EI MS 277.0632, C₁₀H₁₀F₂N₂O₅ requires 277.0636; Found: C 41.73, H 3.89, N 9.81 %, C₁₀H₁₀F₂N₂O₅.0.75H₂O 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-tertbutyldimethylsilyl-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 CHCl₃/H₂O (4:1, 50 mL) and the organic layer was washed with H₂O (2x10 mL) and brine (10 mL), dried (Na₂SO₄) and concentrated *in vacuo* to give the crude products **4a** and **9a** as colourless glasses. Each colourless glass was dissolved in CH₂Cl₂ (3 mL) and applied onto a column of silica gel. The columns were eluted with CH₂Cl₂/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 $^{\circ}$ C for 4 h and then at r.t. for 18 h. The solvent was removed *in vacuo* and the residue was partitioned between CHCl₃/sat. aq. NaHCO₃ (2:1, 60 mL), the organic layer was washed with H₂O (2x10 mL) and brine (10 mL), dried (Na₂SO₄) and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ (3 mL) and applied onto a column of silica gel. The column was eluted with CH₂Cl₂/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'-Odimethoxytrityluridine (9a) (0.578 g, 1 mmol) and diisopropylammonium tetrazolide (0.180 g, 1 mmol) were dissolved in dry CH₂Cl₂ (10 mL) and 2-cyanoethoxy-bis-diisopropylaminophosphine (0.37 g, 0.4 mL, 1.2 mmol) in dry CH₂Cl₂ (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 CH₂Cl₂/Et₃N (99.75:0.25) (50 mL). The solutions were washed with sat. aq. NaHCO₃ (2x10 mL), water (2x10 mL) and brine (20 mL), dried (Na₂SO₄) and concentrated *in vacuo*. Each oily residue was dissolved in CH₂Cl₂/Et₃N (99.75:0.25) (3 mL) and applied onto a silica gel column packed in CH₂Cl₂/Et₃N (99.75:0.25). The column was eluted with CH₂Cl₂/Et₃N/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.; $\delta_{\rm H}$ 1.07-1.24 (m, 12H, iPr), 2.71 (t, 2H, NCCH₂), 3.28(m, 2H, H-5', H-5''), 3.57 (m, 4H, POCH₂, 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); $\delta_{\rm F}$ -82.85 (d, 1F, $J_{\rm F-F}$ =43.5 Hz), -83.89 (m, 1F); $\delta_{\rm P}$ (600 MHz; CDCl₃) 152.53 (s), 152.59 (s); UV $\lambda_{\rm max}$ 234 nm, ε 21540, $\lambda_{\rm min}$ 226 nm, ε 20474; Observed FAB MS 801.2847, C₄₀H₄₅F₂N₄O₈PNa 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.; $\delta_{\rm H}$ (CDCl₃) 1.07-1.29 (m, 12H, iPr), 2.24 (t, 2H, CH₂CN), 3.55 (m, 6H, H-5', H-5'', POCH₂, 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); $\delta_{\rm F}$ (CDCl₃) -79.30 (m, 1F), -80.48 (d, 1F, J_{F-F}=28.3Hz); $\delta_{\rm P}$ (600 MHz; CDCl₃) 152.16 (s), 152.25 (s); UV $\lambda_{\rm max}$ 234 nm, ε 22822, $\lambda_{\rm min}$ 230 nm, ε 21670; Observed FAB MS 801.2845, C₄₀H₄₅F₂N₄O₈PNa requires 801.2841.

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