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New Method for the Preparation of Some 2′- and 3′-Trifluoromethyl-2′,3′-dideoxyuridine Derivatives

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Abstract—Reaction of 5′-*O*-dimethoxytrityl-2′-oxo-3′-*O*-trimethylsilylethoxymethyluridine (**2**) and 5′-*O*-dimethoxytrityl-3′-oxo-2′-*O*-trimethylsilylethoxymethyluridine (**10**), with bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide and zinc gave the corresponding 2′- and 3′-difluoromethylene derivatives **3** and **11**. Attempted removal of the 3′- and 2′-*O*-trimethylsilylethoxymethyl (SEM) groups from compounds **3** and **11**, with tetrabutylammonium fluoride in tetrahydrofuran (THF), resulted in fluorination at the unsaturated difluoromethylene carbon with loss of the SEM group and formation of hitherto unreported 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine (**5**) and 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine (**13**). Detritylation of **5** and **13** gave 2′,3′-didehydro-2′,3′-dideoxy-2′ (3′)-trifluoromethyluridines **6** and **14**. Finally, hydrogenation of **5** and **13** followed by detritylation provided 2′,3′-dideoxy-2′-trifluoromethyluridine (**8a**) and 2′,3′-dideoxy-3′-trifluoromethyluridine (**16a**).

Introduction

The introduction of a fluorine atom into the sugar moiety of some nucleosides resulted in compounds with a broad spectrum of antiviral and anticancer activity.[1] Since fluorine and trifluoromethyl groups have similar inductive effects, σ=0.5 and 0.45, respectively, incorporation of the trifluoromethyl group is likely to provide analogues with potentially interesting biological properties and improved transport characteristics owing to increased lipophilicity.[1,2] However, only a few such compounds have been reported in the nucleoside series which is probably due to the shortcomings of existing synthetic methods. These methods are based on the condensation of appropriate carbohydrate precursors, bearing 2- or 3-trifluoromethyl groups, with various heterocyclic bases. The carbohydrate precursors are obtained via the addition of trifluoromethyltrimethylsilane to suitably protected 2- or 3-oxo sugars.[2–5] This requires several synthetic steps including the difficult and low yielding Barton type deoxygenation of the unreactive tertiary hydroxyl function.[3] Recently, we reported the synthesis of a series of 2′- and 3′-difluoromethylenenucleosides using a convenient method for mild difluoromethylenation of protected 2′- and 3′-oxonucleosides with bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide in the presence of zinc.[6,7] We are currently developing various aspects of the chemistry of these analogues. In this report, we describe nucleophilic substitution at the unsaturated carbon of the difluoromethylene group with a fluoride anion, assisted by the neighbouring trimethylsilylethoxymethyl group. This substitution has resulted in the formation of new 2′- and 3′-trifluoromethyluridine derivatives[8,9] and appears to be the first reported route for the incorporation of trifluoromethyl groups into nucleosides via transformation of the sugar moiety.

Results and Discussion

During the course of our studies concerned with the synthesis of the 2′- and 3′-difluoromethylenenucleosides, we noticed that the reactivity of protected 2′- and 3′-oxonucleosides towards bromodifluoromethyl[tris(dimethylamino)]-phosphonium bromide and zinc depended on the protecting groups at the neighbouring 2′- or 3′-hydroxyl functions. For example, the difluoromethylenation of 2′-oxo-3′,5′-tetraisopropyldisiloxanyluridine required only five equivalents of the quaternary phosphonium salt whereas 5′-*O*-dimethoxytrityl-2′-oxo-3′-*O*-*t*-butyldimethylsilyluridine required 10 equiv. of the reagent and a longer reaction time. Both the tetraisopropyldisiloxanyl and *t*-butyldimethylsilyl protecting groups could be readily removed with tetrabutylammonium fluoride or ammonium fluoride.[6] In view of these differences and potential advantages we decided to evaluate the trimethylsilylethoxymethyl (SEM) as a possible protecting group for the 2′- and 3′-hydroxyl functions in the difluoromethylenation step. According to the literature reports the SEM group could be readily removed with tetrabutylammonium fluoride in

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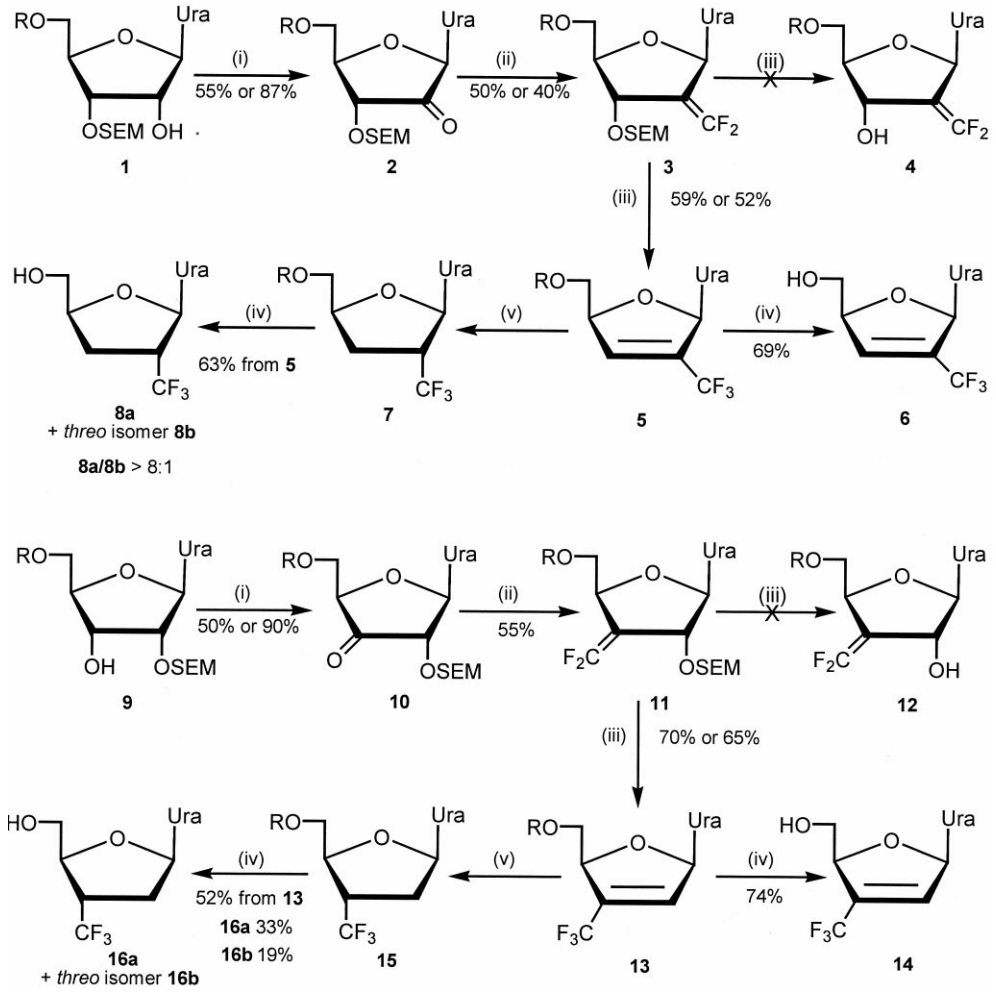


Scheme 1. Synthesis of 2′- and 3′-trifluoromethyl-2′,3′-dideoxyuridine derivatives. R=dimethoxytrityl; SEM=trimethylsilylethoxymethyl; Ura=uracil-1-yl: (i) pyridinium dichromate, molecular sieves 3 Å, CH_2Cl_2 or Dess Martin periodinane; (ii) $[(Me_2N)_3PCF_2Br]Br$, Zn, THF; (iii) Bu_4NF, THF, absence or presence of molecular sieves 3 Å; (iv) 80% aqueous acetic acid; (v) 10% palladium on activated carbon, EtOH.

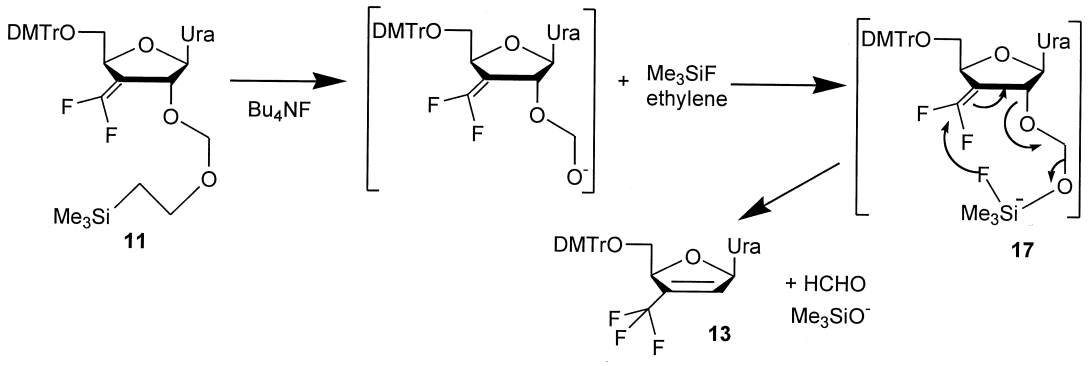
tetrahydrofuran in the presence of molecular sieves. Addition of molecular sieves is crucial as it results in anhydrous conditions in which the removal of the SEM group is favoured over desilylation.[10]

5′-*O*-Dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**1**) and 5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**9**), required as starting materials for the oxidation step, were prepared following the literature procedure.[11] Compounds **1** and **9** were oxidised with pyridinium dichromate in dichloromethane[12] to give the corresponding 2′- and 3′-oxo derivatives **2** and **10**. These derivatives were sufficiently stable to be purified by column chromatography on silica gel and were isolated in 55 and 50% yield, respectively (Scheme 1). An alternative oxidation of **1** and **9** with the Dess Martin reagent[13] gave almost quantitative yields of the crude oxo derivatives **2** and **10** that could be used in the difluoromethylenation step without further purification. Treatment of **2** and **10** with five equivalents of bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide and zinc in THF, either under reflux or in a sonic bath at 40°C, gave the expected 2′- and 3′-difluoromethylene derivatives **3** and **11** in 40–55% yield. Compounds **2** and **10** thus showed similar reactivity to that of the analogous tetraisopropyldisiloxanyl derivatives reported previously.[6]

When 2′-deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**3**) (Scheme 1) was treated with tetrabutylammonium fluoride in THF, in the presence of 3 Å molecular sieves, a clear conversion into a single more polar product was observed. ^1H and ^{19}F NMR studies revealed that hitherto unreported 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine (**5**) was formed as a result of a nucleophilic attack of the fluoride anion at the unsaturated difluoromethylene carbon with concomitant shift of the double bond and loss of the 3′-*O*-SEM group.

None of the products expected from deprotection such as **4** or S_N2 type substitution at the 3′-position were detected. ^{19}F NMR proved particularly diagnostic showing the presence of a characteristic singlet at −61.5 ppm corresponding to a trifluoromethyl group which is consistent with the literature data.[14]

The same product was isolated when tetrabutylammonium fluoride in THF was used in the absence of molecular sieves.



Scheme 2. Proposed mechanism of the fluoride ion reaction.

Subsequently, the 5′-*O*-dimethoxytrityl group was removed under mild, acidic conditions to produce 2′,3′-didehydro-2′,3′-dideoxy-2′-trifluoromethyluridine (**6**).

Interestingly, a similar substitution was observed in the 3′-difluoromethylene series (Scheme 1). Reaction of compound **11** with tetrabutylammonium fluoride in THF, both in the presence and absence of molecular sieves, gave 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine (**13**), again as a result of a likely S_N2' attack of the fluoride anion at the unsaturated difluoromethylene carbon with the expulsion of $SEMO^-$ from the 2′-position. The removal of the 5′-*O*-dimethoxytrityl group from **13** afforded 2′,3′-didehydro-2′,3′-dideoxy-3′-trifluoromethyluridine (**14**) in 74% yield. ^{1}H NMR studies of **6** and **14** confirmed the formation of the double bond between the 2′- and 3′-carbons. The spectra showed signals at around 6.8 and 7.3 ppm corresponding to the vinylic 2′- and 3′-protons.

It is thought that the mechanism of these transformations involves an initial attack of F^- at silicon, with the expulsion of trimethylsilylfluoride and ethylene. Subsequently, the formation of intermediate **17**, followed by a nucleophilic attack of the fluorine ion at $=CF_2$, provide 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine (**13**), formaldehyde and Me_3SiO^-[15,16] (Scheme 2). The literature data confirm that displacement of the fluorine in trimethylsilylfluoride by oxygen nucleophiles has been observed[17,18] but further studies are required to corroborate the proposed mechanism.

S_N2' type substitution, involving the shift of the double bond, was described for both 2′- and 3′-methylene nucleosides.[19–21] Various nucleophiles such as fluoride, azide, iodide and thiophenyl were used in conjunction with leaving groups including mesyl, diphenylphosphine oxide and diethylaminodifluoro-sulphoxide. To the best of our knowledge S_N2' substitution for 2′- and 3′-difluoromethylene-deoxynucleosides is so far unreported although it has been known for some aliphatic difluoroallylic systems[15,22–25] and a 2-difluoromethylene substituted methylglucoside.[26] None of these substitutions, however, proceed with the participation of the neighbouring trimethylsilylethoxymethyl group.

Since there is a selection of methods for derivatisation of the double bond,[27,28] 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′- and 3′-trifluoromethyluridines **5** and **13** appear to be versatile intermediates for further transformations. Thus, hydrogenation of compound **5**, in the presence of palladium on activated carbon (10% Pd), followed by removal of the dimethoxytrityl group with 80% aqueous acetic acid, afforded 2′-deoxy-2′-trifluoromethyluridine (**8a**) and the corresponding 2′-*threo* isomer, **8b**, in the ratio of eight to one. Compound **8a** was obtained pure by preparative HPLC in an excellent 63% yield, for the two steps, but the minor 2′-*threo* isomer, **8b**, could not be isolated owing to the coelution with contaminants. Similar hydrogenation of compound **13** showed lower stereoselectivity and after detritylation of crude **15**, two isomers, 3′-deoxy-3′-trifluoromethyluridine (**16a**)[4,8] and the corresponding 3′-*threo* isomer **16b**, were isolated in the ratio of two to one and fully characterised by their MS and NMR spectra. The configuration at the 2′-carbon and 3′-carbon was assigned by 2D NOESY and NOE studies; for compound **16a** our assignment was consistent with the literature data.[4,8]

Unlike the literature methods discussed earlier, the method presented by us involves only five steps, oxidation, difluoromethylenation, S_N2' mediated fluorination, hydrogenation and detritylation and appears to be a method of choice for the incorporation of trifluoromethyl groups into nucleosides. We are currently studying the substitution in 2′- and 3′-difluoromethylenenucleosides using various nucleophiles in conjunction with different leaving groups and the results will be published at a later date.

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. ^{13}C NMR spectra with ^{1}H decoupling were recorded at 100 MHz using a Bruker AMX 400. Unless otherwise indicated, DMSO-d_6 was used as the solvent. 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. NOE measurements were carried out in DMSO-d_6 solutions at 25°C applying the NOEDIFF mode of the Bruker software package, D1=2 s,

D2=0.5 or 1 s, S3=50 L. 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 s. Observed rotations at the Na_{-D} line were obtained at 20°C using a Perkin–Elmer 141 polarimeter. 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, films, 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) 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 and 280 nm. Columns Apex WP ODS (250×10 mm id, 7 μm, 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. 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-3′-*O*-trimethylsilylethoxymethyluridine (**1**) and 5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**9**) were made according to the procedures described by Wincott et al.[11] The 12-I-5 triacetoxyperiodinane (the Dess–Martin reagent) was obtained as recommended by Dess and Martin.[13] Bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide was prepared essentially as described by Houlton et al.[29] and Riesel et al.[30] Zinc was activated according to Hu et al.[31] Palladium on activated carbon (10% Pd) was purchased from Aldrich.

Oxidation of protected nucleosides 1 and 9 with pyridinium dichromate (general procedure)

A solution of 5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**1**) (3.36 g, 5 mmol) or 5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**9**) (3.36 g, 5 mmol) in CH_2Cl_2 (25 mL) was added by syringe, under argon, to a stirred suspension of pyridinium dichromate (3.75 g, 10 mmol) and powdered 3 Å molecular sieves (3.27 g) in CH_2Cl_2 (37 mL). Each mixture was stirred at rt for 18 h, concentrated in vacuo to half its volume, applied onto a silica gel column and chromatographed eluting with CH_2Cl_2/EtOAc (4:1) to give products **2** and **10** as brown foams.

5′-*O*-Dimethoxytrityl-2′-oxo-3′-*O*-trimethylsilylethoxymethyluridine (2). Yield: 1.87 g (55%); R_f 0.20 (A), 0.38 (D); $[\alpha]_D^{20}$ +37.4 (*c* 0.107, MeOH); ν_{max} (film) 1782, 1694, 1632, 1609, 1583 cm^{-1}; δ_H −0.07 (s, 9H, CH_3), 0.71 (m, 2H, CH_2Si), 3.47 (m, 4H, H-5′, H-5″, CH_2O), 3.71 (s, 6H, OCH_3), 4.17 (m, 1H, H-4′), 4.57 (d, 1H, H-3′, *J*=8.16 Hz), 4.64 (d, 1H, OCH_2O, *J*=6.75 Hz), 4.78 (d, 1H, OCH_2O, *J*=6.75 Hz), 5.56 (s, 1H, H-1′), 5.73 (d, 1H, H-5, *J*=7.84 Hz), 6.80–7.42 (m, 13H, trityl), 7.83 (d, 1H, H-6, *J*=7.84 Hz), 11.61 (s, 1H, NH); Observed ES MS 673.3, $[C_{36}H_{42}N_2O_9Si\text{-}H]^-$ requires 673.25; Found: C 63.00, H 5.97, N 4.28%, $C_{36}H_{42}N_2O_9Si.0.5\ H_2O$ (683.27) requires C 63.33, H 6.34, N 4.09%.

5′-*O*-Dimethoxytrityl-3′-oxo-2′-*O*-trimethylsilylethoxymethyluridine (10). Yield: 1.7 g (50%); R_f 0.61 (A), 0.54 (D); $[\alpha]_D^{20}$ +30.6 (*c* 1.75, MeOH); ν_{max} (film) 1781, 1697, 1609, 1583 cm^{-1}; δ_H 0.01 (s, 9H, CH), 0.80 (t, 2H, CH_2Si, *J*=7.55 Hz), 3.46 (m, 4H, CH_2O, H5′, H-5″), 3.82 (s, 6H, OCH_3), 4.56 (m, 1H, H-4′), 4.72 (m, 3H, OCH_2O, H-2′), 5.67 (d, 1H, H-5, *J*=8.09 Hz), 6.17 (d, 1H, H-1′, *J*=6.93 Hz), 6.82–7.36 (m, 13H, trityl), 7.83 (d, 1H, H-6, *J*=8.09 Hz), 11.52 (s, 1H NH); Observed ES MS 673.4, $[C_{36}H_{42}N_2O_9Si\text{–}H]^-$ requires 673.25; Found: C 63.89 H 6.24 N 3.73%, $C_{36}H_{42}N_2O_9Si$ (674.82) requires C 64.08, H 6.27, N 4.15%.

Oxidation of protected nucleosides 1 and 9 with the Dess–Martin reagent (general procedure)

A solution of 5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**1**) (1.67 g, 2.5 mmol) or 5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**9**) (1.67 g, 2.5 mmol) in dry CH_2Cl_2 (10 mL) was added by syringe, under argon, to a solution of the Dess–Martin reagent (3.21 g, 7.5 mmol) in dry CH_2Cl_2 (30 mL) at 0–5°C. The resulting mixture was stirred at 0–5°C for 20 min and then at rt for 18 h. The reaction was quenched with diethyl ether (100 mL), poured onto an ice cold, saturated aqueous solution of $NaHCO_3$ (70 mL) containing $Na_2S_2O_3{\cdot}5H_2O$ (8.75 g) and stirred for 10 min. The organic layer was washed with saturated aqueous $NaHCO_3$ (2×20 mL), water (20 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated in vacuo to give products **2** and **10** as colourless solids. The crude products were of sufficient purity to be used in the difluoromethylenation step. For analytical purposes they were dissolved in CH_2Cl_2 (2 mL) and chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (5:1) to give compounds **2** and **10** as colourless solids.

5′-*O*-Dimethoxytrityl-2′-oxo-3′-*O*-trimethylsilylethoxymethyluridine (2). Yield:1.45 g (87%); mp 73–80°C.

5′-*O*-Dimethoxytrityl-3′-oxo-2′-*O*-trimethylsilylethoxymethyluridine (10). Yield:1.5 g (90%); mp 72–75°C. The spectroscopic and analytical properties of compounds **2** and **10** were consistent with those quoted above.

Difluoromethylenation of protected ketonucleosides 2 and 10 (general procedure)

5′-*O*-Dimethoxytrityl-2′-oxo-3′-*O*-trimethylsilylethoxymethyluridine (**2**) (0.67 g, 1 mmol) or 5′-*O*-dimethoxytrityl-3′-oxo-2′-*O*-trimethylsilylethoxymethyluridine (**10**) (0.67 g, 1 mmol), bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide (1.85 g, 5 mmol) and powdered activated zinc (0.48 g, 7.5 mmol) were suspended in THF (20 mL) and the mixture was stirred under reflux for 25 min. The solid was filtered off (glass microfibre) and the filtrate concentrated in vacuo. Each residue was partitioned between $CHCl_3$/saturated aqueous $NaHCO_3$ (3:1, 80 mL). The aqueous layer was extracted with $CHCl_3$ (2×10 mL)

and the combined chloroform extracts were washed with water (2×15 mL) and brine (20 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Each oily residue was dissolved in CH_2Cl_2 (3 mL) and chromatographed on silica gel eluting with CH_2Cl_2/EtOH (98.5:1.5) to give products **3** and **11** as colourless solids.

2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (3). Yield: 0.31 g (50%); R_f 0.57 (A), 0.53 (D); mp 63–67°C; δ_H −0.04 (s, 9H, CH_3), 0.71 (m, 2H, CH_2Si), 3.37–3.42 (m, 4H, H-5′, H-5″, CH_2O), 3.74 (s, 6H, OCH_3), 4.15 (m, 1H, H-4′), 4.65 (s, 2H, OCH_2O), 4.96 (bs, 1H, H-3′), 5.47 (d, 1H, H-5, J=8.08 Hz), 6.75 (s, 1H, H-1′), 6.87–7.37 (m, 13H, trityl), 7.60 (d, 1H, H-6, J=8.08 Hz), 11.45 (s, 1H, NH); δ_F −81.02 (d, 1F, J_{F-F}=38.6 Hz), −81.31 (d, 1F, J_{F-F}=38.6 Hz); Observed FAB MS 731.2586, $[C_{37}H_{42}F_2N_2O_8Si+Na]^+$ requires 731.2576.

3′-Deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (11). Yield: 0.39 g (55%); R_f 0.66 (A), 0.54 (D); mp 82–86°C; δ_H −0.03 (s, 9H, CH_3), 0.80 (t, 2H, CH_2Si J=8.25 Hz), 3.49–3.53 (m, 4H, H-5′, H-5″, CH_2O), 3.73 (s, 6H, OCH_3), 4.70 (s, 2H, OCH_2O), 4.95 (bs, 1H, H-4′), 5.10 (bs, 1H, H-2′), 5.28 (d, 1H, H-5, J=8.24 Hz), 5.96 (d, 1H, H-1′, J=3.10 Hz), 6.85–7.36 (m, 13H, trityl), 7.78 (d, 1H, H-6, J=8.24 Hz), 11.49 (s, 1H, NH); δ_F −82.86 (d, 1F, J_{F-F}=46.0 Hz), −83.39 (d, 1F, J_{F-F}=46.0 Hz); Observed FAB MS 731.2590, $[C_{37}H_{42}F_2N_2O_8Si+Na]^+$ requires 731.2576.

Difluoromethylenation of compound 2 in a sonic bath

5′-*O*-Dimethoxytrityl-2′-oxo-3′-*O*-trimethylsilylethoxymethyluridine (**2**) (0.185 g 0.5 mmol) bromodifluoromethyl[tris(dimethylamino)]phosphonium bromide (0.94 g, 2.54 mmol) and powdered activated zinc (0.24 g, 3.75 mmol) were suspended in dry THF (10 mL) and the mixture was sonicated in a sonic bath (Camlab Transsonic T460/H) at 25–40°C for 2 h. The solid was filtered off (glass microfibre filter) and the filtrate was concentrated in vacuo. The yellowish residue was worked up and purified as described above to give product **3** as a pale yellow solid, identical to that obtained by difluoromethylenation under reflux.

2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (3). Yield: 0.140 g (40%). The spectroscopic properties of compound **3** were consistent with those quoted above.

Reaction of protected difluoromethylene nucleosides 3 and 11 with tetrabutylammonium fluoride in THF in the absence of molecular sieves (general procedure)

2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**3**) (0.21 g, 0.29 mmol) or 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**11**) (0.21 g, 0.29 mmol) were dissolved in THF (3.5 mL) and 0.5 M tetrabutylammonium fluoride (1.5 mL, 1.5 mmol) was added by syringe. Each solution was stirred at 50°C, under argon, for 3 h. The solvent was removed in vacuo and the residue partitioned between EtOAc/H_2O (4:1, 50 mL) and the organic layer was washed with H_2O (2×10 mL) and brine (10 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo to give crude products **5** and **13** as colourless glasses. Each colourless glass was dissolved in CH_2Cl_2 (3 mL) and chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (17:1) for **5** and CH_2Cl_2/EtOAc (7:3) for **13** to give **5** and **13**, respectively, as colourless froths.

2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine [1-(2,3-didehydro-2,3-dideoxy-5-*O*-dimethoxytrityl-2-*C*-trifluoromethyl-β-D-*glycero*-pentofuranosyl)uracil] (5). Yield: 0.099 g (59%); R_f 0.54 (A), 0.45 (D); δ_H 3.49–3.63 (m, 2H, H-5′, H-5″), 3.74 (s, 6H, OCH_3), 4.87 (d, 1H, H-5, J=8.04 Hz), 5.14 (bs, 1H, H-4′), 6.81–7.32 (m, 15H, trityl, H-1′, H-3′), 7.77 (d, 1H, H-6, J=8.04 Hz), 11.46 (s, 1H, NH); δ_F −61.35 (s, 3F, CF_3); Observed FAB MS 581.1880, $[C_{31}H_{29}F_3N_2O_6+H]^+$ requires 581.1899.

2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine [1-(2,3-didehydro-2,3-dideoxy-5-*O*-dimethoxytrityl-3-*C*-trifluoromethyl-β-D-*glycero*-pentofuranosyl)uracil] (13). Yield: 0.12 g (70%); R_f 0.35 (A); δ_H 3.39 (m, 2H, H-5′, H-5″), 3.68 (s, 6H, OCH_3), 4.69 (d, 1H, H-5, J=8.03 Hz), 5.19 (s, 1H, H-4′), 6.86–7.34 (m, 15H, trityl, H-1′, H-2′), 7.65 (d, 1H, H-6, J=8.03 Hz), 11.49 (s, 1H, NH); δ_F −61.17 (s, 3 F, CF_3); Observed FAB MS 581.1870, $[C_{31}H_{29}F_3N_2O_6+H]^+$ requires 581.1899.

Reaction of protected difluoromethylene nucleosides 3 and 11 with tetrabutylammonium fluoride in THF in the presence of molecular sieves (general procedure)

2′-Deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**3**) (0.21 g, 0.29 mmol) or 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**11**) (0.21 g, 0.29 mmol) and 3 Å molecular sieves (0.21 g) were suspended in THF (3.5 mL) and 0.5 M tetrabutylammonium fluoride (1.5 mL, 1.5 mmol) was added by syringe. Each suspension was stirred at 50°C under argon for 3 h. The solid was filtered off and the filtrate was concentrated in vacuo. The residue was partitioned between EtOAc/H_2O (4:1, 50 mL) and the organic layer was washed with H_2O (2×10 mL) and brine (10 mL), dried with Na_2SO_4, filtered and concentrated in vacuo to give crude products **5** or **13** as colourless glasses. Each colourless glass was dissolved in CH_2Cl_2 (3 mL) and chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (17:1) for **5** and CH_2Cl_2/EtOAc (7:3) for **13** to give **5** and **13**, respectively, as colourless froths.

2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine (5). Yield: 0.088 g (52%).

2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine (13). Yield: 0.11 g (65%). The spectroscopic properties of compounds **5** and **13** were consistent with those quoted above.

Detritylation of protected nucleosides 5 and 13 with 80% aqueous acetic acid (general procedure)

2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine (**5**) (0.08 g, 0.14 mmol) or 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine (**13**) (0.08 g, 0.14 mmol) were stirred at rt in 80% aqueous acetic acid (2 mL) for 2 h. For **5**, the acid was removed in vacuo and the residue coevaporated with toluene (2×5 mL) and partitioned between $H_2O/CHCl_3$ (4:1, 2.5 mL) and the aqueous layer was extracted with $CHCl_3$ (2×1 mL) and concentrated in vacuo to give crude **6** as a colourless glass. Crude **6** was analysed by reverse phase HPLC (gradient elution; 5% B–60% B over 26 min) and showed the main peak at retention time of 11.63 min. Preparative HPLC followed by freeze drying resulted in compound **6** as a colourless glass.

For **13**, the acid was removed in vacuo and the residue was coevaporated with toluene (2×5 mL) and partitioned between EtOAc/H_2O (4:1). The organic layer was washed with 5% aqueous $NaHCO_3$ (10 mL), H_2O (10 mL) and brine (10 mL), dried (Na_2SO_4), filtered and concentrated in vacuo to yield crude **14** as a cream foam. The foam was dissolved in CH_2Cl_2 (2 mL) and chromatographed on silica gel eluting with CH_2Cl_2/EtOH (19:1) to give **14** as a colourless froth.

Both **6** and **14** were >98% pure by analytical reverse-phase HPLC. Product **14** had retention time of 11.67 min.

2′,3′-Didehydro-2′,3′-dideoxy-2′-trifluoromethyluridine[1-(2,3-didehydro-2,3-dideoxy-2-*C*-trifluoro-methyl-β-D-*glycero*-pentofuranosyl)uracil] (6). Yield: 0.027 g (69%); R_f 0.29 (C); δ_H 3.72 (m, 2H, H-5′, H-5″), 5.02 (m, 1H, H-4′), 5.29 (bs, 1H, OH), 5.69 (d, 1H, H-5, *J*=8.06 Hz), 7.09 (s, 1H, H-1′), 7.30 (d, 1H, H-3′, *J*=0.84 Hz), 7.99 (d, 1H, H-6, *J*=8.06 Hz), 11.44 (s, 1H, NH); δ_F −61.50 (s, 3F, CF_3); UV λ_{max} 257 nm ϵ 9150; λ_{min} 230 nm ϵ 2962; Observed FAB MS 279.0589, $[C_{10}H_9F_3N_2O_4+H]^+$ requires 279.0593.

2′,3′-Didehydro-2′,3′-dideoxy-3′-trifluoromethyluridine[1-(2,3-didehydro-2,3-dideoxy-3-*C*-trifluoro-methyl-β-D-*glycero*-pentofuranosyl)uracil] (14). Yield: 0.028 g (74%); R_f 0.32 (C); δ_H 3.65–3.72 (m, 2H, H-5′, H-5″), 5.00 (bs, 1H, H-4′), 5.28 (t, 1H, 5′-OH, *J*=4.52 Hz), 5.65 (d, 1H, H-5, *J*=7.98 Hz), 6.84 (d, 1H, H-1′, *J*=1.63 Hz), 6.93 (d, 1H, H-2′, *J*=1.63 Hz), 7.70 (d, 1H, H-6, *J*=7.98 Hz), 11.42 (s, 1H, NH); δ_F −61.44 (s, 3F, CF_3); UV λ_{max} 258 nm ϵ 10 200; λ_{min} 230 nm ϵ 3780; Observed FAB MS 279.0616, $[C_{10}H_9F_3N_2O_4+H]^+$ requires 279.0593.

Hydrogenation of compounds 5 and 13 and detritylation of resulting compounds 7 and 15 (general procedure)

To a solution of 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine (**5**) (0.05 g, 0.085 mmol) or 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine (**13**) (0.160 g, 0.27 mmol) in dry ethanol (5 mL for **5** and 15 mL for **13**) palladium on activated carbon (10% Pd) (0.025 g for **5** and 0.08 g for **13**) was added. The apparatus was evacuated and flushed with hydrogen three times. The solution was then stirred under an atmosphere of hydrogen for 18 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. Each colourless residue was partitioned between $CHCl_3/H_2O$ (4:1, 12 mL for **5** and 25 mL for **13**) and the organic layer was washed with H_2O (2×5 mL) for **5** and (2×10 mL) for **13** and brine (10 mL), dried with Na_2SO_4, filtered and concentrated in vacuo to give crude products **7** (0.046 g) and **15** (0.14 g) as colourless glasses.

Products **7** (0.046 g) and **15** (0.14 g) were dissolved in 80% aqueous acetic acid (2 mL for **7** and 5 mL for **15**) and each solution was stirred at rt for 90 min. The solvent was removed in vacuo, each residue was coevaporated with toluene (2×5 mL) and partitioned between $CHCl_3/H_2O$ (1:4, 12 mL for **7** and 25 mL for **15**). The aqueous layer was extracted with chloroform and concentrated in vacuo to give crude products **8** and **16** as colourless glasses. Each glass was analysed by reverse phase HPLC (gradient elution: 5% B–60% B over 26 min).

Crude product **8** showed the main peak at retention time of 11.33 min and minor peaks at 10.58–11.10 min. Preparative HPLC followed by freeze drying afforded compound **8a** and a presumed *threo* isomer **8b** coeluting with a contaminant at 11.08 min. ^1H NMR of the contaminated fraction showed, inter alia, signals at 5.63 (d, 1H, H-5, *J*=8.06 Hz), 6.02 (d, 1H, H-1′, *J*=6.05 Hz), 7.95 (d, 1H, H-6, *J*=8.06 Hz) indicating the presence of minor isomer **8b**. The estimated ratio of **8a**/**8b** (peak areas) was greater than 8:1. Crude product **16** showed the main peak at retention time of 12.30 min and a minor peak at 11.18 min. Preparative HPLC followed by freeze drying resulted in compounds **16a** and the isomeric **16b**. Compounds **8a**, **16a** and **16b**, obtained as colourless glasses, were found to be >98% pure by analytical reverse-phase HPLC.

The stereochemistry of the products was confirmed by a series of 2D NOESY and NOE experiments. **8a**—2D NOESY showed a cross peak between H-2′ and H-6 and at the same time the lack of a cross peak between H-1′ and H-2′. This is only possible if the H-2′ proton is on the β face. **16a**—Irradiation of the H-3′ proton gave a 2% enhancement of H-5′ and H-5″. Irradiation of the H-5′ and H-5″ protons gave a 2.5% enhancement of H-3′. This is only possible if both protons are on the β face. **16b**—Irradiation of the H-1′ proton gave a 2% enhancement of H-3′. Irradiation of the H-3″ gave a 3% enhancement of H-1′. This is only possible if both protons are on the α face.

2′,3′-Dideoxy-2′-trifluoromethyluridine[1-(2,3-dideoxy-2-*C*-trifluoromethyl-β-D-*erythro*-pentofuranosyl)uracil] (8a). Yield: 0.015 g (63%); R_f 0.26 (C); $[\alpha]_D^{20}$ +17.9 (*c* 0.15, MeOH); ν_{max} (film), 3387, 1693, 1462, 1411, 1384, 1321, 1284, 1225, 1169, 1117, 1079, 1027 cm^{-1}; δ_H (400 MHz, DMSO-d6/D_2O) 2.28 (m, 1H, H-3′), 3.15 (m, 1H, H-3″), 3.47 (m, 1H, H-2′), 3.59 (m, 1H, H-5′), 3.68 (m, 1H, H-5″), 4.22 (m, 1H, H-4′), 5.77 (d, 1H, H-5, *J*=8.11 Hz), 6.16 (d, 1H, H-1′, *J*=5.52 Hz), 7.89 (d, 1H, H-6, *J*=8.11 Hz); δ_C 26.58 (q, J_{C-F}=1.8 Hz, C-3′) 47.39 (q, J_{C-F}=27.7 Hz, C-2′), 62.40 (C-5′), 80.36 (C-4′), 83.90 (q, J_{C-F}=2.9Hz, C-1′), 102.73 (C-5), 126.93 (q, J_{C-F}=278.5 Hz, CF_3),

140.88 (C-6), 150.54 (C-2), 163.38 (C-4); δ_F −67.99 (d, 3F, CF_3, J_{H-F}=10.08 Hz); UV λ_{max} 258 nm ϵ 9070; λ_{min} 229 nm ϵ 2506; Observed FAB MS 281.0750, $[C_{10}H_{11}F_3N_2O_4+H]^+$ requires 281.0744.

2′,3′-Dideoxy-3′-trifluoromethyluridine[1-(2,3-dideoxy-3-*C*-trifluoromethyl-β-D-*erythro*-pentofuranosyl)uracil] (16a). Yield: 0.022 g (33%); R_f 0.29 (C); $[\alpha]_D^{20}$ +22.1 (*c* 0.2, MeOH); ν_{max} (film) 3423, 1689, 1624, 1463, 1401, 1326, 1266, 1234, 1197, 1164, 1112, 1089, 1066 cm^{-1}; δ_H 2.25–2.41 (m, 2H, H-2′, H-2″), 3.29 (m,1H, H-3′), 3.51 (m, 2H, H-5′, H-5″), 4.15 (m, 1H, H-4′), 5.28 (m, 1H, 5′–OH), 5.63 (d, 1H, H-5, *J*=7.23 Hz), 6.01 (t, 1H, H-1′, *J*=6.49 Hz), 7.82 (d, 1H, H-6, *J*=7.23 Hz), 11.15 (bs, 1H, NH); δ_C 31.73 (C-2′) 42.09 (q, J_{C-F}=27 Hz, C-3′), 61.90 (C-5′), 79.78 (C-4′), 84.56 (C-1′), 102.16 (C-5), 125.99 (q, J_{C-F}=276 Hz, CF_3), 140.85 (C-6), 150.75 (C-2), 163.48 (C-4); δ_F −68.19 (d, 3F, CF_3 J_{H-F}=10.1 Hz); The ^1H and ^{19}F NMR spectra recorded in acetone-d_6 were consistent with the literature[4]; UV λ_{max} 260 nm ϵ 10462; λ_{min} 230 nm ϵ 2566; Observed FAB MS 281.0738, $[C_{10}H_{11}F_3N_2O_4+H]^+$ requires 281.0749.

1-(2,3-Dideoxy-3-*C*-trifluoromethyl-β-D-*threo*-pentofuranosyl)uracil (16b). Yield: 0.013 g (19%); R_f 0.26 (C); gum; ν_{max} (film) 3387, 1694, 1510, 1485, 1404, 1279, 1165, 1119, 1057 cm^{-1}; δ_H 2.07 (m, 1H, H-2′), 2.65 (m, 1H, H-2″), 3.42 (m, 1H, H-3′), 3.67 (m, 2H, H-5′, H-5″), 4.19 (m, 1H, H-4′), 5.05 (m, 1H, 5′-OH), 5.67 (d, 1H, H-5, *J*=7.98 Hz), 5.95 (t, 1H, H-1′, *J*=6.86 Hz), 7.73 (d, 1H, H-6, *J*=7.98 Hz), 11.35 (bs, 1H, NH); δ_C 32.74 (C-2′), 42.05 (q, J_{C-F}=29 Hz, C-3′), 61.91 (C-5′), 79.92 (C-4′), 84.85 (C-1′), 103.80 (C-5), 141.72 (C-6), 152.30 (C-2), 164.15 (C-4)[32]; δ_F −63.66 (d, 3F, CF_3, J_{H-F}=10.1 Hz); UV λ_{max} 260 nm ϵ 9224; λ_{min} 230 nm ϵ 2385; Observed FAB MS 281.0738, $[C_{10}H_{11}F_3N_2O_4+H]^+$ requires 281.0749.

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