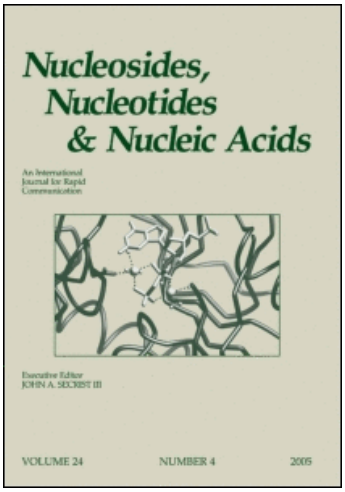

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SYNTHESIS OF SOME 2′- AND 3′-FLUOROALKYL SUBSTITUTED NUCLEOSIDES AND OLIGONUCLEOTIDES

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As part of a programme on the development of antisense oligonucleotides, as selective inhibitors of oncogene expression, it was intended to investigate the properties of oligonucleotides modified at the 2′- or 3′-position with difluoromethylene, difluoromethyl and trifluoromethyl groups. It was expected that such oligonucleotides might possess increased stability against nucleases as well as improved hybridisation properties and transport characteristics (1,2,3).

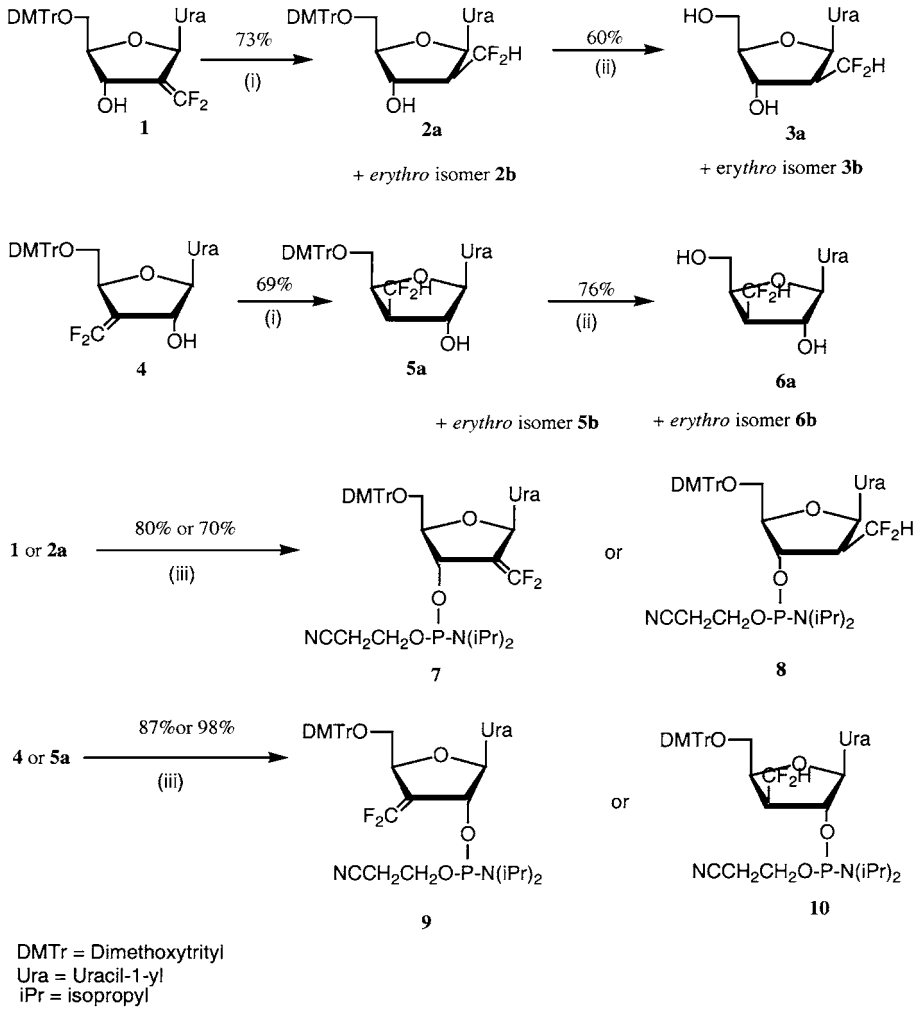
The synthesis of target oligonucleotides entailed prior preparation of the appropriate nucleoside precursors. It was found that reaction of suitably protected 2′- and 3′-ketonucleosides with crystalline bromodifluoromethyl[tris (dimethylamino)]-phosphonium bromide in the presence of zinc gave 2′- and 3′-difluoromethylene nucleosides in high yields (4–6). Subsequently, these compounds were used as starting materials for further transformations.

Thus, hydrogenation of 2′-deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**1**) (4) and 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine (**4**) (4), gave the corresponding 2′- and 3′-difluoromethyluridine derivatives **2a/2b** (*threo*/*erythro* 6:1) and **5a/5b** (*threo*/*erythro* 8:1), respectively. Detritylation of compounds **2a/2b** and **5a/5b** provided two pairs of diastereoisomers, **3a/3b** (7) and **6a/6b** (7), that could be separated by HPLC.

Interestingly, reaction of 2′-deoxy-2′-difluoromethylene-5′-*O*-dimethoxytrityl-3′-*O*-trimethylsilylethoxymethyluridine (**11**) (6) and 3′-deoxy-3′ difluoromethylene-5′-*O*-dimethoxytrityl-2′-*O*-trimethylsilylethoxymethyluridine (**17**) (6) with tetrabutylammonium fluoride, resulted in fluorination at the unsaturated difluoromethylene carbon with loss of the trimethylsilylethoxymethyl

group and formation of 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-2′-trifluoromethyluridine (**13**) and 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-3′-trifluoromethyluridine **19**, respectively.

No products of expected desilylation such as **12** and **18** were detected. Detritylation of **13** and **19** afforded the expected 2′,3′-didehydro-2′,3′-dideoxy-2′ (3′)-trifluoromethyluridines **14** and **20**. Hydrogenation of compounds **13** and **19** followed by detritylation provided 2′,3′-dideoxy-2′-trifluoromethyluri dine (**16a**) and



Scheme 1. Synthesis of 3′- and 2′-*O*-Phosphoramidites of 2′- and 3′-Difluoromethyleneuridine and 2′- and 3′-Difluoromethyluridine.

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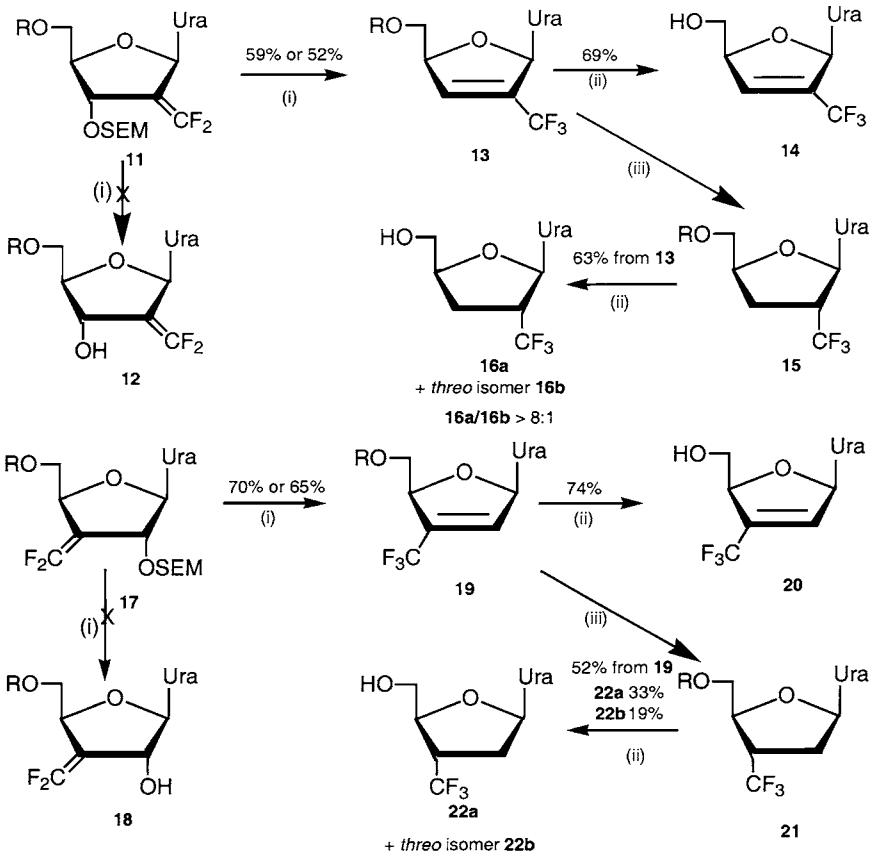

Scheme 2. Synthesis of 2′- and 3′-trifluoromethyluridine derivatives.

2′,3′-dideoxy-3′-trifluoromethyluridine (**22a**), along with the corresponding threo isomers **16b** and **22b**, respectively (6). (Scheme 2).

Finally, phosphitylation of compounds **1**, **2a**, **4** and **5a** furnished the corresponding 2′- and 3′-*O*-phosphoramidites (**7**) (4), (**8**) (7), (**9**) (4) and (**10**) (7). (Scheme 1) Attempted incorporation of 3′-deoxy-3′-difluoromethylene-5′-*O*-dimethoxytrityluridine-2′-*O*-phosphoramidite (**9**) into oligonucleotide sequences was only possible after detailed studies in solution to customise the standard solid phase protocol. Replacement of iodine with t-butylhydroperoxide and the succinyl linker with oxalyl linker enabled the synthesis of short alternating oligonucleotides (8).

The incorporation of 2′-deoxy-2′-difluoromethyl-5′-*O*-dimethoxytrityluridine-2′-*O*-phosphoramidite (**8**), and its 3′-difluoromethyl counterpart **10**, required

fewer changes in the standard protocol and resulted in (2′–5′) and (3′–5′) linked oligonucleotides modified with 3′- or 2′-difluoromethyl groups.

ACKNOWLEDGMENTS

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7. All the NMR spectra were recorded in DMSO-d6 and UV spectra in 95% EtOH. **3a** NMR δ_H 3.02 (m, 1H, H-2′), 3.63 (m, 3H, H-4′, H-5′, H-5″), 4. 33 (t, 1H, H-3′ J = 7.69 Hz), 4.95 (bs, 1H, 5′ -OH), 5.61 (d, 1H, H-6, J = 8.12 Hz), 5.83 (d, 1H, 3′ -OH, J = 7.25 Hz), 6.02 (t of d, 1H, CF_2H, J_{HF} = 45.6 Hz, J_{HH} = 4.47 Hz), 6.21 (d, 1H, H-1′, J = 7.75 Hz) 7.84 (d, 1H, H-6, *J* =8.12 Hz), 11.41 (bs, 1H, NH); δ_C 51.60 (t, $J_{C\text{-}F}$ = 19 Hz, C-2′), 57.96 (C-5′), 66.31 (C-3′), 81.04 (C-1′), 83.84 (C-4′), 100.44 (C-5), 114.54 (t, $J_{C\text{-}F}$ = 240 Hz, CF_2H), 140.18 (C-6), 149.31 (C-2), 162.13 (C-4); δ_F −117.17 (1F, d (J_{FF} = 292 Hz) of d ($J_{Hgem\text{-}F}$ = 54.6 Hz), −121.46 (1F, d (J_{FF} = 292 Hz) of d ($J_{Hgem\text{-}F}$ = 53.53 Hz); UV λ_{max} 259 nm ε_{max} 6468, λ_{min} 229 nm ε_{min} 1564; Observed FAB MS 279.0770, $[C_{10}H_{12}F_2N_2O_5 + H]^+$ requires 279.0793. 3b NMR δ_H 2.89 (m, 1H, H-2′), 3.59 (m, 2H, H-5′, H-5″), 3.85 (m, 1H, H-4′), 4. 36 (d, 1H,-3′, J = 4.48 Hz), 5.12 (bs, 1H, 5′-OH), 5.69 (d, 1H, H-5, J = 8.13 Hz), 5.79 (bs, 1H, 3′-OH), 6.33 (d, 1H, H-1′, J = 8.53 Hz), 6.19 (t of d, 1H, CF_2H, J_{HF} = 55.4 Hz, J_{HH} = 6.80 Hz), 7.85 (d, 1H, H-6, *J* = 8.13 Hz), 11.32 (bs, 1H, NH); δ_F −114.58 (1F, d (J_{FF} = 295 Hz) of q ($J_{Hgem\text{-}F}$ = 54.1 Hz, $J_{H2'\text{-}F}$ = 10.0 Hz), −123.51 (1F d (J_{FF} = 295 Hz) of q ($J_{Hgem\text{-}F}$ = 55.9 Hz, $J_{H2'\text{-}F}$ = 14.3 Hz); UV λ_{max} 260 nm ε_{max} 7426, λ_{min} 230 nm ε_{min} 1098; Observed FAB MS 279.0702, $[C_{10}H_{12}F_2N_2O_5{}^+ H]^+$requires 279.0793. **6a** NMR δ_H2.87 (m, 1H, H-3′), 3. 57 (m, 2H, H-5′, H-5″), 4.30 (m, 2H, H-2′, H-4′), 5.32 (bs, 1H, 5′-OH), 5.71(m, 3H, H-1′, 2′-OH, H-5), 6.31 (t of d, 1H, CF_2H, J_{HF} = 49.08 Hz, J_{HH} = 6.88 Hz), 7.84 (d, 1H, H-6, J = 8.16 Hz), 11.34 (bs, 1H, NHi);); δ_C C-2′), 49.38 (t, $J_{C\text{-}F}$ = 19.5 Hz, C-3′), 60.58 (C-5′), 76.90 (C-4′), 87.96 (C-1′), 102.40 (C-5), 116.96 (t, $J_{C\text{-}F}$ = 251 Hz, CF_2H), 140.69 (C-6), 150.92 (C-2), 163.03 (C-4); δF −112.08 1F d (J_{FF} = 294 Hz) of q ($J_{Hgem\text{-}F}$ = 55.55 Hz, $J_{H\text{-}3'\text{-}F}$ = 11.57 Hz), −116.75 1F d (J_{FF} = 294 Hz) of q (J_{Hgem_F} = 56.3 Hz, $J_{H\text{-}3\text{-}F}$ = 15.08 Hz); UV λ_{max} 260 nm ε_{min} 8778 λ_{min} 229 nm ε_{min} 3436; Observed ES MS 279.0802, $[C_{10}H_{12}F_2N_2O_5 + H]^+$ requires 279.0793. **6b** NMR δ_H 2.73 (m, 1H, H-3′), 3.52 (m, 1H, H-5′), 3.75 (m, 1H, H-5″), 4. 34 (m, 3H, H-2′, H-4′, 5′-OH), 5.61 (d, 1H, J = 8.24 Hz, H-5), 5.68 (bs, 2H, 3′-OH, H-1′), 6.20 (t , J_{HF} = 56.1 Hz of d J_{HH} = 5.28 Hz, 1H, CF_2H), 7.98 (d, 1H, H-6, *J* = 8.24 Hz), 11.30 (bs, 1H, NH); δ_F −116.03 1F d (J_{FF} = 290 Hz) of q ($J_{Hgem\text{-}F}$ =

55.6 Hz, $J_{H3\text{-}F} = 10.06$ Hz) , −122.70 1F d ($J_{FF} = 290$ Hz) of q ($J_{Hgem\text{-}F} = 57.1$ Hz, $J_{H3'\text{-}F} = 18.7$ Hz); UV λ_{max} 262 nm ε_{max} 8885 ε_{min} 230 nm ε_{min} 2020; Observed FAB MS 301.0600, $[C_{10}H_{12}F_2N_2O_5 + Na]^+$ requires 301.0612. **3a** - 2D NOESY showed a cross peak between H-2′ and H-4′ and at the same time the lack of a cross peak between H-2′ and H-5′. There was also a cross peak between H-6 and CF_2H. This is only possible if the H-2′ proton is on the α face. **3b** - 2D NOESY showed a cross peak between H-2′ and H-5 and at the same time the lack of a cross peak between H-2′ and H-4′. This is only possible if the H-2′ proton is on the β face. **6a** - 2D NOESY showed a cross peak between between H-5′ and CF_2H. and at the same time the lack of a cross peak between H-3′ and H-5′. There was also a cross peak between H-6 and CF_2H. This is only possible if the H-3′ proton is on the α face. **6b** - 2D NOESY showed a cross peak between H-4′ and CF_2H and at the same time the lack of a cross peak between H-5′ and CF_2H. There was also a cross peak between H-3′ and H-6. This is only possible if the H-3′ proton is on the β face.

8. NMR δ_H 1.05–1.14H (m, 14H, iPr), 2.59 (m, 1H, H-2′), 2.72 (t, 2H, J = 5.50 Hz, $OCH_2\underline{CH_2}CN$), 3.45 (m, 2H, iPr), 3.51 (m, 2H, H-5′, H-5″), 3.73 (s, 6H, OCH_3), 4.04 (m, 3H, $O\underline{CH_2}CH_2CN$, H-4′), 4.67 (m, 1H, H-3′), 5.31 (d, 1H, H-5, $J = 9.13$ Hz), 6.10 t ($J_{HF} = 56$ Hz) of m, 1H, CF_2H), 6.30(m, 1H, H-1′), 6.87–7.42 (m, 13H, trityl), 7.65 (2 d, unresolved, H-6), 11.39 (bs, 1H, NH); δ_{F-} 117.9 (d of m, 1F, CF_2H), −122.2 (d of m, 1F, CF_2H) δ_P 150.18 (s), 150.64 (s); Observed FAB MS 779.3050, $[C_{40}H_{47}F_2N_4O_8P–H]$ requires 779.3021. **10** NMR $\delta_{\mathbf{H}}$, (1.21 , m, 14H, iPr), 2.88 (t, 2H, J = 6.82 Hz, $OCH_2\underline{CH_2}CN$), 2.99 (m, 1H, H-3′), 3.48(m, 4H, H-5′, H-5″, iPr), 3. 71 (s, 6H, OCH_3), 4.04 (m, 3H, $O\underline{CH_2}CH_2CN$, H-4′), 4.51 (m, 1H, H-2′), 5.59 (d, 1H, H-5, $J = 8.11$ Hz), 5.89 (d, 1H, H-1′, $J = 5.62$ Hz), 6.18 t ($J_{HF} = 48.5$ Hz) of m, 1H, CF_2H), 6.82–7.45 (m, 13H, trityl), 7.55 (d, 1H, H-6, $J = 8.11$ Hz), 11.34 (s, 1H, NH); δ_F −112.58–(-114.86) (m, 1F, CF_2H) −117.11–(-118.99) (m, 1F, CF_2H); δ_P 152.502 (s), 152.409 (s) ; Observed FAB MS 779.3063, $[C_{40}H_{47}F_2N_4O_8P\text{-}H]$ requires 779.3021.

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