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Direct Synthesis, Substitution, and Structure of 1-(2'-Deoxy-β-D-*erythro*-pentofuranosyl)-4-pentafluorophenylpyrimidin-2*H*-one

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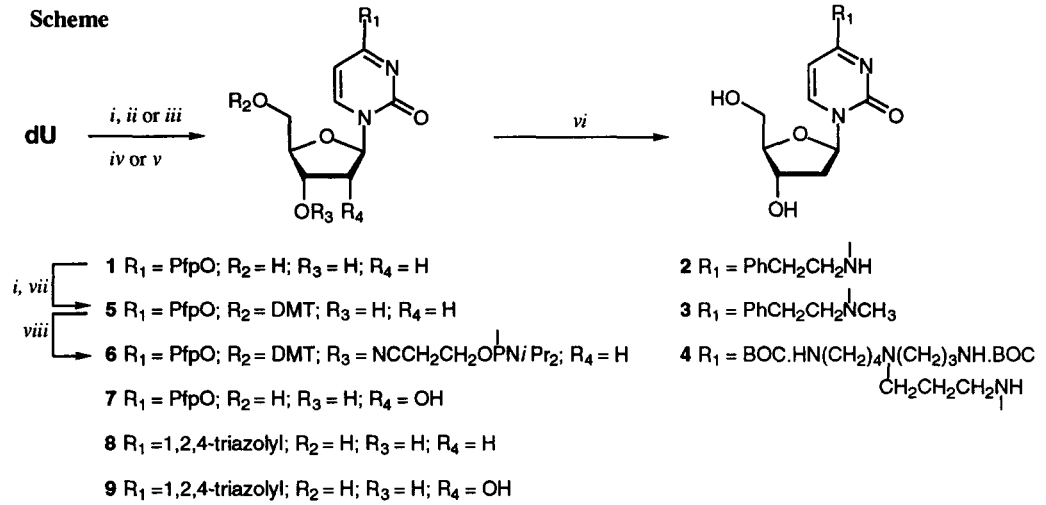
Abstract: Direct methods have been developed to access the title nucleoside **1** from 2'-deoxyuridine (**dU**). The C-4 pentafluorophenyl group of **1** is readily displaced by amine nucleophiles forming N-4 substituted cytosines in good to excellent yields.

Our interest in the development of antisense and antigene oligonucleotides has prompted an investigation of cytosine nucleosides modified at N-4 necessitating efficient syntheses of these adducts. The presence of N-methyl substituents attached at the amino groups of the bases usually has a destabilising effect on the base pairing of oligonucleotides[1]. However, the observation that inclusion of aralkyl substituents[2] in hexose-DNA[3] significantly enhances hybridisation warrants a closer study of pentose oligonucleotides containing these substituents. We chose to substitute the N-4 of cytosine initially since modification of this position for other purposes has already attracted attention. Binding of DNA by proteins has been studied using cytosine with a ^{15}N label at C-4, for example[4]. Introduction of ^{15}N and protected amino groups at C-4 can be achieved at the nucleoside level using C-4 azolyl derivatives of uridines[5]. Isotopic labelling and substitution at the C-4 position is also possible at the oligonucleotide level[6]. To date, successful formation of C-4 azolyl derivatives of deoxy nucleosides has required time consuming protection/deprotection strategies for the reactive 5'-OH and 3'-OH positions. Protecting groups have included; acetoyl, benzoyl, di-*t*-butylmethylsilyl, trityl, dimethoxytrityl (DMT) and phosphoramidite[5-7]. An additional complication is the requirement for protection of the 2'-OH during the formation of C-4 azolyl derivatives of ribose nucleosides which has been met by the use of the tetrahydropyranyl group[5].

Our aim was to develop an efficient, direct method for the synthesis of N-4 modified cytosines requiring only *in situ* protection of the sugar hydroxyls. Thus, we have established two convenient one-pot syntheses which give direct access to the cytosine precursor **1** in good yield from 2'-deoxyuridine (**dU**). We chose the pentafluorophenyl (Pfp) group for subsequent displacement instead of the more familiar azolyl[5]. Transient protection of the reactive 5'-OH and 3'-OH groups was achieved through formation of either trifluoroactetate esters[8] or trimethylsilyl (TMS) ethers[9]. The first one-pot transient protection method we investigated involved treatment of **dU** in pyridine with TMSCl (2.2 eq.) at room temperature (Scheme). After 30 minutes,

Scheme



Reagents: *i* C_5H_5N; *ii* TMSCl, $ClC_6H_4OP(O)Cl_2$; *iii* $(CF_3CO)_2O$; *iv* PfpOH; *v* 1,2,4-1*H*-triazole; *vi* amine, 1,4-dioxane; *vii* DMTCl; *viii* $NCCH_2CH_2OP(Cl)NiPr_2$, THF, $(CH_3CH_2)_3N$.

4-chlorophenylphosphodichloridate (2.5 eq.) was added at 0°C and the mixture allowed to warm to room temperature. After 24 h, pentafluorophenol (PfpOH, 5 eq.) was added and the solution left to stir for 72 h. The product mixture was concentrated and the residual oil subjected to flash chromatography (EtOAc-MeOH 9:1) giving **1** in 65% yield[10]. A second, higher yielding synthesis of **1** was possible involving addition of trifluoroacetic anhydride (4 eq.) to a solution of **dU** in pyridine at 0°C. The solution was stirred for 24 h at room temperature before PfpOH (10 eq.) was added[11]. After a further 48 h the product solution was concentrated and the residual oil purified as before giving **1** in 78% yield. Recrystallization of **1** from a mixture of methanol and toluene gave crystals suitable for x-ray analysis. An ORTEP[12] plot of **1** is shown in the Figure and the relevant crystallographic data given in the Table.

Figure X-ray Crystallographic Structure of **1**.

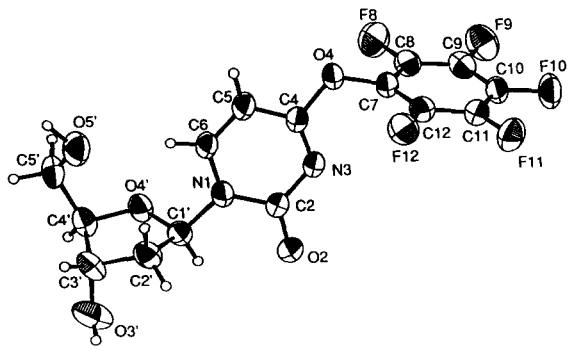


Table Crystallographic Data[13] for **1**.

Formula	$C_{15}H_{11}N_2F_5O_5$
System	monoclinic
Space group	$P2_1$
Cell parameters (Å)	
a	7.739(2)
b	5.502(1)
c	18.546(4)
β (°)	98.54(2)
Volume *V* (Å^3)	780.9(3)
Z	2

The bridging oxygen at O-4 is less conjugated with the Pfp ring than with the pyrimidine, which is oriented *anti* to the sugar ring with its C-2' *endo*, C-3' *exo* pucker and *gauche-gauche* 5'-OH group.[14] The Pfp group in **1** can be readily displaced by amine nucleophiles[15] to give the N-4 substituted 2'-deoxycytosines **2** to **4** (Scheme)[16]. Reaction of **1** with *N*-phenethylamine in dioxane at 80°C overnight gave **2** in 85% yield after purification by flash column chromatography. Under similar conditions **1** reacted with *N*-methyl-*N*-phenethylamine to give **3** in excellent yield (98%). The Pfp group of **1** was also displaced by the N-protected polyamine derivative; *N*1, *N*8-*bis*-Boc-*N*4-propylaminospermidine[17] giving **4** but in more modest yield (58%). In order to make modified cytosine bases after oligomer synthesis, we have made available phosphoramidite **6**. Dimethoxytritylation of **1** to give **5** (85%) followed by conversion to the phosphoramidite **6** (75%) was achieved using standard methods[9,18]. Phosphoramidite **6** is fully compatible with automated solid-supported oligonucleotide synthesis giving coupling efficiencies of >98% following standard protocols.[18] Synthesis of oligonucleotides containing **2** to **4** and post-synthetic substitution of oligonucleotides containing **1** are currently under investigation.

The one-pot transient protection procedure may be extended to the ribose series without interference from the 2'-OH. Uridine (**U**) was treated with trifluoroacetic anhydride and PfpOH as described and the ribose nucleoside **7** isolated in 59% yield. We have recently reported the crystal structure[19] of a protected analogue of AZT; 1-(3'-azido-2',3'-dideoxy-5'-*O*-acetyl-β-D-*erythro*-pentofuranosyl-5-methyl-4-(1,2,4-1*H*-triazolyl)-pyrimidin-2*H*-one (ATAZT), which has anti-HIV properties. In order to make unprotected C-4 azolyl analogues directly for comparison with ATAZT, we applied the one-pot transient protection methodology to **dU** and **U**. Thus, **dU** was treated with trifluoroacetic anhydride and with 1,2,4-1*H*-triazole in place of PfpOH giving **8** in good yield (79%). Similarly, the ribose analogue **9** was directly available from **U** (56%). The antiviral properties of a series of C-4 modified analogues are currently being evaluated and will be reported elsewhere.

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10. (a) Analytical data for **1**: mp 176°C; TLC (EtOAc-MeOH) R_f 0.50; UV (95% EtOH) λ_{max} 286 nm (ε 6730); IR (KBr) υ_{max} 3479, 3403, 3083, 2940, 1654, 1556, 1450, 1295 and 1114 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 2.07 (m, 1H, CH_2-2'), 2.29 (m, 1H, CH_2-2'), 3.62 (s, 2H, CH_2-5'), 3.88 (d, 1H, J = 3.4 Hz, CH-4') 4.23 (s, 1H, CH-3'), 5.14 (s, 1H, OH-5'), 5.28 (s, 1H, OH-3'), 6.06 (t, 1H, J = 6.1 Hz, CH-1'), 6.59 (d, 1H, J = 7.3 Hz, CH-5), 8.59 (d, 1H, J = 7.3 Hz, CH-6); ^{13}C NMR [$(CD_3)_2SO$] δ 41.2 (CH_2-2'), 61.0 (CH_2-5'), 70.0 (CH-4'), 87.2 (CH-3'), 88.5 (CH-5), 93.0 (CH-1'), 148.0 (CH-6), 154.0 (C-4), 168.3 (C-2); ^{19}F NMR [$(CD_3)_2SO$] δ -14.72 (t, 2F, J = 20.2 Hz, 2 x *meta* CF), -19.57 (t, 1F, J = 21.2 Hz, *para* CF), -21.12 (d, 2F, J = 24.2 Hz, 2 x *ortho* CF); Mass spectrum (FAB) *m/z* (I_r) 417 (M + Na^+, 15%), 395 (M + H^+, 22%), 305 (14%) and 279 (100%) [Found: *m/z* 395.067 (M + H^+). $C_{15}H_{12}F_5N_2O_5$ requires 395.068]; Anal. Calcd for $C_{15}H_{11}F_5N_2O_5$: C, 45.69%; H, 2.79%; F, 24.11%; N, 7.11%. Found: C, 45.64%; H, 2.56%; F, 24.14%; N, 7.06%. (b) Positive chemical shifts are downfield of the TMS reference for ^1H and ^{13}C NMR and negative chemical shifts are upfield of the CF_3CCl_3 reference for ^{19}F NMR.
11. An NMR investigation showed that formation of the O-5' and O-3' trifluoroacetate esters occurred in minutes whereas formation of the intermediate C-4 pyridinium adduct was much slower. The product yield was lowered if addition of PfpOH was not delayed until approximately 24 h after initial reaction of **dU** with triflouroacetic anhydride.
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13. Enraf-Nonius CAD4 diffractometer, 2969 reflections measured, final R = 0.0361 for 2609 observed data [$I>2\sigma(I)$], $wR(F^2)$ = 0.113 for 2734 independent data.
14. C4-O4 = 1.365(2)Å, O4-O7 = 1.386(3)Å, N3-C4-O4-C7 = -1.4(3)°, C4-O4-C7-C8 = 104.7(2)°, C2-N1-C1'-O4' = -124.7(2)°; pseudorotation parameters P = 174.4°, t_m = 35.5°; O4'-C4'-C5'-O5' = -63.0(3)°, C3'-C4'-C5'-O5' = 55.9(3)°.
15. General method: To a solution of **1** (0.5 mmol) in 1,4-dioxane (10 cm^{-3}), the appropriate amine (1 to 1.2 eq.) was added and the mixture stirred at 80°C overnight. The product solution was concentrated and subjected to flash column chromatography to give the pure product.
16. All reported compounds gave satisfactory TLC, ^1H NMR, ^{13}C NMR, ^{19}F NMR, mass spectral, and elemental analysis data, in full agreement with their assigned structures.
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