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EFFECT OF C5-AMINO SUBSTITUENT ON 2'-DEOXYURIDINE BASE PAIRING WITH 2'-DEOXYADENOSINE: INVESTIGATION BY ^{1}H AND ^{13}C NMR SPECTROSCOPY+

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Abstract: *Substituents at 5-position of a pyrimidine base are known to affect its base pairing properties with complementary purines, either by altering the imino N3-H acidity or by modifying the acceptor strength of C2 and C4 carbonyls. This paper reports comparative base pair property of 5-methyl-2'-deoxyuridine (dT) and 5-amino-2'-deoxyuridine (dUNH$_2$) with 2'-deoxyadenosine (dA) as their 3',5'-di-t-butyldimethylsilyl derivatives in chloroform-d. Using ^{1}H and ^{13}C NMR, it is demonstrated that the 5-NH$_2$ substituent in 2'-deoxyuridine results in (i) decreased association (lower K$_a$) with 2'-deoxyadenosine compared to dA:dT complexation and (ii) increased receptor strength of C2 carbonyl compared to C4 carbonyl and (iii) lower temperature for separation of 6-amino protons of dA due to its complexation with dUNH$_2$ compared to that with dT.*

INTRODUCTION

Hydrogen bonding between complementary base pairs of nucleic acids plays a crucial role in defining DNA structure and regulating the information transfer process during replication and transcription processes[1]. Past studies have demonstrated that by use of soluble monomers and base derivatives, the complexation can be studied in low dielectric solvent such as chloroform[2] and dimethylsulfoxide[3], where bases associate primarily via hydrogen bonds. The lipophilic derivatives of nucleosides cytidine and guanosine have been shown by ^{1}H NMR study to form heterodimers, trimers and tetramers at appropriate stoichiometries via Watson-Crick and Hoogsteen bonding schemes[4]. Substitution on nucleobase affects the hydrogen bonding affinity of monomer complementary pairs[2]. For example, substituents at 5-position of a pyrimidine base may alter either the acidity of imino N3 proton or affect the polarizability of C4 carbonyl; both cases lead to marked changes in association with complementary base via hydrogen bond involving these acceptor/donor sites. Similarly, substituents at 2 and 8 positions of a purine base may change their complementation strength.

Recently, there has been renewed interest in modified nucleosides and nucleotides, not only for their antiviral properties, but also for their applications in oligonucleotide therapeutics[5]. 5-aminouridine and its acyl derivatives are known to posses a wide range of biological effects[6], including antibacterial and antiviral activities and interference in purine biosynthesis. Further, 5-amino pyrimidine which possesses additional

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hydrogen bonding sites, on incorporation into an oligonucleotide may have potential to modulate major groove interactions of DNA and hence their biochemical and biophysical properties. In this paper, we report the differential ability of 2'-deoxyadenosine (dA) to hydrogen bond with 5-amino-2'-deoxyuridine ($dUNH_2$) and 5-methyl-2'-deoxyuridine (dT) in chloroform (Figure 1). By employing ^1H and ^{13}C NMR to investigate the association process, it is demonstrated that at a monomeric level complementation of dA with $dUNH_2$ is weaker compared to that with dT.

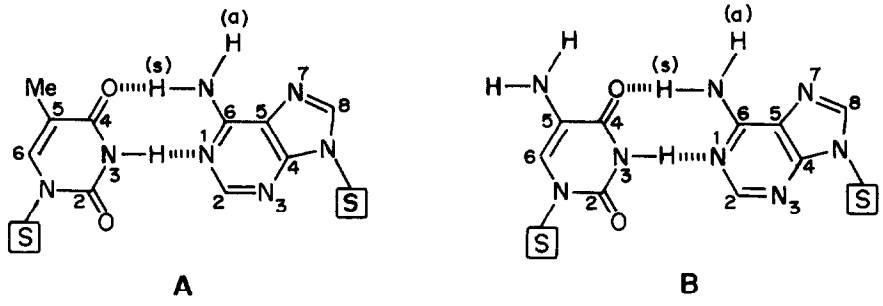


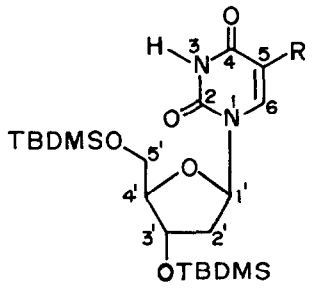
Figure 1. Watson-Crick hydrogen bonding scheme in (A) dT:dA and (B) $dUNH_2$:dA complexes. *s* and *a* refers to *syn* (hydrogen bonded) *anti* (non hydrogen bonded) protons on 6-NH_2 function of dA. S refers to 2'-deoxysugar of nucleosides.

RESULTS AND DISCUSSION

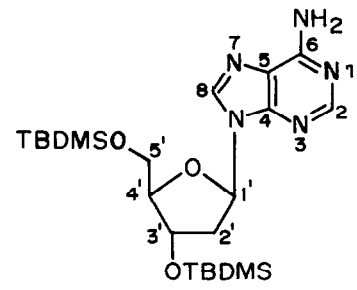
Complexation titrimetry and evaluation of binding constants

In view of the demonstrated utility[4] of lipophilic 3',5'-bis(triisopropyl) derivatives of 2'-deoxycytidine (dC) and 2'-deoxyguanosine (dG) for ^1H NMR study of base pair formation through hydrogen bonding in non-aqueous solvents, we have used the corresponding bis(t-butyldimethylsilyl) (TBDMS) derivatives of $dUNH_2$ (1), dT(2), and dA(3) in the present studies. $dUNH_2$ was synthesised from dU by a known procedure[7] and characterized by preparation of suitable derivatives. The nucleosides were converted into their corresponding 3',5'-bis(TBDMS) derivatives according to literature[8] and purified by chromatography to spectroscopic purity. The association constants between the complementary pairs dA:dT and dA:$dUNH_2$ were determined by titrimetry involving incremental, stoichiometric additions of dA to the pyrimidine component in chloroform-d. The titration was monitored by ^1H NMR spectral changes after each addition. In both pairs, the imino N3 proton (N3-H) of pyrimidine exhibited significant downfield shifts whose magnitude varied as a function of purine concentration (Figure 2). In contrast to N3-H, other protons on nucleobase and sugar moieties showed negligible shifts during titration. The selective downfield shift of N3-H is indicative of its involvment in hydrogen bonding (Figure 1). This is further confirmed by low temperature ^1H NMR experiments (see later) where it exhibited similar downfield shifts upon cooling. The protons of the exocyclic 6-NH_2 group of dA also shifted downfield compared to free dA but as titration proceeded, an upfield shift was noticed due to a gradual increase in the fractional concentration of free purine. Figure 2 shows mole ratio plot for N3-H shifts in pairwise complexation of dA:dT and dA:$dUNH_2$. The binding

isotherms clearly point to a complexation stoichiometry of 1:1, beyond which the induced shifts reach a plateau to a limiting value. The binding constants computed for 1:1 complexation using HOSTEST II programme[9], incorporating a linear regression algorithm were for dA:dT 330 M^{-1} and for dA:dUNH$_2$ 245 M^{-1}. Thus the dUNH$_2$ complexation with dA is about 30% weaker than dA:dT complexation.



1 R = NH_2 ; **2** R = CH_3



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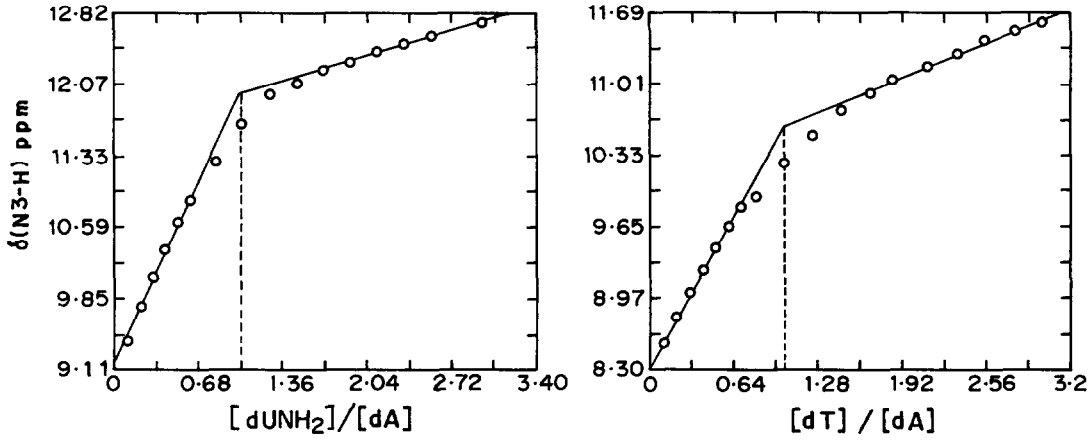

Figure 2. Plots of shifts in ^{1}H resonance of N3-H as a function of added dA (**3**). Vertical dotted line at break point corresponds to 1:1 stoichiometry.

Temperature dependant ^{1}H NMR spectral studies

In order to check whether the additional hydrogen bonding function (5-NH_2) promotes any non Watson-Crick patterns, temperature dependant ^{1}H NMR of a 1:1 stoichiometric mixture of individual pairs dA:dT and dA:dUNH$_2$ were recorded upon mixing equimolar amounts of purine and pyrimidine components in chloroform-d. The N3-H of dT and 6-NH_2 of dA moved downfield as expected for association through Watson-Crick hydrogen bonding scheme[3]. The 5-NH_2 protons did not show any such shift indicating their non-involvment in base pairing. As the temperature of the mixture was lowered, all the exchangeable protons (N3-H of dT and dUNH$_2$, 6-NH_2 of dA and 5-NH_2 of dU NH_2) suffered downfield

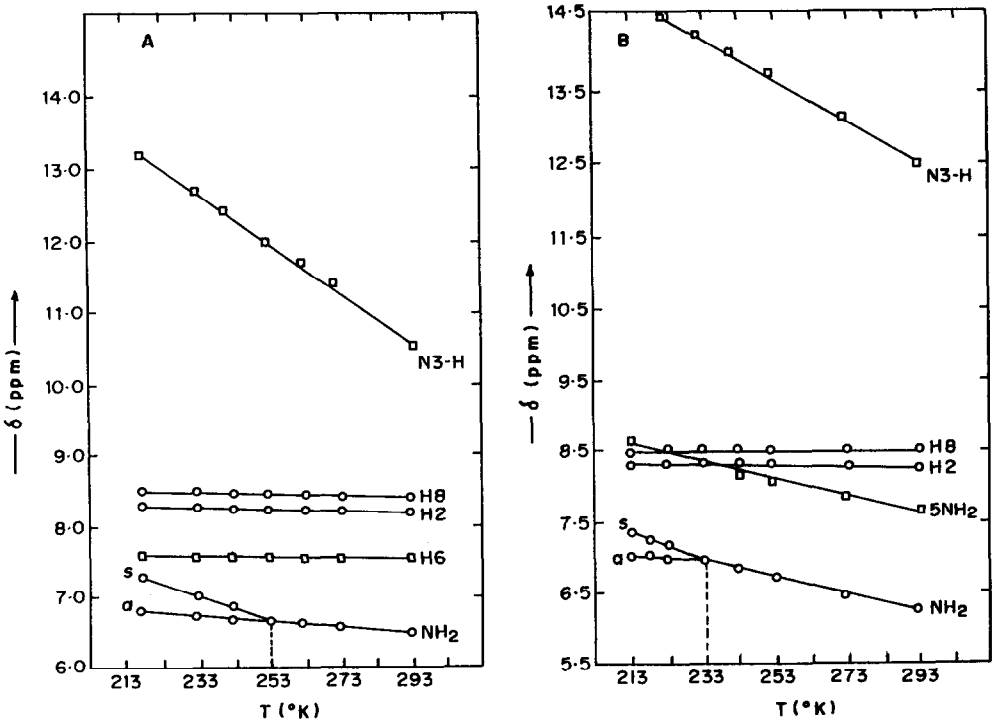

Figure 3. Plot of shift of various ^1H resonances in (A) dA:dT and (B) dA:dUNH$_2$ complexes at 1:1 stoichiometry as a function of temperature. ○ indicates protons in dA and □ refers to protons in dT (A) and dUNH$_2$ (B). The corresponding proton assignments are shown on right side. Splitting temperature of 6-NH$_2$ protons in each is indicated by vertical dotted line.

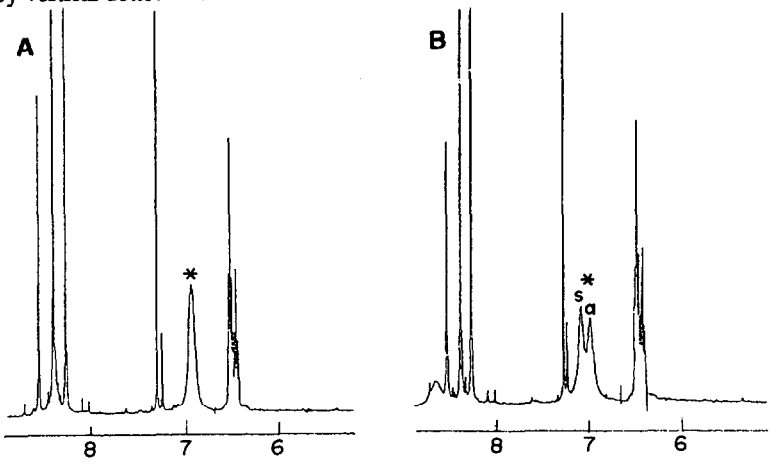

Figure 4. Partial ^1H NMR spectra of dA:dUNH$_2$ (1:1) complex at (A) 233°K and (B) 223°K. The 6-NH$_2$ resonances are indicated by *.

shifts (Figure 3). In contrast to exchangeable protons (amino and imino), H2 and H8 of purine and H6 of pyrimidine did not shift significantly. This confirms that selective downfield shift observed for 6-NH_2 (purines) and N3-H (pyrimidines) protons are a consequence of hydrogen bonding rather than the possible stacking or aggregation at lower temperature. In both pairs, the observed shifts had a linear dependence on temperature and the magnitude of shifts observed for N3-H protons was approximately twice that seen for 6-NH_2 protons. This is characteristic of complexation by Watson-Crick hydrogen bonding mode.

Significant differences were noticed for 6-NH_2 protons of dA upon cooling the 1:1 complexes. At low temperatures the 6-NH_2 protons exhibited separated signals (Figure 4) in both complexes and this is due to hindered rotation about N6-C6 bond. The hindered rotation is perhaps assisted by hydrogen bonding of 6-NH_2 group with C4 carbonyl of dT. Such an effect has been earlier noticed for 4-NH_2 protons of cytidine[10], 2-NH_2 protons of guanosine[4] and 6-NH_2 protons of adenosine[11] upon base pair complexation. Interestingly the 6-NH_2 protons of dA exhibited separated signals below -20°C in dA:dT complex, whereas in dA:dUNH_2 complex, similar splitting occurred only at much lower temperature (-40°C) (Figure 3). This change in 6-NH_2 proton splitting temperature may be directly attributed to difference in the 6-NH_2--O=C hydrogen bond strength among the two complexes; a stronger hydrogen bond leads to a slower rotation of 6-NH_2 group and hence a higher coalescence temperature. The observed results imply that in dA:dUNH_2 complex, the 6-NH_2--O=C hydrogen bond is weaker compared to analogous bonding in dA:dT complex. Thus 5-NH_2 substituent in a pyrimidine lowers the strength of the amino-carbonyl hydrogen bond in Watson-Crick base pairing mode. This fact is in agreement with a lower association constant obtained for dA:dUNH_2 pairing compared to dA:dT complex.

Table 1 shows the temparature coefficients ($\Delta\delta/\Delta T$) for the exchangable protons N3-H, 5-NH_2 and 6-NH_2 in dA:dT and dA:dUNH_2 complexes, as computed from experimental results. The imino proton (N3-H) in both complexes have almost identical values and are higher than those for 5- and 6-amino protons. The temperature coefficients of the 5-NH_2 protons was identical to that 6-NH_2 protons under rapid rotation regime (column 1). Among the separated 6-NH_2 protons (column 2), the downfield proton (syn) which is hydrogen bonded has a higher value than the non hydrogen bonded (anti) protons. Although no significant differences were noticed in the pattern of temperature coefficients observed for analogous protons in the two complexes, the variations seen among the imino and amino protons are quite diagnostic of their relative hydrogen bonding strengths.

Table 1: Temperature coefficients ($\Delta\delta/\Delta T$, ppm/deg)*

Complex	N3-H	5-NH_2	----------6-NH_2----------	
			1+	2#
dA:dT	0.03	-	0.01,	0.018(s),0.006(a)
dA:dUNH_2	0.029	0.01	0.012,	0.02 (s),0.006(a)

* Signs are ignored since in all cases a downfield shift is observed.
+ Column 1: temperature coefficient upto break point in Fig 3.
# Column 2: s, syn; a, anti

^{13}C NMR spectral studies of complexation

^{13}C chemical shifts directly reflect the electron density at carbon and are therefore useful as analytical probes for study of hydrogen bond formation involving amide carbonyls in nucleosides[12]. Hydrogen bonding promotes polarization of the carbonyl group which drains electron density from the carbon, leading to a downfield shift. The electron density at C4 may be influenced by a C5 substituent in pyrimidines and this inturn may purturb the acceptor capacity of C4 carbonyl group. A comparison of the induced shifts of C4 and C2 carbonyls in pyrimidines enables delineation of association through Watson-Crick (C4) or reversed Watson-Crick (C2) base pairing modes. The results were interpreted as due to simultaneous existence of both Watson-Crick and reverse Watson-Crick bonding patterns and in dA:dT complex there exists a greater population of molecules using C4 carbonyl as acceptor site, compared to C2 carbonyl, while in dA:dUBr complex, the reverse situation is seen. Such ^{13}C shift derived results also have support from IR data[2].

Table 2 gives the magnitude of induced ^{13}C NMR shifts for selected nucleobase carbons in dA:dT and dA:dUNH$_2$ complexes. It is observed that C2 and C4 signals of pyrimidine component shift downfield by larger amounts than other signals, clearly attributable to their direct participation in base pairing. In dA:dT complex, C4 exhibits a downfield shift (0.8ppm) higher than C2 (0.53ppm), while in dA:dUNH$_2$, the situation is reversed (C4, 0.68; C2, 0.76 ppm). The results for dA:dT complex compares well with that reported earlier[12] while the pattern obtained for dA:dUNH$_2$ complex resembles that seen in case of C5-Br nucleosides[12]. Although the differences seen in ^{13}C induced shifts among C4 and C2 carbonyls in dA:dUNH$_2$ complex is not as pronounced as corresponding values in dA:dUBr case, the observed qualitative identity suggests a predominance of reverse Watson-Crick base pairing in dA:dUNH$_2$ complex. The induced changes seen for dA (C5, C6 and C8) in both complexes are similar indicating identical electron density changes in dA upon complexation with dT and dUNH$_2$.

Table 2: Complexation induced shifts in ^{13}C NMR*

	--------dT/dUNH$_2$----------				---------dA----------		
Complex	**C2**	**C4**	**C5**	**C6**	**C5**	**C6**	**C8**
dA:dT	-0.53	-0.79	-0.04	+0.005	+0.009	-0.016	-0.07
dA:dUNH$_2$	-0.76	-0.68	+0.16	-0.11	+0.36	-0.16	-0.1

* Signs of induced shifts; - indicates downfield, + indicates upfield

Effect of 5-NH$_2$ substitution on dA:dU complexation

It is known from earlier literature that electronic effect of C5 substituents on pyrimidine ring markedly influence the base pairing patterns[1,2]. C5-CH$_3$ substituent (in dT) which is a weak electron releasing group, pushes electron into pyrimidine ring, thus increasing the electron density at C4 and consequently enhancing polarisation of C4 carbonyl. Thus C4 carbonyl becomes a better electron acceptor for hydrogen bonding compared to C2 carbonyl. On the other hand, Br being more electronegative, C5 substitution with Br

drains electron density from the ring, thus decreasing the polarizability of C4 carbonyl leading to a weaker hydrogen bond compared to C2 carbonyl. However, this is accompanied by an increase in N3-H acidity, which enhances its hydrogen bond donor property, leading to an overall increase in association constant. NH_2 group, although not as much electronegative as Br, is a better electron releasing function (by resonance) and it is therefore interesting to see its effect on base pairing. Further, it provides additional hydrogen bonding sites and being a small hydrophilic group, it has the potential to modulate major groove interactions when incorporated as a C5 substituent in DNA. Our present study shows following interesting effects of C5-NH_2 pyrimidine substituents on base pair association with dA even at monomer level.

1. Compared to dT, $dUNH_2$ has a weaker association constant with dA. This can be attributed to a decrease in polarizability of C4 carbonyl, thereby leading to a weaker 6-NH_2(dA)--O=C4($dUNH_2$) hydrogen bonding as evidenced by a lower splitting temperature for 6-NH_2 protons. The 5-NH_2 function has a rapid rotation around C5-N5 bond and no separation of 5-NH_2 protons is observed even at -50°C. Although no direct evidence could be obtained for its participation in intramolecular hydrogen bonding with C4 carbonyl, such a possibility cannot be ruled out since 5-NH_2 group shows a reasonable temperature coefficient. If such intramolecular hydrogen bonding does exist, it will certainly contribute to alter the acceptor strength of C4 carbonyl and may assume significance at DNA level.

2. The electron releasing mesomeric effect of C5-NH_2 group does not contribute to enhance the C4 carbonyl polarization. Existence of such an effect is borne by a marked upfield shift of C6 in $dUNH_2$ compared to dU or dT.

3. C5-NH_2 substitution causes an increase in population of molecules with 6NH_2(dA)--O=C2(dU) hydrogen bonding (reverse Watson-Crick) compared to 6NH_2(dA)--O=C4(dU) bonding mode (Watson-Crick). As this is sterically more hindered, the overall association by base pair complementation is a weaker process.

Thus C5-NH_2 substituent can cause subtle changes of base pairing properties of pyrimidine nucleobase. In addition to the above, the 5-NH_2 function can be a suitable centre to covalently anchor extraneous ligands for modulation of DNA properties. We have recently incorporated $dUNH_2$ into synthetic oligonucleotides[13] and further work is in progress to study their biophysical properties, nuclease resistance, chemical derivatization, enzymatic incorporation and their ability to induce directed mutation in biological systems.

EXPERIMENTAL

All 2'-deoxynucleosides and t-butyldimethylchlorosilane (TBDMC) were obtained from Aldrich. TLC was carried out using E. Merck precoated silica gel 60 F_{254} plates (Cat No 5554) and using the following solvent system: A, CH_2Cl_2:MeOH (85:15); B, CH_2Cl_2:MeOH (95:5). Compounds were visualised on TLC plate either under UV light and\or as dark spots after spray with perchloric acid in ethanol (60%) followed by charring. Column chromatographic separations were done using silica gel (100-200 mesh, Loba-Chemie).

^1H (200 MHz) and ^{13}C (80 MHz) NMR spectra were recorded on Bruker ACF200 MHz spectrometer, fitted with an Aspect 3000 computer. Low temperature ^1H NMR were recorded on Bruker MSL300 NMR spectrometer using BVT1000 temperature controller. For ^1H and ^{13}C NMR, TMS and solvent chloroform-d resepectively were used as internal standards and chemical shifts expressed in δ scale (ppm). The ^1H NMR titration data were analysed after volume corrections, using HOSTEST II software[9]. The confidence level in the analysis were more than 0.95.

5-Amino-2'-deoxyuridine

5-Bromo-2'-deoxyuridine (3.10 gm, 10 mmol) was treated with liquid ammonia (25 ml) in stainless steel bomb and heated at 55°C for 24 hr. After removal of excess ammonia the resulting product was treated with pyridine (25 ml) when a solid separated out. This was filtered and the filtrate was concentrated to a gum, which was then chromatographed on silica gel. The column was eluted with CH_2Cl_2 containing increasing proportions of MeOH, when the required compound eluted at 20% MeOH in CH_2Cl_2 (1.8 gm, yield 74%). Rf (System A) = 0.2, Ninhydrin positive, **^1H NMR** (D_2O): 7.25 (s, 1H, H6), 6.25 (t, 1H, H1', J = 7 Hz), 4.4 (m, 1H, H3'), 3.95 (m, 1H, H4'), 3.7 (m, 2H, H5'and H5''), 2.25 (m, 2H, H2' and H2''). **^{13}C NMR** (DMSO-d6): 160.6 (C4), 148.9 (C2), 122.8 (C5), 115.1 (C6), 87 (C4), 83.6 (C1'), 70.6 (C3'), 61.7 (C5'), 38.7 (C2').

5-N-Acetyl-3',5'-di-O-acetyl-5'-amino-2'-deoxyuridine

5-Amino-2'-deoxyuridine (0.24 gm, 1mmol) was taken in dry pyridine (2 ml) to which acetic anhydride (1.4 ml, 15mmol) was added and kept stirred for 2 hr. It was then concentrated to a gum, which was chromatographed on silica gel and on elution with 8% MeOH in CH_2Cl_2 gave the desired triacetate as white solid, (0.26 gm, yield 70%), m.p., 241 - 242°C, Rf (System A) = 0.83, Ninhydrin negative, **^1H NMR** ($CDCl_3$-$DMSOd_6$): 11.15 (bs, 1H, N3-H), 8.4 (s, 1H, H6), 8.05 (s, 1H, 5-NHCO), 6.15 (t, 1H, H1', J = 6.9 Hz), 5.05 (m, 1H, H3'), 4.1 (m, 2H, H5' and 5"), 3.9 (m, 1H, H4'), 2.15 (m, 2H, H2'and 2"), 1.9 and 1.85 (s, 9H, 3 x$COCH_3$). **^{13}C NMR** ($CDCl_3$-$DMSOd_6$): 169.5 and 170.1 (2x$\underline{C}OCH_3$), 168 ($NH\underline{C}OCH_3$), 159.1 (C4), 148.2 (C2), 124.5 (C5), 114.8 (C6), 84.2 (C4'), 81.4 (C1'), 73.7 (C3'), 63.1 (C5'), 36.2 (C2'), 20.1, 20.2 and 23.1 (3 x$CO\underline{C}H_3$).

5-Amino-3',5'-di-O-(t-butyldimethylsilyl)-2'-deoxyuridine (1)

5-Amino-2'-deoxyuridine (1.20 gm, 5 mmol), was taken in dry pyridine (2 ml) to which TBDMC (3 gm, 20 mmol), was added and kept stirred at r.t. for 24 hr. The reaction was monitored by TLC and after completion it was concentrated to an oil and chromatographed on silica gel. Elution with n-Hexane:CH_2Cl_2 (1:4) followed by increasing amounts of MeOH in CH_2Cl_2 gave the required disilyl derivative **1**, (1.40 gm, yield 60%), m.p., 198 - 200°C, Rf (System B) = 0.56, Ninhydrin positive. **^1H NMR** ($CDCl_3$): 9.81 (s, 1H, N3-H), 8.63 (s, 1H, H6), 7.73 (s, 2H, 5-NH_2), 6.3 (t, 1H, H1', J = 6.5 Hz), 4.4 (m, 1H, H3'), 3.9 (m, 1H, H4'), 3.73 and 3.83 (m, 2H, H5' and 5"), 2.05 and 2.3 (2xddd, 1H each, H2' and 2", J = 3.2, 6.5 and 13.5 Hz), 0.90 and 0.93 (2xs, 18H, 2x$C(CH_3)_3$), 0.08 and 0.10 (2xs, 12H, 2x$Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$): 159.5 (C4), 148.2 (C2), 126.4 (C5), 114.7 (C6), 87.9 (C4'), 85.7 (C1'), 72.3 (C3'), 63.2 (C5'), 40.1 (C2'). Analysis, Calc. $C_{21}H_{41}O_5N_3Si_2$: C, 53.47; H, 8.75; N, 8.90. Found: C, 53.60; H, 9.20; N, 8.45.

3',5'-di-O-(t-butyldimethylsilyl)-2'-deoxythymidine, (2) and 3',5'-di-O-(t-butyldimethylsilyl)-2'-deoxyadenosine, (3)

These were synthesised according to general procedure reported in literature[8].

Compound **2**, **^{1}H NMR** ($CDCl_3$): 8.85 (s, 1H, N3-H), 7.4 (s, 1H, H6), 6.33 (dd, 1H, H1', J = 5.9, 7.8 Hz), 4.41 (m, 1H, H3', J = 2.7 Hz), 3.92 (m, 1H, H4'), 3.8 and 3.7 (ddd, 2H, H5' and 5"), 2.0 and 2.2 (ddd, 2H, H2' and 2", J = 2.7 and 5.9 Hz), 1.9 (s, 3H, 5-CH3), 0.9 and 0.92 (2xs, 18H, 2x$C(CH_3)_3$), 0.06 and 0.13 (2xs, 12H, 2x$Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$): 163.5(C4), 150.1 (C2), 135.4 (C6), 110.7 (C5), 87.8 (C4'), 84.8 (C1'), 72.2 (C3'), 62.9 (C5'), 41.3 (C2'). Analysis, Calc. $C_{22}H_{42}O_5N_2Si_2$: C, 56.13; H, 8.98; N, 5.95. Found: C, 55.94; H, 9.5; N, 5.75.

Compound **3**, **^{1}H NMR** ($CDCl_3$): 8.35 (s, 1H, H8), 8.15 (s, 1H, H2), 6.45 (t, 1H, H1', J = 6.4 Hz) 5.9 (s, 2H, $6NH_2$) , 4.61 (m, 1H, H3'),4.01 (m, 1H, H4'), 3.7 and 3.8 (ddd, 2H, H5' and 5"), 2.4 and 2.5 (ddd, 2H, H2' and 2", J = 6.4, 4.1 and 13.0 Hz), 0.93 (s, 18H, 2x$C(CH_3)_3$), 0.15 (s, 12H, 2x$Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$): 155.3 (C6), 152.9 (C2), 149.6 (C4), 139.1 (C8), 120.1 (C5), 87.9 (C4'), 84.3 (C1'), 71.9 (C3'), 62.8 (C5'), 41.2 (C2'). Analysis, Calc. $C_{22}H_{41}O_3N_5Si_2$: C, 55.08; H, 8.60; N, 14.59. Found: C, 55; H, 8.50; N, 14.36.

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REFERENCES

1. Saenger, W.; *"Principles of Nucleic Acid Structures"*, 1984, Springer-Verlag, NY

2. Koyogoku, Y.; Lord, R. C.; Rich, A. *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 250-257

3. (a) Katz, L.; Penman, S. *J. Mol. Biol.* **1966**, *15*, 220-231.
   (b) Newmark, R. A.; Cantor, C. R. *J. Am. Chem. Soc.* **1968**, *90*, 5010-5017.

4. (a) Williams, L. D.; Chawla, B.; Shaw, B. R. *Biopolymers*. **1987**, *26*, 591-603.
   (b) Williams, N. G.; Williams, L. D.; Shaw, B. R. *J. Am. Chem. Soc.* **1989**, *111*, 7205-7209.

5. (a) Ulhmann, E.; Peyman, A. *Chemical. Rev.* **1990**, *90*, 544-584.
   (b) Tamm, C.; Strazewski, P. *Angew. Chem. Int. Ed. Eng.* **1990**, *29*, 36-57.
   (c) Englisch, U.; Guass, D. H. *Angew. Chem. Int. Ed. Eng.* **1991**, *30*, 613-629.

6. (a) Visser, D. W.; Roberts, M. *J. Biol. Chem.* **1951**, *194*, 695-701.
   (b) Smith, D. A.; Visser, D. W. *Biochim. Biophys. Acta.* **1966**, *119*, 221-226.
   (c) Ivanovics, G. A.; Rousseau, R. J.; Robins, R. K. *J. Med. Chem.* **1971**, *14*, 1155-1158.

7. Visser, D. W.; *"Synthetic Procedures in Nucleic Acid Chemistry"*. John-Wiley, NY

8. Ogilvie, K. K. *Can. J. Chem.* **1973**. *51*, 3799-3807.

9. Wilcox, C. S. in "*Frontiers in Supramolecular Organic Chemistry and Photochemistry*", Schneider.H.; Durr.H.; Ed.; VCH. Weinheim, **1990**.

10. (a) Becker, E. D.; Miles, H. T.; Bradley, R. B. *J. Am. Chem. Soc.* **1965**, *87*, 557
(b) Raszka, M.; Kaplan, N. O. *Proc. Natl. Acad. Sci. USA*. **1972**, *69*, 2025-2029.

11. Kyogoku, Y.; Iwahashi, H.; Sugeta, H. *Biochemistry*. **1982**, *21*, 631-638.

12. (a) Jones, A. J.; Grant, D. M.; Winkley, M. W.; Robins, R. K. *J. Phy. Chem.* **1970**, *74*, 2684-2689.
(b) Kyogoku, Y.; Iwahashi, H. *J. Am. Chem. Soc.* **1977**, *99*, 7761-7765.
(c) Jones, A. J.; Winkley, M. W.; Grant, D. M.; Robins, R. K. *Proc. Natl. Acad. Sci. USA*. **1970**, *65*, 27-30.

13. This was done by using ß-cyanoethyl phosphoramidite chemistry on Pharmacia Gene Asembler Plus. Barawkar, D. A; Ganesh, K. N.; Unpublished results.