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SOLID PHASE SYNTHESIS OF DNA CONTAINING 5-NH_2-2'-DEOXYURIDINE+

Dinesh A Barawkar and K.N.Ganesh*
Division of Organic Chemistry, Bio-Organic Unit
National Chemical Laboratory, Pune 411008, INDIA.

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Abstract: Solid phase synthesis of DNA containing 5-amino-2'-deoxyuridine is reported using trifluoroacetyl as a 5-NH_2 protector.

There is growing interest in oligonucleotides containing modified bases and base analogues due to their applications as oligonucleotide probes and antisense inhibitors useful in oligonucleotide therapeutics.[1-4] Substituents on nucleobases (C5 of pyrimidines, C2 and C8 of purines) may affect their complementation properties by altering the acidity of hydrogen bond donor sites and polarizability of acceptor groups.[5] We have recently shown that, at monomer level, compared to dA:dT complexation the 5-NH_2 substituent in dU leads to slightly decreased association with dA and increased receptor strength of C2 carbonyl over C4 carbonyl.[6] 5-NH_2 Uridine and its N^5-acyl derivatives are known to possess a wide range of biological effects, including antibacterial and antiviral activities.[7] When 5-NH_2 pyrimidine is incorporated into an oligonucleotide, the additional hydrogen bonding sites present at C5 are directed into the major groove and may thereby influence the biophysical properties and interactions of DNA with groove binding ligands. Further, 5-NH_2 group can also be a suitable chemical center for covalent anchoring of extraneous ligands such as fluorophores and DNA cleaving moieties. In view of these attractive possibilities, herein we demonstrate a convenient strategy for the incorporation of 5-NH_2-dU into DNA. This needed development of a selective protector for 5-NH_2 group which is stable to chemistry of oligonucleotide synthesis. To the best of our knowledge, no previous report exists on the synthesis of either an appropriately protected precursor monomer such as **8** required for phosphoramidite approach or 5-NH_2 pyrimidine consisting DNA.

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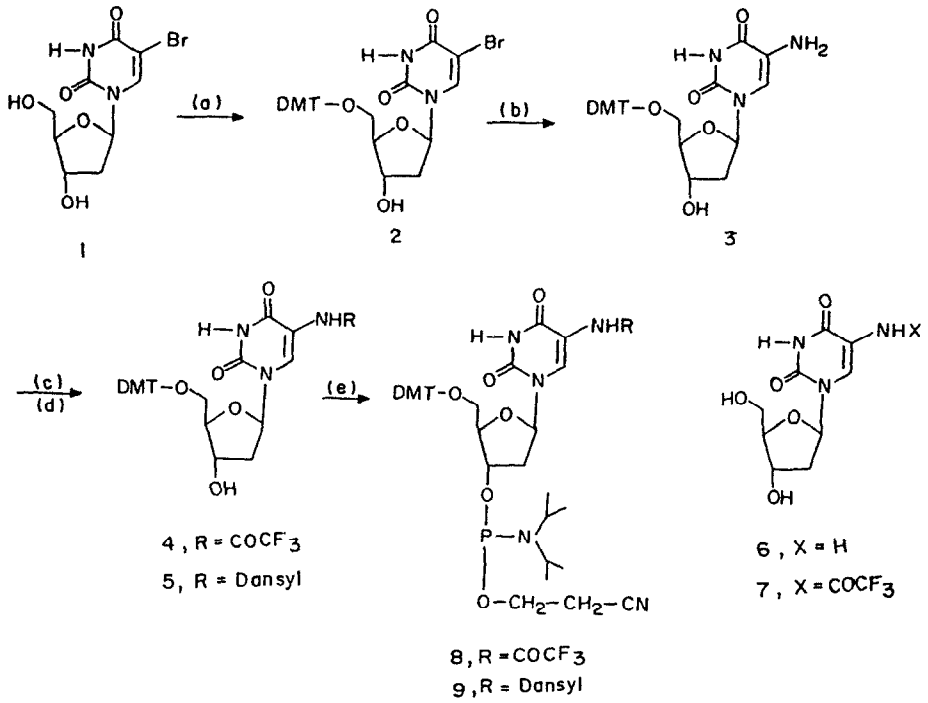

Scheme 1: Reagents (a) DMTCl/Pyridine. (b) Liquid NH_3, 60°C, 24 hr. (c) CF_3COOEt, MeOH/TEA, RT. (d) Dansylchloride, DMF/$NaHCO_3$. (e) 2-Cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite.

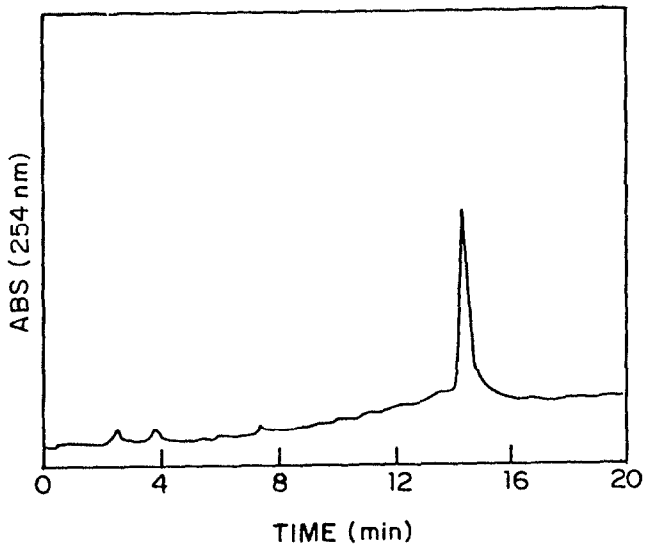

Figure 1: Reverse phase HPLC[10] of **11**.

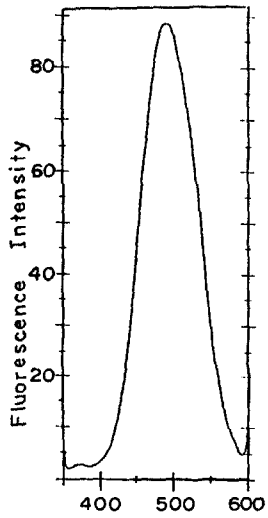

Figure 2: Fluorescence spectrum of **13** in H_2O \ex 335nm, \em 500 nm.

The chemical reactivity of 5-NH_2 group of pyrimidines is different from that of exocyclic 4-NH_2 group in dC. We found that the standard amino protecting groups on nucleobases[8,9] such as benzoyl, acetyl etc. were not suitable for 5-NH_2 function since the final N-deprotection with ammonia was inefficient.[10] We resorted to the use of trifluoroacetyl group (TFA) as a 5-NH_2 protector. So far, this group has not been used as a protecting group for nucleobase exocyclic amino functions in DNA synthesis due to its high lability. We found that $COCF_3$ as a protector of 5-NH_2 group had right stability and ammoniacal lability (aq NH_3, 1 hr, 60°C) for use in oligonucleotide synthesis and so the target compound **8** was synthesised according to Scheme **1**.

5-Br-dU **1**, was converted to the 5'O-DMT derivative **2**. This was treated with liquid NH_3 in a steel bomb and the removal of excess NH_3 gave compound **3** in quantitative yield (95%). **3** was reacted with ethyltrifluoroacetate[11] in MeOH/TEA to obtain 5'O-DMT-N^5-TFA-2'-dU **4** in 75% yield. Alternatively, **4** could be synthesised by first converting **1** into 5-NH_2 derivative[7] **6** followed by N^5-trifluoroacetylation to give **7** and subsequent dimethoxytritylation. The route shown in Scheme **1** had two advantages: (i) it gave a better overall yield (52%) than the alternative method (26%) and (ii) practical isolation of **3** from liquid ammonia reaction of **2** was easier than that of **6** from **1**. Compound **4** was converted into the desired ß-cyanoethyl phosphoramidite **8** by using standard procedure.[12] All compounds were purified to homogeneity by either chromatography or crystallisation and characterised by ^1H and ^{13}C NMR spectroscopy.[13] The monomer **8** gave ^{31}P NMR (δ, 149.6 and 149.4 ppm, ref, 85% H_3PO_4) characteristic of 3'O-phosphoramidite and no N^5-phosphonylation was observed. A fluorescent analog, 5-NH_2-dansyl derivative **9** was also synthesised from **3** via **5**.

10 d(C G C G A A T $U^{\#}$ C G C G) $U^{\#}$ = 5-NH_2-dU
11 d(C G C G A A $U^{\#}$ $U^{\#}$ C G C G)
12 d(C G C G A A T U^{*} C G C G) U^{*} = 5-NH-dansyl-dU
13 d(C G C G A A U^{*} U^{*} C G C G)

Compounds **8** and **9** were individually incorporated at desired sites into DNA oligomers **10-13** corresponding to analogs of well studied Dickerson's dodecamer by usual protocol on an automated DNA synthesiser (Pharmacia GA plus). The coupling efficiencies of modified amidites were similar to the commercial phosphoramidites of normal nucleosides. After the completion of synthesis, final on-column detritylation was followed by NH_3 treatment to yield fully deprotected oligonucleotides. These were purified by FPLC (Pharmacia) on RPC column[14] and rechecked by HPLC (Figure 1).

The compounds **10** and **11** possessing 5-NH_2-dU residues gave slightly lower T_m (**10**, 57°C; **11**, 55°C in 10 mM Tris.HCl, pH 7.6, 80 mM NaCl and 20 mM $MgCl_2$) as compared to that of unmodified dodecamer (T_m, 60°C). Duplex generated by an equimolar mixture of **12** and unmodified Dickerson's dodecamer gave a T_m of 53°C. Thus dansylation of 5-NH_2 group in dU has only marginal effect on stability of duplex. Oligonucleotides **10** and **11** were found to be stable to digestion by Eco R1 restriction enzyme confirming modification within the recognition site (**G A A T T C**), while the digestion product with snake venom phosphodiesterase gave the expected base composition. The correct incorporation and retention of 5-NH_2-dansyluridine in DNA oligomers **12** and **13** was indicated by the UV absorbance at 335 nm (broad band) along with that due to DNA at 260 nm and the fluorescence with emmision at 500 nm on excitation at 335 nm (Figure 2).

In this communication we have demonstrated successful solid phase synthesis of DNA containing 5-NH_2-dU and using TFA as a 5-NH_2 protector. The method has also been extended to prepare the corresponding fluorescent derivatives. The reactivity of 5-NH_2 group may permit a direct post-synthetic conjugation of modified DNA with useful ligands.[15] The only other methods for synthesis of C5 substituted dU derivatives are by Pd(II) mediated coupling of either 5-HgCl derivative with α,ß-unsaturated esters[16] and allylamines[17] or Pd(0) coupling of 5-iodo nucleosides with alkynylamines in presence of added CuI[18]. The present method is not only comparable to the reported method in terms of ease and overall yields, but also offers possibilities of direct conjugation of ligand to C5 via amide linkage with or without a spacer chain. Direct conjugation of ligands with short linker chains have useful consequences in control of fluorescence properties[19] and precision in footprinting experiments.[20] Biophysical studies of oligonucleotides containing mono and multiple 5-NH_2-dU units are in progress to evaluate their potential for future applications as oligonucleotide probes, antisense inhibitors and for conjugation with intelligent ligands.

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13. *Typical procedures and spectral data*: **Compound 2, ^{1}H NMR** ($CDCl_3$): δ 8.15 (s, 1H, H6), 7.25 to 7.75 (m, 9H, DMT), 6.85 (m, 4H, DMT), 6.35 (q, 1H, H1'), 4.6 (m, 1H, H3'), 4.15 (m, 1H, H4'), 3.8 (s, 6H, 2xOCH_3), 3.4 (m, 2H, H5'and H5"), 2.6 and 2.35 (m, 2H, H2'and H2"). **^{13}C NMR** ($CDCL_3$): 159.4 (C4), 149.1 (C2), 139.3 (C6), 97.1 (C5), 86.6 (C4'), 85.7 (C1'), 72.1 (C3'), 63.4 (C5'), 55.1 (OCH_3), 41.2 (C2').

Compound 3, ^{1}H NMR ($CDCl_3$): δ 7.7 to 7.2 (m, 9H, DMT), 6.85 (m, 4H, DMT), 6.5 (t, J=6.5Hz, 1H, H1'), 4.65 (m, 1H, H3'), 4.1 (m, 1H, H4'), 3.75 (S, 6H, 2xOCH_3), 3.45 (m, 2H, H5' and H5"), 2.4 (m, 2H, H2' and H2"). **^{13}C NMR**: ($CDCl_3$) 160.7 (C4), 150 (C2), 127 (C6), 116.6 (C5), 86.1 (C4'), 84.6 (C1'), 72 (C3'), 63.6 (C5'), 55.1 (OCH_3), 40.4 (C2').

Compound 4, **3** (0.5 g, 1mmol) was treated with CF_3COOEt (1.2 mL, 10mmol) and TEA (0.69 mL, 10mmol) in MeOH (12 mL) at r.t for 18 h. Work up using CH_2Cl_2 gave **4** (0.48 g,75%), Rf (3% MeOH in CH_2Cl_2) 0.4; **^{1}H NMR** ($CDCl_3$): δ 8.6 (s, 1H, H6), 7.75 to 7.2 (m, 9H, DMT), 6.8 (m, 4H, DMT), 6.35 (t, J=6Hz, 1H, H1'), 4.4 (m, 1H, H3'), 4.1 (m, 1H, H4'), 3.8 (s, 6H, 2xOCH_3), 4.45 to 3.35 (m, 2H, H5'and H5"), 2.45 to 2.2 (m, 2H, H2' and H2"). **^{13}C NMR**, ($CDCl_3$): 159.2 (C4), 148.6 (C2), 144.5 ($\underline{C}OCF_3$), 136.4 (C6), 129 (C5), 112.3 (CF_3), 86 (C4'), 85.7 (C1'), 71.6 (C3'), 63.(C5'),55.2 (OCH_3), 39.9 (C2').

Compound 5, 3 (0.4 g, 0.7 mmol) was treated with dansyl chloride (0.1 g, 0.8 mmol) in DMF (2 mL) containing saturated $NaHCO_3$ (1.2 mL) at r.t for 6 h, purified by column chromatography, gave **5** (0.28 g, 50%), Rf (3% MeOH in CH_2Cl_2) 0.5; **^{1}H NMR** ($CDCl_3$): δ 8.65 to 7.9 (m, 6H, Dansyl), 7.65 (s, 1H, H6), 7.55-7.0 (m, 9H, DMT), 6.85 (m, 4H, DMT), 6.16 (t, J=6.3Hz, 1H, H1'), 4.36 (m, 1H, H3'), 4.1 (m, 1H, H4'), 3.8 (s, 1H, 2xOCH_3), 3.53-3.35 (m, 2H, H5' and H5"), 2.8 (s, 6H, 2xNCH_3), 2.45-2.0 (m, 2H, H2'and H2"). **^{13}C NMR** ($CDCl_3$): 159.6 (C4), 148.5 (C2), 85.7 (C4'), 85.1 (C1'), 71.5 (C3'), 63.7 (C5'), 54.8 (OCH_3), 44.8 (NCH_3), 39.7 (C2').

Compound 7, m.p 96-98°C, **^{1}H NMR** (DMSO-D_6): δ 8.25 (s, 1H, H6), 6.15 (t, J=8 Hz, 1H, H1'), 4.2 (m, 1H, H3'), 3.8 (m, 1H, H4'), 3.55 (m, 2H, H5'and H5"), 2.2-2.0 (m, 2H, H2'and

H2"). **13C NMR** (DMSO-D_6): 159.2 (C4), 149.1 (C2), 144.1 ($\underline{C}OCF_3$), 142.8 (C6), 130.5 (C5), 112 (CF_3), 87.7 (C4'), 85.7 (C1'), 71.1 (C3'), 62.1 (C5'), 40.5 (C2').

Compound 8, Synthesised from 4 according to ref 12 and purified by precipitation from DCM by cold hexane. **^{1}H NMR** ($CDCl_3$): δ 8.6 (s, 1H, H6), 7.15 to 7.55 (m, 9H, DMT), 6.8 (m, 4H, DMT), 6.35 (t, 1H, H1'), 4.5 (m, 1H, H3'), 4.2 (m, 1H, H4'), 3.7 (s, 6H, 2xOCH_3), 3.2 to 3.6 (m, 6H, OCH_2, 2xNCH, H5' and H5"), 2.4 to 2.8 (m, 4H, CH_2CN, H2' and H2"), 1.25 (d, 12H, 2x$NC(CH_3)_2$).

14. FPLC, Buffer A: 5% CH_3CN, 0.1 M TEAA. Buffer B: 30% CH_3CN, 0.1 M TEAA. Gradient: 0%B, 3 min; 0-15%B, 5 min; 15-75%B, 35 min; 75-100%B, 1 min. HPLC, Buffer A and B: same as above. Gradient: A to B, 20 min.
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21. Abbreviations: dA, 2'-deoxyadenosine; dU, 2'-deoxyuridine; dT, thymidine; dansyl, 5-dimethylamino-1-naphthalenesulphonyl; DMT, 4:4'-dimethoxytrityl; TFA, trifluoroacetyl; TEAA, triethylammonium acetate.