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8-AMINO(2-AMINOETHYL)-2'-DEOXYADENOSINE INCORPORATION INTO DNA BY SOLID PHASE SYNTHESIS+

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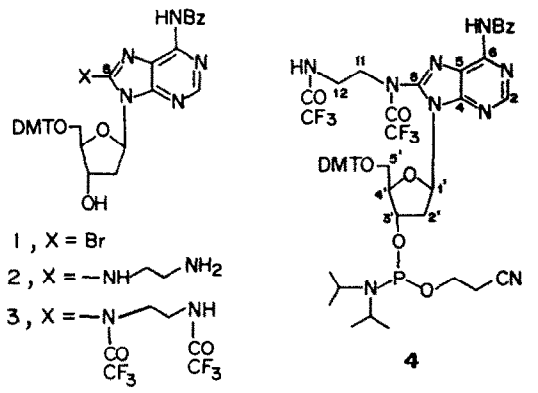
Abstract: Solid phase synthesis of modified oligonucleotide **5** *containing 8-amino(2-aminoethyl)-2'-deoxyadenosine unit is reported using trifluoroacetyl as a C8-side chain amino protector.*

There has been growing interest in synthetic DNA containing unnatural ligands linked at specific sites to act as reporter groups or as DNA molecular scissors.[1-3] Such DNA conjugates have been obtained either by chemical incorporation of modified nucleotides carrying ligands at base residues (N^4 of dC/C5 of T) or by synthetic conjugation at 5' or 3' DNA terminus. Through "convertible nucleoside approach," a series of functional groups have been installed at N^4 of dC and N^6 of dA by post-synthetic aminoalkylation of a single precursor.[4,5] Recently, site-specific DNA backbone modifications have been effected by oxidative amination of internucleotide H-phosphonate bonds.[6,7] Among various strategies for functional tethering of DNA, approaches for ligand conjugation at C8 of purines has been singularly lacking.[8,9] In view of the interesting DNA structural modifications that this may offer, we demonstrate here, the synthesis of hitherto unknown C8-amino(2-aminoethyl)-dA phosphoramidite synthon **4** and its site-specific incorporation into DNA sequences.

The target synthon **4** was prepared from the previously reported[9] 5'O,N^6-protected-8-bromo-2'-dA **1** in 3 steps. Aminoalkylation at C8 was achieved by reaction of $H_2N(CH_2)_2NH_2$ with **1** in EtOH (25 °C, 20 h) to obtain **2** in 70% yield. Acetyl and benzoyl groups as C8 side chain amino protectors proved to be too stable for final deblocking with aq. NH_3 (60 °C, 17h) and could only be deprotected[3] by 1M NaOH (60 °C, 8h.). The choice of trifluoroacetyl as a protecting group for C8-side chain amino groups was dictated by its stability to solid phase DNA synthesis by phosphoramidite chemistry and lability to final deprotecting reagents. The N,N'-bis(trifluoroacetyl) derivative **3** was synthesised from **2** by reaction with CF_3COOEt in MeOH/TEA.[10] **3** was transformed into the required ß-cyanoethyl phosphoramidite **4** by using standard method.[11] No N-phosphonylation was observed as seen by ^{31}P NMR of **4** that gave signals only at 149.9 and 149.5 ppm which are characterastic of 3'-O-amidite. All compounds were purified by chromatography and characterised by ^1H and ^{13}C NMR.[12]

+NCL Communication Number 5638



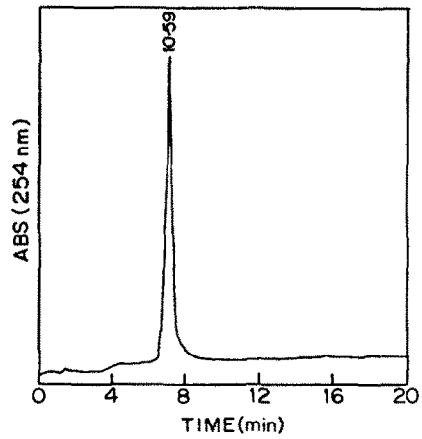


Figure 1: Reverse phase HPLC of **5**. For conditions see ref. 13

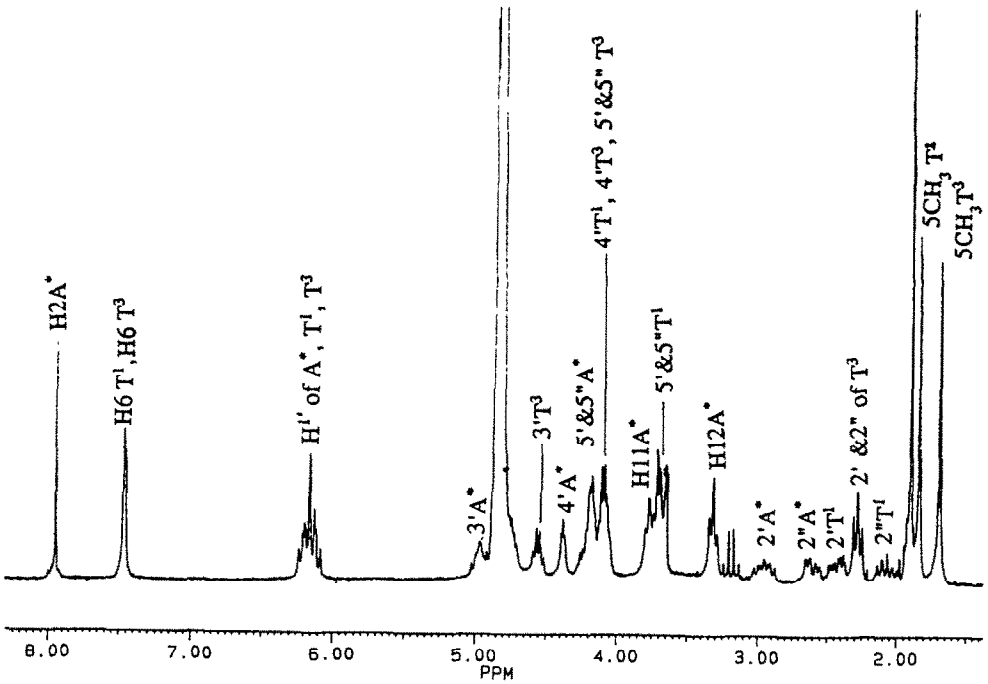
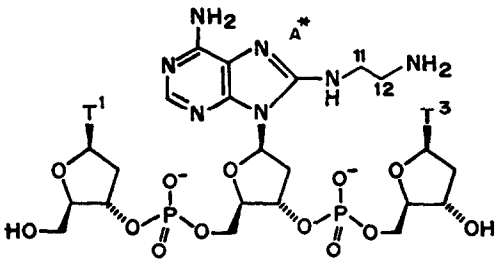


Figure 2: 200 MHz NMR spectrum of **6** in D_2O. All assignments confirmed by 2D COSY.

Compound **4** was incorporated into the well studied Dickerson's dodecamer sequence at defined sites by usual procedure on an automated DNA synthesiser (Pharmacia GA Plus). The coupling efficiencies of modified amidite **4** was similar to the commercial phosphoramidites of normal nucleosides. After completion of synthesis, final on-column detritylation was followed by aq. NH_3 treatment (60 °C, 18h.) to yield the fully deprotected oligonucleotide **5**. This was desalted on NAP-25 gel filtration column and purity checked by reverse phase HPLC (Figure 1).[13]

5, **d(C G C G A* A T T C G C G)** **A* = 8-amino(2-aminoethyl)adenine**

6,



The successful incorporation of **4** and retention of C8 modified dA in oligonucleotide synthesis was proved unambiguously by solid phase synthesis of a trinucleotide **6** (10 μmol scale) and its characterisation by ^1H NMR spectra (Figure 2). The diagnostic signals due to H2 (7.95 ppm) and the two CH_2's from C8-amino(2-aminoethyl) side chain of dA (H11, 3.8 & H12, 3.3 ppm) and H6 (7.45 ppm, overlapping) and 5-CH_3 (1.88 & 1.68 ppm) of T^1& T^3 could be clearly assigned in ^1H NMR. **6** gave a single ^{31}P NMR signal at -0.49 ppm (ref. 85% H_3PO_4). Compared to Dickerson's dodecamer, the modified oligonucleotide **5** showed a lower T_m ($\Delta T \simeq 15°$), perhaps due to repulsion of two positively charged ω-NH_3^+ groups situated spatialy close in this self complementary duplex DNA or due to a modified glycosidic torsion.[14] **5** was also 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 convenient synthetic approach presented here would enable access to a number of hitherto unknown C8-ligand conjugated DNA oligomers, with potential to alter stability of DNA duplexes and triplexes (for eg. see ref. 5). Further, since C8 side chains protrude into the major groove of DNA helix, interesting possibilities exist for derivatization with amino acids,[15] peptides and metal chelators for structural modulation and site-specific cleavage of DNA.

References and Notes

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and 3 cited therein.

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12. *Typical procedures and selected spectral data*: **Compound 2**: **1** (0.8 g, 1.1 mmol) in absolute EtOH (20 ml) was stirred with $NH_2(CH_2)_2NH_2$ (0.5 ml, 7.8 mmol) at 25 °C for 20 h. Solvent removal followed by silica gel chromatography gave **2** (0.55 g, 70%). Rf (MeOH:CH_2Cl_2, 1:9 V/V) = 0.18, λmax (MeOH), 308 nm (ϵ 5 x 10^4); **^{1}H NMR** ($CDCl_3$:DMSO-d_6:D_2O, 5:3:1) δ 8.3 (s, 1H, H2), 6.38 (t, J = 7.0 Hz, 1H, H1'), 4.5 (m, 1H, H3'), 4.0 (m, 1H, H4') 3.7 (s, 6H, 2 x OMe), 3.4 (dd, J = 10.7 and 5.0 Hz, 1H, H5"), 3.26 (dd, J = 10.7 and 5.0 Hz, 1H, H5'), 3.1 (t, J = 5 Hz, 2H, H11), 3.0 (m, 1H, H2' overlap with H11), 2.73 (t, J = 5 Hz, 2H, H12), 2.25 (m, 1H, H2"). **^{13}C NMR** ($CDCl_3$:DMSO-d_6, 5:3) δ 85.6 (C1'), 42.6 (C2'), 70.8 (C3'), 82.9 (C4'), 63.1 (C5'), 41 (C11), 36.9 (C12), 123.4 (C5), 153 (C2), 147.4 (C4), 153.7 (C6) and 144 (C8).

Compound 3: **2** (0.4 g, 0.6 mmol) was treated with CF_3COOEt (1 ml, 9 mmol) and TEA (1.3 ml, 9 mmol) in dry EtOH (7 ml) at r.t for 18 h. Work-up using CH_2Cl_2 gave **3** (0.4 g, 80 %), Rf (MeOH:H_2O, 1:9, V/V) 0.6; **^{1}H NMR** (DMSO-d_6) δ 9.5 (brs, 2H, NH), 6.1 (t, J = 6.3 Hz, 1H, H1'), 4.6 (m, 1H, H3'), 3.9 (m, 1H, H4'), 3.7 (s, 6H, 2 x OCH_3), 3.4 (m, 4H, H11 and H12), 3.2 (dd, J = 4.2 and 3.8 Hz, H5'), 3.1 (dd, J = 5.9 and 6.6 Hz, H5"), 3.0 (m, 1H, H2'), 2.1 (m, 1H, H2"); **^{13}C NMR** (DMSO-d_6) δ 157.4 (N$\underline{C}$O$\underline{C}F_3$), 156.7 (NH$\underline{C}$O$\underline{C}F_3$), 152.9 (C6), 152.3 (C2), 149.1 (C8), 127 (C5), 119.3 (NCO$\underline{C}F_3$), 117.8 (NHCO$\underline{C}F_3$), 85.8 (C1'), 83.0 (C4'), 71.2 (C3'), 64.2 (C5'), 55.4 (OCH_3), 46.3 (C11), 41.5 (C2') and 39.7 (C12).
13. Column: Novapak C18, Buffer A: 5% CH_3CN, 0.1 M triethylammoniumacetate , Buffer B: 30% CH_3CN in 0.1 M triethylammoniumacetate, Gradient: A to B 20 min.
14. Studies on a series of C8-amino(alkylamino)-5'-AMP have shown dependence of glycosidic torsion upon the length of the alkyl chain.[14a,b] While in ethylenediamine derivatives, the strong electrostatic interactions possible between 5'-phosphate and the w-amino function forces the glycosidic conformation to anti, higher alkyledene derivatives adopt a syn form. Whether such conformational effects are also true for DNA oligomers containing C8 substituents is not clear. (a) Lavayre, J.; Ptak, M. and Leng, M., *Biochem. Biophys. Res. Commun.*, **1975**, *65*, 1335. (b) Saenger, W., *Principles of Nucleic Acid Structure*, Springer- Verlag, New York, **1983**.
15. We have prepared C8-histaminoadenosine derivatives by a similar procedure for designing ribonuclease mimics and will be reported elsewhere.
16. T.P.P acknowledges CSIR (New Delhi) for a research fellowship.