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Synthesis and Conformational Studies of d(TpA) and r(UpA) Conjugated with Histamine and Ethylenediamine#

T.P. Prakash, R. Krishna Kumar and K.N. Ganesh*

Bio-Organic Unit, Organic Chemistry Division,
National Chemical Laboratory, Pune 411008, India

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Abstract: Dinucleotides (Figure 1b-d) possessing histamino/ethylenediamino substituents at C8 of adenine have been synthesised for modelling the molecular interactions that occur at catalytic site of nucleases. These compounds have been designed for putative molecular recognition of internucleotide phosphate by a complementary group (imidazole/-NH_2) in the pendant C-8 side chains. ^1H NMR spectroscopic analysis of synthesised model compounds indicate that C-8 modification leads to increase in percentage of S conformation of modified sugar while still maintaining an anti glycosyl torsion as in unmodified analog d(TpA). The C-8 side chain functionality (histamine/ethylenediamine) is probably involved in intramolecular interaction (electrostatic/ hydrogen bond) with the phosphate and/or 2'OH in (14). Such predisposition of side chain catalytic groups is important in developing appropriate models for active site of nucleases.

INTRODUCTION

Binding and hydrolytic cleavage of phosphate diesters, particularly biological phosphates such as DNA and RNA are important processes[1,2]. Enzymes that act on nucleic acids such as ribonuclease,[3] DNA polymerase I,[4] *E.Coli* alkaline phosphatase,[5] endo and exo restriction nucleases,[4] contain either metal ions[6] (Zn^{2+}, Mg^{2+}) or positively charged amino acid residues such as arginine, lysine or protonated histidine in their active sites for recognition-interaction with negatively charged phosphate group. The active sites also contain amino acids with nucleophilic side chains such as aspartic acid, tyrosine and histidine which mediate phosphate hydrolysis by either general or specific base catalysis. Recently, several attempts have been made to mimic hydrolytic nuclease action by synthetic models such as oligonucleotide-peptides[7] and drugs.[8]

Ribonuclease A, in particular functions through two histidine and a lysine at the active site.[3] In our approaches towards development of synthetic nucleases, we have attempted step-wise modelling of the molecular interactions that occur at the catalytic site of ribonuclease. In this paper, we present synthetic and spectroscopic studies on three dinucleotides (Figure 1b-d) which contain substitution of either imidazole (histidine mimic) or an amino ethylamino group (lysine mimic) at C8 of adenine unit. The 2'-deoxy–

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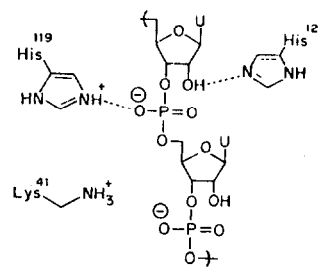
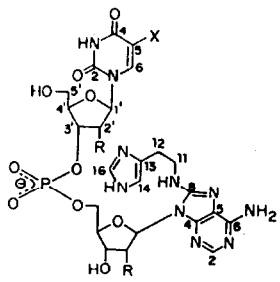
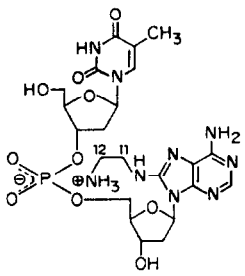


(a)



(b) R = H, X = CH_3

(c) R = OH, X = H



(d)

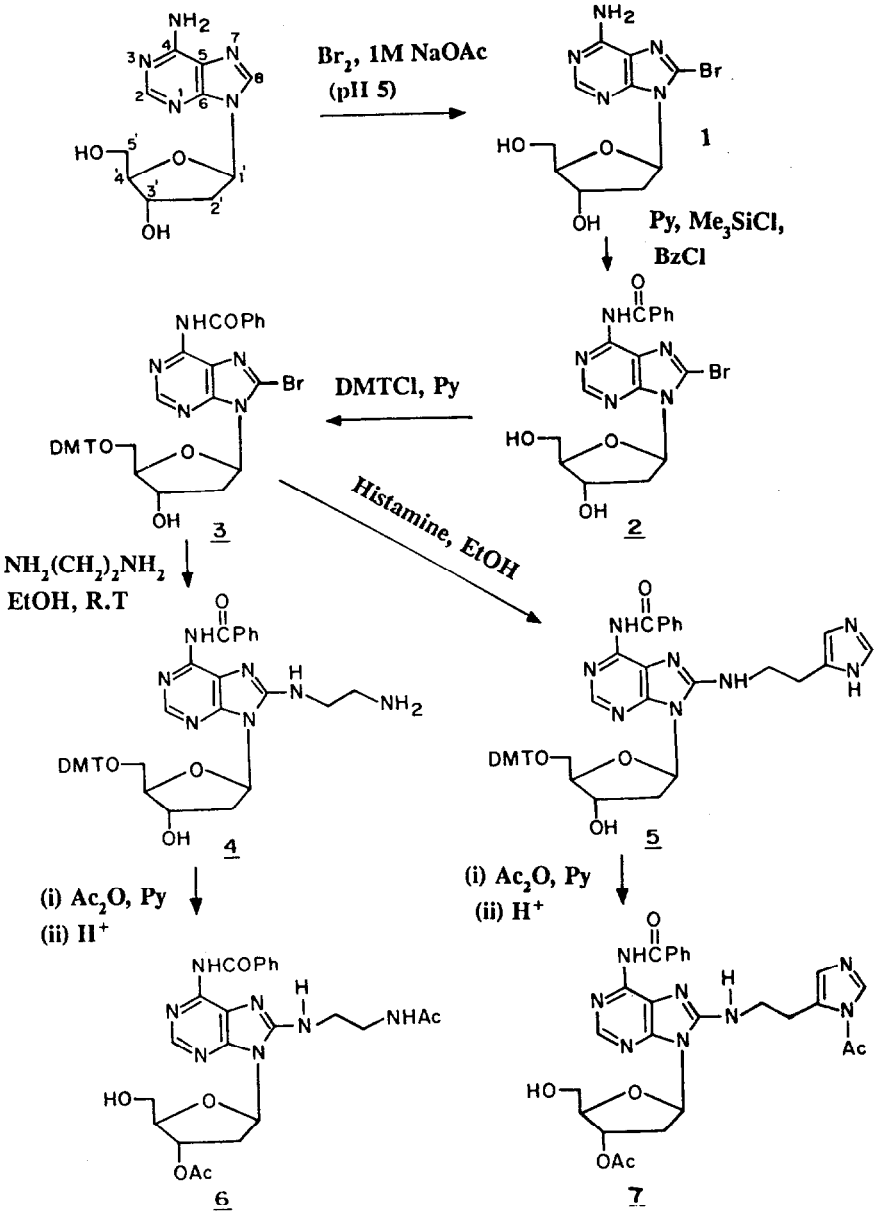
Figure 1:

dinucleotides in Figure 1b and 1d would exclusively model the phosphate-amine interactions whereas the r(UpA) analog (Figure 1c) contain in addition a 2′-OH group on 5′-nucleotidyl unit, as in natural substrate. Such spatial positioning of an imidazole/amino group near the internucleotide phosphodiester linkage is expected to effect or accelerate the phosphate cleavage when the full complement of catalytic groups are incorporated into the model for testing the cleavage reaction.

RESULTS AND DISCUSSION

Synthesis of C8 conjugated histamine and ethylenediamine dinucleotides. The C8 position of adenine is easily amenable for bromination and hence suitable for linking a side chain functional group via an aminoalkyl spacer chain. We have previously employed such a strategy for synthesis of fluorescent adenine derivatives[9]. The synthetic route used at present is similar but with minor modifications. Treatment of 2′-deoxyadenosine with bromine-water in sodium acetate buffer at pH 5.0 gave 8-bromo-2′-deoxyadenosine **1**[10]. This was N^6 benzoylated by the transient protection method[11] to yield **2** which was then 5′-O-protected with 4,4′-dimethoxytrityl (DMT) group to give **3**. Subsequent reaction with excess of either 1,2-diaminoethane or histamine gave the corresponding C8 conjugates **4** and **5** respectively. These were then converted into their N,3′O-acetyl derivatives by reacting with acetic anhydride-pyridine, followed by detritylation with 2% dichloroacetic acid (DCA) in dichloromethane (DCM) to generate the 5′-hydroxyl terminal blocks **6** and **7**.

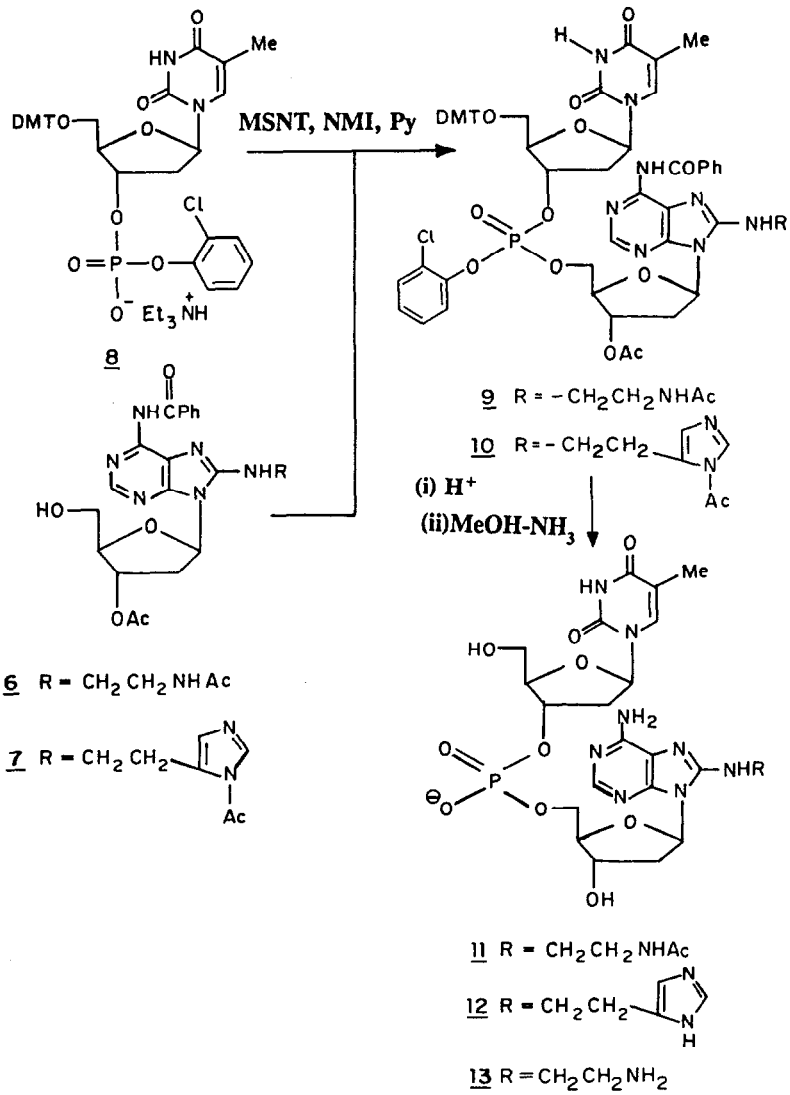
The triethylammonium[5′-O-DMT-thymidine-3′O-(2-chlorophenyl)phosphate][12] **8** was then condensed with either **6** or **7** in presence of mesitylenesulphonyl-3-nitrotriazole (MSNT) and N-methylimidazole (NMI) to generate the protected dinucleotides **9** and **10** respectively. No side products due to N-phosphonylation (as seen in ^{31}P NMR) or 5′-sulphonylation were observed. The dinucleotides **9** and **10** purified by silica gel column chromatography were then completely deprotected by treatment with 2% DCA-DCM to remove 5′-DMT group followed by reaction with MeOH-NH_3 to effect cleavage of the N^6-benzoyl, 3′-O-acetyl and 2-chlorophenyl groups, to yield the products **11** and **12**. These compounds were purified by ion-exchange column chromatography. The ^1H NMR clearly indicated that while the N-acetyl group of C8 side chain in **12** was completely removed during ammonolysis, the N-acetate of ethylenediamine chain in **11** remained intact (see experimental). This N-acetyl group was

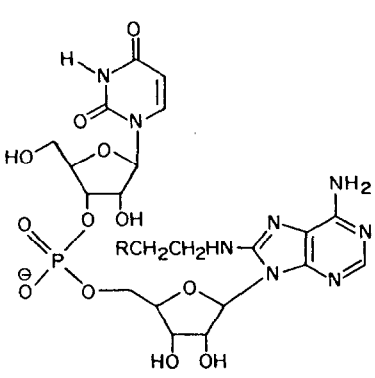


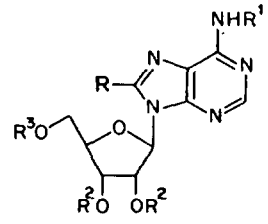
hydrolysed by a final treatment of **11** with aq. NaOH (1M) at 60°C to give **13** whose analysis by HPLC showed that the internucleotide phosphate link was unaffected under these reaction conditions. Control experiment with unmodified d(TpA) confirmed the stability of phosphate linkage. These results are also supported by a literature precedent[13] where the N-acetyl group of a dC analog could be deblocked only under the above conditions without affecting the internucleotide linkage. Figure 2 shows the HPLC purity

of the dinucleotides **12** and **13** which were further characterized by ^{1}H and ^{31}P NMR spectroscopy. The above synthetic route clearly established that the C8 aminoalkyl chain is stable to phosphotriester oligonucleotide synthesis and deprotection conditions, without accompanying any chemical modifications.

The target ribodinucleotide **14** was synthesised similarly by condensation of the 5′-OH compound **19** (prepared from 8-bromoadenosine **15** in 4 steps, see experimental) with the ribo 3′-phosphodiester **20** possessing TBDMS as a 2′-OH protector. We have earlier demonstrated the synthesis and utility of monomers such as **20** in RNA synthesis by phosphotriester approach[14]. The condensation product **21** was successively treated with 2% DCA-DCM, sat. MeOH-NH_3 and tetrabutylammoniumfluoride (TBAF), to





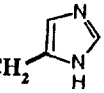


15 $R_1 = R_2 = R_3 = H$, R = Br

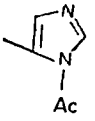
16 R_1 = Bz, $R_2 = R_3 = H$, R = Br

17 R_1 = Bz, $R_2 = H$, R_3 = DMT, R = Br

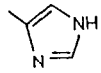
18 R_1 = Bz, $R_2 = H$, R_3 = DMT, R = $NHCH_2CH_2$

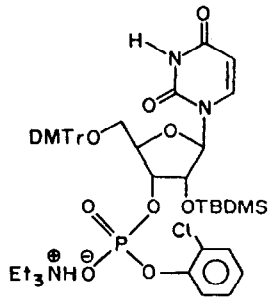


19 R_1 = Bz, R_2 = Ac, $R_3 = H$, R = $NHCH_2CH_2$



14 R =





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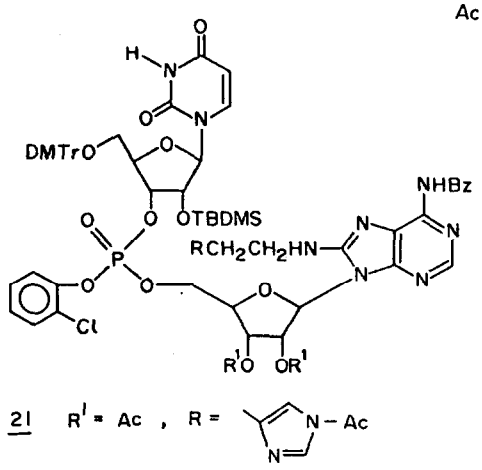

21 R^1 = Ac , R =

effect removal of 5′-DMT, phosphate and amino protecting groups and cleavage of 2′O-silyl group respectively. The completely deprotected ribodinucleotide **14** was purified by ion-exchange chromatography (Figure 2) and characterised by spectroscopy.

Spectroscopic characterisation- ^1H and ^{31}P NMR Studies.

One of the main requirements for successful intramolecular interaction of internucleotide phosphate group with the C8-conjugated ligand in dinucleotides is their relative spatial predisposition. This is indirectly influenced by the nucleoside sugar pucker, the glycosyl torsion and phosphate conformation. In order to understand the possible variations in the above structural features, we have analysed the ^1H and ^{31}P NMR of the dinucleotides **12-14.**

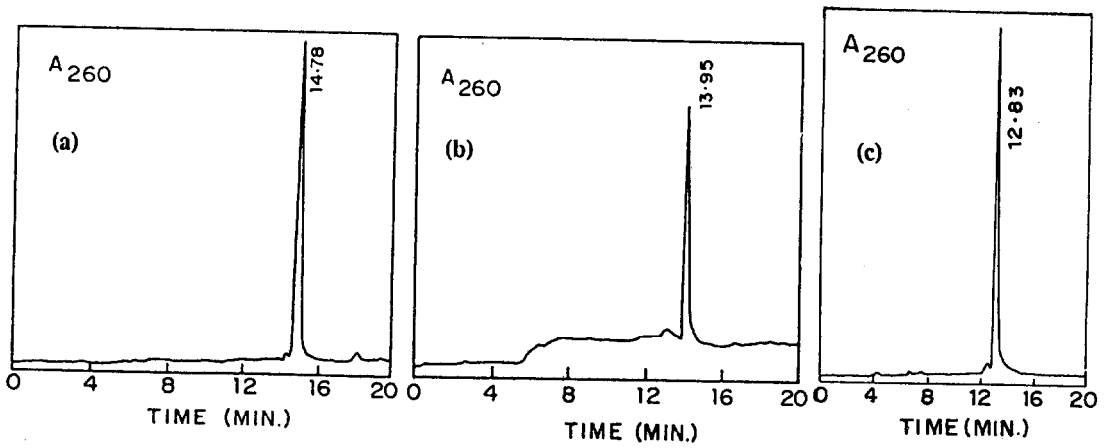

Figure 2: Reverse phase HPLC of (a) 12 (b) 13 and (c) 14. For conditions see experimental.

^1H NMR spectroscopy. Rinkel and Altona[15] have previously demonstrated that the conformational analysis of the sugar ring in nucleic acids can be performed by using sums of coupling constants viz., Σ 1′ (J1′2′+ J1′2″), Σ2′ (J1′2′+ J2′2″ + J2′3′), Σ2″ (J1′2″ + J2″3′ + J2′2″) and Σ3′(J2′3′ + J2″3′ + J3′4′). A Σ1′ value of 13.3 Hz or more was shown to arise by a predominant S-type conformation (2′-endo, >60%) wherein J1′2′>J1′2″ and Σ2′>Σ2″. In case of ribose sugars the J1′2′ and J3′4′ can be used to estimate population of N-type conformer. Crucial to the success of this empirical method is the unambiguous assignments of all the protons of different sugar residues to obtain ^1H-^1H vicinal coupling constants of different sugar residues.

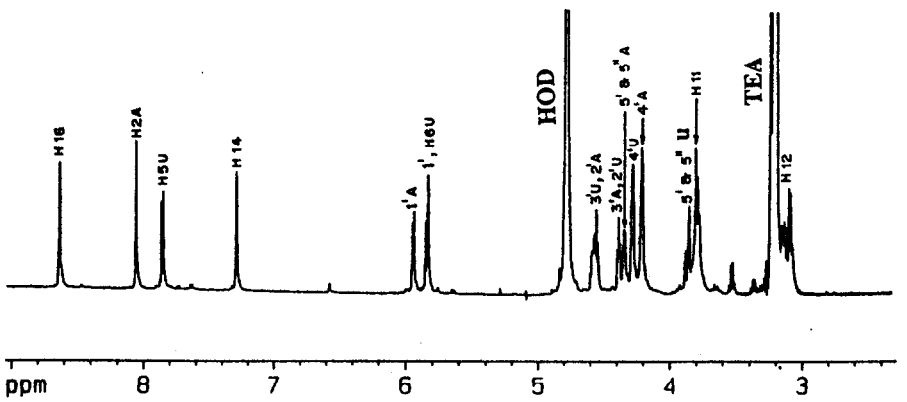

Figure 3: 500 MHz ^1H NMR spectrum of 14 (2 mM) in D_2O.

The complete proton chemical shift assignments of both sugar residues in the three dinucleotides was achieved by a combination of 1D and 2D NMR spectroscopy (Figure 3 and 4). The 2D NMR spectra were particularly needed for assignment of H3' and H5', H5" which are normally masked in 1D spectra by the residual solvent HOD signal and the side chain methylene protons. 2D NMR was also helpful for assignment of H17 and H18 from imidazole moieties in **12** and **14**. Coupling constant values (J1'2' and J2'3') were computed by means of a first order analysis. For all the deoxyribose units, the H1' resonance appeared as a triplet and hence individual J1'2' and J1'2" couplings could not be obtained from this part of the spectrum. Alternatively, this information was sought from the total width of H2" multiplet (=Σ2") which explicitly showed all the eight lines arising from its simultaneous coupling to H2', H1' and H3' protons. In dinucleotide **13** an extensive overlap with methylene resonances of histamine side chain excluded clear identification of H2" signal while in ribodinucleotide **14**, overlap of H2' and H3' signals prevented extraction of J1'2' and J3'4' coupling constants. Table-1 summarises the coupling constants and %S conformer calculated for various dinucleotides using the empirical method of Rinkel and Altona.[16]

It is observed that in all dinucleotides, the sugar units in general show a clear preference to populate in S-type conformation (>60%). The variation in S conformer population of 5'-nucleotidyl units

Table I: *Coupling Constants and %S Conformers*[+] *of Dinucleotides* **11-13**

Dimers		J1'2'	J1'2"	Σ1'	Σ2'	Σ2"	%S
dTpA	dTp-	8.1	6.1	14.2	-	24.4	75
	-pdA	7.3	6.0	13.3	-	22.2	59
11	dTp-	7.6	6.1	13.7	28.9	23.3	66
	-pdA	7.9	6.7	14.6	29.5	25.1	81
12	dTp-	7.4	6.2	13.6	28.1	23.8	64
	-pdA	8.0	6.6	14.6	29.6	23.8	81
13	dTp-	7.8	6.0	13.8	27.8	-	68
	-pdA	7.7	6.7	14.4	-	-	78

[+] $\%S = \frac{\Sigma 1' - 9.8}{5.9}$, (see ref 16 and 17)

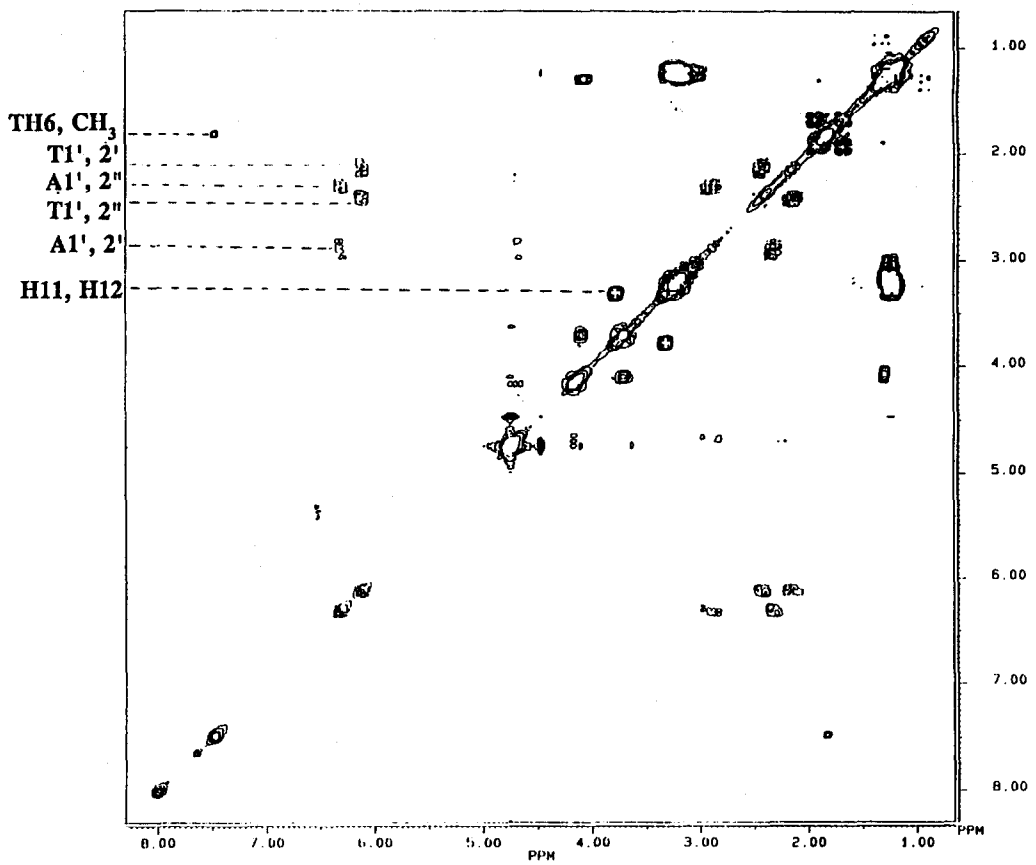

Figure 4: 2D COSY-45 spectrum of **13**. Experiment was carried out on Bruker AC 200 spectrometer attached with Aspect 3000 computer with 1024 and 256 datapoints along W_2 and W_1 dimensions respectively. FT was performed after zero-filling in W_1 axis and with suitable sine bell functions. Cross peaks assigned on left horizontal axis.

Table 2: ^{31}P Chemical Shifts of Dinucleotides 11-14

Dimer	dTpA	11	12	13	14
ppm	-0.49	-0.47	-0.50	-0.45	-0.21

among various dinucleotides was less than 15% (range 65-80 %) compared to a larger variation of 35% (range 59-94%) among 3′-nucleotidyl units. In the unmodified dimer (dTpA), the 5′-sugar residue has a greater preference for the S-type conformation compared to the 3′-sugar residue (Table-1). C8 modification leads to a reversal of situation and among the modified dimers, the S-conformer population is highest in case of C8-EDA modified dimer **13**. The computed data indicate that C8 modification does not change the sugar conformation, but instead increases the population of existing S-conformer. This is consistent with the previous observations[16] that in unmodified dinucleotides, irrespective of the nature of base and sequence, the pentose ring shows a clear preference to exist in S-conformation. Our present data extends the above rule to the effect that purine C8 modification leads to a greater increase in S-conformer population.

Inspection of relative proton chemical shifts shows a consistent trend in C8 modification induced shifts of H1′, H2′ and H2" protons. Modification of base in 3′-nucleotidyl unit (-pdA) imparts a through-space deshielding effect on H1′ and H2′ of neighbouring 5′-nucleotidyl unit (dTp-) resulting in a downfield shift of these protons by ≃ 0.2 and 0.6 ppm respectively, as compared to the corresponding protons in unmodified d(TpA). On the other hand, H2" of dTp- residues in **12-14** is upfield shifted by 0.2 ppm compared to H2" of d(TpA). The H1′ and H2′ protons of 3′-nucleotidyl unit in modified dinucleotides shows a reverse effect: upfield shifts of H1′ and a downfield shift of H2" (0.1 -0.2 ppm). Although changes in shifts for ^{1}H of modified 3′-dA unit would originate partly from electronic changes at C1′ and C2′, the nature of shifts observed for ^{1}H of dTp- suggests an anti conformation for the modified nucleoside (dA). In this conformation the C8-conjugated ligand is spatially close to the protons of adjacent 5′-sugar unit which thus exhibit induced shifts arising from through-space dipolar effects. This is substantiated by the generally larger induced shifts in histamine conjugates compared to EDA derivatives which may be attributed to histamine ring current effects. Thus C8 modification does not significantly affect the glycosyl torsion (anti disposition) found in natural nucleotides while the sugar residues exhibit enhanced population of S-conformers. 2D NOSEY spectra although gave the expected characterestic cross peaks from both nucleotidyl units but indicated no NOE's useful for internucleotidyl conformational assignment.

^{31}P NMR spectroscopy. ^{31}P chemical shifts can potentially provide a probe for the analysis of conformation of phosphate ester backbone in nucleic acids, since the predominant contribution to chemical shifts arises from stereoelectronic effects.[17] It has been generally observed that phosphodiesters in stereoelectronically favoured g$^-$g$^-$ conformation exhibit upfield shifts compared to those in gt or tg forms. An intramolecular interaction between the conjugated ligand and the internucleotidic phosphate group in **12-14**, either through electrostatic or H-bonding is expected to alter both the electronic density at phosphorus and the phosphate geometry. Evidence for such induced effects was sought from their ^{31}P NMR spectral shifts.

Table-2 shows comparative ^{31}P chemical shifts of various dinucleotides synthesised in the present work. It is seen that the observed chemical shifts of **11-13** (-0.44 to -0.50 ppm) is upfield to that of **14** (-0.21ppm). This indicates that in **11-13**, the phosphate conformation is the stereoelectronically favoured g$^-$g$^-$ form similar to that observed in normal (unmodified) nucleic acids while in **14**, there may exist significant

contribution from trans conformers (g⁻t or tt) or intramolecular environmental effects or both. In **14**, where an electrostatic interaction involving C8 side chain is less likely, a trans geometry for phosphate may arise from intramolecular hydrogen bond of the imidazole NH with phosphate oxygen or the imidazole ring N with 2'-OH. The former possibility is expected even in the deoxydimer **12**. Although the present data can not distinguish between the two possibilities, it appears that imidazole on C8 side chain in **14** is involved in intramolecular interactions thus affecting conformation of phosphate group. It may be mentioned that an intramolecular phosphate-NH_3^+ interactions has been seen in C8-EDA adenosine monophosphate.[18]

CONCLUSIONS

In this paper we have demonstrated the synthetic possibility of conjugating side chains containing intelligent functions (potentially catalytic) to C8 of adenine. Spectroscopic results suggest an intramolecular interaction of the conjugated ligands with internucleotidic phosphate group as manifested by altering the sugar and phosphate conformations. These dinucleotides are first of a group of compounds designed to model electrostatic and hydrogen bonding interactions present in the active site of ribonucleases. We have also recently incorporated C8-EDA conjugated 2'-dA nucleosides site specifically into oligonucleotides to generate higher members of this series[19]. Further studies are aimed at the introduction of putative catalytic groups (such as polyamines, arginine etc.) for promoting intramolecular interaction with phosphate and construction of the full complement of catalytic groups to test for the cleavage reaction.

EXPERIMENTAL

2'-Deoxynucleosides and 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT) were obtained from Aldrich,-U.K. 1-Methylimidazole (Fluka), histamine (Sigma) and ethylenediamine (SRL, India) were used without further purification. 8-Bromo-2'-deoxyadenosine[10], 8-bromo-5'-O-(4,4'-DMT)-2'-deoxyadenosine[9], and the nucleotide monomers[9] **8**, **20** were synthesised by reported procedures. Pyridine was refluxed and distilled from ninhydrin followed by distillation over CaH_2. Column chromatography was carried out on silica gel (100-200 mesh, Loba Chemie, India), and TLC was performed on precoated plates (silica gel GF254, Merck 5554) Solvent; A: DCM-MeOH, 9:1 (V/V), Solvent B: DCM-MeOH, 9.5:0.5(V/V). The spots were visualised under UV light and by spraying 60% perchloric acid-ethanol (3:1) followed by charring. UV spectra were recorded on Perkin-Elmer Lambda 15 spectrometer. HPLC: Buffer A 0.1 M TEAA, Buffer B 30% CH_3CN in 0.1 M TEAA, gradient A to B 20 min., flow rate 2 ml/min., UV detector at 260 nm. The melting points reported are uncorrected. NMR spectra were recorded on Bruker MSL 300 or AC 200 spectrometers. All chemical shifts are expressed in ppm. ^1H NMR spectra are reported downfield from TMS in $CDCl_3$ and in the case of D_2O downfield from DSS. ^{31}P NMR spectra are recorded at 81 MHz with 85% H_3PO_4 as external reference. ^{13}C NMR were recorded at 50 MHz in both ^1H decoupled and INEPT modes.

N^6-Benzoyl-5'-O-[4,4'dimethoxytrityl-8-amino(2-aminoethyl)]-2'deoxyadenosine (4). Compound (3) (800 mg, 1.1 mmol) was taken in absolute ethanol (20 ml) into which ethylenediamine (0.52 ml, 7.8 mmol) was added. The reaction mixture was stirred at room temperature for 20 h. The excess solvent was removed under vaccum and the product purified by silica gel column chromatography. Elution with DCM:MeOH gave compound (4) (530 mg, 68%) as a white solid (mp 123 °C). Rf (solvent A) = 0.18; Ninhydrin positive; λ = 308 nm (MeOH, ϵ 5.4 x 10^4); ^{1}H NMR ($CDCl_3$:$DMSOd_6$: D_2O, 5:3:1) δ 8.28(s, 1H, H2), 7.98 (dd, J = 7.9 Hz, 2H, ArH), 7.53-7.11 (m, 12H, ArH), 6.70 (dd, J = 8.8Hz, J = 3.5 Hz, 4H, ArH), 6.38 (t, J = 6.9 Hz, 1H, H1'), 4.56 (m, 1H, H3'), 4.03 (m, 1H, H4'), 3.69 (s, 6H, 2 x OCH_3), 3.4 (dd, J = 10.7 and 4.9 Hz, 1H, H5''), 3.26 (dd, J = 10.7 and 4.5 Hz, 1H, H5'), 3.08 (t, J = 5 Hz, 2H, H11), 3.0 (m, 1H, H2', overlapping with H11), 2.73 (t, J = 5 Hz, 2H, H12), 2.25 (m, 1H, H2"); ^{13}C NMR ($CDCl_3$:$DMSOd_6$, 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), 144 (C8), 165, 133.6, 127.7, 126.8, 131.5 (all from Bz).

N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-8-histamino-2'-deoxyadenosine (5). N^6 Benzoyl-8-bromo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (3) (0.35 g, 0.48 mmol) was dissolved in dry ethanol (10 ml). Histamine (0.11 g, 0.95 mmol) was added into this and the reaction mixture was stirred at room temperature for 18 h. Evaporation of ethanol gave a residue which was purified by silica gel column chromatography. Elution with DCM containing incremental amounts of MeOH gave (5), which eluted with 6% MeOH-DCM (0.29 g, 79%) as colourless solid (M.P. 134 °C). Rf(solvent A) = 0.37; λ= 311 nm (MeOH, ϵ 4.6 x 10^4); ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1H, H2), 8.04 (d, J = 6.8 Hz, 2H, ArH), 7.52 (s, H16 overlapping with ArH), 7.58-7.22 (m, 13H, ArH), 6.77 (dd, J = 8.9 Hz, 4H, ArH) 6.61 (t, J =7.2 Hz, 2H, H1', overlapping with H14), 4.7(m, 1H, H3'), 4.14 (d, J = 3Hz, H4'), 3.77 (s, 6H, 2xOCH_3), 3.60 (m, 2H, H11), 3.4 (dd, J =2.8 Hz, 2H, H5' and 5"), 2.87 (m, 1H, H2'), 2.6 (m, 2H, H12), 2.36 (m, 1H, H2''); ^{13}C NMR ($CDCl_3$) δ 85.9 (C1'), 42 (C2'), 71.3 (C3'), 86.3 (C4'), 63.2 (C5'), 153 (C2), 147.9 (C4), 117.8 (C5), 153.6 (C6), 143.9 (C8), 38.1 (C11), 26 (C12), 129.9 (C13), 122.8 (C14), 134.2 (C16).

3'O-Acetyl-N^6-benzoyl-8-amino(2-N-acetylaminoethyl)-2'-deoxyadenosine (6). Compound (4) (180 mg, 0.27 mmol) was acetylated as above using dry pyridine (2.6 ml) and acetic anhydride (150 µl, 1.60 mmol) for 14 h. Aqueous work-up afforded 3'O, N^{13}-diacetate of 4 (182 mg, 86%). Rf (solvent A) = 0.56; Ninhydrin negative. This compound (180 mg, 0.23 mmol) was dissolved in DCM (1 ml) to which 2% DCA in DCM (10 ml) was added while cooling in an ice bath. The reaction was complete in 5 min. after which the mixture was extracted with DCM (30 ml) and washed with aq. sat. $NaHCO_3$. On concentration, a white foam was obtained which was purified by column chromatography to afford (6) (96 mg, 83%) (mp 138 °C). Rf (solvent A) = 0.42; λ= 308 (MeOH, ϵ 5.4 x 10^4). ^{1}H NMR ($CDCl_3$) δ 8.38 (S, 1H, H2), 7.9 (dd, J =7.0 Hz and 2.0 Hz, 2H, ArH), 7.61-7.4 (m, 3H, ArH), 6.55 (dd, J = 10.0 and 5.4 Hz, 1H, H1'), 5.36 (d, J = 6.3 Hz, 1H, H3'), 4.09 (brs, 1H, H4'), 3.92 (d, J = 10.8 Hz, 1H, H5''), 3.83 (dd, J = 11.6 and 2.2 Hz, 1H, H5'), 3.56 (t, J = 5 Hz, 2H, H11), 3.34 (m, 2H, H12), 2.67 (m, 1H, 2'), 2.15 (m, 1H, H2''), 2.04 (s, 3H, $OCOCH_3$), 1.89 (s, 3H,

$NCOCH_3$); ^{13}C NMR ($CDCl_3$) δ 85.2 C1', 41.9 C2', 75.6 C3', 83.4 C4', 61.7 C5', 153.4 C6, 147.8 C4, 143.0 C8, 153.1 C2, 123.1 C5, 39.9 C11, 35.2 C12, 171.6 O$\underline{C}OCH_3$, 170.3 N$\underline{C}OCH_3$ 22.7 OCO$\underline{C}H_3$, 20.8 NCO$\underline{C}H_3$, 165.3, 133.7, 132.1, 128.4, 127.6, (all from Bz).

3'O-Acetyl-N^6-benzoyl-8-(1-N-acetylhistamino)-2'-deoxyadenosine (7). Compound (**5**) (0.36 g, 0.47 mmol) was co-evaporated with dry pyridine (5ml), followed by dissolution in same solvent (5 ml). Acetic anhydride (0.27 ml, 2.8 mmol) was added and stirred for 6h at room temperature. The reaction mixture on work-up afforded 3'O, N^{15}-diacetate of **5** (0.33 g, 83%) as a white solid (mp 136-137 °C). Rf (solvent A) = 0.58. ^1H NMR ($CDCl_3$) δ 8.4 (s, 1H, H2), 8.06 (d, J = 7 Hz, 2H, ArH), 7.53 (s, 1H, H16 overlapping with ArH), 7.57-7.24 (m, 12H, ArH), 6.81(m, 4H, ArH), 6.78 (s, 1H, overlapping with ArH, H14), 6.48 (dd, 1H, H1'), 5.52 (t, 1H, H3'), 4.17 (m, 1H, H4'), 3.78 (s, 6H, 2xOCH_3), 3.54 (m, 2H, H11), 3.43 (m, 2H, H5'), 2.89 (m, 2H, H12), 2.74 (m, 1H, H2'), 2.17 (m, 1H, H2"), 2.1 (s, 3H, OCO-CH_3), 2.06 (s, 3H, $NCOCH_3$). This compound (**7**) (160 mg, 0.19 mmol) was detritylated using 2% DCA in DCM as above to obtain (**7**) (90 mg, 87%) as a white solid (mp 146 °C). Rf (solvent A) = 0.47; λ= 312 nm (MeOH, ϵ 11.6 x 10^4). ^1H NMR ($CDCl_3$) δ 8.4 (s, 1H, H2), 7.95 (d, J = 7Hz, 2H, ArH), 7.64(s, 1H, H16), 6.67 (s, 1H, H14), 6.58 (dd, J = 10.1 and 5.6 Hz, 1H, H1'), 5.37 (d, J = 6 Hz, 1H, H3'), 4.05 (brs, 1H, H4'), 3.95 (d, J = 9.6 Hz, 1H, H5''), 3.81 (d, J = 9.8 Hz, 1H, H5'), 3.61 (t, J = 10 Hz, 2H, H11), 2.9 (t, J = 10 Hz, 2H, H12), 2.64 (m, 1H, H2'), 2.16 (m, 1H, H2"), 2.08 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $NCOCH_3$).

Dimer (9). The 3'-phosphodiester (**8**) (346 mg, 0.41 mmol) and the 5'-hydroxyl compound (**6**) (90 mg, 0.18 mmol) were dried by co-evaporation with dry pyridine (2 ml) and the residue dissolved in dry pyridine (1 ml). After the addition of 1-methylimidazole (350 µl, 4.28 mmol) and MSNT (400 mg, 1.35 mmol) the reaction mixture was kept stirred at room temperature for 25 min. when TLC showed its completion. It was quenched with sat. aq. $NaHCO_3$ (3 ml) and extracted with DCM (2x10 ml). The dried organic layer was concentrated and the resultant residue was purified by column chromatography when the dimer (**9**) eluted with DCM/MeOH (120 mg, 55%). Rf (solvent A) = 0.53; ^{31}P NMR ($CDCl_3$) ppm -6.7 and -7.6.

Dimer (10). This was synthesised by a similar condensation of (**8**) (142 mg, 0.17 mmol) and (**7**) (50 mg, 0.09 mmol) in dry pyridine (1 ml) containing 1-methylimidazole (130 µl, 1.62 mmol) and MSNT (151 mg, 0.51 mmol). Yield 61% (70 mg). Rf (solvent A) = 0.62; ^{31}P NMR ($CDCl_3$) ppm -6.5 and -7.6.

Dimer (11). The dimer (**9**) (120 mg, 0.1 mmol) after 5'-detritylation with 2% DCA in DCM was dissolved in freshly prepared sat. MeOH-NH_3 (15 ml) and heated in a sealed tube at 60 °C for 24h. It was cooled and removal of excess of MeOH-NH_3 gave a residue which was purified on DEAE Sephadex A-25 column (2cmx12cm). Elution was performed using TEAA buffer (pH 7.0) using a gradient from 0.01 M to 0.2 M (200 ml each) with a flow rate of 1 ml/min. The fractions containing desired product was pooled and lyophilised to obtain (**15**) as a white solid (50 mg, 76%). HPLC retention time 13.7 min.; λ= 272 nm (H_2O, ϵ 6.38 x 10^4), ^{31}P NMR ppm -0.47; ^1H NMR (D_2O) δ 6.11

(t, 1H, H1'T), 6.27 (t, 1H, H1'A), 2.14 (m, 1H, H2'T), 2.47 (m, 1H, H2"T), 2.79 (m, 1H, H2'A) 2.34 (m, 1H, H2"A), 4.73 (m, 1H, H3'T, overlapping with HOD signal) 4.65 (m, 1H, H3'A, overlapping with HOD signal), 4.12 (m, 1H, H4'T), 4.15 (brs, 1H, H4'A), 3.74 (m, 2H, H5'&5"T), 4.19 (m, 2H, H 5' and 5"A, overlapping with 4'A), 7.49 (s, 1H, H6), 1.87 (s, 3H, 5-CH_3), 7.96 (s, 1H, H8), 3.59 (m, 2H, H11), 3.43 (m, 2H, H12), 1.99 (s, 3H, $NCOCH_3$).

Dimer (12). Dimer (**10**) (70 mg, 0.06 ml) was first 5'O-deprotected with 2% DCA followed by reaction with sat. MeOH-NH_3 as above to obtain (**12**) (44 mg, 70%), as white solid. HPLC retention time 14.78 min.; λ = 271 nm (H_2O, ϵ 10.9 x 10^4); ^{31}P NMR (D_2O) ppm -0.50; ^1H NMR (D_2O) δ 6.04 (t, 1H, H1'T), 6.17 (t, 1H, H1'A), 2.13 (m, 1H, H2'T), 2.40 (m, 1H, H2"T), 2.53 (m, 1H, H2'A), 2.22 (m, 1H, H2"A), 4.8 (m, H3'T, overlapping with HOD signal), 4.46 (m, 1H, H3'A), 4.08 (m, 4H, H4'A, H4'T and H5' & 5"A), 3.69 (m, 4H, H5'& 5" T and H11), 3.05 (m, 2H, H12), 8.54 (s, 1H, H16), 7.23 (s, H, H14), 7.85 (s, 1H, H2A), 7.45 (s, 1H, H6T), 1.89 (s, 3H, 5-CH_3).

Dimer (13). The compound (**11**) (50 mg, 0.07 mmol) was dissolved in aq. NaOH (1 M, 2 ml) and heated over a water bath at 60 °C for 6 h. the reaction was monitored by HPLC and after completion the reaction mixture was concentrated to 1 ml and loaded into a DEAE Sephadex A-25 column (2 cm x 12 cm). Elution was performed with TEAB buffer (pH 7.3) using gradient from 0.01 M to 0.2 M (200 ml each) with a flow rate of 1 ml/min. The fractions containing desired products were pooled and lyophilised to get (**13**) (32 mg, 67%). HPLC retention time 13.95 min.; ^{31}P NMR ppm -0.45; ^1H NMR (D_2O) δ 8 (s, 1H, AH2), 7.48 (s, 1H, TH6), 6.31 (t, J = 6.8, 1H, H1'A), 6.12 (t, J = 7.4 Hz, 1H, H1'T), 4.8(m, H3'T overlapping with HOD signals), 4.72 (m, H3'A overlapping with HOD signal), 4.15 (m, 3H, H5', 5" and 4' A), 4.1 (m, 1H, 4'T), 3.77 (t, J = 4.4 Hz, 2H, H11), 3.7(m, 2H, H5'T), 3.31(m, H12, overlapping with TEA signals), 2.89 (m, 1H, 2'A) 2.44(m, 1H, H2"T), 2.37 (m, 1H, H2"A), 2.13 (m, 1H, H 2'T), 1.84 (s, 3H, 5-CH_3).

8-Bromoadenosine[10] (15). Adenosine (4 g, 15 mmol) was dissolved in NaOAc buffer (1.0 M, pH 4, 100 ml) at 50 °C. Then the solution was cooled to room temperature and Br_2-water (115 ml) was added. After 4 h at room temperature excess bromine was distroyed by adding $NaHSO_3$ and pH of the solution was adjusted to 7 with 5N NaOH solution. Reaction mixture was then kept at 4°C overnight and the precipitated **15** was filtered to get an yellow solid (4.07 gm, 78%) (mp 234 °C, decomp). Rf (solvent A) = 0.24, λ = 264 nm (MeOH, ϵ 4.8 x 10^4). ^1H NMR ($DMSOd_6$) δ 8.08 (s, 1H, H2), 7.41 (s, 2H, NH), 5.9 (d, J = 7.7 Hz, 1H, H1'), 5.1 (t, J = 7.7 Hz, 1H, H2'), 4,25 (brs, 1H, H3'), 4.03 (brs, 1H, H4'), 3.66 (d, J = 10.5 Hz, 1H, H5"), 3.55 (d, J = 10.5 Hz, 1H, H5'), 5.7, 5.5, 5.3 (brs, OH); ^{13}C NMR ($DMSOd_6$) δ 155.2 (C6), 152.4 (C4), 149.9 (C2), 127.1 (C8), 119.8 (C5), 90.6 (C1'), 86.8 (C4'), 71.4 (C2'), 71.0 (C3'), 62.2 (C5'). M/e, 213 (100%, $C_5N_5H_3Br$).

N^6-Benzoyl-8-bromoadenosine (16). 8-Bromoadenosine (**15**) (3.8 g, 11 mmol) was benzoylated using transient protection procedure[11] using pyridine (90 ml), TMSCl (14 ml, 165 mmol) and benzoyl chloride (6.4 ml, 55 mmol) the residue obtained after work-up was purified by column chromatography.

Eluting with DCM containing incremental amounts of MeOH afforded **16** (2.50 g, 66%) as a light brown solid (mp 129 °C). Rf (solvent A) = 0.43; λ = 282.3 nm (MeOH, ϵ 8.2 x 10^4). ^{1}H NMR ($CDCl_3$+ $DMSOd_6$, 5:3) δ 8.7 (s, 1H, H2), 8.10 (m, 2H, ArH), 7.2-7.7 (m, 3H, ArH), 6.1 (d, J = 7.0 Hz, 1H, H1′), 5.06 (dd, J = 5.9, 1H, H2′), 4.47 (d, J = 5.9 Hz, 1H, H3′), 4.36 (brs, 1H, H4′), 3.95 (d, J = 14.7 Hz, 1H, H5′′), 3.75 (d, J = 11.8 Hz, 1H, H5′); ^{13}C NMR ($DMSOd_6$) δ 90.6 (C1′), 69.8 (C2′), 70.8 (C3′), 89.4 (C4′), 63.6 (C5′), 152.6 (C2), 149.9 (C4), 119.4 (C5), 154.9 (C6), 136.9 (C8), 165.4, 133.1, 127.3, 127, 132.4 (all from Bz).

N^6-Benzoyl-8-bromo-5′-O-(4,4′-dimethoxytrityl)adenosine (17). Compound (**16**) (2.40 g, 5.4 mmol) was dried by co-evaporation with dry pyridine and was redissolved in anhydrous pyridine (20 ml) and DMTCl (2.2 g, 6.6 mmol) was added. The reaction mixture was stirred at 25 °C for 18 h after which water (20 ml) was added and extracted with DCM (3x15 ml). The organic layer upon concentration gave an oily residue which was purified by column chromatography using DCM:MeOH as eluent to obtain (**18**) (2.90 g, 72%), as a white solid (mp 124 °C). Rf (solvent A) = 0.67; λ = 282.0 nm (MeOH, ϵ 10.9 x 10^4); ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, H2), 7.92 (d, J = 7.3 Hz, 2H, ArH), 7.52-7.08 (m, 12H, ArH), 6.64 (dd, J = 8.6 and 4 Hz, 4H, ArH), 5.99 (d, J = 4.8 Hz, 1H, H1′), 5.41 (m, 1H, H2′), 4.63 (m, 1H, H3′), 4.19 (m, 1H, H4′), 3.65 and 3.64 (2xs, 6H, OCH_3), 3.32 (m, 2H, H5′); ^{13}C NMR ($CDCl_3$) δ, 90.2 (C1′), 71.1 (C2′), 71.5 (C3′), 84.2 (C4′), 63.5 (C5′), 152.0 (C2 and C6), 147.9 (C4), 121.5 (C5), 127.5 (C8), 136, 133.3, 132.7, 128.7, 164.5 (all from Bz), 55 (DMT-OCH_3), 112.9, 158.4, 128.1, 128.7, 129.1, 130, 133.7, 135.8, 158.3 (all from DMT)

N^6-Benzoyl-5′-O-(4,4′dimethoxytrityl)-8-histaminoadenosine (18). Compound (**17**) (0.93 g, 1.24 mmol) was reacted with histamine (280 mg, 2.52 mmol) in dry ethanol (25 ml) as above. The product was purified by column chromatography to afford (**18**) (0.76 g, 79%) as a solid (mp 137 °C). Rf (solvent A) = 0.27; λ = 310 nm (MeOH, ϵ 11.0 x 10^4); ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H, H2), 7.88 (d, J = 7.9 Hz, 2H, ArH), 7.41 (s, H16, overlapping with Ar-H), 7.4-7.1 (m, 12H, ArH), 6.70 (dd, J = 8.6 Hz and 1.8 Hz, 4H, ArH), 6.4 (s, 1H, H14) 6.09 (d, J = 5.9 HZ, 1H, H1′), 4.92 (m, 1H, H2′), 4.45 (m, 1H, H3′), 4.18 (m, 1H, H4′), 3.68 (s, 6H, 2xOCH_3), 3.54 (d, J = 9.9 Hz, 1H, H5"), 3.33 (d, J = 6.4 Hz, 1H, H5′), 3.15 (m, 2H, H11), 2.50 (m, 2H, H12); ^{13}C NMR ($CDCl_3$) δ 153.4 (C2), 148.1 (C4), 118.9 (C5), 153.7 (C6), 144 (C8), 88.1 (C1′), 70.8 (C2′), 71.7 (C3′), 84.7 (C4′), 63.3 (C5′), 37.3 (C11), 26.2 (C12), 130.5 (C13), 122.9 (C14), 135.2 (C16), 165.7, 133.7, 127, 127.8, 132.3 (all from Bz), 158.7, 135.2, 133.6, 130.5, 129.2, 128.4, 128.2, 113.2, (all from DMT), 55.1 (DMT-OCH_3).

N^6-Benzoyl-2′,3′-O-diacetyl-8-(15N-acetyl)histaminoadenosine (19). Compound (**18**) (0.68 g, 0.87 mmol) was acetylated with pyridine (10 ml) and aecetic anhydride (0.7 ml, 7 mmol) by usual procedure to obtain the peracetate (0.72 g, 91%). This was detritylated by 2% DCA in DCM to obtain (**19**) (0.28 g, 58%), as a white solid (mp 157 °C). Rf (solvent A) = 0.26; λ = 311 (MeOH, ϵ 10.7 x 10^4). ^{1}H NMR ($CDCl_3$+$DMSOd_6$, 5:3) δ 8.5 (s, 1H, H2), 8.07 (d, J = 6.5 Hz, 2H, ArH), 7.6-7.4 (m, 3H, H16, ArH overlapping with s), 6.83 (s, 1H, H14), 6.46 (d, J = 8.4 Hz, 1H, H1'), 5.69 (dd, J = 5.9 Hz

and 5.8 Hz, 1H, H2'), 5.55 (d, J = 5.9 Hz, 1H, H3'), 4.28 (brs, 1H, H4'), 3.90 (d, J = 11.5 Hz, 1H, H5"), 3.82 (d, J = 11.5, 1H, H5'), 3.66 (t, J = 6.3 Hz, 2H, H11), 2.97 (t, J = 6.5 Hz, H12), 2.18 (s, 6H, $OCOCH_3$), 1.96 (s, 3H, $NHCOCH_3$); ^{13}C NMR ($CDCl_3$+$DMSOd_6$, 5:3) δ 168.9 and 168.4 (NHCOR, R = CH_3, Ar), 152.6 (C2), 147.3 (C4), 129.2 (C5), 152.1 (C6), 143.0 (C8), 83.3 (C1'), 71.1 (C3'), 69.4 (C2'), 83.0 (C4'), 60.1 (C5'), 19.3 ($-NCOCH_3$), 19.8 (2',3'-$OCOCH_3$), 25.6 (NHCOCH3), 42.2 (C11), 25.5 (C12), 115.9 (C13), 122.2 (C14), 133.8 (C16), 133.5, 127.3, 131.2, 127.6 (all from Bz) M/e 564 (M^+-CH_3CO).

Dimer (21). The 3'-phosphodiester (**20**) (205 mg, 0.22 mmol) and 5' hydroxyl component (**19**) (100 mg, 0.17 mmol) were condensed together in presence of MSNT (190 mg, 0.65 mmol) and 1-methylimidazole (100 µl, 1.29 mmol) in pyridine (1.6 ml) as above to afford (**21**). ^{31}P NMR ($CDCl_3$) ppm -5.46 and -5.86.

Dimer (14). The dimer (**21**) was 5'-detritylated with 2% DCA in DCM as described before. This was followed by treatment with sat. MeOH-NH_3 (15 ml) at 50 °C in a sealed tube for 18-20 h. Removal of excess MeOH-NH_3 followed by co-evaporation with dry THF (2x2 ml) gave a residue which was treated with 1 M TBAF in dry THF (0.9 ml), at room temperature for 4 h. The reaction was quenched with autoclaved TEAA buffer (2ml, 0.05 M, pH 7), evaporated and the residue was loaded on a column of DEAE sephadex A-25 (2cm x 12 cm). Elution was performed with TEAA buffer using gradient from 0.01 M TEAA to 0.2 M TEAA (200 ml each, pH 7.0). The fractions corresponding to major peak were pooled and lyophilised to afford (**30**) (47 mg, 36 % overall yield). HPLC retention time 12.83 min.; λ= 271 nm (H_2O, ε5.5 x 10^4); ^{31}P NMR (D_2O) ppm -0.21; ^1H NMR (D_2O, 500 MHz) δ 5.95 (d, 1H, H1'A), 5.84 (d, 1H, H1'U,overlapping with H5), 4.57 (m, 2H, H2'A and H3'U), 4.39 (t, 2H, H2'U and H3'A), 4.35 (q, 2H, H5'and H5" A), 4.29 (brs, 1H, 4'U), 4.22 (brs, 1H, 4'A), 3.84 (dd, 2H, H5'and H5" U), 8.64 (s, 1H, H16), 8.07 (s, 1H, H2A), 7.86 (d, 1H, H6U), 7.3 (s, 1H, H14), 5.86 (d, H5U, overlapping with H1'U), 3.79 (t, 2H, H11), 3.2 (m, H12, overlapping with TEA).

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20. Abbreviation:- TEA, triethylammonium; THF, tetrahydrofuran; TEAA, triethylammonium acetate; DCM, dichloromethane; DCA, dichloroacetic acid; TBDMS, t-butyldimethylsilyl; TBAF, tetrabutylammoniumfluoride; NMI, N-methylimidozole; Py, pyridine.