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**APPROACHES TO NUCLEASE MODELS: SYNTHESIS AND
CONFORMATIONAL STUDIES OF dT_prA CONJUGATED WITH
HISTAMINE AT C8(A) AND ETHYLENEDIAMINE AT C5'(T)**

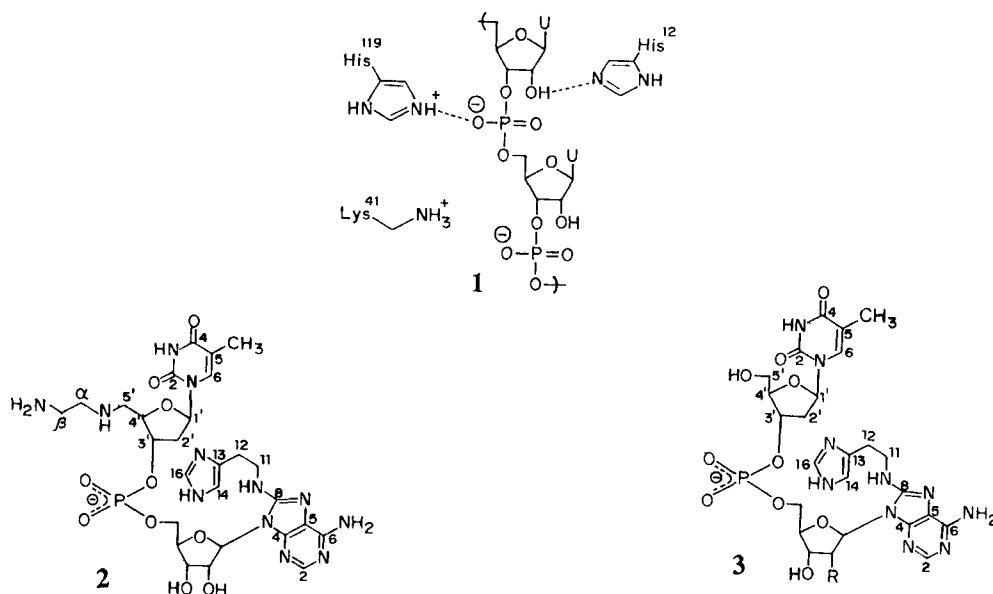
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Abstract: Synthesis and spectroscopic characterization of a dinucleotide dT_prA 2 having imidazole containing side chain at C8 of adenine and aminoethylamino side chain at C5' of nucleosugar is presented. Spectroscopic results suggest that such modifications do not drastically change the conformation of the new molecule relative to that of parent derivative. An anti glycosyl torsion would place the C8-conjugated histamine close to phosphate in right orientation for putative intramolecular interaction as in ribonuclease catalytic site.

INTRODUCTION

Molecular recognition of phosphate anions¹, particularly those present in biological systems such as ATP, DNA and RNA and their hydrolytic cleavage² are assuming importance. The strategies for successful phosphodiester hydrolysis are intimately related to chemical recognition of phosphates, which in principle may be achieved by host molecules through electrostatic and hydrogen bonding mechanisms. The catalytic sites in nucleases^{3,4} possess either metal ions (Zn²⁺, Mg²⁺) or positively charged organic residues (arginine, lysine or protonated histidine) for recognition of negatively charged phosphate groups. In addition, they also contain amino acids with nucleophilic side chains such as aspartic acid, tyrosine and histidine which trigger reactions leading to phosphate hydrolysis or transesterification.

Ribonuclease A⁴, in particular functions through two histidines and a lysine at the active site **1**. As a first step towards modeling the molecular interactions in such a catalytic site, we herein report the design, synthesis and conformational analysis of a dinucleotide dT_prA **2** containing histamine conjugation at C8 of 3'-A unit and an aminoethylamino group (lysine mimic)



linked at C5' of 5'-T unit. Since spatial orientational effects are of great importance in molecular catalysis, a juxtaposition of primary catalytic groups near nucleotidyl phosphate for molecular recognition is expected to effect or accelerate the phosphate cleavage when full complement of catalytic groups are incorporated into the model for testing the cleavage reaction.

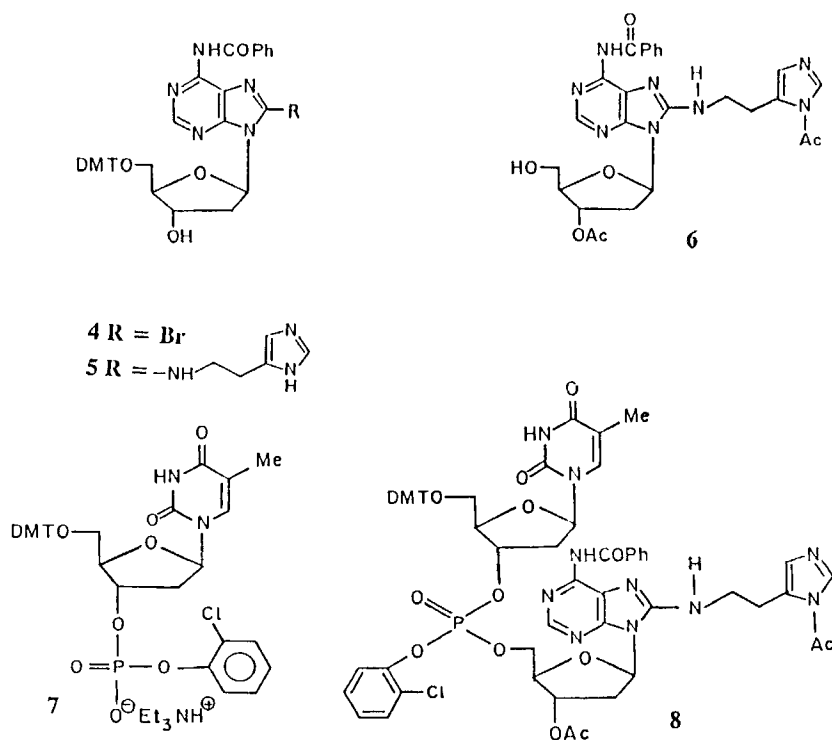
RESULTS AND DISCUSSION

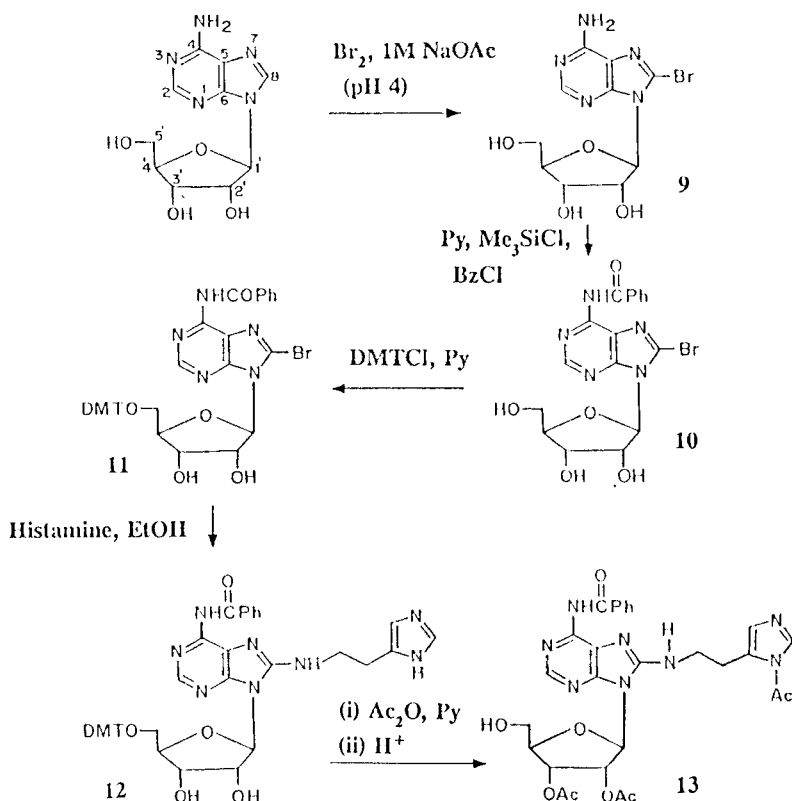
Synthesis of C8 histamine conjugated dinucleotides **2** and **3**

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. Aminoalkylation at C8 of adenine is well known in literature⁵. We have previously employed this strategy for synthesis of fluorescent adenosine derivatives^{5c}. Before the synthesis of target dinucleotide **2**, its simpler analog **3** was first synthesised to test the stability of histamino side chain to synthetic protocols. Treatment of 2'-deoxyadenosine with bromine-water in sodium acetate buffer at

pH 5.0 gave 8-bromo-2'-deoxyadenosine.⁶ This was N⁶-benzoylated by the transient protection method⁷ and then 5'-O-protected with 4,4'-dimethoxytrityl (DMT) group to give **4**. Subsequent reaction with excess histamine in EtOH gave the corresponding C8 conjugate **5** in 79% yield. This product was first converted into the corresponding N¹m,3'-O-diacetate by reaction with acetic anhydride-pyridine and converted to the 5'-OH terminal block **6** by detritylation with 2%-dichloroacetic acid (DCA) in dichloromethane (DCM).

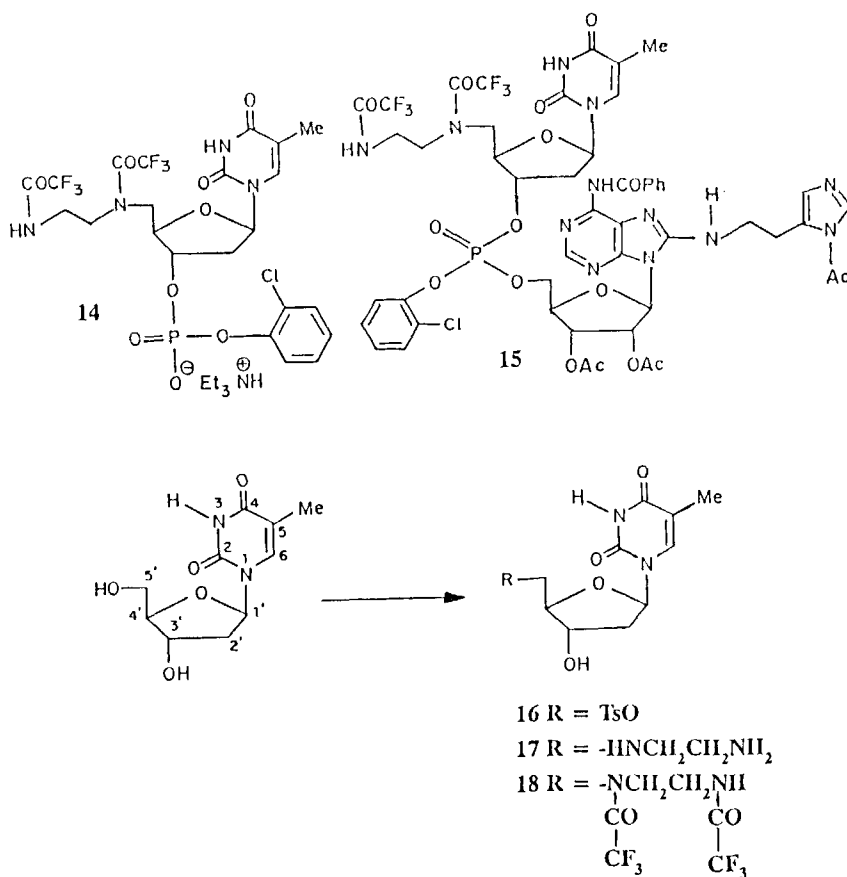
The triethylammonium [5'-O-DMT-thymidine-3'-O-(2-chlorophenyl) phosphate]⁸ **7** was then condensed with **6** in the presence of mesitylenesulphonyl-3-nitrotriazole (MSNT) and N-methylimidazole (NMI) to generate the protected dinucleotide **8**. No side products arising due to N-phosphorylation (³¹P NMR) or 5'-sulphonylation (¹H NMR) were detected. The dinucleotide **8** was purified by silica gel column chromatography and then completely deprotected by successive treatment with 2% DCA-DCM for 5'-detritylation and MeOH-NH₃ to effect removal of benzoyl, acetyl and 2-chlorophenyl groups. This yielded **3** as ammonium salt which was purified by ion-





exchange column chromatography by elution with triethylammonium carbonate (TEAB) and isolated as its triethylammonium salt. Its purity was checked by HPLC and characterized by ^1H and ^{31}P NMR. The successful preparation of **3** demonstrated that C8-histamino chain is stable to phosphotriester oligonucleotide synthesis, deprotection conditions and proceeded without any accompanying chemical modifications.

The desired model dinucleotide **2** carrying double conjugation at C5' of T and C8 of 3'rA unit was synthesised as follows: 8-Bromoadenosine **9**, obtained from adenosine by bromination,⁶ was first N⁶-benzoylated by transient protection procedure⁷ to obtain **10**. This was converted to its 5'-O-DMT derivative **11** which on treatment with histamine in EtOH as before gave the C8-histamino derivative **12** in 80% yield. The required 5'-OH terminal block **13** was generated from **12** in two steps consisting of acetylation and then deprotection with acid. The phosphodiester **14** was condensed with **13** in presence of MSNT and to obtain the protected dinucleotide **15**. This on global deprotection with aq. NH_3 gave the desired target dinucleotide **2**. This was purified by ion-exchange chromatography

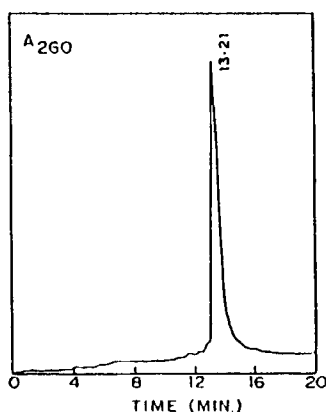


and elution with TEAB yielded its triethylammonium salt whose homogeneity was checked by reverse phase HPLC (Figure 1). Its structure was confirmed by ¹H NMR.

The phosphodiester **14** was synthesised as follows: 5'-O-tosylthymidine¹³ **16** was treated with ethylenediamine at room temperature to yield 5'-amino-5'-deoxythymidine **17**, followed by trifluoroacetylation⁹ to obtain **18**. Subsequent 3'-O-phosphorylation using 2-chlorophenyldichlorophosphate gave **14**. The normally employed acyl protecting groups (benzoyl, acetyl) defied deprotection with ammoniacal reagents. The trifluoroacetate group as an amino protector for 5'-aminoalkylamino function was found to be compatible with phosphotriester condensation chemistry and final deprotection conditions.

Spectroscopic characterization

One of the main requirements for successful intramolecular interaction involving internucleotide phosphate group and C8-conjugated ligand in

Figure 1: Reverse phase HPLC of **2**.

dinucleotides is their relative spatial predisposition. This is indirectly determined by the nucleoside sugar pucker, the glycosyl torsion and phosphate conformation. In order to understand the effect of C8 and C5'-modifications on the dinucleotide conformation, we have analysed the ^1H NMR spectra of **2** and **3** using the method of Rinkel and Altona¹⁰. According to these authors, the conformational analysis of sugar ring in nucleic acids can be performed by using the sums of coupling constants viz., $\Sigma 1'$ ($J_{1'2'} + J_{1'2''}$), $\Sigma 2'$ ($J_{1'2'} + J_{2'2''} + J_{2'3'}$), $\Sigma 2''$ ($J_{1'2''} + J_{2'3'} + J_{2'2''}$) and $\Sigma 3'$ ($J_{2'3'} + J_{2'3''} + J_{3'4'}$). A $\Sigma 1'$ value of 13.3 Hz or more with $J_{1'2'} > J_{1'2''}$ and $\Sigma 2' > \Sigma 2''$ was shown to arise by a predominant S-type conformation (2'-endo, >60%). In case of ribose sugars the $J_{1'2'}$ and $J_{3'4'}$ can be used to estimate percentage population of N-type conformer¹¹. Crucial to the success of these empirical methods is the unambiguous assignments of all these protons of different sugar residues to obtain ^1H - ^1H vicinal coupling constants in individual sugar residues.

The complete proton chemical shift assignment of both sugar residues in **2** and **3** was achieved by a combination of 1D and 2D NMR spectroscopy (Figure 2). In particular, 2D NMR was employed for unambiguous assignment of H3' and H5'5'' (which are normally masked in 1D spectra by the residual HOD signal), the C8 side chain methylene protons and the imidazole protons H17 and H18 of histidine moiety. The coupling constants $J_{1'2'}$ and $J_{2'3'}$ were calculated by means of a first order analysis. For the deoxyribose units, the H1' resonance appeared as a triplet and hence individual $J_{1'2'}$ and $J_{1'2''}$ couplings could not be obtained

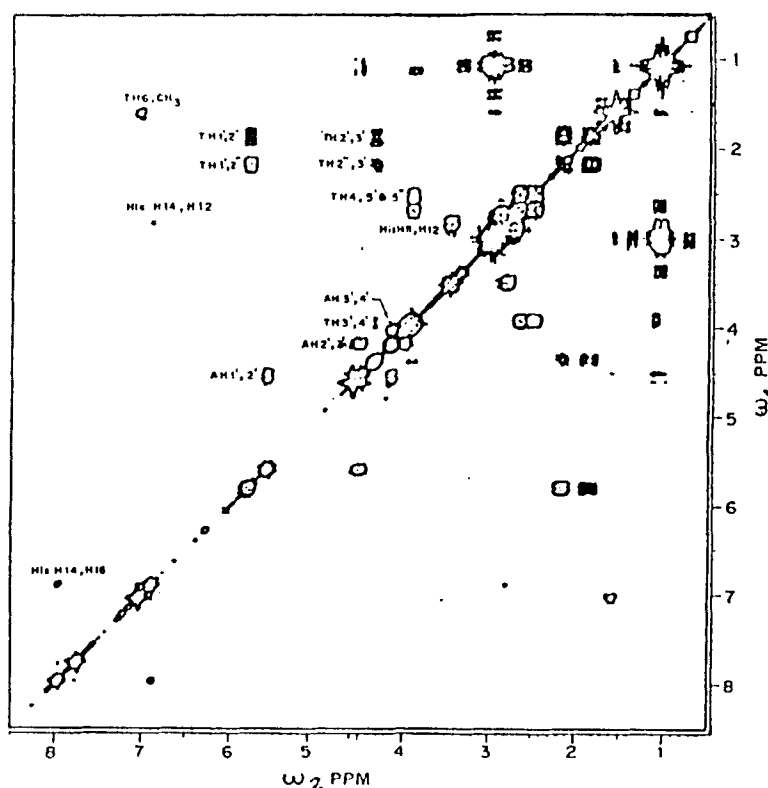


Figure 2: 2D COSY-45 spectrum of 2.

from H1' signals in the spectrum. Alternatively, this information was sought from the total width of H2'' multiplet ($=\Sigma 2''$) which explicitly showed all eight lines arising from its simultaneous coupling to H2', H1' and H3' protons. Table-1 summarises the coupling constants and %S conformer calculated for various dinucleotides using the empirical method of Rinkel and Altona¹⁰.

It has been previously observed^{10,11} that in unmodified dinucleotides, irrespective of the nature of base and sequence, the pentose ring prefers to exist in S conformation. Accordingly, in the unmodified dimer d(TpA) both 5' and 3' sugar residues have a preference for the S-type conformation and among these the 5'-sugar exhibits a greater bias for S-type compared to the 3'-sugar residue. (Table-1). C8 modification in **3** leads to a reversal of this situation with %S of 3' sugar being enhanced over that of 5'-sugar. Thus C8 modification does not drastically alter the sugar conformation, but instead increases the population

Table 1: Coupling constants and %S conformers⁺ of dinucleotides 2 and 3

Compound		J1'2'	J1'2''	Σ1'	Σ2'	Σ2''	Σ3'	%S
dTpA	dTp-	8.1	6.1	14.2	---	24.4	---	75
	-pdA	7.3	6.0	13.3	---	22.2	---	59
2	dTp-	7.9	6.1	14.0	28.4	22.9	9.3	71
	-pdA	6.4	---	---	12.7	---	10.3	62
3	dTp-	7.4	6.2	13.6	28.1	23.8	---	64
	-pdA	8.0	6.6	14.6	29.6	23.8	7.4	81
$+ \%S = \frac{\Sigma 1'-9.8}{5.9}, \quad \%N = \frac{J3'4'}{J1'2' + J3'4'}, \quad (\text{ref 10 and 11})$								

of the existing %S conformer. In contrast, the 5'-modification in **2** effects negligible consequences on sugar conformation and the relative %S population of the 5' and 3' sugar residues in **2** are similar to that in the unmodified d(TpA).

Inspection of the relative proton chemical shifts (experimental) shows a consistent trend in C8 modification induced shifts of H1', H2' and H2'' protons. Base modification 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 otherhand, H2'' of 5'dTp- residues in **2** and **3** is upfield shifted by 0.2 ppm compared to H2'' of d(TpA). The H1' and H2' protons of 3'-nucleotidyl unit in modified dinucleotidyl units show a reverse effect: upfield shifts of H1' and a downfield shift of H2'' (0.1-0.2 ppm). Although the altered shifts for ¹H of modified 3'-dA unit would partly originate from electronic changes at C1' and C2', the nature of shifts observed for H2'' sugar proton of dTp suggests an anti conformation for the modified nucleoside dA. In this conformation, the C8- conjugated histamino residue is spatially close to the protons of adjacent 5'-sugar unit which thus exhibits induced shifts arising from through space dipolar effects. It has been noticed earlier¹² that for ribonucleosides, J1'2' is a good indication for determining the glycosyl conformation preference, with J1'2' (anti) > J1'2' (syn) by ~1Hz. The magnitude of J1'2' observed for **2** points to an anti conformation for 3'-rA unit. Thus ¹H NMR studies indicate that C8 modification as in **2** does not significantly affect the

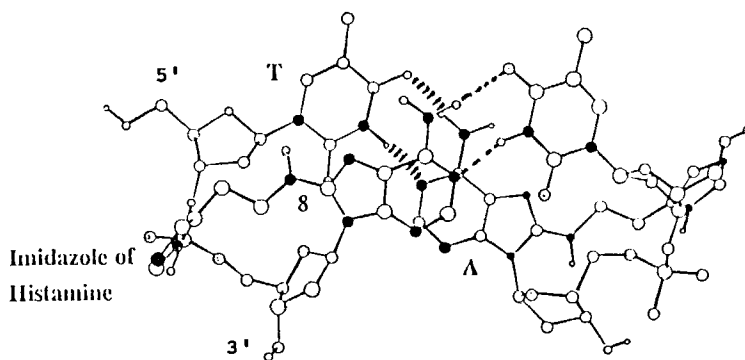


Figure 3

glycosyl torsion (anti disposition) found in natural nucleotides while the sugar residues exhibit an enhanced population of S-conformer. Although 2D NOESY spectra gave the expected characteristic cross peaks from both nucleotidyl units, it indicated no NOE's useful for internucleotidyl conformational assignment. It may be pointed out that H8 of purines which is a good marker for syn/anti conformational assignment is non-available in the present compounds as it is replaced by the histamine side chain.

We also examined the CD spectra of dTpA and the modified dinucleotides **2** and **3**. No significant changes in CD profile were observed suggesting an absence of gross structural changes in the modified dimers. It has been earlier observed^{5a} that in 8-substituted purine nucleotides, syn to anti conversion is accompanied by changes in CD spectra. Figure 3 shows the energy minimised (Amber programme) structure of **3** generated from the coordinates of the crystal structure of dTpA and modified by introduction of histamine side chain at C8 of dA. It is seen that the imidazole moiety has a close approach to the phosphate residue with a potential for intramolecular interaction through hydrogen bonding either to phosphate or to 5'O of dA. Such possibility has favourable implications for inducing phosphate hydrolysis in dinucleotides containing the full complement of the catalytic site.

CONCLUSIONS

In this paper we have demonstrated the possibility of the synthesis of dinucleotides with imidazole containing side chains linked to C8 of adenine and

ethylenediamine ligand to C5' of nucleosugar. ^1H NMR spectroscopic results suggest that such modifications do not drastically change the conformation of the new molecules relative to that of the parent derivative. An anti glycosyl torsion would place the conjugated ligand and internucleotidic phosphate in right orientation for possible intramolecular interaction. These dinucleotides are first of a group of compounds designed to model electrostatic and hydrogen bonding interactions present in the active site of ribonucleases. Further studies are aimed at the introduction of putative cationic groups such as amines¹³, arginine etc., for promoting electrostatic interaction with phosphate and generating the full complement of the catalytic site to test for cleavage reactions.

EXPERIMENTAL SECTION

2'-Deoxynucleosides and MSNT were obtained from Aldrich (UK). 1-Methylimidazole (Fluka) and ethylenediamine (SRL, India) were used without further purification. 8-Bromo-2'-deoxyadenosine⁶, 8-bromo-5'-O-DMT-2'-deoxyadenosine⁵ **4**, 5'-O-tosylthymidine¹⁴ **16** and the nucleotide monomers⁵ were prepared from the corresponding nucleosides according to the literature 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 with 60% perchloric acid-EtOH (3:1) followed by charring. All solid compounds gave satisfactory elemental analyses. UV spectra were recorded on Perkin-Elmer Lambda 15 spectrometer. HPLC: Buffer A, 0.1M Triethylammonium acetate (TEAA); Buffer B, 30% CH_3CN in 0.1M TEAA, gradient A to B in 20 min., flow rate 2 ml/min., UV detector at 260 nm. The melting points reported are uncorrected. NMR spectra were recorded on Bruker AC-F 200 spectrometer fitted with Aspect 3000 computer. All chemical shifts expressed are in ppm downfield from TMS in CDCl_3 and in case of D_2O , downfield from DSS. ^{31}P NMR spectra were 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 and the assignments were done by comparison of these spectra, chemical shift arguments and wherever permissible by analogy with assignments in known compounds.

N⁶-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-8-histamino-2'-deoxyadenosine (5)

Compound **4** (0.35 g, 0.48 mmol) was dissolved in dry EtOH (10 ml) and histamine (0.11 g, 0.95 mmol) was added into this. The reaction mixture was stirred at 25°C for 18 h. Evaporation of excess EtOH gave a residue which was purified by silica gel column chromatography. Elution with DCM containing incremental amounts of MeOH gave **5** (0.29 g, 79%), which eluted with 6% MeOH-DCM, as a colourless solid, M.P 134°C, R_f (Solvent A) 0.37, λ = 311 nm (MeOH, ϵ 4.6 \times 10⁴), ¹H NMR (CDCl₃) 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, H_{1'} overlapping with H₁₄), 4.7 (m, 1H, H_{3'}), 4.14 (d, J=3 Hz, H_{4'}), 3.77 (s, 6H, 2xOMe), 3.60 (m, 2H, H₁₁), 3.4 (dd, J=2.8 Hz, 2H, H_{5'5''}), 2.87 (m, 1H, H_{2'}), 2.6 (m, 2H, H₁₂), 2.36 (m, 1H, H_{2''}); ¹³C NMR (CDCl₃) δ , 85.9 (C_{1'}), 42.0 (C_{2'}), 71.3 (C_{3'}), 86.3 (C_{4'}), 63.2 (C_{5'}), 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), 55.2 (DMT-OCH₃).

N⁶-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-8-histaminoadenosine (12)

Compound **11** (0.93 g, 1.24 mmol) was reacted with histamine (0.28 g, 2.52 mmol) in dry EtOH (25 ml) for 18 h. followed by evaporation of EtOH and column chromatography over silica gel as above gave **12** (0.76 g, 80%) as a solid, M.P 137°C, R_f (Solvent A)=0.27, λ = 310 nm (MeOH, ϵ 11 \times 10⁴), ¹H NMR (CDCl₃) δ 8.29 (s, 1H, H₂), 7.88 (d, J=7.9 Hz, 2H, ArH), 7.41 (s, H₁₆ overlapping with ArH), 7.4-7.1 (m, 12H, ArH), 6.7 (dd, J=8.6 and 1.8 Hz, 4H, ArH), 6.4 (s, 1H, H₁₄), 6.09 (d, J=5.9 Hz, 1H, H_{1'}), 4.92 (m, 1H, H_{2'}), 4.45 (m, 1H, H_{3'}), 4.18 (m, 1H, H_{4'}), 3.68 (s, 6H, 2xOCH₃), 3.54 (d, J=9.9 Hz, 1H, H_{5''}), 3.33 (d, J=6.4 Hz, 1H, H_{5'}) 3.15 (m, 2H, H₁₁), 2.50 (m, 2H, H₁₂). ¹³C NMR (CDCl₃) δ , 153.4 (C₂), 148.1 (C₄), 118.9 (C₅), 153.7 (C₆), 144 (C₈), 88.1 (C_{1'}), 70.8 (C_{2'}), 71.7 (C_{3'}), 84.7 (C_{4'}), 63.3 (C_{5'}), 37.3 (C₁₁), 26.2 (C₁₂), 130.5 (C₁₃), 122.9 (C₁₄), 135.2 (C₁₆), 55.1 (DMT-OCH₃).

3'-O-Acetyl-N⁶-benzoyl-8-(1N-acetylhistamino)-2'-deoxyadenosine (6)

Compound **5** (0.36 g, 0.47 mmol) was co-evaporated with dry pyridine (5 ml) followed by dissolution in same solvent (5 ml). Acetic anhydride (0.3 ml, 2.8 mmol) was added and stirred for 6h at room temperature. the reaction mixture on work-up afforded 3'-O, N¹⁵-diacetate of **5** (0.33 g, 83%) as a white solid, M.P 136°C, R_f (Solvent A) =0.58, ¹H NMR (CDCl₃) 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, H_{1'}), 5.52 (t, 1H, H_{3'}), 4.17 (m, 1H, H_{4'}), 3.78 (s, 6H, 2xOCH₃), 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, OCOCH₃), 2.06 (s, 3H, NCOCH₃). This compound (160 mg, 0.19 mmol) was detritylated using 2% DCA-DCM (10 ml, 3 min) and aq. NaHCO₃ work-up gave **6** (90 mg, 87%) as a white solid, M.P. 146°C, R_f (Solvent A) = 0.47, λ = 312 nm (MeOH, ϵ 11.6 \times 10⁴). ¹H NMR (CDCl₃) δ 8.4 (s, 1H, H2), 7.95 (d, J=7 Hz, 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, OCOCH₃), 2.03 (s, 3H, NCOCH₃)

N⁶-Benzoyl-2',3-O-diacetyl-8-(15N-acetyl)histaminoadenosine (13)

Compound **12** (0.68 g, 0.87 mmol) on acetylation as above using acetic anhydride (0.7 ml, 7 mmol) in pyridine (10 ml) gave the peracetate (0.72 g, 91%). This was detritylated with 2% DCA-DCM to yield **13** (0.28 g, 58%) as a white solid, M.P. 157°C, R_f (Solvent A) = 0.26, λ = 311 nm (MeOH, ϵ 10.7 \times 10⁴). ¹H NMR (CDCl₃:DMSO-d₆, 5:3, v/v) δ 8.5 (s, 1H, H2), 8.07 (d, J=6.5 Hz, 2H, ArH), 7.6-7.4 (m, 3H, ArH overlapping with H16), 6.83 (s, 1H, H14), 6.46 (d, J=8.4 Hz, 1H, H1'), 5.69 (dd, J=5.9 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 Hz, 1H, H5'), 3.66 (t, J=6.3 Hz, 2H, H11), 2.97 (t, J=6.5 Hz, H12), 2.18 (s, 6H, 2xOCOCH₃), 1.96 (s, 3H, NHCOCH₃). ¹³C NMR (CDCl₃:DMSO-d₆, 5:3, v/v) δ 168.9 and 168.4 (NHCOCH₃, R=Ar, CH₃), 152.6 (C2), 147.3 (C4), 129.2 (C5), 152.1 (C6), 143 (C8), 83.3 (C1'), 71.1 (C3'), 69.4 (C2'), 83 (C4'), 60.1 (C5'), 19.3 (NCOCH₃), 19.8 (2',3'-OCOCH₃), 25.6 (NHCOCH₃), 42.2 (C11), 25.5 (C12), 115.9 (C13), 122.2 (C14), 133.8 (C16), m/e, 564 (M⁺-COCH₃).

5'-(2-Aminoethyl)amino-5'-deoxythymidine (17)

5'-Tosylthymidine (**16**, 4.86 g, 12.3 mmol) was treated with ethylenediamine (15 ml) at room temperature for 22 h. after which excess ethylenediamine was removed under reduced pressure to get a gum. This was purified by silica gel chromatography and elution with isopropanol-aq. NH₃ (9:1, v/v) gave **17** (2.58 g, 74%) as a hygroscopic solid, M.P. 86°C, R_f (isopropanol:aq. NH₃:H₂O, 6:1:1, v/v) = 0.33, λ = 266 nm (MeOH, ϵ 3 \times 10⁴). ¹H NMR (D₂O) δ 7.4 (s, 1H, H6), 6.26 (t, J=6.9 Hz, 1H, H1'), 4.34 (m, 1H, H3'), 4.01 (m, 1H, H4'), 3.02-2.75 (m, 6H, H5', H and H β), 2.35 (m, 2H, H2' and H2''), 1.87 (s, 3H, 5-CH₃). ¹³C NMR (D₂O) δ 155.5 (C2), 171 (C4), 138 (C6), 112.6 (C5), 13.3 (5-CH₃), 85.8 (C1'), 40.1 (C2'), 73.2 (C3'), 86.4 (C4'), 51.8 (C5'), 48.6 (C α), 39.2 (C β). m/e, 254 (M⁺-CH₂NH₂), 225 (M⁺-NHCH₂CH₂NH₂), 159 (C₇H₁₅N₂O₂).

5'-(2N-Trifluoroacetyl-aminoethyl)-N-trifluoroethyl-amino-5'-deoxythymidine (18)

Compound **17** (2.22 g, 5 mmol) was dried by co-evaporation with

anhydrous pyridine and dissolved in same solvent (50 ml). To this, chlorotrimethyl silane (4.4 ml, 50 mmol) was added while cooling at 0°C and then kept stirred for 1 h. This was followed by addition of trifluoroacetic anhydride (4.3 ml, 30 mmol) and stirred for a further 2h at 5°C. The reaction was then quenched with water (20 ml) and the product was extracted with DCM (3x25 ml). The dried organic layer was concentrated to a residue which on purification by column chromatography (DCM-MeOH elution) gave **18** (2.23 g, 60%) as a solid, M.P 97°C, R_f (Solvent A) =0.45. λ , 264 nm (MeOH, ϵ =5.1x10⁴). ¹H NMR (CD₃COCD₃) δ 7.45 (s, 1H, H₆), 6.2 (q, J=6.5 Hz, 1H, H_{1'}), 4.34 (m, 1H, H_{3'}), 4.15 (m, 1H, H_{4'}), 3.95 (dd, J=4.7 Hz, 1H, H_{5''}), 3.95 (dd, J=4.7Hz, 1H, H_{5'}), 3.85 (dd, J=4.7Hz, 1H, H_{5'}), 2.30 (m, 2H, H_{2'}), 1.84 (s, 3H, CH₃), 3.57-3.79 (m, 4H, H and H_B). ¹³C NMR (CD₃COCD₃) δ 164.2 (C₄), 158.2 and 157.5 (NCOCF₃), 151 (C₂), 137 (C₆), 119.9 and 114.2 (NCOCF₃), 111.1 (C₅), 83.5 (C_{1'}), 39.3 (C_{2'}), 73 (C_{3'}), 86 (C_{4'}), 50.7 (C_{5'}), 12 (5CH₃), 49.2 (C₄), 46.9 (C_B), m/e 476 (M⁺).

Triethylammonium[5'-(2N-trifluoroacetyl aminoethyl)-N-trifluoroacetyl amino-5'-deoxythymidine-3'O-(2-chlorophenylphosphate)] (14)

Compound **18** (0.47 g, 0.9 mmol) was dried by coevaporation with anhydrous pyridine (10 ml). In a separate flask, 2-chlophenyldichlorophosphate (1.6 ml, 9.1 mmol) was dissolved in dry pyridine (10 ml) to which water (0.16 ml) was added slowly while cooling. The mixture was allowed to reach room temperature during 10 min. The precipitated pyridinium chloride was removed by filtration and the clear filtrate was added to dried **18**. The mixture was concentrated to half its volume and kept stirred for 0.5 h at room temperature. The reaction was quenched by addition of TEAA (pH 6.3, 1M, 20 ml), extracted into DCM (2x25 ml), washed with TEAA (0.1M, 2x15 ml) and the dried organic layer was concentrated. The residue was purified by column chromatography over silica gel and elution with DCM-MeOH gave **14** (0.46 g, 61%), R_f (Solvent A)=0.21, ³¹P NMR (CDCl₃) δ =-6.14 and -6.86.

Dimer 8: The 5'O-DMT- 3'-phosphodiester **7** (0.14 g, 0.17 mmol) and the 5'-OH component **6** (0.05 g, 0.09 mmol) were dried by coevaporation with dry pyridine (2 ml) and the residue dissolved in dry pyridine (1 ml). To this, MSNT (0.15 g, 0.51 mmol) and NMI (130 μ l, 1.62 mmol) was added and the reaction mixture was kept stirred under dry conditions at room temperature for 0.5 h when TLC showed it to be complete. It was then quenched with saturated aq. NaHCO₃ (2 ml) nad extracted into DCM (2x10 ml). The dried organic layer was concentrated and the resultant residue was purified by column chromatography to yield **8** (0.07 g, 61%) as a gum. R_f (Solvent A)=0.62, ³¹P NMR (CDCl₃), -6.5 and -7.6 ppm.

Dimer 15: This was prepared by condensation of the 3'-phosphodiester **14** (0.2 g, 0.26 mmol) with the 5'-OH component **13** (0.12 g, 0.2 mmol) in dry pyridine (2.6 ml) in presence of MSNT (0.23 g, 0.78 mmol) and NMI (0.12 ml, 1.6 mmol). The reaction carried out as above gave a gum on work-up which was purified by silica gel column chromatography (DCM-MeOH elution) to yield **15** (0.15 g, 60%) as a white foam, R_f (Solvent A) = 0.61. ^{31}P NMR (CDCl_3) δ -6.71 and -7.55 ppm.

Dimer 2: The dimer **15** (0.15 g, 0.12 mmol) was heated with sat. MeOH-NH₃ (10 ml) in a sealed tube at 60°C for 24 h. The reaction mixture was cooled and the excess NH₃ was evaporated off to obtain a residue which was purified by ion-exchange column chromatography over DEAE Sephadex A25 (Pharmacia) using a column of size 2cmx10cm. The column was eluted with triethylammonium bicarbonate buffer (pH 7.3) with a gradient from 0.01M to 0.2M and a total volume of 400 ml. The dimer **15** was the major peak as detected at 254 nm and eluted last. The appropriate fractions were pooled and lyophilised to get a white residue which was lyophilised 3 times with addition of water to obtain the desired dimer **15** as the corresponding triethylammonium salt. HPLC (C18): retention time 13.2 min. $\lambda = 271$ nm (H_2O , $\epsilon = 7.8 \times 10^4$), ^1H NMR (D_2O) δ 8.17 (s, 1H, H16), 7.95 (s, 1H, H2 of A), 7.23 (s, 1H, H6 of T), 7.09 (s, 1H, H14), 5.99 (t, 1H, H1'T), 5.78 (d, 1H, H1'A), 4.72 (t, 1H, H2'A), 4.56 (m, 1H, H3'T), 4.36 (t, 1H, H3'A), 4.20 (brs, 1H, H4'A), 4.13 (brs, H5' and H5''A overlapping with H4'A), 4.11 (brs, 1H, 4'T overlapping with 5'A), 2.85 (m, 1H, H5''T), 2.71 (dd, 1H, H5'T), 2.39 (m, 1H, 2''T), 2.05 (m, 1H, H2'T), 3.68 (t, 2H, H11), 2.95 (t, 2H, H12), 3.1 (m, 2H, α H), 2.86 (m, 2H, β H), 1.79 (s, 3H, CH₃). ^{31}P NMR (D_2O) δ = -0.49 ppm.

Dimer 3: The dimer **8** (0.07 g, 0.06 mmol) was first 5'-O-detritylated with 2%DCA-DCM and then treated with sat. MeOH-NH₃ in a sealed tube at 60°C for 24 h. This was followed by ion-exchange purification as described above to obtain **3** (0.044 g, 70%) as a white fluffy solid. HPLC (C18) ret. time 14.78 min. $\lambda = 271$ nm (H_2O , $\epsilon = 10.9 \times 10^4$). ^1H NMR (D_2O) δ 8.54 (s, 1H, H16), 7.23 (s, 1H, H14), 7.85 (s, 1H, H2 of A), 7.45 (s, 1H, H6 of T), 6.04 (t, 1H, H1'T), 6.17 (t, 1H, H1'A), 4.8 (m, H3'T overlapping with HOD), 4.46 (m, 1H, H3'A), 4.08 (m, 4H, H4'A, H4'T, H5'5''T and H11), 3.69 (m, 4H, H5'5''T and H11), 3.05 (m, 2H, H12), 2.40 (m, 1H, H2''T), 2.53 (m, 1H, H2'A), 2.22 (m, 1H, H2''A), 2.13 (m, 1H, H2'T). ^{31}P NMR (D_2O) δ = -0.5 ppm.

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