# A DIRECT ASSIGNMENT OF ALL BASE AND ANOMERIC HI′ SIGNALS IN THE PROTON SPECTRUM OF A TRINUCLEOSIDE DIPHOSPHATE, ApApA: STRUCTURE IMPLICATIONS

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## 1. Introduction

Trinucleotides can be considered as primitive models for triplet codons of t-RNA and there is accordingly considerable interest in defining structures and conformations for these molecules. An important step in this direction was made recently [1] with the first successful crystal structure determination for a trinucleoside dephosphate, adenylyl-(3′-5′)-adenylyl-(3′-5′)-adenosine, ApAp$^{+}$A$^{+}$. Because it is difficult to predict what effect solution forces will have on structure, particularly for smaller molecules with conformational flexibility, it is essential to obtain comparable structural information in solution. However, the use of NMR spectroscopy for this purpose is severely restricted because of extensive signal overlap, especially in homo-oligonucleotides, i.e. XpXpX. Selective labeling of nucleotidyl units [2,3] is an obvious solution to overlap problems and in this communication we describe the use of this approach to achieve the first direct assignment of base and anomeric HI′ protons for a homo-oligonucleotide, ApApA.

## 2. Experimental

### 2.1. *Materials and methods*

The sodium salts of 3′-adenosine monophosphate and adenylyl-(3′-5′)adenosine, ApA, were purchased from Sigma Chemical, St. Louis, Missouri, and were used without further purification. Polynucleotide phosphorylase from *Micrococcus lysodeitkticus* and 5′-adenosine diphosphate (sodium salt) were purchased from Miles Laboratories, Elhart, Ind., and alkaline phosphatase from *E. coli* was obtained from Worthington Biochemical, Freehold, N. J. 100% $D_2O$ was purchased from Stohler Isotopes.

### 2.2. *Synthesis of labeled trimers*

Selectively deuterated trinucleoside diphosphates were synthesized enzymatically (polynucleotide phosphorylase) from appropriate labeled dinucleoside monophosphates *ApA (A$\overset{*}{p}$A)† and ADP following procedures of Thach [4]. A complete assignment of all the base H2 and exchangeable H8 proton and anomeric H1′ signals required synthesis of a minimum of three selectively deuterated trimers, i.e., *ApApA, D-8-A$\overset{*}{p}$ApA‡ and ApApA-D-8. The labeled dimers *ApA and D-8-A$\overset{*}{p}$A used as starting materials were synthesized chemically as described elsewhere [2]; D-8-AMP-3′ employed for synthesis of D-8-A$\overset{*}{p}$A was prepared by heating AMP-3′ in $D_2O$ for 3 hr at 85–90°C, while labeled mononucleotides were extracted from fully deuterated blue-green algae, *Synechococcus lividus*, grown in $D_2O$. The final trimer product was isolated from the reaction mixture by paper chromatography and was further purified by DEAE cellulose chromatography.

### 2.3. *Measurement of NMR spectra*

Proton spectra were recorded at probe temperature (16 ± 2°C) on a Varian HR-220 spectrometer equipped with a Nicolet Technology Fourier transform accessory.

†Nucleotide symbols denoted with an asterisk are fully deuterated except at the C8 position.
‡In D-8-A$\overset{*}{p}$ApA the H8 proton of Ap- is replaced with D-8.

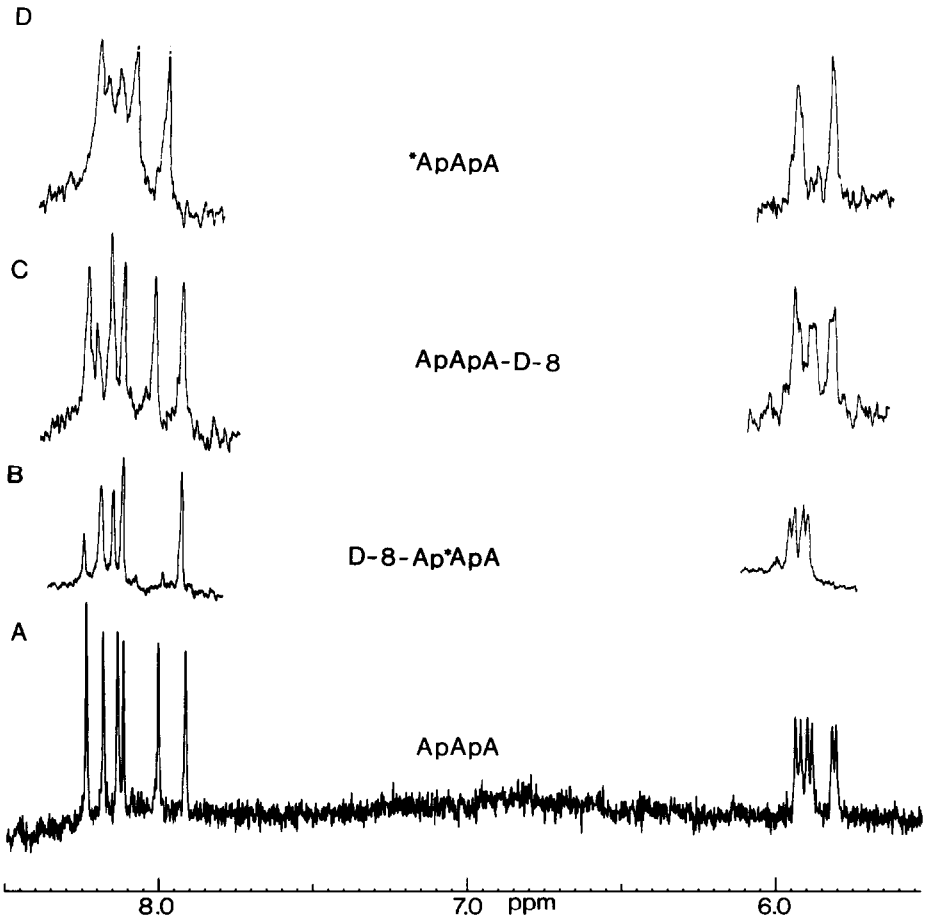


Fig.1. 200 MHz PMR spectra of ApApA and its selectively deuterated analogues in $D_2O$ at 16°C. A) ApApA; conc. 0.005 M. B) D-8-Ap*ApA; conc. ~0.01 M. C) ApApA-D-8; conc. ~0.01 M. D) *ApApA; conc. ~0.01 M. Note that the trimer spectrum shows a measurable concentration dependence; however, no cross-over of base or H1′ signals occurred in the range covered.

All solutions were prepared by lyophilization from 100% $D_2O$ three times prior to final dissolution in 100% $D_2O$. The pD of the solution was in the range of 6.5–6.8. A small amount of TSP ($< 10^{-3}$ M) was added as internal reference, and measured shifts are accurate to ± 0.005 ppm.

## 3. Results and discussion

### 3.1. *Assignment*

Fig.1. shows the base and anomeric proton regions of the spectrum for ApApA along with relevant portions of spectra for the three selectively deuterated trimers *ApApA, D-8-Ap*ApA, and ApApA-D-8. The six signals at ~8.1–8.2 ppm, fig.1A can be attributed to H2 and H8 signals of the bases, while the three doublets at higher field arise from the anomeric H1′ protons. Deuterium exchange measurements at elevated temperatures permit assignment of the three signals at lowest field to H8 protons.

Further signal assignments to individual nucleotidyl units are made by intercomparison of spectrum A with corresponding spectra for selectively labeled trimers. For example, the spectrum of D-8-Ap*ApA shows an absence of signals at 5.82, 8.02 ppm and a diminution in intensity of the signal at 8.25 ppm relative to the other two H8 signals, thereby identifying H1′, H2 for

Table 1
Assignments and chemical shifts of base and anomeric protons of ApApA*

| Proton | Assignment and chemical shifts, ppm | | |
|---|---|---|---|
| | Ap- | -pAp- | -pA |
| H2 | 7.918 | 8.016 | 8.132 |
| H8 | 8.250 | 8.150 | 8.196 |
| H1′ | 5.904 | 5.825 | 5.939 |

* Shifts are measured for a 0.005 M solution at 16°C, relative to internal TSP.

Table 2
Chemical shift differences between ApA and ApApA (T = 16°C)

| Proton | Δδ*, ppm | |
|---|---|---|
| | Ap- | -pA |
| H2 | 0.114 | 0.063 |
| H8 | .041 | .113 |
| H1′ | .025 | .295 |

* Δδ = (trimer shift−dimer shift); positive values denote upfield shifts. Dimer and trimer are adjusted for intermolecular stacking contributions.

-pAp- and H8 for Ap-. Similarly, comparison with spectra C and D leads to assignment of H8 for -pA and H2, and H1′ signals for Ap-. The remaining assignments follow directly. A summary of assignments and chemical shifts for all the base and anomeric protons is given in table 1.

### 3.2. *Structural implications*

The base and anomeric proton assignments reveal several interesting features relating to ApApA conformation. Firstly, the present data show the danger of basing signal assignments for oligonucleotides on shifts expected for likely oligomer conformations. For example, on the assumption that the base ring orientations in ApApA are an extension of the stacked ApA conformation [5–8] (*anti, anti,* right hand helix), then predicted H2 shieldings† should increase in order -pA < Ap < -pAp-; the greater shielding of H2-pAp reflecting shielding contributions from two adjacent stacked base rings. Actually, the observed H2 shieldings increase as -pA < -pAp- < Ap-. This trend coupled with the observation that all of the base and H1′ signals of ApApA shift upfield relative to ApA, table 2, though in smaller magnitudes and different patterns than for the monomer → dimer transition, leads us to conclude that the additional adenylyl moiety produces an increase in overall base stacking relative to ApA accompanied by a change in base-ring orientations in the stacked trimer. Additionally, the H2 shift data suggest that Ap- is more closely stacked with -pAp than is -pA. In this regard, the NMR results support a solution conformation similar to that for $ApA\overset{+}{p}A^{+}$ in the crystalline state [1] where adenosine rings of $ApA^{+}$- are stacked helically while the third adenylyl group -$pA^{+}$ is folded over in a non-helical arrangement. An analogous non-helical -pApA segment in solution would account for the smaller than expected shielding of H2 -pAp-.

Further conformational information is derivable from H1′ splittings which arise from spin coupling to H2′. Since virtual coupling effects† are undoubtedly present, a direct evaluation of $J_{H1'H2'}$ from doublet separations cannot be made. Nevertheless, if H2′ and H3′ shifts in ApApA do not differ significantly from ApA, as seems to be true from spectral comparisons, then $J_{H1'H2'}$ values of 3.4, 2.8, 3.5 Hz are estimated for Ap-, -pAp-, and -pA respectively. The trimer values are comparable to corresponding couplings in ApA 3.1 (Ap-) and 4.0 Hz (-pA) [9] and much lower than in monomers, i.e., 6.3, 5.9 Hz [10,11]. From this it is concluded that the N(C3′-*endo*) $\rightleftharpoons$ S(C2′-*endo*) ribose ring conformational equilibrium [10,12] favors the N conformer in all three ApApA nucleotides, the preference being somewhat greater in -pAp- than the others. Since stacking is known to produce a shift from C2′-*endo* to C3′-*endo* ribose conformations [6,9], the coupling data offer further support for the ApApA structure proposed from shift trends.

† H2 shifts serve as good markers for base orientations since they are only marginally affected by phosphate ionization state and glycosidic torsion angle change.

† Both $J_{H1'H2'}$ coupling of ApA showed presence of virtual coupling, the problem being most pronounced for Ap- where shifts for 2′ and 3′ protons were nearly equal [9].

In summary, the synthesis of selectively deuterated ApApA derivatives has permitted unambiguous assignment of all base and anomeric H1′ PMR signals for the trimer. Additionally, the derived chemical shifts and couplings are consistent with an equilibrium solution conformation (base-stacked, C3′-*endo* ribose rings) with overall structural features similar to those reported for $ApAp^{+}A^{+}$ in the crystalline state. Finally, some circumspection is advisable in utilizing monomer and dimer conformational data for structure predictions of higher oligonucleotides.

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