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Synthesis, Physical, Chemical, and Enzyme Studies on bis-2, β -Diaminopurine β -D-Ribofuranoside p1, p4-Tetraphosphate

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SYNTHESIS, PHYSICAL, CHEMICAL, AND ENZYME STUDIES ON BIS-2,6-DIAMINOPURINE β -D-RIBOFURANOSIDE P^1 , P^4 -TETRAPHOSPHATE.

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Abstract The biological rôle of diadenosine-5',5" P^1 , P^4 -tetraphosphate, Ap₄A, can be explored by the use of *bis*-2-aminoadenosine-5',5" P^1 , P^4 -tetraphosphate, Dp₄D, and its P^2 , P^3 -methylene analogues. Syntheses and characteristics of six Dp₄D species are described.

Diadenosine-5',5"'P1,P4-tetraphosphate Ap₄A (Fig.1) is a ubiquitous component of all cells. Since its discovery in 1965 by Paul Zamecnik,¹ Ap₄A has been proposed to play a part: (a) in the response of cells to various forms of shock and was thus designated² as an 'alarmone'; (b) as a pleiotypic regulatory molecule with roles in the initiation of DNA replication;³ and (c) with related nucleotides, in blood platelet function.⁴ However, the precise function of Ap₄A in such areas has yet to be resolved.⁵

As part of our programme⁶ to identify the role(s) of Ap₄A, we have synthesised a number of 2,6-diaminopurine 9-β-D-ribofuranoside analogues, Dp₄Ds (1a-f).

The most successful procedure involves converting 2,6-diaminopurine riboside⁷ into its 5'-phosphoromorpholidate (2) for coupling with pyrophosphoric acid (3a) or one of a range of methylene-bisphosphonic acids (3b-f) (Scheme). Under appropriate conditions, this affords both

Scheme

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Table 1 1H NMR of Dp4D Compounds.

Compound 1	δН8	δH ^{1'}	δ H2*	δ H ³ '	δ H ⁴ '	δ H ⁵	Bridge-H
DPPPPD a	8.00 s	5.80 s	4.67 dd	4.49 dd	4.26 m	4.18 m	-
DPPCF2PPD b	8.00 s	5.86 d	4.70 t	4.50 m	4.30 m	4.20 m	
DPPCHFPPD c	7.98 s	5.85 d	4.68 t	4.50 m	4.30 m	4.18 m	5.17 dt
DPPCCI2PPD d	8.03 s	5.83 d	4.68 t	4.52 dd	4.30 m	4.23 m	-
DPPCHCIPPD e	8.06 s	5.90 d	4.70 dd	4.55 dd	4.33 m	4.20 m	n.d.
DPPCH2PPD f	8.06 s	5.90 d	4.70 dd	4.53 dd	4.33 m	4.20 m	2.53 t

Table 2 31P NMR of Dp4D analogues (N.B. $^2J_{12} = ^2J_{34}$, and $^4J_{13} = ^4J_{24}$)

Compound 1		δ P ¹ (P ⁴)	δ P ² (P ³)	² / ₁₂	⁴ <i>J</i> 13	² / ₂₃	² JPF
DPPPPD a		-10.929	-22.366	17.7	0.5	15.3	-
DPPCF2PPD b		-10.876	-6.150	21.3	6.7	10.2	83
DPPCHFPPD c	\Box	-10.852	0.438	22.9	1.6	7.1	61
DPPCCI2PPD d		-10.821	-1.480	23.9	4.2	72	-
DPPCHCIPPD e		-10.651	2.744	25.5	-0.35	1.5	-
DPPCH2PPD f		-10.713	8.108	25.6	-0.3	5.5	-

Dp₄D (1a) and DTP (4a) and their analogues, which can be separated efficiently by chromatography on DEAE Sephadex with gradient elution using TEAB at pH 8.5.

The ¹H (Table 1) and ³¹P (Table 2) NMR spectra of these compounds along with positive ion FABMS analysis and tlc homogeneity confirm their identity. The characteristic twelve-line AA'XX' ³¹P NMR spectra for the Dp₄D analogues were analysed using the "Panic" routine on a Bruker WP80SY machine at 32MHz.

Preliminary studies on the stability of interaction of Dp₄D with (pdT)₁₀ suggest that the T_m is less than 5°C (pH 7.0, μ =0.1). Results of kinetic analysis of the interaction of the Ap₄A hydrolase from lupin⁸ with Dp₄D and its inhibition by the analogues (1b-f) will be reported elsewhere.

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