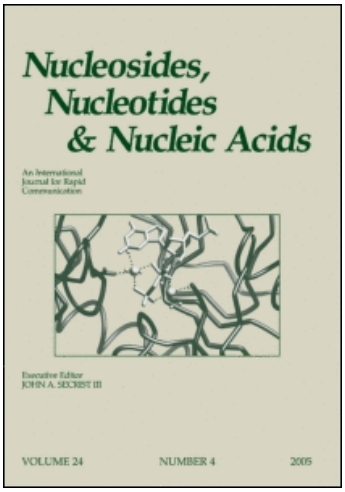

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Synthesis, Physical, Chemical, and Enzyme Studies on bis-2,β-Diaminopurine β-D-Ribofuranoside p1, p4-Tetraphosphate

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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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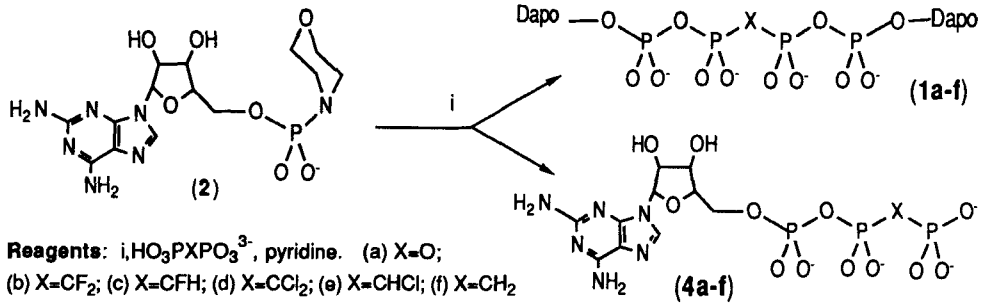
Abstract The biological rôle of diadenosine-5',5''' P^1,P^4-tetraphosphate, Ap_4A, can be explored by the use of *bis*-2-aminoadenosine-5',5''' P^1,P^4-tetraphosphate, Dp_4D, and its P^2,P^3-methylene analogues. Syntheses and characteristics of six Dp_4D species are described.

Diadenosine-5',5'''P^1,P^4-tetraphosphate Ap_4A (Fig.1) is a ubiquitous component of all cells. Since its discovery in 1965 by Paul Zamecnik,[1] Ap_4A has been proposed to play a part: (a) in the response of cells to various forms of shock and was thus designated[2] 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 Ap_4A in such areas has yet to be resolved.[5]

As part of our programme[6] to identify the role(s) of Ap_4A, we have synthesised a number of 2,6-diaminopurine 9-β-D-ribofuranoside analogues, Dp_4Ds (1a-f).

The most successful procedure involves converting 2,6-diaminopurine riboside[7] 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



Reagents: i,$HO_3PXPO_3^{3-}$, pyridine. (a) X=O;
(b) X=CF_2; (c) X=CFH; (d) X=CCl_2; (e) X=CHCl; (f) X=CH_2

Scheme

Table 1 ^{1}H NMR of Dp$_4$D Compounds .

Compound 1	δ H^8	δ H$^{1'}$	δ H$^{2'}$	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	-
DPPCF$_2$PPD **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
DPPCCl$_2$PPD **d**	8.03 s	5.83 d	4.68 t	4.52 dd	4.30 m	4.23 m	-
DPPCHClPPD **e**	8.06 s	5.90 d	4.70 dd	4.55 dd	4.33 m	4.20 m	*nd*
DPPCH$_2$PPD **f**	8.06 s	5.90 d	4.70 dd	4.53 dd	4.33 m	4.20 m	2.53 t

Table 2 ^{31}P NMR of Dp$_4$D analogues (N.B. $^2J_{12} = {}^2J_{34}$, and $^4J_{13} = {}^4J_{24}$)

Compound 1	δ P^1 (P^4)	δ P^2 (P^3)	$^2J_{12}$	$^4J_{13}$	$^2J_{23}$	$^2J_{PF}$
DPPPPD **a**	-10.929	-22.366	17.7	0.5	15.3	-
DPPCF$_2$PPD **b**	-10.876	-6.150	21.3	6.7	10.2	83
DPPCHFPPD **c**	-10.852	0.438	22.9	1.6	7.1	61
DPPCCl$_2$PPD **d**	-10.821	-1.480	23.9	4.2	7.2	-
DPPCHClPPD **e**	-10.651	2.744	25.5	-0.35	1.5	-
DPPCH$_2$PPD **f**	-10.713	8.108	25.6	-0.3	5.5	-

Dp$_4$D (**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 ^{1}H (Table 1) and ^{31}P (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' ^{31}P NMR spectra for the Dp$_4$D analogues were analysed using the "Panic" routine on a Bruker WP80SY machine at 32MHz.

Preliminary studies on the stability of interaction of Dp$_4$D 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 Ap$_4$A hydrolase from lupin[8] with Dp$_4$D and its inhibition by the analogues (**1b-f**) will be reported elsewhere.

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