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Diadenylated polyols as new non-isopolar analogues of diadenosine tri- and tetraphosphates

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Abstract

A series of diadenosine polyphosphate-mimics were prepared based upon phosphorothioylation of the hydroxyl functions of polyols **1** and 1,3,2-oxathiaphospholane ring-opening condensation methodology.

Keywords: phosphorothioylation; polyols; oxathiaphospholanes ring-opening condensation.

Since the discovery by Zamecnik in 1966[1] that diadenosine polyphosphates ($A_{pn}As$) are by-products of the lysyl-*t*RNA synthetase, dinucleoside polyphosphates have excited strong interest and have been well studied.[2] Their biological functions are not yet adequately understood. Adenosine-containing dinucleoside tri- and tetraphosphates have been proposed to have various intracellular functions, including regulation of DNA replication and signalling stress responses. There is considerable current interest in an extracellular role for $A_{p4}A$ and $A_{p3}A$ in blood platelet physiology.[3] Recently it was demonstrated that the tumour suppressor protein, Fhit,[4a] is an $A_{p3}A$ hydrolase,[4b] whose signalling appears to depend on $A_{pn}A$ binding.[5] That result suggests that $A_{p3}A$ or similar dinucleoside polyphosphates (DNPP) may be factors in tumourigenesis.[6] A major problem in understanding the cellular function of $A_{pn}As$ is their rapid degradation by both specific and non-specific hydrolases and phosphorylases. For some years, much effort has been put into chemical synthesis of DNPPs that are stable to enzymatic cleavage.

Recent publications of Blackburn et al.[7] presenting a new generation of so-called 'supercharged' analogues of diadenosine polyphosphates and data demonstrating their biological activities prompted us to publish our results on the synthesis of a novel class of non-isopolar analogues of $A_{pn}As$ which are generally isosteric[8] with the parent Ap_nA nucleotides. Our concept is based upon phosphorothioylation of hydroxyl functions of polyols **1** with $N^6,N^6,O^{2'},O^{3'}$-tetrabenzoyladenosine 3′-*O*-(2-thiono-1,3,2-

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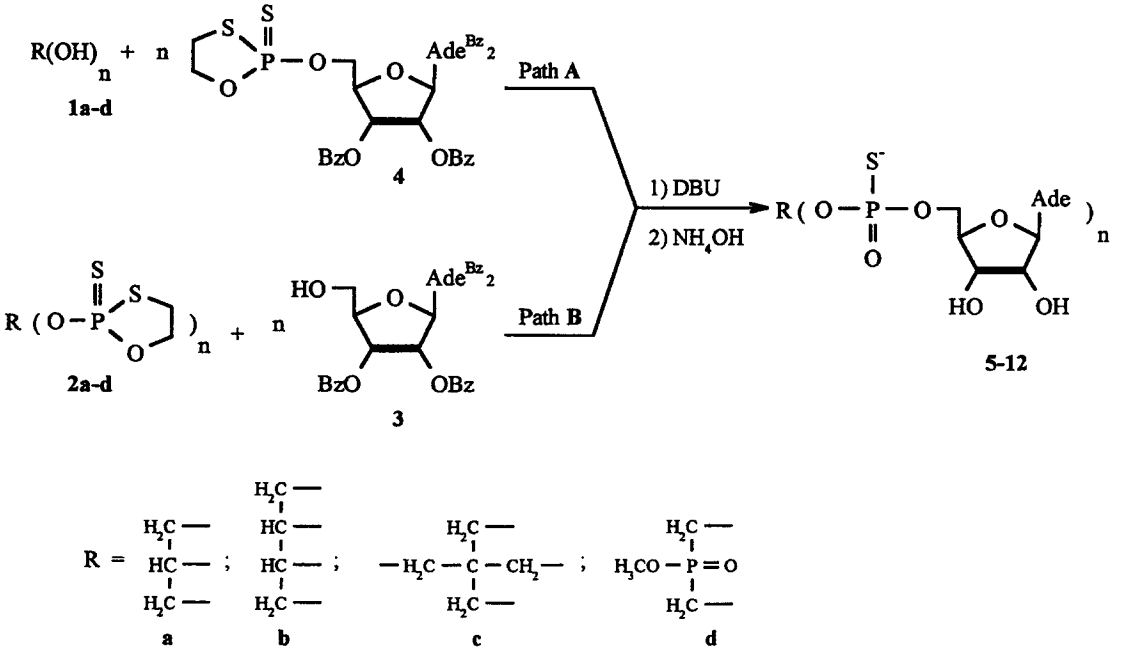
oxathiaphospholane) (**4**, as a mixture of two unseparated diastereomers).† Alternatively, phosphorothioylation of polyols **1** by means of 2-chloro-1,3,2-oxathiaphospholane followed by sulfurisation provides by-products **2**,‡ which undergo further reaction with $N^6,N^6,O^{2'},O^{3'}$-tetrabenzoyladenosine (**3**).

Both approaches are depicted in Scheme 1 as Path A and Path B, respectively. In both scenarios (Path A and Path B) equimolar amounts of DBU have been used as the reagent of choice for accelerating the 1,3,2-oxathiaphospholane ring-opening condensation process.[9]



Scheme 1.

The crude reaction mixtures were treated with an excess of aqueous ammonia in order to remove benzoyl and, alternatively, *O*-(β-cyanoethyl) (vide infra) protecting groups, providing **5–12**. Compounds **5–12** were purified by means of silica gel chromatography (PTLC)§ and Sephadex A-25 ion-exchange chromatography.¶ Although substrates **2** and **4** possess *P*-chiral centres, racemic **2** and diastereomeric mixtures of **4** were used in all reactions presented in Scheme 1. Therefore, compounds **5–12** are mixtures of corresponding diastereomers. The primary physico-chemical characteristics of the final products, together with an indication of the ratio of substrates used in condensation reactions, and the preparative yield of final products (triethylammonium salts; calculated on substrate **2** or **1**) are presented in Table 1. It should be explained that the syntheses of compounds **10–12** have been performed via Path B in such

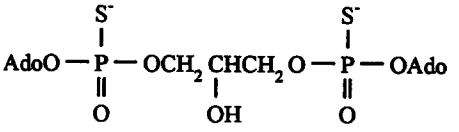
† Compound **4** was obtained in the reaction of $N^6,N^6,O^{2'},O^{3'}$,-tetrabenzoyladenosine (**3**) with 2-chloro-1,3,2-oxathiaphospholane in pyridine solution in the presence of S_8. The crude product was purified by silica gel column chromatography using chloroform:hexane (8:2) as an eluent to give **4** in 72% yield.

‡ The synthesis of compound **2a**: to a suspension of elemental sulfur (0.38 g, 1.5 mmol) in pyridine (5 ml) was added 2-chloro-1,3,2-oxathiaphospholane (0.9 g, 6.3 mmol). Then solution of glycerol (0.138 g, 1.5 mmol) in pyridine (1 ml) was introduced to the reaction mixture which was stirred for 12 h at room temperature. The crude product was purified by silica gel column chromatography using chloroform: *n*-hexane (7:3) to give **2a** in 77% yield [^{31}P NMR ($CDCl_3$) 105.6 ppm (m); FAB-MS (m–1) *m/z* 505].

§ The developing system isopropanol:ammonia:water (7:1:2) was used.

¶ Columns were eluted with a linear gradient of ammonium bicarbonate buffer (pH 7.5) from 0.2 to 1 M.

a manner that pre-determined equivalents of **3** (lower than *n*) were mixed with DBU and **2**; then the unreacted oxathiaphospholanes were subjected to DBU-assisted ring-opening process by means of 3-hydroxypropionitrile.[10]



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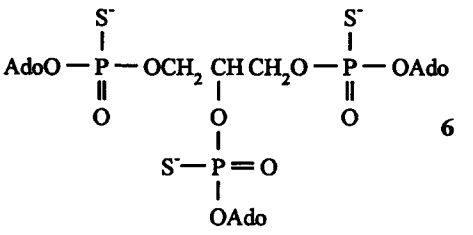

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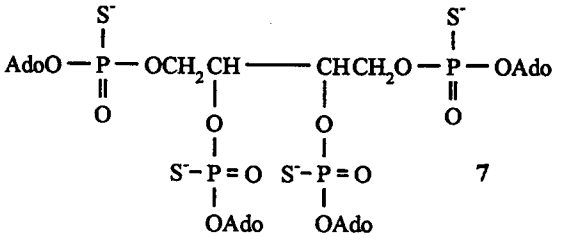

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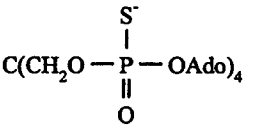

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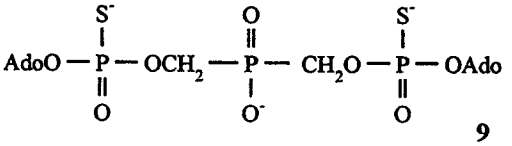

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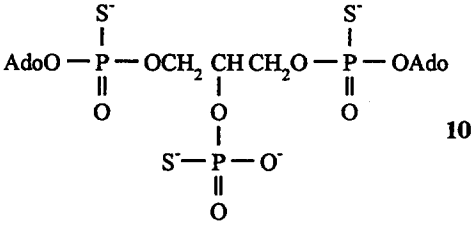

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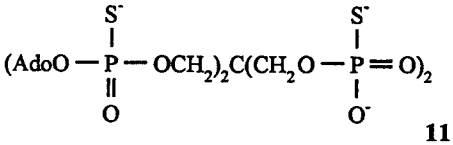

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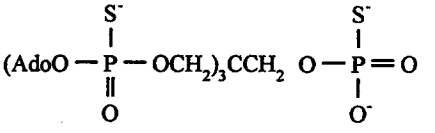

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Compounds **5**, **6** and **8** were tested as inhibitors of Ap_3A and Ap_4A hydrolases and, independently (plus compound **9**) as inhibitors of platelet aggregation. Preliminary results indicate that they do not inhibit lupin Ap_4A hydrolase, but, interestingly, serve as inhibitors of Fhit Ap_3A hydrolase.[11] The strongest inhibitory effect for platelet aggregation possesses compound **9**.[12] Corresponding phosphate analogues of **5–12** are now under similar evaluation, and results on the biological activity of *P*-achiral compounds will be incorporated into further development of the present concept. Since the 1,3,2-oxathiaphospholane ring-opening condensation is known to be stereospecific process,[13] separation of **4** into *P*-diastereomers should provide the route to the *P*-stereocontrolled synthesis of **5–12**.

Acknowledgements

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Table 1
The physico-chemical characteristics of compounds **5–12**

Comp. No	Substrates	Substrates Ratio	Product	
			^{31}P NMR [ppm] Chemical shifts (H_3PO_4)	MS-MALDI (M-1) m/z
5	**1a+4**	1:2	56.83	782
6	**2a+3**	1:3	57.56	1127
7	**1b*+4**	1:4	57.32	1502
7	**2b*+3**	1:4	57.32	1502
8	**1c+3**	1:4	56.28	1516
9	**2d+3**	1:2	58.46, 58.24, 30.28; $^3J_{P-P}$=35.5 Hz	815
9	**1d+4**	1:2		815
10	**2a+3**	1:2	56.61, 45.95	877
11	**2c+3**	1:2	57.12, 46.71	1018
12	**2c+3**	1:3	56.94, 46.82	1267

**meso*-erythritol has been used as the starting matherial

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