Adenine Nucleoside 3'-Tetraphosphates Are Novel and Potent Inhibitors of Adenyl Cyclases*

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| 3'-di < 3'-triphosphate and Ado < 2',5'-deoxyadenosine < 2',5'-dd-Ado. Given the dependence of potency on 3'-phosphate group content and because the effectiveness of nucleotides to induce conformational transitions in some proteins has been proportional to the number of phosphates (11), the possibility is suggested that 3'-tetra-phosphate derivatives would be yet more potent inhibitors of adenyl cyclases. Consequently, 2'-deoxy- and 2',5'-deoxyadenosine 3'-tetraphosphates (2'-d-3'-A, P and 2',5'-dd-3'-A, P) were synthesized and tested for inhibition of crude and purified forms of adenylyl cyclase.

EXPERIMENTAL PROCEDURES
Preparation and Assay of Adenyl Cyclase—Detergent-solubilized preparations of adenylyl cyclase from rat and bovine brains were prepared and assayed as described previously (4, 12, 13). Bovine brain adenylyl cyclase was purified as described by Pfeuffer et al. (14). Inhibition kinetics were determined on enzyme assayed in duplicate or triplicate, with concentrations of divalent cation fixed in excess of the ATP concentration as described previously (12, 15). IC₅₀ values were derived from inhibition curves and Kᵢ values from replots of slopes from Lineweaver-Burk plots comprising six to eight concentrations of inhibitor in at least two experiments for each condition. CAMP formation was linear with respect to reaction time and enzyme concentration under these reaction conditions.

Na⁺/K⁺-ATPase—Activity of Na⁺/K⁺-ATPase was determined in a reaction mixture containing 50 mM triethanolamine-HCl, pH 7.5, 100 mM NaCl, 2 mM KCl, 5 mM MgCl₂, 100 nM 5'-ATP, and ~10⁶ cpm [γ-³²P]ATP in a volume of 100 μl. Reactions were for 10 min at 30 oC and were terminated by the addition of a 1-ml slurry of ice-cold 0.1 m H₃PO₄ containing 25 mg of charcoal/ml. These suspensions were centrifuged, and a measured portion of the supernatant fraction containing the released ³²P was quantified by Chernenkov radiation in a liquid scintillation counter. The rate of ³²P release was linear with reaction time and enzyme concentrations under the conditions of these experiments.

C₄⁺/Calmodulin-dependent Cyclic Nucleotide Phosphodiesterase—Activity was determined as described previously (16) in a reaction mixture containing 50 mM triethanolamine-HCl, pH 7.5, 2 mM MgCl₂, 40 μM CAMP, 1 mg of bovine serum albumin/ml, 1 mM dithiothreitol, 50 μM CaCl₂, and ~10⁶ cpm [³²P]cAMP in a volume of 100 μl. Reactions were for 10 min at 30 oC and were stopped by the addition of 20 μl of a solution containing 100 mM triethanolamine-HCl, 25 mM EDTA, 2.5 mM 3-isobutyl-1-methylxanthine, and 4.53 mM cAMP. 5'-Nucleotidase from Crotalus atrox snake venom was then added to a final concentration of 0.8 mg/ml, and samples were incubated an additional 15 min at 30 oC. This second reaction was terminated by the addition of a 1-ml slurry of ice-cold 0.1 m H₃PO₄ containing 25 mg of charcoal/ml. These suspensions were centrifuged, and a measured portion of the supernatant fraction containing the released ³²P was quantified by Chernenkov ra-

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The abbreviations used are: Ado, adenosine; 2',5'-dideoxyadenosine; 2'-d-3'-AMP, 2'-dideoxyadenosine 3'-monophosphate; 2'-d-3'-ADP, 2'-dideoxyadenosine 3'-diphosphate; 2'-d-3'-ATP, 2'-dideoxyadenosine 3'-triphosphate; 2',5'-d-3'-AMP, 2',5'-dideoxyadenosine 3'-monophosphate; 2',5'-dd-3'-ADP, 2',5'-dideoxyadenosine 3'-diphosphate; 2',5'-dd-3'-ATP, 2',5'-dideoxyadenosine 3'-triphosphate; 2'-3'-A, P, 2'-dideoxyadenosine 3'-tetraphosphate; 2',5'-dd-3'-A, P, 2',5'-dideoxyadenosine 3'-tetraphosphate; HPLC, high performance liquid chromatography; 3':5'-cAMP, adenosine-3':5'-cylic monophosphate.
diation in a liquid scintillation counter. Calmodulin (~0.7 μM) caused a 3.1 ± 0.1-fold (n = 8) increase in the rate of cAMP hydrolysis by this phosphodiesterase preparation. This rate was linear with reaction time and enzyme concentrations under the conditions of these experiments.

cAMP-dependent Protein Kinase A—Holozyme was isolated from bovine brain and enriched by ammonium sulfate precipitation and chromatography on DEAE-Sephadex as established procedures (17). Activity was measured in a reaction mixture containing 10 mM Na-phosphate, pH 7.5, 1 mg of bovine serum albumin/ml, 5 mM MgCl₂, 100 μM ATP, and 10 μg of histone, in a volume of 70 μl. Reactions were started with the addition of protein kinase A and were for 15 min at 30 °C. ATP was determined by spotting 5 μl portions of each reaction on Whatman P81 phosphocellulose paper filters (25 mm diameter) and placing these in ice-cold 75 mM phosphoric acid. Filters were then washed three times with the phosphoric acid and one wash with ethanol, each after perhaps 10 min. Filters were air dried, and adsorbed 32P-histone was quantified by Chenerykov radiation in a liquid scintillation counter. Histone phosphorylation was linear with enzyme concentration and reaction time under these assay conditions, with ATP utilization always less than 2%. The addition of 1 μM cAMP caused a 9.5 ± 0.4-fold (n = 9) increase in the rate of histone phosphorylation by this preparation of protein kinase A.

Quantification of Nucleotides by High Performance Liquid Chromatography—Nucleotides were quantified after high performance liquid chromatography (HPLC) as areas under peaks determined with a Waters 996 photo-diode array detector and the accompanying Millennium software (Version 2.10). Ion exchange chromatography was on an Altex Spherogel TSK DEAE-5PW column (5 μm, 7.5 × 75 mm) developed with sequential step gradients of triethylammonium bicarbonate, pH 8.5, to separate nucleoside 3′-monophosphate, 3′-diphosphate, 3′′-triphosphate, and 3′′′-tetraphosphates.

Sodium Salt of Triphosphoric Acid—Pentadoxotriphosphate (40 g, 56.7 mmol) was dissolved in 200 ml of cold water, and the resulting solution was added to a Büchner funnel loaded with 3H-5 m, 1H, H-3

2.5′-dd-3′A-P—2′,5′-Dideoxyadenosine 3′-O-[(2,2,2-trichloroethyl)morpholinophosphonate] (0.89 g, 1.5 mmol) was added to a solution of pyridine (80 ml) containing activated zinc (0.15 g) (18) and tributylammonium salt of triphosphoric acid (15 g, 15 mmol), under the exclusion of moisture. The mixture was stirred at room temperature for 2 days. The reaction was then concentrated in vacuo, diluted with cold water (300 ml), filtered, and then purified by chromatography on QAE-Sephadex (HCO₃⁻ form) with a linear gradient of triethylammonium bicarbonate (0.1–1 M). The appropriate fractions were lyophilized and then coevaporated several times with methanol, yielding 0.18 mmol of 2′,5′-dd-3′-A-P. This mixture was isolated as its sodium salt by addition of 1 m sodium iodide in acetone to a methanol solution of the triethylammonium nucleotide. The precipitate was collected by centrifugation and washed with cold acetone and dried in vacuo giving the sodium salt of 2′,5′-dd-3′-A-P. 3′H NMR (D₂O) δ 1.41 (d, 3H, J = 6.5 Hz, 3H-5′), 2.80–3.00 (m, 2H, H-2′, and H-6′), 6.52 (t, 1H, J = 6.9 Hz, H-1′), 8.28 (s, 1H, H-2), 8.47 (s, 1H, H-8). 32P NMR (D₂O) δ 18.55 (t, J = 17.1 Hz, H-3′), −17.80 (t, J = 19.2 Hz, P-2), −8.09 (dd, J₁₂ = 9.1 Hz, J₁₋₁ = 18.1 Hz, P-1), −1.55 (d, J = 13.8 Hz, P-4). 2′-d-3′A-P—(Dimethoxytrityl)-2′-dideoxyadenosine 3′-O-[(2,2,2-trichloroethyl)morpholinophosphonate] (1.42 g, 2.5 mmol) was added to a solution of pyridine (50 ml) containing activated zinc (0.5 g) (19) and tributylammonium salt of triphosphoric acid (15 g, 15 mmol), under the exclusion of moisture. The mixture was stirred at room temperature for 2 days. The reaction mixture was then concentrated in vacuo, diluted with 80% acetic acid at room temperature for 30 min. The medium was then neutralized with a cold solution of 0.5 M NaHCO₃, diluted to two liters, filtered, and then purified by chromatography on QAE-Sephadex as above, yielding 0.21 mmol of 2′-d-3′-A-P. This nucleotide was also isolated as its sodium salt as above. No impurities were noted on anion exchange HPLC. 3′H NMR (D₂O) δ 2.81–2.99 (m, 2H, H-2′ and H-6′), 3.88 (d, 2H, H-2′), 4.40–4.45 (m, 1H, H-4′), 5.06–5.19 (m, 1H, H-5′), 6.7 (d, J = 5.4 Hz, H-3′), 8.25 (s, 1H, H-2), 8.26 (s, 1H, H-8). 32P NMR (D₂O) δ 18.28 (t, J = 17.1 Hz, P-3′), −17.24 (t, J = 19.7 Hz, P-2′), −8.07 (dd, J₁₋₁ = 7.7 Hz, J₁₋₂ = 19.2 Hz, P-1′), −1.44 (d, J = 18.4 Hz, P-4′).

Materials—[α-32P]ATP and [γ-32P]ATP were purchased from ICN Pharmaceuticals. Lubrol-PX (from Sigma, L-3753), used for solubilizing the enzyme, was filtered through alumina (Neutral, AG7, from Bio-Rad) to remove peroxides. [α-32P]ATP was prepared from

![Scheme 1. Reagents and conditions.](image-url)

**Scheme 1. Reagents and conditions.** i, tert-ButMgCl, tetrahydrofuran, room temperature; ii, phosphorylating agent, room temperature; iii, zinc, (Bu₃N)₂H₂P₂O₁₀pyridine, room temperature, followed by AcOH 80%, room temperature, 30 min for 2.**

Results—The previously described synthesis of a family of adenine nucleoside 3′- polyphosphates combined the method of alkoxyide activation with the use of triboemoxy phosphoromorpholino-chloride as an initial phosphorylating agent (5, 6, 20). This method was extended for the synthesis of 2′-d-3′-A-P and 2′,5′-dd-3′-A-P by the second stage use of the tributylammonium salt of inorganic triphosphate (Scheme 1). Although yields for these syntheses were low (8.4 and 12%, respectively), they were sufficient to permit the preparation of research quantities of these 3′-nucleotides.

Inhibition of Adenyl Cyclases—The 3′-triphosphates, 2′-d-3′-A-P and 2′,5′-dd-3′-A-P, exhibited notably more potent inhibition of adenyl cyclase than did the respective 3′-triphosphates (Fig. 1). A comparison of potencies of these nucleotides, determined from several experiments, are given in Table 1. The addition of the fourth phosphate increased potency for the 2′-deoxy derivative more than for the 2′,5′-dideoxy derivative, whereas in both cases, the increase in potency was not quite an order of magnitude. With IC₅₀ values of 32 and 106 nM (Table 1), respectively, for 2′,5′-dd-3′-ATP and 2′-d-3′-ATP, the 3′-triphosphates exhibited potencies with this rat brain extract similar to those previously reported (6). The 3′-tetraphosphates exhibited IC₅₀ values of 7.4 nM for 2′-d-3′-A-P and 2′,5′-dd-3′-A-P and Kᵢ values of 53 and 23 nM, respectively, making them the most potent known regulators of adenylyl cyclase activity. This inhibition occurred at an estimated enzyme concentration of approximately 0.9 nM, with the assumptions of a mass of 116 kDa and a specific activity of 7 μmol/min·mg of protein for the purified type I adenylyl cyclase (14, 21). This is not a large excess of inhibitor relative to
enzyme, and slightly lower IC\textsubscript{50} values might be observed with lower enzyme concentrations.

Inhibition was rapid in onset for inhibition by 2',5'-dd-3'-ATP or by either of two concentrations of 2',5'-dd-3'-A\textsubscript{4}P (Fig. 2). As expected from their IC\textsubscript{50} values, inhibition by 10 nM 2',5'-dd-3'-A\textsubscript{4}P elicited an inhibited rate similar to that achieved with 50 nM 2',5'-dd-3'-ATP; 100 nM 2',5'-dd-3'-ATP almost completely suppressed activity. The lack of lag phase argues for a rapid equilibrium mechanism of interaction of adenosine 3'-polyphosphates with the enzyme.

In our previously reported kinetic determinations for inhibition conducted either with adenine nucleosides or with adenine nucleoside 3'-phosphates, inhibition typically conformed to a linear noncompetitive mechanism (5, 6, 12). Although some exceptions to this observation have been noted (7, 12, 22–24), this behavior has become a characteristic of P-site inhibition. Both 2'-d-3'-A\textsubscript{4}P and 2',5'-dd-3'-A\textsubscript{4}P (Fig. 3) elicited a nonlinear noncompetitive inhibition of the detergent-extracted adenylyl cyclase from rat brain, the preparation we have used for many comparisons of P-site ligands (cf. Refs. 2, 4–6, 10, 12, and 13). By comparison, a linear noncompetitive inhibition was observed with either 3'-tetraphosphate with the enzyme purified from bovine brain, shown in Fig. 4 for 2',5'-dd-3'-A\textsubscript{4}P. The noncompetitive character of this inhibition is perhaps more clearly shown with a Hofstee plot (V/S) (Fig. 5). For this plot, a series of parallel lines is expected for straightforward noncompetitive inhibition, whereas intersecting lines would be consistent with competitive inhibition. The reason for the apparent discrepancy in kinetic behavior between adenylyl cyclases in the rat brain and purified bovine brain preparations is not known. It does not lie in nonlinear behavior of the reaction per se (cf. Fig. 2) but likely lies in the complex character of the crude detergent extract or differences in the conformations of the enzymes being tested. Nor was the nonlinear behavior because of an effect of breakdown products of the 3'-tetraphosphates. No significant hydrolysis of either 2'-d-3'-A\textsubscript{4}P or 2',5'-dd-3'-A\textsubscript{4}P was noted during the course of typical

| Nucleotide | Rat Brain IC\textsubscript{50} (nM) | Rat Brain K\textsubscript{i} (nM) | Bovine Brain IC\textsubscript{50} (nM) | Bovine Brain K\textsubscript{i} (nM) |
|------------|-----------------------------------|---------------------------------|-----------------------------------|---------------------------------|
| 2'-d-3'-ATP | 106 ± 6\textsuperscript{a} | 90\textsuperscript{b} | 2',5'-dd-3'-A\textsubscript{4}P | 10.5 ± 2.4\textsuperscript{c} | 15 ± 5\textsuperscript{c} | 53 ± 18\textsuperscript{c} |
| 2',5'-dd-3'-ATP | 32 ± 2\textsuperscript{c} | 40\textsuperscript{d} | 2',5'-dd-3'-A\textsubscript{4}P | 7.4 ± 1.5\textsuperscript{c} | 9.3 ± 1.3\textsuperscript{c} | 23 ± 3\textsuperscript{c} |

\textsuperscript{a} Values are averages ± range from two experiments, each comprising six concentrations of inhibitor and assayed in duplicate.

\textsuperscript{b} From Ref. 6.

\textsuperscript{c} Values are averages ± S.E. from four experiments, each comprising six concentrations of inhibitor and assayed in duplicate.

\textsuperscript{d} Values derived from data previously published (5, 6).
adenyl cyclase incubations (Table II). This lack of hydrolysis, whether enzymatic or nonenzymatic, is consistent with the established stability of both 2′-d-3′-ATP and 2′,5′-dd-3′-ATP that we reported earlier (6).

**Effects on Other Enzymes**—Because few adenine nucleotides interact solely with a single protein, the possibility was considered that adenosine 3′-polyphosphates might affect enzymes other than adenylyl cyclase. As an initial investigation in this direction, we tested effects on Na+/K+ATPase and on two enzymes participating in the cAMP signaling cascade, cAMP phosphodiesterase and cAMP-dependent protein kinase.

Under conditions with which strophanthidin exhibited an IC,

![Image](image-url)

**FIG. 4.** Double reciprocal plot for inhibition of adenylyl cyclase purified from bovine brain by 2′,5′-dd-3′-A4P. Enzyme was prepared and assayed as described under “Experimental Procedures.” Units for velocity are pmol of cAMP formed/min/100 μl of assay volume). Concentrations of 2′,5′-dd-3′-A4P were as indicated.

![Image](image-url)

**FIG. 5.** Eadie-Hofstee plot for inhibition of adenylyl cyclase purified from bovine brain by 2′,5′-dd-3′-A4P. Data are from the experiment represented in Fig. 4. Units for velocity are pmol of cAMP formed/min/100 μl of assay volume. Concentrations of 2′,5′-dd-3′-A4P were as indicated.

2′,5′-dd-3′-A4P at concentrations from 10 nM to 11 μM exhibited any effect whatsoever on enzyme activity.

Ca2+/calmodulin-sensitive cAMP phosphodiesterase was tested in the absence or presence of 0.7 μM calmodulin, which elicited >3-fold activation of this enzyme preparation. Neither 2′,5′-dd-3′-A4P at concentrations from 0.1 to 10 μM nor 2′,5′-dd-3′-ATP at concentrations from 3 nM to 10 μM had any effect on phosphodiesterase activity, without or with Ca2+/calmodulin.

Although Flockhart et al. (25) evaluated effects of numerous nucleosides and nucleotides on both cAMP- and cGMP-dependent protein kinases, none was a nucleoside 3′-phosphate. The rabbit muscle cAMP-dependent protein kinase we tested was unaffected by either 2′,5′-dd-3′-A4P or 2′,5′-dd-3′-ATP, at concentrations from 10 nM to 10 μM, whether in the absence or presence of 1 μM cAMP, which elicited >9-fold activation with this enzyme preparation (Fig. 6). Even though these experiments were conducted with a concentration of substrate tenfold greater than that of the 3′-nucleotides, it was clear that these 3′-nucleotides had no effect on either catalytic (5′ATP) or regulatory (cAMP) domains of this protein kinase.

**DISCUSSION**

2′-Deoxy- and 2′,5′-dideoxadenosine 3′-tetraphosphates represent important additions to the family of 3′-polyphosphates constituting the most potent known regulators of adenylyl cyclases. The rapid onset and linear noncompetitive nature of inhibition are characteristics that conform to those expected for P-site ligands. Inhibition by either 2′-d-3′-A4P (IC95 = 10.5 nM) or 2′,5′-dd-3′-A4P (IC95 = 7.4 nM) was not because of the formation of inorganic polyphosphates, whether by enzymatic or nonenzymatic means. Significant hydrolysis of the 3′-tetraphosphates did not occur, notwithstanding the crude nature of some of the adenylyl cyclase preparations with which they were tested. This stability was consistent with that previously reported for the homologous 3′-triphosphates (6) and implies a lack of significant 3′-nucleotidase activity in these preparations. Moreover, the possibly resulting inorganic polyphosphates, i.e. PP, P2, P3, and P4, are not potent inhibitors (6). PP, P2, and P3 were three orders of magnitude less potent (IC95 = 55 μM) than were the title compounds, and inhibition was competitive with respect to substrate (6). PP, which also had no effect (IC95 = 1.4 mM) and inhibition was competitive with respect to substrate (6).

These observations argue convincingly that it is 2′-d-3′-A4P and 2′,5′-dd-3′-A4P that cause inhibition in the present study.

The striking inhibitory potency of 2′-d-3′-A4P and 2′,5′-dd-3′-A4P is due both to the specificity of interaction of the adenine moiety, for which there is nearly an absolute requirement, and to the binding energy contributed by the addition of phosphates.

| Time | 5′-ATP | 2′-d-3′-A4P | 2′,5′-dd-3′-A4P |
|------|--------|-------------|----------------|
| 5    | 98.4   | 95.6        | 100.1          |
| 15   | 94.5   | 91.1        | 99.2           |
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Leaving occurs and would prevent a subsequent conformational shift to a form capable of interacting again with substrate metal-5′-ATP.

Inhibition by adenosine 3′-polyphosphates is a characteristic conserved in all mammalian adenylyl cyclases sequenced to date. Although examples from bacteria and possibly sperm indicate that catalysis can occur without susceptibility to P-site ligands (2, 31), it is nonetheless a characteristic that might well have been lost by natural selection did it not serve an important function. It provides an exquisite means for inhibition of this crucial signal transduction pathway. Both 2′-d-3′-AMP and 3′-AMP occur naturally, their levels may be chronically regulated (39), and 3′-polyphorylation of nucleoside 3′-monophosphates is known to occur in mammalian systems (40). There are, however, very few reports related to nucleoside-3′-polyphosphates in animal cells. If nucleoside 3′-tri- or 3′-tetraphosphates were to exist naturally in animal cells and to exert a physiological effect in the regulation of adenylyl cyclases, they might be expected to exist in minute quantities, in a range consistent with their effects on adenylyl cyclases. For this reason alone, their presence may not have been detected. By comparison, adenosine 5′-tetraphosphate occurs naturally (e.g. Refs. 35 and 36), and there is considerable literature on diadenosine tetraphosphates (A(5′)p(4′)A; e.g. Ref. 37). However, the former has not been tested as a P-site ligand, and the latter is not an inhibitor (4) although a number of 3′ → 5′-dinucleotides do inhibit the enzyme (cf. Table II of Ref. 4). That adenosine 3′-polyphosphates can inhibit adenylyl cyclase in intact cells was confirmed by Hempel et al. (38), who observed complete obliteration of serotonin-induced elevations in cAMP and the accompanying hyperpolarization response in lobster stomatogastric ganglion cells. Because of the nature of microinjection experiments, though, it was not possible to determine precisely the actual intracellular concentration of 2′,5′-dd-3′-ATP that was effective.

Given that few if any adenine nucleotides interact solely with one protein, it is also likely that, as part of their overall action in cells, adenosine 3′-polyphosphates will affect proteins other than adenylyl cyclases. Neither 2′,5′-dd-3′-ATP nor 2′,5′-dd-3′-A P affected activities of the three other adenine nucleotide binding enzymes we tested. This does not preclude effects on other enzymes. DNA polymerase is an interesting example and comparisons of it and adenylyl cyclase may suggest structural motifs required for regulation by this class of nucleotide. Prokaryotic DNA polymerase is well known to bind 2′-deoxyribose 3′-triphosphates, and even 3′-tetraphosphates, via the triphosphate domain (41). The affinity for nucleotide (2′-d-3′-ATP: \( K_D \approx 38–68 \mu M \) (41)), though, is almost 3 orders of magnitude less than that exhibited by adenylyl cyclase, implying at once that the 3′-nucleotide binding domains of these two enzyme families exhibit similarities and important differences. That adenylyl cyclases share structural as well as catalytic characteristics with “palm” domains of the polymerase I family of prokaryotic DNA polymerases is also evident from comparisons of three-dimensional topologies of both proteins, as recently pointed out by Artyumiuk et al. (42), though qualified by Bryant et al. (43). These observations would suggest that other enzymes sharing this topology and/or yielding nucleoside

**Fig. 6.** Lack of effect of 2′,5′-dd-3′-ATP or 2′,5′-dd-3′-A P on cAMP-dependent protein kinase. Activities were determined as pmol of 2′,5′-dd-3′-ATP or 2′,5′-dd-3′-A P transferred from \( [\gamma ^{32}P]ATP \) to histone per min. Additions of 3′-nucleotides and cAMP (1 \( \mu M \)) were as indicated.

2 Although we reported kinetic evidence consistent with this mechanism, a characteristic uncompetitive inhibition for inhibition in the presence of GSs (12) also supported by studies of Dessauer and Gilman (32) with recombinant soluble enzyme, we observed no effect of inorganic pyrophosphate on the affinity of the enzyme for the P-site ligand 2′-d-3′-AMP (12).

3 These solved crystal structures also do not show the two metal-binding sites known to participate in catalysis with native enzyme (12, 15).

4 The nomenclature for adenosine 3′,5′-bis-polyphosphates implies 5′-substitution to the left of the base and 3′-substitution to the right of the base, e.g. pppp5′/A3′/ppp, pppApp, papApp, or alternatively p Ap or p dAp. By this norm, 3′ → 5′-linked dinucleotide polyphosphates are correctly denoted as Apn–A or dApn–dA. Unfortunately, this latter abbreviation (e.g. AppApp or ApA) is used inappropriately also for 5′ → 5′-linked didadenosine polyphosphates by many authors and can lead to considerable confusion, causing some data base searching protocols to cite incorrect references.
monophosphate and inorganic pyrophosphate as products would be potential targets for additional interactions with nucleoside 3’-polyphosphates.

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