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One-Flask Synthesis of Dinucleoside Tetra- and Pentaphosphates

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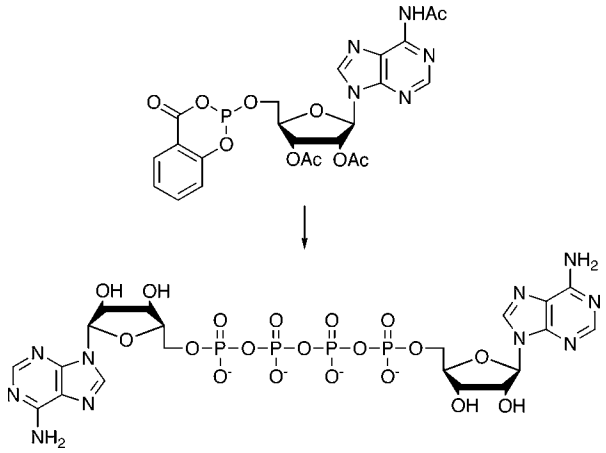
ABSTRACT



We report a one-flask route for the synthesis of dinucleoside tetra- and pentaphosphates, in isolated yields of 50–85%. This route relies on a mixture of P(III) and P(V) chemistries, using phosphitylation of a protected nucleoside with 2-chloro-4*H*-l,3,2-benzo-dioxaphosphorin-4-one (salicylchlorophosphite), followed by sequential reaction with inorganic pyrophosphate and a nucleoside 5′ mono- or diphosphate.

Dinucleoside polyphosphates (5′-5‴-Np$_n$N, $n = 2-7$) have been proposed as signaling and regulatory molecules for many different biological functions in most forms of life.[1] Although the most abundant and best characterized of these specialized RNA molecules are Ap$_3$A, Ap$_4$A, and Ap$_5$A, examples with other nucleosides are known but are typically found at lower concentrations. Gp$_3$G and Gp$_4$G are exceptional in occurring at high concentrations in the dehydrated embryonic cysts of brine shrimp.[2] The main source of most cytoplasmic Ap$_4$N is the "back-reaction" of NTPs with various adenylated intermediates, such as aminoacyl-adenylate, catalyzed by aminoacyl−tRNA synthetase,[3] and luciferin, catalyzed by firefly luciferase.[4] The intracellular levels of Np$_n$N are controlled by a variety of hydrolyzing enzymes, including Ap$_4$A hydrolase (a MuT motif protein) and Ap$_3$A hydrolase (a product of the FHIT tumor suppressor gene).[5] Potent extracellular activities for Ap$_4$A and Ap$_5$A are well-known,[6] and many of their receptors have been established.[7] Two examples with important therapeutic potential are inhibition of platelet aggregation[8] and regulation of vasoactivity.[9] A high-yield synthesis for Np$_n$N and their analogues would facilitate studies of their possible medical applications.

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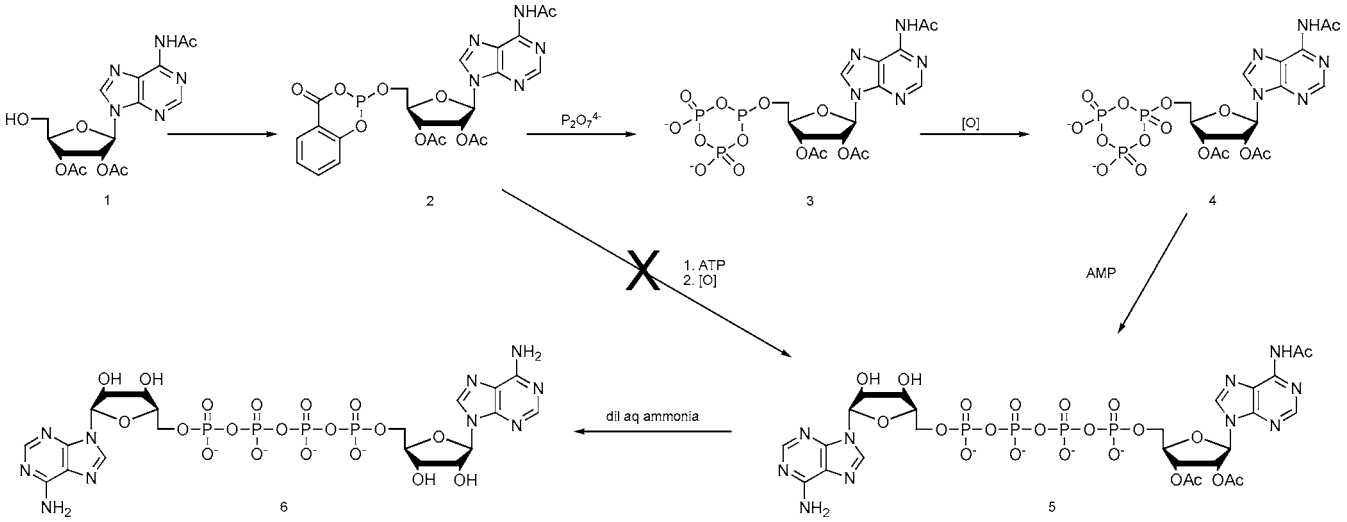
Scheme 1. Synthesis of Ap_4A



Enzymatic approaches[10] are limited by scale and to naturally occurring nucleosides. The most widely used chemical approach for the synthesis of Np_4N has been the reaction of a nucleoside triphosphate with a nucleotide activated as the morpholidate, diphenylphosphorochloridate, or imidazolate,[11] but the yields have been modest. Blackburn pioneered the synthesis of Np_4N analogues, for the most part by more specialized routes and also in modest yields.[12]

Orgel reported many years ago that treatment of adenosine 5′-tetraphosphate with a carbodiimide formed a cyclic trimetaphosphate intermediate that could hydrolyze back to the starting material or eliminate inorganic trimetaphosphate to give adenosine monophosphate.[13] Nucleoside triphosphates have also been cyclized to the trimetaphosphate using carbodiimides,[14] and recently, a series of nucleoside-dye oligophosphates were prepared using intermediates made in this way.[15]

We have developed a new, one-flask route, shown in Scheme 1, for the preparation of Ap_4A that makes the trimetaphosphate in a more efficient synthesis. Our route begins with the Eckstein procedure for the preparation of triphosphates by phosphitylation of triacetyl adenosine (**1**) with 2-chloro-4*H*-l,3,2-benzo-dioxaphosphorin-4-one (salicylchlorophosphite) followed by reaction with inorganic pyrophosphate to give the cyclic derivative (**3**). For triphosphate synthesis, **3** is oxidized to **4** with concomitant hydrolysis of **4**,[16] and modified di-[17] and triphosphates[18] have also been made using this approach. We first tried reaction of **2** with adenosine 5′-triphosphate (ATP), followed by oxidation, but this route gave complex mixtures in which only traces of **5** could be detected.

We found instead that careful oxidation of **3** to **4**, under conditions that do not bring about hydrolysis, followed by

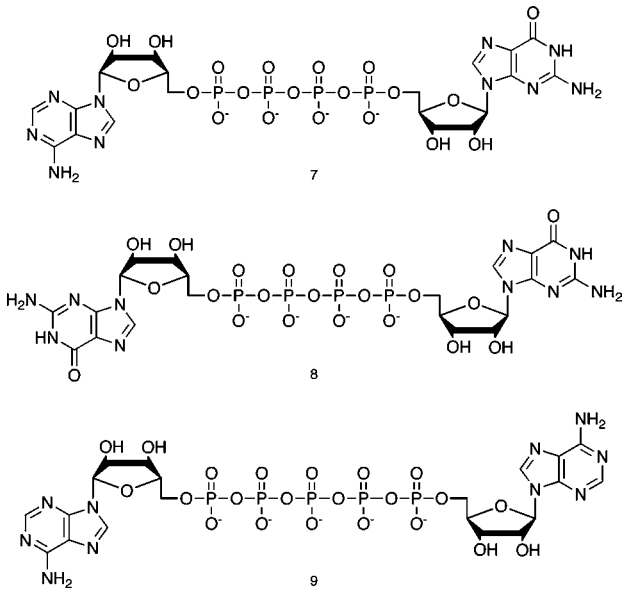


Figure 1. Ap_4G (**7**), Gp_4G (**8**), and Ap_5A (**9**), prepared by the route shown in Scheme 1.

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reaction of **4** with AMP in dry DMF, catalyzed by $ZnCl_2$, gives clean conversion to the partially protected tetraphosphate **5**. $MgCl_2$ was less effective than $ZnCl_2$ but better than no catalyst. After mild ammonia treatment to remove the acetyl groups and ion exchange to remove the Zn^{2+} while it is solubilized as an ammonium complex, the final tetraphosphate **6** was isolated in yields of 85%.[19] This yield compares very well to previously reported tetraphosphate syntheses, with which we were seldom able to obtain yields as high as 25%. Ap_4G[20] (**7**) and Gp_4G[21] (**8**) are prepared in a similar manner, and the Ap_5A[22] (**9**) is prepared by addition of ADP to **4** (Figure 1). NMR characterization for Ap_4A agrees with previously published data.[23] In the complex second-order ^{31}P NMR spectra for all four compounds, resonances for the end phosphates are well separated from those of the middle phosphates. In the case of the pentaphosphate Ap_5A, the second and third phosphates are not resolved, and the envelope appears as a broad singlet. This result is consistent with ^{31}P NMR data for $Na_7P_5O_{16}$ in a study of a series of polyphosphates, in which the difference in chemical shifts for the second and third phosphates was less than their coupling constant.[24]

The approach described here can also be used to prepare a variety of modified dinucleoside polyphosphates, and such work is underway in our laboratory.

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Supporting Information Available: Spectra (UV, MS, ^1H NMR, ^{13}C NMR, and ^{31}P NMR). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Preparation of Ap_4A. To a solution of 2′,3′-*O*-6-*N*-triacetyladenosine (0.13 g, 0.33 mmol) in 2 mL of anhydrous *N*,*N*-dimethylformamide (DMF) was added 2-chloro-4*H*-l,3,2-benzo-dioxaphosphorin-4-one (0.13 g, 0.64 mmol, 1.9 equiv). The solution was stirred for 15 min at room temperature under N_2. A 0.5 M solution of bis(tri-*n*-butylammonium) pyrophosphate in anhydrous DMF (1.3 mL, 0.65 mmol, 2.0 equiv) was vortexed with tri-*n*-butylamine (0.60 mL, 2.5 mmol, 7.6 equiv) and immediately added to the reaction mixture. After 20 min, a solution of iodine (0.12 g, 0.47 mmol, 1.4 equiv) in 1.5 mL of pyridine and 0.01 mL of water was added. After 15 min, a mixture of adenosine monophosphate monohydrate, in proton form (0.45 g, 1.23 mmol, 3.7 equiv), and zinc chloride (0.42 g, 3.1 mmol, 9.4 equiv) that had been dried together by evaporation of pyridine and DMF was added with stirring. After 16 h, 10% aqueous ammonia (20 mL, 118 mmol, 358 equiv) was added, and the deprotection was complete after 1 h. The dilute basic solution was applied to a sodium cation-exchange resin (50WX2, 10 mL, 18 equiv) to remove Zn^{2+}. The product was concentrated and purified by preparative reverse-phase HPLC using 0.1 M ammonium bicarbonate in acetonitrile to give 0.25 g of Ap_4A in the ammonium form (0.28 mmol, 85%): UV λ max = 260 nm; ^1H NMR δ (D_2O, 400 MHz) 8.40 (s, 2H), 8.15 (s, 2H), 6.01 (d, J = 5.73 Hz, 2H), 4.69 (t, J = 5.40 Hz, 2H), 4.54 (t, J = 4.35 Hz, 2H), 4.39−4.34 (m, 2H), 4.33−4.21 (m, 4H); ^{31}P NMR (D_2O, 400 MHz) δ −10.16, −21.90. The mass was confirmed by ESI-MS in negative mode as *m*/*z* (M − 1) 835.33 amu (calcd for $C_{20}H_{27}N_{10}O_{19}P_4^-$, 835.04).

(20) Preparation of Ap_4G. Starting with 2′,3′-*O*-2-*N*-triacetylguanosine (0.14 g, 0.34 mmol) to make the trimetaphosphate intermediate, Ap_4G was prepared by the same procedure as that described above. Following HPLC purification, 0.20 g of Ap_4G in the ammonium form was obtained (0.22 mmol, 65%): UV λ max = 256 nm; ^1H NMR δ (D_2O, 400 MHz) 8.40 (s, 1H), 8.12 (s, 1H), 8.00 (s, 1H), 6.04 (d, J = 5.51 Hz, 1H), 5.80 (d, J = 5.94 Hz, 1H), 4.71 (t, J = 5.45 Hz, 2H), 4.58−4.50 (m, 2H), 4.39−4.18 (m, 6H); ^{31}P NMR δ (D_2O, 400 MHz) d −10.17, −21.82. The mass was confirmed by ESI-MS in negative mode as *m*/*z* (M − 1) 851.22 amu (calcd for $C_{20}H_{27}N_{10}O_{20}P_4^-$, 851.04).

(21) Preparation of Gp_4G. Starting with 2′,3′-*O*-2-*N*-triacetylguanosine (0.13 g, 0.32 mmol), Gp_4G was prepared by the same procedure as that described above, except that the cation-exchange resin was in the Li^+ form rather than in the Na^+ form to minimize aggregation of the product. Following HPLC purification, 0.14 g of Gp_4G in the ammonium form was obtained (0.15 mmol, 47%): UV λ max = 253 nm with the shoulder at 275 nm; ^1H NMR δ (D_2O, 400 MHz) 8.04 (s, 2H), 5.84 (d, J = 5.93 Hz, 2H), 4.74 (t, J = 5.80 Hz, 2H), 4.54 (t, J = 4.11 Hz, 2H), 4.35−4.30 (m, 2H), 4.30−4.20 (m, 4H); ^{31}P NMR δ (D_2O, 400 MHz) d −9.15, −20.84. The mass was confirmed by ESI-MS in negative mode as *m*/*z* (M − 1) 867.37 amu (calcd for $C_{20}H_{27}N_{10}O_{21}P_4^-$, 867.03).

(22) Preparation of Ap_5A. Starting with 2′,3′-*O*-6-*N*-triacetyladenosine (0.12 g, 0.31 mmol), the intermediate trimetaphosphate was prepared as described above. Adenosine diphosphate (0.29 g, 0.68 mmol, 2.2 equiv) was used in the coupling instead of AMP. Following HPLC purification, 0.15 g of Ap_5A in the ammonium form was obtained (0.15 mmol, 48%): UV λ = max 259 nm; ^1H NMR δ (D_2O, 400 MHz) 8.45 (s, 2H), 8.16 (s, 2H), 6.02 (d, J = 5.76 Hz, 2H), 4.70 (t, J = 5.42 Hz, 2H), 4.56 (t, J = 4.24 Hz, 2H), 4.43−4.35 (m, 2H), 4.34−4.20 (m, 4H); ^{31}P NMR δ (D_2O, 400 MHz) d −10.18, −21.61. The mass was confirmed by ESI-MS in negative mode as *m*/*z* (M − 1) 915.17 amu (calcd for $C_{20}H_{28}N_{10}O_{22}P_5^-$, 915.01).

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