A Novel Synthesis of Nucleoside 5′-Triphosphates

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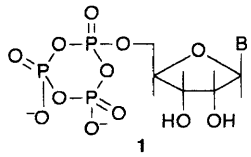
Facile syntheses of ribonucleoside 5′-triphosphates have been accomplished in good yield (>60%) in a one-pot reaction of unprotected nucleosides with phosphoryl chloride followed by treatment of the resulting phosphorodichloridate with tri-*n*-butylammonium phosphate in the presence of dimethylformamide.

The importance of ribo- and deoxy-ribonucleoside 5′-triphosphates in biological systems, and their involvement in a host of biochemical processes, have led to the development of a number of methods for their synthesis. Most of these involve the conversion of mononucleotides to reactive intermediates such as the morpholidate,[1] imidazolidate,[2] phosphoramidate,[3] or 8-quinolate[4] followed by displacement of the leaving group with pyrophosphate. Formation of phosphoric anhydrides using diphenyl phosphorochloridate[5] or di-*n*-butylphosphinothioyl bromide[6] followed by displacement with pyrophosphate represent methods which have found substantial use. The widely used Yoshikawa procedure,[7] which affords a nucleoside 5′-phosphorodichloridate upon treatment of an unprotected nucleoside with phosphoryl chloride in trimethyl phosphate in the presence of a trace of water, has been extended by reaction of the dichloridate with pyrophosphate to give nucleoside triphosphates directly.[8,9]

For a number of years, nucleoside diphosphates have been prepared in this laboratory[10] according to the procedure of Yoshikawa[7] to form the monophosphate followed by the diphenyl phosphorochloridate procedure of Michelson[5] to afford the pyrophosphate. However, when this sequence was applied to 1-amino-6-thioinosine[11] **2a** the reaction with diphenylphosphorochloridate under Michelson's anhydrous basic conditions[5] gave rise to phosphoramidate formation at the *N*-amino group in addition to pyrophosphate formation, leading to a complex mixture after displacement with inorganic phosphate. It was, therefore, decided that the use of the Yoshikawa intermediate as outlined by Ludwig,[8] but substituting tributylammonium phosphate for tributylammonium pyrophosphate, should give rise to the desired 5′-diphosphate in a simple one-pot reaction. Upon completion of this reaction, the reaction mixture was examined using a strong anion exchange (SAX) HPLC system. The primary product of the reaction was eluted from the column at a time inconsistent with a 5′-diphosphate but remarkably like that of a 5′-triphosphate. Formation of the 5′-triphosphate as the major product was confirmed using ^{31}P NMR spectroscopy. The spectrum revealed a pair of doublets with an upfield triplet very similar to those reported for ATP.[12,13] The reaction was repeated using adenosine **2b**. HPLC and ^{31}P NMR spectroscopy revealed the major product to be ATP.



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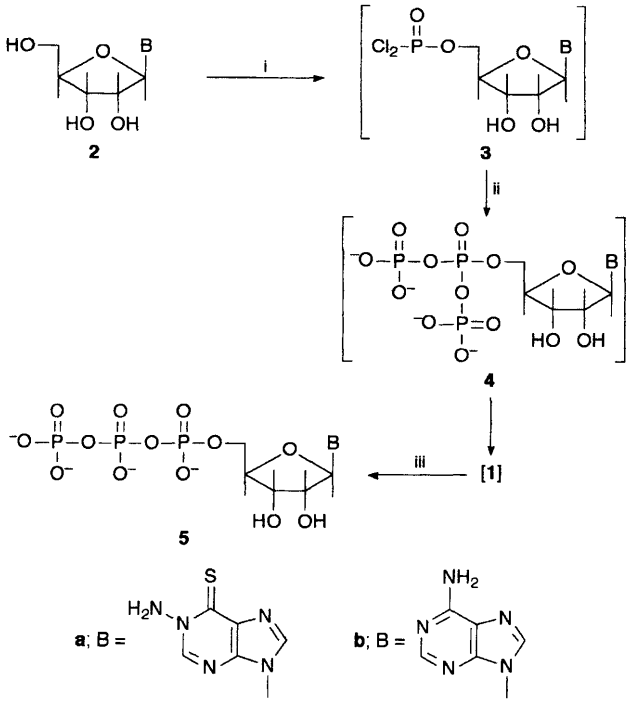
The procedure involved dissolution of the nucleoside (0.2 mmol) in trimethyl phosphate (1 ml) followed by treatment with phosphoryl chloride (0.6 mmol) for three hours. The reaction mixture was then treated directly with a solution of tri-*n*-butylammonium phosphate (2 mmol) in anhydrous dimethylformamide (DMF; 3 ml) with the further addition of 0.6 ml of tri-*n*-butylamine. After one minute, the reaction was terminated with the addition of aqueous 1 mol l^{-1} triethylammonium hydrogen carbonate buffer (pH 7.5; 20 ml). The crude product was purified on a DEAE cellulose (HCO_3^-) column with a linear gradient from 0 to 1 mol dm^{-3} of



Scheme 1 *Reagents and conditions*: i, $POCl_3$ (3 equiv), trimethyl phosphate, 0 °C, 3 h; ii, $Bu^n_3N^+H$–OPO_3H_2 (10 equiv), Bu^n_3N, DMF, 0 °C, 1 min; iii, aq. Et_3N^+H HCO_3^-, pH 7.5

$Et_3NH^+HCO_3^-$ (pH 7.5). The 5′-triphosphate was converted to the tetrasodium salt using the procedure of Hoard and Ott[2] to give an isolated yield of 60–65%. The purity of the 5′-triphosphates was ascertained using ^1H and ^{31}P NMR spectroscopy, and by chromatography on an SAX HPLC column (Whatman Partisil 10, 25 cm × 4.6 mm) using a multistep gradient: 10 mmol dm^{-3} acetic acid + 6 mmol dm^{-3} KH_2PO_4 buffer (pH 4.0) to 0.6 mol dm^{-3} KH_2PO_4 buffer (pH 5.0) over a period of 60 min at a flow rate of 1 ml min^{-1} with UV monitoring at 260 and 318 nm.

The HPLC analysis revelaed that the reaction mixture contained at least 85% of the triphosphate along with 11% of the diphosphate. No other component exceeded 2% of the total products.

These observations constitute a novel, convenient and quick route to nucleoside 5′-triphosphates in which even groups sensitive to phosphorylation (N–NH_2, secondary OH) need not be protected. They further support a hypothesis, advanced over forty years ago by Michelson and Todd,[14] which invoked the intermediacy of a cyclic metatriphosphate **1** in the synthesis of triphosphates from monophosphates. This hypothesis, also put foward some years later by Smith and Khorana,[15] was supported by an elegant study from the Hecht group[16] in which 'pseudo ATP' **4b** was isolated, albeit in low yield, and was shown to be converted in part to ATP with carbonyl diimidazole.

The results are consistent with the sequence shown in Scheme 1. The phosphorodichloridate intermediate **3** in the Yoshikawa phosphorylation[7] would be expected to form Hecht's 'pseudo NTP' with the large excess of inorganic phosphate present. The reaction mixture contains an excess of phosphoryl chloride, which would react rapidly with the added DMF to give the powerful Vilsmeier–Haack condensing agent[17] *in situ*. Very rapid intramolecular dehydration would form **1** which upon addition of water would rapidly hydrolyse to the more thermodynamically stable linear triphosphate **5**.

The method described herein should provide a simple and quick alternative to existing methods of nucleoside 5′-triphosphate synthesis.

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