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A review on the chemical synthesis of pyrophosphate bonds in bioactive nucleoside diphosphate analogs

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ABSTRACT

Currently, there is an ongoing interest in the synthesis of nucleoside diphosphate analogs as important regulators in catabolism/anabolism, and their potential applications as mechanistic probes and chemical tools for bioassays. However, the pyrophosphate bond formation step remains as the bottleneck. In this Digest, the chemical synthesis of the pyrophosphate bonds of representative bioactive nucleoside diphosphate analogs, i.e. phosphorus-modified analogs, nucleoside cyclic diphosphates, and nucleoside diphosphate conjugates, will be described.

Nucleoside diphosphate (NDP) analogs are important intermediates in the catabolism and anabolism processes in biological systems. For example, the release of ADP and UDP is often involved in the initial step to trigger the purinergic signaling during injury, stress, apoptosis, or chemical stimuli, and understanding the regulatory mechanisms of this signaling cascade has been an important medical area.[1] ADP is a potent agonist for the human $P2Y_1$ receptor, and UDP is a potent agonist for the $P2Y_6$ receptor (P2Y receptors: G protein-coupled receptors).[2] Stimulations by ADP can lead to platelet shape change/aggregation and thromboxane A2 generation.[3] UDP was reported to induce the expression of chemokines (critical in inflammation) in microglia and astrocytes.[4] Recently, GDP inhibition of proton leak was reported.[5] One mM GDP stimulation of respiration and decrease of the membrane potential in isolated mitochondria were observed.[6] Cyclic nucleoside diphosphate ribose, such as cyclic adenosine 5′-diphosphate ribose (cADPR), a potent intracellular-Ca^{2+}-mobilizing second messenger, is produced enzymatically from nicotinamide adenine dinucleotide (NAD^+) by ADP-ribosyl cyclases. This cADPR/Ca^{2+} signaling system exists in diverse mammalian, protozoa and plant cells.[7] Sugar nucleoside diphosphates or glycosyl esters of nucleoside diphosphates (NDP–sugars) are precursors of a number of modified sugars, branched sugars, and glycoconjugates, glycosyl donors of glycosyltransferases in oligo- and polysaccharide biosynthesis. Many NDP–sugars have been isolated from natural resources.[8] Uridine 5′-pyrophosphate analogs are the most common natural sugar nucleotides involved in a number of biological processes. For example, the α-D-galactofuranosyl derivative is an intermediate in the biosynthesis of the galactocarolose produced by *Penicillium charlesii* G. Smith. Guanosine 5′-(α-D-mannopyranosyl)pyrophosphate, a precursor of the respective esters of various uronic acids and deoxy sugars, has been isolated from yeast, many microorganisms, higher plants and animals. A number of cytidine 5′-glycosyl pyrophosphates were isolated from mutant Gram-negative bacterial cells which were defective in lipopolysaccharide synthesis. TDP–β-L-rhamnose, found in numerous Gram-negative bacterial cell surfaces, is important for the biosynthesis of lipopolysaccharides and the cell walls of microorganisms,[9] and it is considered as a target for antibiotic development. Dinucleoside 5,5′-polyphosphates (also called dinucleotides, with 2–8 phosphate groups) are important extracellular signaling compounds; they are substrates for the P2 nucleotide receptor family, and several DNA polymerases from viruses, bacteria and humans. In some cases, the phosphate chain length of the dinucleotides may determine the specificity for the receptor subtypes, and even agonist/antagonist activities at the same receptor.[2] Dinucleoside diphosphates Ap_2A, Ap_2G and Gp_2G, isolated from human tissues, exhibited a P2Y-mediated promoting effect on the vascular smooth muscle cell proliferation.[10]

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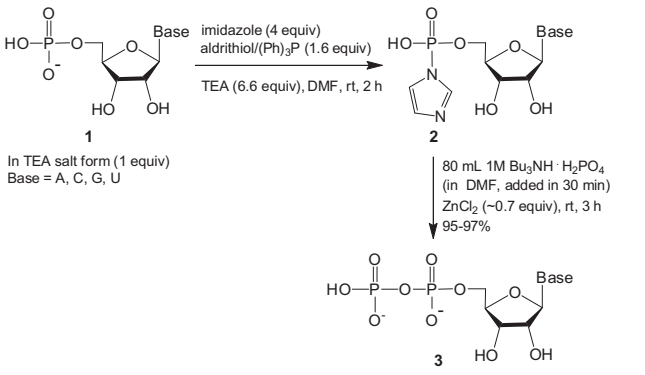
Based on the biological importance of natural nucleoside diphosphate analogs, various non-natural analogs have been synthesized and studied as potential therapeutic candidates and/or inhibitors/promoters of therapeutically significant enzymes. They can also serve as chemical biology tools for assay developments[11] and probe enzyme and/or receptor functional mechanisms. Recently, Wagner and co-workers discovered a base-modified (5-formylthien-2-yl) UDP–galactose that decreases galactosyl transfer with a new inhibition mode (K_i values in the low to sub-micromolar range).[12] The Wagner group also reported a C-glycosidic analogue of UDP–galactose that presented anti-bacterial UDP–Gal 4′-epimerase activity at micromolar concentrations.[13] Hostetler et al. found that phospholipid prodrugs such as 3′-deoxythymidine (3dT) diphosphate dimyristoylglycerol was 18- to 50-fold more active than 3dT in antiretroviral activity in LAV-infected cells,[14a] and acyclovir diphosphate dimyristoylglycerol was quite potent against acyclovir-resistant herpes simplex virus.[14b] Phosphorus modification of NDP analogs has been an active area, and nucleoside phosphorothioate ((d)NDPαS or (d)NDPβS) and α-*P*-boranodiphosphate ((d)NDPαB) analogs, wherein one of the nonbridging oxygens in the phosphate is replaced by a sulfur or borane moiety, are typical examples studied in the Eckstein and Shaw labs.[15] Some unique properties imparted by the modified diphosphate bonds have been reported. For example, the phosphorothioate and the α-*P*-borano moieties lead to increased stability toward enzymes[15a,16] and improved phosphorylation by nucleoside diphosphate kinase.[17] The stable UDPβS, a terminal phosphorothioate of uridine, was found more potent than the parent UDP in the P2Y receptor-mediated vasoconstrictions.[18] The presence of an α-*P*-borano group resulted in a 10-fold enhancement of the catalytic efficiency of the phosphorylation for the active *R*p isomer of dideoxynucleoside α-*P*-boranodiphosphates (ddNDPαBs) relative to unsubstituted AZTDP or d4TDP, compared to a 2.5-fold enhancement for the active *S*p isomer of α-*P*-thio ddNDP.[17] Boron containing nucleoside analogs have potential therapeutic and diagnostic applications in boron neutron-capture therapy, as antiviral/anticancer drugs and other clinically useful agents. Ginsburg-Shmuel et al. revealed that the *R*p isomer of α-*P*-borano-5-OMe-UDP was a potent (19-fold greater than UDP) and selective $P2Y_6$ nucleotide receptor agonist.[19] The Fischer lab further demonstrated that nucleoside borano or thiophosphate derivatives could be useful in the treatment of a number of diseases such as type 2 diabetes, pain, ocular hypertension and/or glaucoma, neurodegenerative disorders, and osteoarthritis/calcium pyrophosphate dehydrate deposition disease.[20] Recently, an α-*P*-borano-2-Cl-ADP isomer, a promising neuroprotectant, was found to be a potent $P2Y_1$-receptor agonist (EC_{50} 7 nM),[21] while ADPβS and GDPβS were reported to be much better neuroprotectants than the corresponding parent nucleoside phosphorothioates against oxidative stress.[22]

Diphosphate bond chemical synthesis can be accessed via the condensation of two pentavalent monophosphates {P(V)–P(V)}, a pentavalent monophosphate with a trivalent phosphite {P(V)–P(III)}, or two phosphites {P(III)–P(III)}. In early years, nucleoside pyrophosphate bond formation was mainly through P(V)–P(V) approaches, including a protected nucleoside benzyl phosphorochloridate condensation with a second monophosphate ester; direct condensation of two unprotected phosphomonoesters in the presence of trifluoroacetic anhydride or coupling reagents like dicyclohexylcarbodiimide (DCC) and PPh_3/$(PyS)_2$/*N*-methylimidazole; and the attack of a monophosphate analog on an activated nucleoside monophosphate (activated NMP). Phosphoramidates such as phosphoromorpholidates and phosphoroimidazolidates are good examples of the activated NMPs often used, and the two starting phosphate partners in the coupling reactions can be used either with or without protection. Recently, Kore et al. reported[23] that nucleoside diphosphates could be effectively synthesized via the coupling of the corresponding ribonucleoside-5′-phosphoroimidazolide with tributylammonium orthophosphate in the presence of a catalytic amount of zinc chloride ($ZnCl_2$). As outlined in Scheme 1, starting from the unprotected ribonucleoside monophosphate **1**, the activated NMP **2** (phosphoroimidazolide) was prepared first and, after the addition of the orthophosphate and $ZnCl_2$, the condensation to generate nucleoside diphosphate **3** was completed in 3 h at room temperature (rt) with high reaction yields (95-97%). The application of a number of nucleoside phosphoramidates as activated NMPs will be further discussed in the section of *NDP-conjugates* of this Digest. Recent work on the synthesis of nucleoside pyrophosphate analogs has been more focused on the improvement of the effectiveness (reaction time reduction and yield increase) by applying new activated NMPs and condensing reagents, and the development of new approaches in addition to the P(V)-P(V) condensations. However, the availability of nucleoside diphosphate analogs remains challenging, and the pyrophosphate bond synthesis is the bottleneck step. This Digest will highlight major developments of recent reports on the chemical synthesis of diphosphate bonds in representative bioactive nucleoside pyrophosphate analogs including phosphorus-modified nucleotides, cyclopyrophosphate derivatives, and various nucleoside diphosphate conjugates (NDP-conjugates).

Phosphorus-modified nucleoside diphosphates: Michelson's anion exchange procedure[24] has been widely used for pyrophosphate bond synthesis of a number of nucleotide analogs. Nucleoside 5'-*O*-(2-thiodiphosphates) or terminal phosphorothioates may be obtained through S-protected monophosphorothioate and P^1-diphenyl P^2-nucleoside 5′-pyrophosphate following the Michelson procedure (isolated yields up to 35%),[16c] or via the coupling of a diphenyl phosphorochloridate activated NMPS with a protected NMP in pyridine, followed by the cleavage of unprotected ribose ring with periodate-base treatment (yield 78%).[25a] These compounds may also be made from an activated nucleoside phosphoroimidazolidate coupling with an excess thiophosphate triethylammonium salt in DMF in the presence of $ZnCl_2$ (isolated yields ~70%); in the absence of $ZnCl_2$, the desired product was not obtained.[25b] Nucleoside 5′-*O*-(l-thiodiphosphates) or α-*P*-thiodiphosphates are generally prepared by activation of nucleoside 5′-phosphorothioates with diphenylphosphorochloridate before the coupling with a monophosphate.[15a] Recently, Strenkowska et al. reported a one-pot synthesis of modified nucleotides using cyanoethyl *P*-imidazolides **4** (phosphorylating reagents) and microwave oven (dynamic power, max. 10 W, 45 ± 1 °C and 2450 MHz),[26] where nucleoside thiodiphosphates or borano analogs could be synthesized from the corresponding monophosphate analogs in a short



Scheme 1. Synthesis of ribonucleoside-5′-diphosphates via the corresponding phosphoroimidazolides and $ZnCl_2$ catalysis.[23]