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Synthesis of α,δ-Disubstituted Tetraphosphates and Terminally-Functionalized Nucleoside Pentaphosphates

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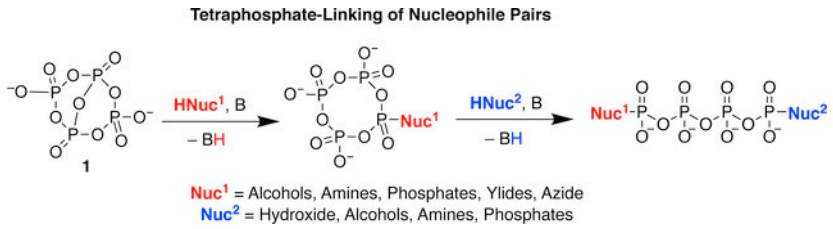
Abstract

The anion $[P_4O_{11}]^{2-}$, employed as its bis(triphenylphosphine)iminium (PPN) salt, is shown herein to be a versatile reagent for nucleophile tetraphosphorylation. Treatment under anhydrous conditions with an alkylamine base and a nucleophile ($HNuc^1$), such as an alcohol (neopentanol, cyclohexanol, 4-methylumbelliferone, and Boc-Tyr-OMe), an amine (propargylamine, diethylamine, morpholine, 3,5-dimethylaniline, and isopropylamine), dihydrogen phosphate, phenylphosphonate, azide ion, or methylidene triphenylphosphorane, results in nucleophile substituted tetrametaphosphates ($[P_4O_{11}Nuc^1]^{3-}$) as mixed PPN and alkylammonium salts in 59% to 99% yield. Treatment of the resulting functionalized tetrametaphosphates with a second nucleophile ($HNuc^2$), such as hydroxide, a phenol (4-methylumbelliferone), an amine (propargylamine and ethanolamine), fluoride, or a nucleoside monophosphate (uridine monophosphate, deoxyadenosine monophosphate, and adenosine monophosphate), results in ring opening to linear tetraphosphates bearing one nucleophile on each end ($[Nuc^1(PO_3)_3PO_2Nuc^2]^{4-}$). When necessary, these linear tetraphosphates are purified by reverse phase or anion exchange HPLC, yielding triethylammonium or ammonium salts in 32% to 92% yield from $[PPN]_2[P_4O_{11}]$. Phosphorylation of methylidene triphenylphosphorane as Nuc^1 yields a new tetrametaphosphate-based ylide ($[Ph_3PCHP_4O_{11}]^{3-}$, 94% yield). Wittig olefination of 2',3'-*O*-isopropylidene-5'-deoxy-5'-uridylaldehyde using this ylide results in a 3'-deoxy-3',4'-didehydronucleotide derivative, isolated as the triethylammonium salt in 54% yield.

Graphical Abstract



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Synthetic details, spectra, and crystallographic details Structure of **9**: CCDC 1991326

Introduction

Oligophosphates permeate biology, with roles in energy transfer,[1] signaling,[2] phosphate storage,[3] and enzymatic regulation.[4,5] However, the syntheses of such molecules are complex, historically requiring iterative phosphorylation procedures[6] or tedious separation of complex mixtures.[7] While enzymatic methods are utilized for the preparation of canonical compounds such as 5'-adenosine triphosphate (ATP), these procedures typically cannot be applied to non-natural nucleotides, which are essential to medical and biochemical research.[8] In recent years, significant progress has been made to efficiently introduce oligophosphate groups in a single operation.[9–14] With the greater availability of these compounds, increased research has been conducted on the roles of oligophosphates in biology, such as elucidating the human PolyP-ome.[15,16] A dominant motif in these new oligophosphorylation procedures has been the introduction of a triphosphate chain from readily available trimetaphosphate.[10–13,17]

Activated forms of trimetaphosphate have been used in conjunction with monophosphate substrates to synthesize various tetraphosphates, such as Taylor's work on the synthesis of nucleoside 5'-tetraphosphates (Figure 1).[11,12] More recently, Jessen reported the synthesis of bifunctional tetraphosphates from a P(III) phosphoramidite reagent and various phosphate nucleophiles.[9] Jessen's methodology utilizes an oxidation step to form a cyclic trimetaphosphate that is subsequently ring opened with another nucleophile (Figure 1). The redox-neutral synthesis of tetraphosphate derivatives from an activated tetrametaphosphate reagent would be highly efficient, resulting in a simplified procedure and allowing phosphorylation of oxidizable substrates. For example, utilizing a tetrametaphosphate based reagent allows synthesis of nucleoside tetraphosphate derivatives from nucleosides rather than first requiring synthesis of the corresponding nucleoside monophosphate and subsequent treatment with a trimetaphosphate based reagent. We recently demonstrated this chemistry by introducing an activated tetrametaphosphate reagent ($[PPN]_2[P_4O_{11}]$, $[PPN]_2[\mathbf{1}]$)[18] and reporting the synthesis of nucleoside tetra- and pentaphosphates.[19] Herein, we expand the reaction scope of this reagent to a range of C, N, and O nucleophiles (Figure 1). Furthermore, we adapt and expand the existing ring opening chemistry of substituted trimetaphosphate derivatives[9–14] to the synthesized tetrametaphosphate derivatives. Mixing and matching the two nucleophiles in these reactions leads to a variety of terminally disubstituted tetra- and pentaphosphates.

Results

Synthesis of Substituted Metaphosphates ($[P_4O_{11}Nuc^1]^{3-}$)

Under anhydrous conditions, **1** reacts rapidly and cleanly with many nucleophiles. Salts of the products, substituted metaphosphates, are isolated easily and in high yield by precipitation from the reaction mixture with diethyl ether. We have previously reported the treatment of anion **1** with methanol[18] and nucleosides.[19] We therefore treated anion **1** with various alcohols in the presence of triethylamine, to prevent side reactions by scavenging the acidic proton that is produced. In this manner, mixed PPN and triethylammonium salts of anions **2** and **3** were obtained by treating anion **1** with neopentanol, a hindered primary alcohol, and cyclohexanol (Figure 2A). Likewise, anion **1** reacts rapidly with

phenol derivatives such as 4-methylumbelliferone, a common fluorophore, in the presence of triethylamine. The resulting mixed PPN and triethylammonium salt of anion **4** was isolated in 91% yield by precipitation from acetonitrile with diethyl ether. The isolated alcohol derivatives of tetrametaphosphate formed oils upon precipitation rather than crystalline material, and removing solvent under vacuum yielded amorphous solids.

Anion **1** also reacts readily with primary and secondary amines resulting in tetrametaphosphate phosphoramidate derivatives containing a P–N bond. Near quantitative reactivity was observed between anion **1** and propargylamine, diethylamine, morpholine, 3,5-dimethylaniline, and isopropylamine resulting in crystalline compounds **5**-**9** (Figure 2). In the case of alkyl amine substrates, a second equivalent of amine is consumed by the acidic proton that is generated to yield a mixed PPN and alkylammonium salt.

Compounds **5**-**9** were obtained as crystalline salts, such as $[PPN]_2[H_3N^iPr][\mathbf{9}]$, which was crystallographically characterized. This structure is similar to that of amine functionalized trimetaphosphates we have previously reported,[10] with planar phosphoramidate nitrogens (Figure 2B). Furthermore, these phosphoramidate N-H groups are found to engage in intramolecular hydrogen bonds that likely help to lock in the observed conformation. The acidic isopropylammonium N-H groups are hydrogen bonded to basic phosphate oxygens on two molecules of **9** resulting in a dimeric structure in the solid state. The resulting dimeric tetraanion is such that it presents four lipophilic isopropyl groups on its surface in a manner that may aid solubility and ability to crystallize.

Anion **1** is also a suitable reagent for tetraphosphorylation of inorganic nucleophiles. Treatment with tetrabutylammonium azide ($[TBA][N_3]$) cleanly forms compound **10** in 24 hours. Relatedly, phosphoazidate analogs of nucleoside 5’-triphosphates have been used as photolabile affinity labels, extruding N_2 under irradiation and covalently attaching to a protein binding site.[20] Interestingly, anion **10** is an isomer of tetrametaphosphate consisting of an azidophosphate substituted trimetaphosphate. The isomer observed in **10** ostensibly results from nucleophile attack at a Q^2 phosphorus rather than attack at a Q^3 phosphorus (Figure 2A).[21] Interestingly, adding DMAP (4-dimethylaminopyridine) to **1** promotes formation of this isomer, allowing phosphorylation of diethylamine to give **11**.

Recalling our previous work on functionalized trimetaphosphates,[10] the parent monophosphate substituted tetrametaphosphate can be obtained by treatment of anion **1** with $[PPN][H_2PO_4]$, immediately leading to the PPN salt of anion **12a** (Figure 2A). Similarly, treatment of anion **1** with the TBA salt of monohydrogen phenylphosphonate[22] yields anion **12b**.

Treatment of **1** with benzylmercaptan and triethylamine yields anion **13** in manner similar to that of the alcohol substrates. Organothiophosphates have widespread applications and typically must be prepared from a P(V) species, as is the case here.[23] In a P(III) based synthesis, such as the oligophosphorylation methodology employed by Jessen,[9,14] the oxidation of P(III) to P(V) would also be expected to oxidize the sulfur atom, preventing the isolation of species with an oxidized phosphorus and reduced sulfur.

We also sought to achieve tetraphosphorylation of an amino acid side chain as oligophosphorylation of protein side chains has recently been discovered as a post-translational modification.[5,24] Tyrosine was chosen due to the UV absorbance of the aromatic ring and the good nucleophilicity of phenolate under basic conditions. Protection of the amine moiety was found to be necessary for an efficient reaction due to the improved solubility of protected amino acids and inhibition of side reactivity at the amine position. Accordingly, treatment of **1** with Boc-Tyr-OMe (N-(*tert*-butoxycarbonyl)-L-tyrosine methyl ester) in acetonitrile in the presence of triethylamine provides anion **14**.

Synthesis of Linear Tetraphosphate Derivatives ($[Nuc^1(PO_3)_3{-}PO_2Nuc^2]^{4-}$)

In one-pot reactions, we synthesized a variety of disubstituted tetraphosphates ($[Nuc^1(PO_3)_3PO_2Nuc^2]^{4-}$) by in situ generation of $[P_4O_{11}Nuc^1]^{3-}$ species followed by treatment with a second nucleophile. When necessary, the resulting compounds were purified by RP-HPLC with a triethylammonium (TEA) acetate buffer or AX-HPLC with ammonium bicarbonate buffer, giving TEA or ammonium salts after lyophilization. Ring opening of these metaphosphate derivatives selectively gives linear tetraphosphate derivatives as opposed to branched phosphates, a result that has been investigated computationally by Jessen for trimetaphosphate derivatives.[14] This result is logical as ring opening reactions that would give rise to a highly charged terminal $-[PO_3]^{2-}$ group should be disfavored compared to those that produce a terminal $-[PO_3R]^-$group. Ring opening reactions that have been demonstrated for trimetaphosphate derivatives[9,14] appear to be generally applicable to the tetrametaphosphate derivatives presented here, albeit often with increased reaction times, suggesting that larger metaphosphate rings are more difficult to ring open.

Ring opening of substituted metaphosphates with hydroxide or water gives monofunctionalized tetraphosphate products. In a prototypical example, treatment of the phenolic tetrametaphosphate intermediate **4** with aqueous tetrabutylammonium hydroxide ([TBA][OH]) yields a phenolic linear tetraphosphate (**16**) and replaces the PPN cations with water soluble TBA. HPLC purification, facilitated by the strong UV absorbance of this umbelliferone-derived substrate, provided the TEA salt in 49% yield from $[PPN]_2$[**1**] (Figure 3A). The ring opening reaction is not perfectly efficient, necessitating HPLC purification and resulting in a lower yield. Additionally, cation exchange from water insoluble PPN salts to soluble TBA or sodium salts for HPLC purification involves precipitation and filtration steps which may diminish yields. In contrast, ring opening of thiol derivative **13** with [TBA][OH] proceeds cleanly, and the TBA salt of **17** was isolated without HPLC purification. The moderate 71% yield of **17** is likely a result of the cation exchange from PPN to TBA. Tyrosine derivative **14** is also cleanly ring opened to **18** with saponification of the methyl ester upon introduction of [TBA][OH]. Protection of the carboxylate moiety as well as having a base stable amine protecting group such as Boc were necessary for this reaction to proceed. Treatment of **1** with either Boc-Tyr-OH or Fmoc-Tyr-OMe and subsequent treatment with [TBA][OH] yielded only tetrametaphosphate instead of the desired linear tetraphosphate derivative. Additionally, the P–N bond in **10** is remarkably water tolerant, and this compound can be converted accordingly to a linear phosphate as well. Cation exchange

and dissolution of **10** in neutral water results in formation of compound **19** in 24 hours with only minor hydrolytic cleavage of the azide group (Figure 3A).

Ring opening is also possible with primary amines, and in a one-pot reaction, a disubstituted tetraphosphate bearing a 4-methylumbelliferone-derived group at one end and a propargylamine-derived phosphoramidate group at the other was synthesized via intermediate **4** and purified by HPLC, providing the TEA salt of anion **20** in 62% yield from $[PPN]_2$[**1**] (Figure 3A). Similar alkyne substituted tetraphosphates have been functionalized further through cycloaddition "click" chemistry.[9] We found that amine substituted tetrametaphosphate species are also amenable to ring opening with a second nucleophile. In a one-pot reaction from $[PPN]_2$[**1**], treating intermediate **8** with ethanolamine rapidly and cleanly leads to a disubstituted tetraphosphate bearing two different phosphoramidate groups at the termini (**21**, Figure 3B). Other phosphoramidate P-N bond containing compounds such as anion **20** are stable in neutral water at room temperature for days. However, anion **21** was found to hydrolyze rapidly in room temperature neutral water, demonstrating the variable water stability of phosphoramidates.[25] However, anion **21** forms cleanly and was isolated as a mixed PPN and hydroxyethylammonium salt in 92% yield from $[PPN]_2$[**1**] without HPLC purification. Additionally, treatment of **1** with adenosine and subsequent ring opening with propargylamine results in a terminally amino functionalized adenosine tetraphosphate (**22**) after HPLC purification.

Previous syntheses of tetraphosphates have utilized the reaction of substituted trimetaphosphates with nucleoside monophosphates in the presence of $MgCl_2$ as a promoter.[9,11,12] In an analogous fashion, treatment of substituted tetrametaphosphate intermediate **4** with a nucleoside monophosphate and $MgCl_2$ yields ϵ-fluorophore labelled nucleoside pentaphosphates **23** and **24** in 5 hours. These compounds were isolated as their TEA salts in 54% and 60% yield respectively after HPLC purification in a one-pot reaction from **1** (Figure 3C). It has been shown that although γ-fluorophore labelled nucleoside triphosphates are poor substrates for DNA and RNA polymerases, ϵ-labelled nucleoside pentaphosphates exhibit significantly higher activity.[26,27] Therefore, related compounds have been used in high-throughput DNA sequencing, and single nucleotide polymorphism (SNP) assays.[26–30] The sole previous synthesis of ϵ-fluorophore labelled nucleoside pentaphosphates is low yielding and tedious, requiring expensive nucleoside triphosphates (NTP) and taking 7 days to complete.[26] This facile synthesis of such compounds may facilitate their use in such biochemical studies. $MgCl_2$ promoted ring opening with nucleoside monophosphates is also amenable to amine substituted tetrametaphosphates. Treatment of **5** with AMP and $MgCl_2$ gives the ammonium salt of pentaphosphate derivative **25** in 32% yield from **1** after AX-HPLC purification (Figure 3C).

$MgCl_2$ promoted ring opening is also applicable to nucleophiles other than phosphates. Treating intermediate **4** with an additional equivalent of 4-methylumbelliferone in the presence of triethyamine, yielded the symmetric tetraphosphate **26**, isolated as its TEA salt in 45% yield from $[PPN]_2$[**1**] after HPLC purification (Figure 3A).

Conditions for ring opening trimetaphosphate derivatives with cesium fluoride were adapted from Jessen[14] to achieve ring opening of amine derivative **5** to fluoro substituted anion **27**.

In contrast to similar trimetaphosphate derivatives which are ring opened in minutes under these conditions,[14] this reaction reached completion in approximately 24 hours.

Phosphorylation of Methylenetriphenylphosphorane and Wittig Olefination Chemistry

In a previous report, we detailed the triphosphorylation of the phosphorus ylide methylene triphenylphosphorane as a new route to oligophosphate analogues containing nonhydrolyzable P–C bonds.[10] Anion **1** similarly reacted with methylene triphenylphosphorane[31] (2 equiv) to give the mixed PPN and methyltriphenylphosphonium salt of tetrametaphosphate based ylide **15**. This new ylide effected Wittig olefination of paraformaldehyde, yielding vinyl tetrametaphosphate **28** (Figure 4). Hydrolysis of **15** led to methyl tetrametaphosphate derivative **29**. In order to synthesize a nucleotide analogue, **15** was treated with 2',3'-*O*-isopropylidene-5'-deoxy-5'-uridylaldehyde,[32] a reaction which surprisingly resulted in removal of the isopropylidene protecting group and dehydration to a 3'-deoxy-3',4'-didehydronucleoside derivative whose formula and structure we confirmed by 2D-NMR spectroscopy and ESI-MS. Ring opening of this metaphosphate derivative with [TBA][OH] yielded the linear form **30**, which was purified by HPLC to give the TEA salt. A number of syntheses of 3',4'-didehydronucleosides have been reported,[33,34] including from Wittig chemistry.[35,36] Some of these derivatives have found biological utility, including as a tumor suppressor.[33,34,37] Furthermore, nucleosides containing a 5'-(*E*)-vinylphosphonate moiety have been used to synthesize modified single-stranded small interfering RNAs (ss-siRNAs) that bind to Argonaute-2 (Ago2), an enzyme responsible for cleaving mRNA.[38,39]

Discussion

NMR Characterization of Oligophosphates

The phosphates synthesized in this study are characterized by multinuclear NMR and ESI-MS, which together give definitive assignment of these species. The sensitivity of $^{31}P\{^{1}H\}$ NMR and the highly diagnostic chemical shift of different phosphate environments facilitate rapid identification of these species from crude $^{31}P\{^{1}H\}$ NMR spectra in non-deuterated solvents (Figure 5). Orthophosphate and its esters appear in the range 5 to −5 ppm, pyrophosphate and other terminal linear phosphates from −5 to −15 ppm, bridging linear phosphates as well as metaphosphates from −15 to −25 ppm, and ultraphosphates appear from −25 to −40 ppm.[40] While the chemical shifts of terminal phosphates are highly sensitive to changes in pH, the other groups are almost invariant to pH. Furthermore, aliphatic alcohol derived phosphoesters show almost no chemical shift change from that of the unfunctionalized phosphate, while phenol derived phosphoesters show an upfield shift by ~5 ppm. Aliphatic phosphoramidates show a downfield shift of ~10 ppm, and aryl phosphoramidates show a downfield shift of ~2 ppm. In combination with $^{31}P–^{31}P$ coupling patterns inherent in oligophosphates, the species in Figure 5 can be assigned readily even without the $^{31}P–^{1}H$ coupling information gained from ^{31}P NMR.

Limitations of Reagent $[PPN]_2[1]$

Anion **1** is an active reagent for the tetraphosphorylation of a wide range of anhydrous nucleophiles, but it is highly moisture sensitive. Adventitious water reacts to form dihydrogen tetrametaphosphate ($[P_4O_{12}H_2]^{2-}$)[18] which is difficult to separate from

$[P_4O_{11}Nuc^1]^{3-}$ compounds. Therefore, when possible, stock solutions of nucleophiles in solvents such as acetonitrile and dimethylformamide were stored over 4Å molecular sieves for at least 12 hours before being utilized. The phosphorylation procedures in this report were carried out under a dry N_2 atmosphere in a glovebox, although we have also reported the use of **1** with Schlenk procedures.[19]

Some substrates were found to be unsuitable for this tetraphosphorylation protocol. No reactivity was observed between anion **1** and stoichiometric *tert*-butanol, a tertiary alcohol, at ambient or elevated temperatures. Similarly, no reactivity was observed with adamantanethiol, a tertiary thiol. These results suggest an upper limit on the steric bulk of **Nuc**[1].

Additionally, some substrates upon treatment with anion **1** give reversible addition. In acetonitrile, treatment with TBA acetate generates acetyl tetrametaphosphate nearly quantitatively by $^{31}P\{^1H\}$ NMR (See SI). However, primarily tetrametaphosphate or anion **1** is isolated upon work up. We obtained similar results with TBA bisulfate, and these observations could be explained by equilibria that favor the products in acetonitrile. Reagent **1** reacts with nucleophiles to give an intramolecular leaving group that is part of the metaphosphate ring, and it is thus well poised to reversibly expel weak nucleophiles. Attempting to precipitate putative equilibrium species ($[P_4O_{11}Nuc^1]^{3-}$) with diethyl ether results in precipitation of anion **1**. The more soluble nucleophile remains in solution, driving the equilibria towards the reactants. In contrast, phosphorylation of substrates such as neutral alcohols and primary or secondary amines results in a proton transfer step to an external base. The favorability of this proton transfer step is likely key to the irreversibility of these reactions.

We have also been unable to identify conditions for ring opening compounds **12a** and **12b** to linear pentaphosphate derivatives. Treatment of **12a** and **12b** with hydroxide or amines instead results in cleavage to tetrametaphosphate and a monophosphate derivative, indicating that tetrametaphosphate is a better leaving group than the linear phosphate that would result from ring opening. No reactivity was observed between these compounds and nucleoside monophosphates in the presence of $MgCl_2$. Furthermore, substituted tetrametaphosphate derivatives such as **4** did not undergo a reaction with the TBA salt of monohydrogen phenylphosphonate ([TBA][HPO_3Ph]) even in the presence of $MgCl_2$. This difference in reactivity between [TBA][HPO_3Ph][22] and $[TBA]_2[AMP]$[45] is likely due to the difference in charge. $[TBA]_2[AMP]$, the TBA salt of a dianion, is readily isolated and soluble in solvents such as acetonitrile and dimethylformamide, possibly due to stabilization of the deprotonated phosphate group through hydrogen bonding interactions to the sugar moiety. However, we were unable to isolate $[TBA]_2[PO_3Ph]$ in organic solvents. The lower charge of monohydrogen [TBA][HPO_3Ph] may correspond to lower nucleophilicity.

Phosphorylation Mechanism and Isomerism

Treatment of anion **1** with most nucleophiles yields a substituted tetrametaphosphate derivative. However, we have observed reversible addition of some nucleophiles as well as rare formation of another product isomer (**10** and **11**), prompting a computational

investigation of the reaction mechanism. The reaction coordinate was calculated for the addition of inorganic azide to **1**, leading to both a substituted tetrametaphosphate derivative via nucleophilic attack at a Q^3 phosphorus or the observed orthophosphoryl-trimetaphosphate isomer, **10**, via nucleophilic attack at a Q^2 phosphorus (Figure 6). Geometries were optimized and frequency calculations performed at the B3LYP-D3BJ/ma-def2-TZVP(-f) level of theory[41–43] with an acetonitrile CPCM solvation model in Orca 4.2.[44] The resulting DFT thermochemistry data for this reaction is presented at 298.15 K. For azide, the orthophosphoryl-trimetaphosphate isomer (**10**) is thermodynamically downhill from the starting material by 2.0 kcal/mol while the tetrametaphosphate isomer (**iso-10** is uphill by 2.4 kcal/mol, explaining the observed selectivity. However, this energy difference is small. The energies of the possible products are close enough to that of the starting materials to explain the reversible addition of some substrates. A transition state was also located corresponding to conversion between the two isomers without the intermediacy of **1**, **TS3**. However, this transition state is thermodynamically uphill from the starting materials by 31.1 kcal/mol, indicating that this direct interconversion is unfavorable. In the case of protic substrates, the transfer of an acidic proton to an amine scavenger after phosphorylation results in an irreversible reaction. In reactions where no proton transfer occurs, the phosphorylation step is reversible and solubility differences between phosphates and the substrate can be exploited to regenerate **1**.

Notably, the thermodynamic barrier for forming the tetrametaphosphate isomer is less than the barrier to form the thermodynamically preferred orthophosphoryl-trimetaphosphate isomer. Therefore, the irreversibility of reactions involving a proton transfer (phosphorylation of alcohols, primary amines, etc.) suggests that many of our isolated tetrametaphosphate derivatives may have been trapped as the kinetic product rather than proceeding to the thermodynamically prefered orthophosphoryl-trimetaphosphate isomer. We therefore sought to selectively synthesize a derivative of the orthophosphoryl-trimetaphosphate isomer by exploiting the reversible addition of a neutral, aprotic nucleophile. Accordingly, treating **1** with DMAP reversibly forms an adduct of the desired orthophosphoryl-trimetaphosphate isomer. Subsequent addition of diethylamine results in phosphorylation and irreversible proton transfer to give the isolable orthophosphoryl-trimetaphosphate isomer **11** (Figure 2A) in contrast to the tetrametaphosphate isomer **6** that is obtained in the absence of DMAP. Such orthophosphoryl-trimetaphosphate derivatives and their ring opening reactions have been well studied as intermediates in the synthesis of oligophosphates.[9,45]

Conclusion

We have demonstrated that anion **1** is an effective tetraphosphorylation reagent for C, N, and O nucleophiles. This reagent therefore joins the nascent body of literature detailing oligophosphate syntheses to meet the growing demand for such compounds in biochemical applications. Furthermore, this reagent extends beyond most previous metaphosphate based phosphorylation reagents, which have focused on trimetaphosphate derivatives. In contrast to such reported methods, we are able to synthesize linear tetraphosphate derivatives without requiring a phosphate nucleophile by utilizing tetrametaphosphate as the starting material. Furthermore, anion **1** has allowed us to easily obtain ϵ-modified nucleoside

5’-pentaphosphates, a class of compounds that have heretofore been difficult and time-consuming to synthesize, via a one-pot reaction sequence. Having demonstrated the scope of the reactivity of anion **1** towards simple nucleophiles and nucleoside derivatives, we are now exploring tetraphosphorylation of complex biologically relevant molecules to enable the study of pressing biochemical questions concerning the roles and utility of oligophosphates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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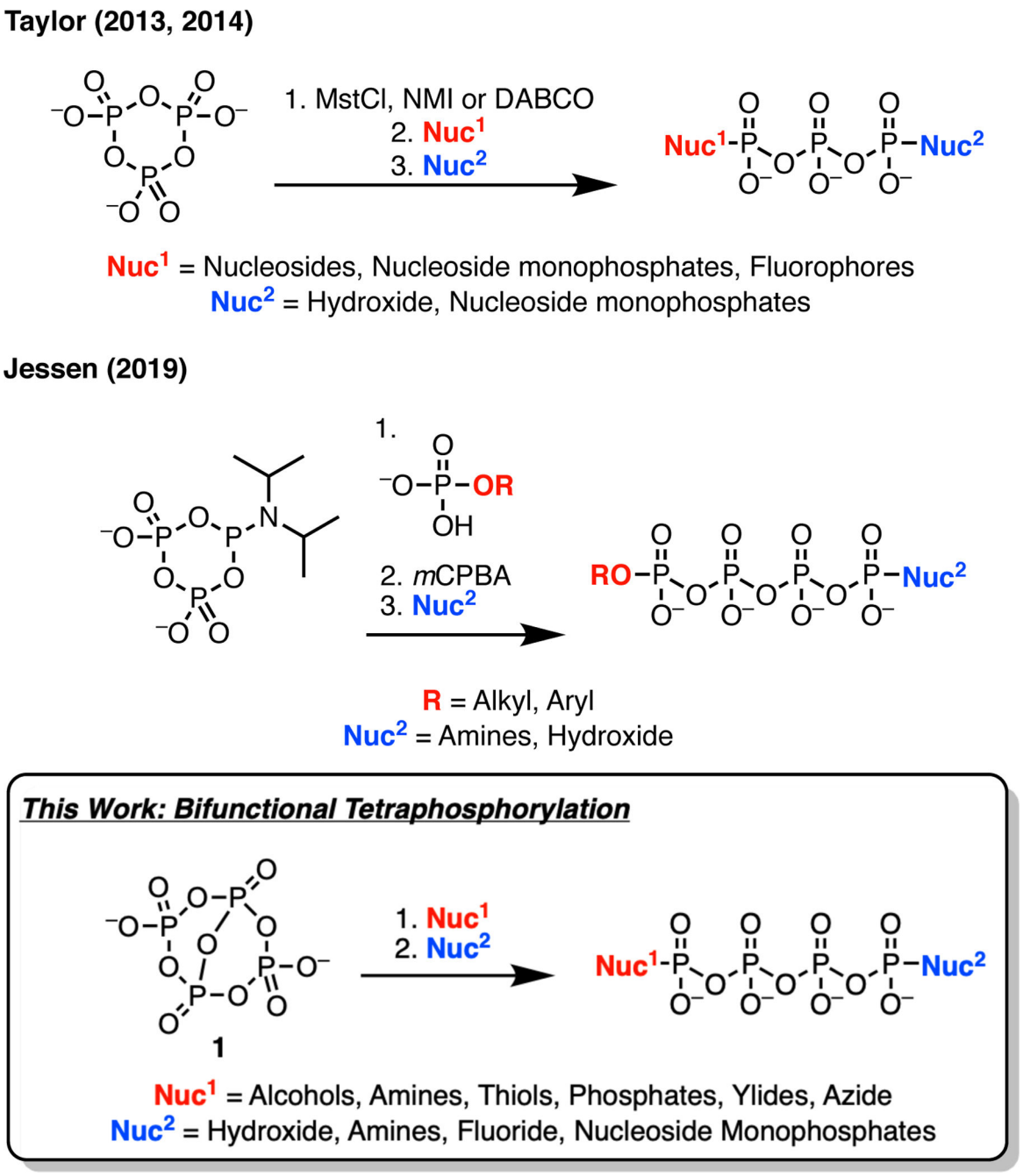

Figure 1.

Representative syntheses of bifunctional tetraphosphates from Taylor,[11,12] Jessen,[9] and this work on the tetraphosphorylation of a diverse range of nucleophiles to make mono- and disubstituted tetraphosphates.

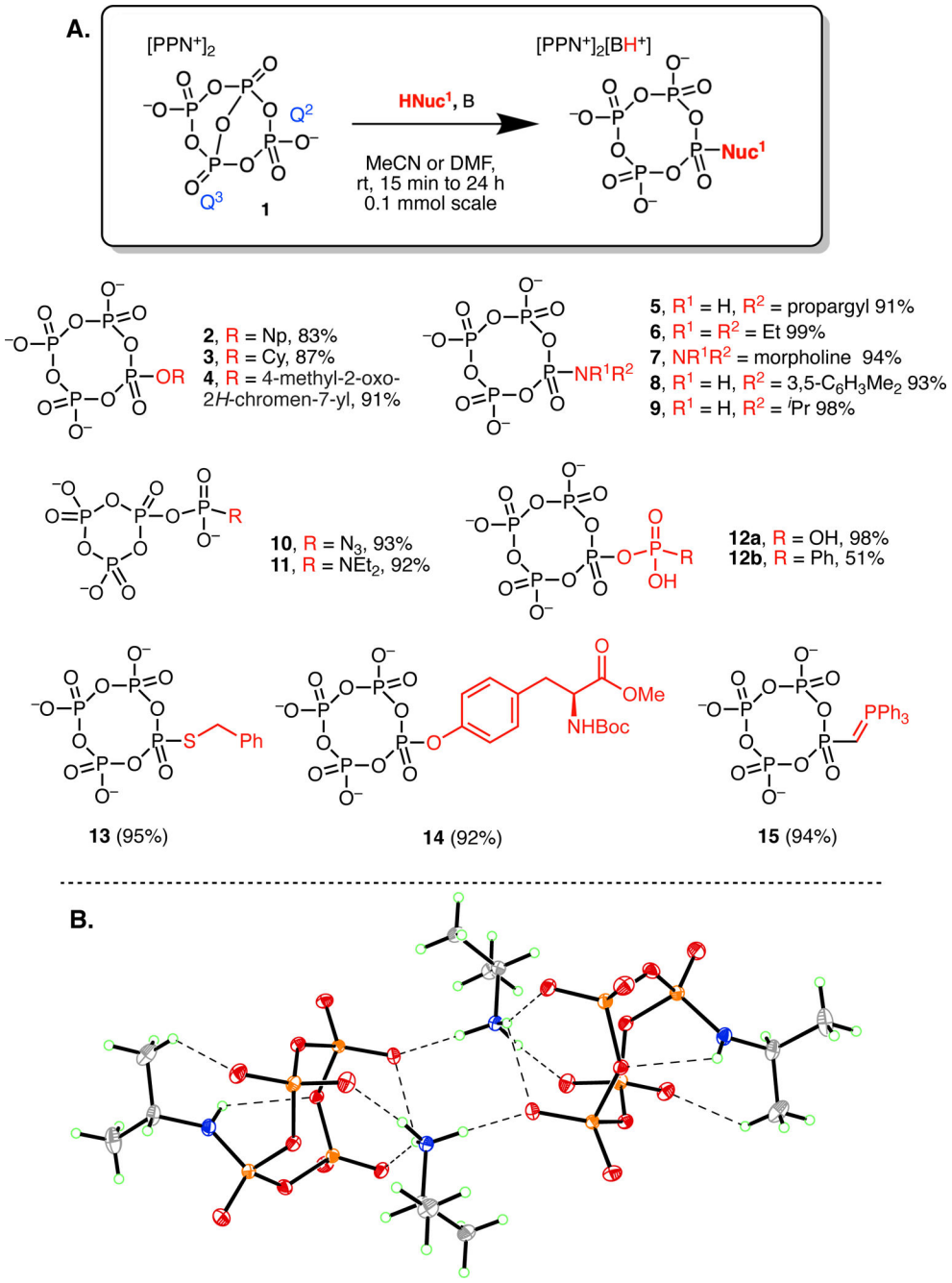

Figure 2.

A. Synthesis of anions **2** to **15** from anion **1** B. X-ray crystal structure of $[PPN]_2[H_3N^iPr][\mathbf{9}]$ with thermal ellipsoids set at 50% probability, the PPN counterions omitted for clarity, and hydrogen bonds denoted by dashed lines (P = orange, O = red, N = blue, C = grey, H = green).

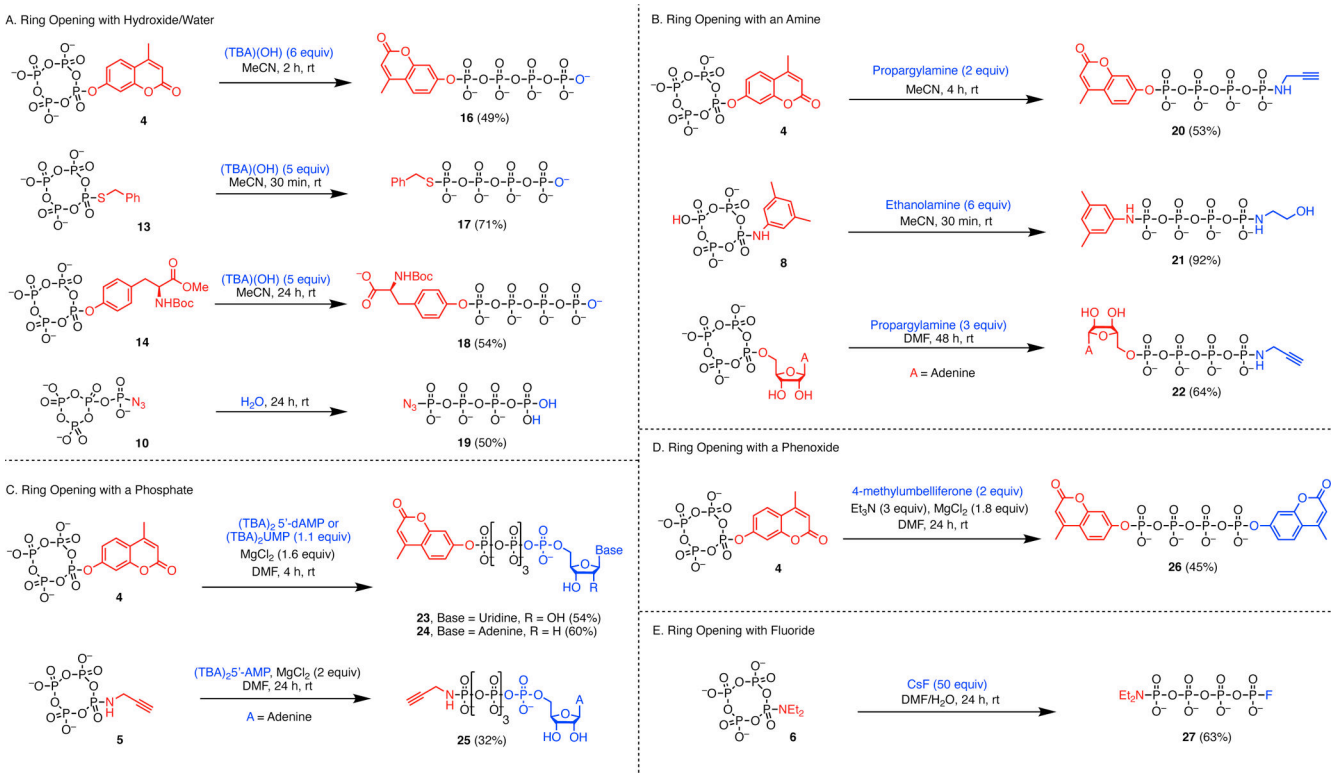

Figure 3.

Synthesis of terminally disubstituted linear tetra- and pentaphosphates (**16**–**27**). The reported yields are for one-pot reactions from **1**.

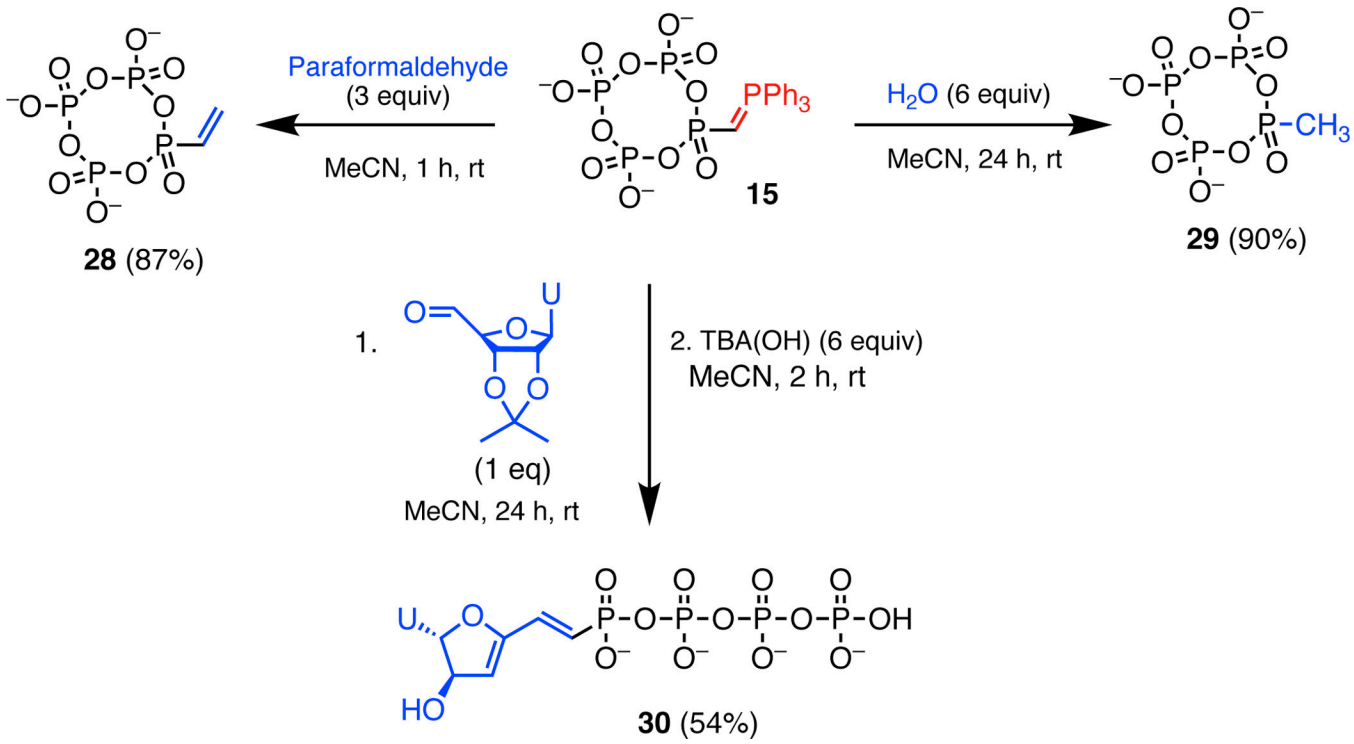

Figure 4.

Synthesis of tetrametaphosphates **28**–**29**, and a linear 3'-deoxy-3',4'-didehydronucleoside derivative **30** upon treatment of **15** with 2',3'-*O*-isopropylidene-5'-deoxy-5'-uridylaldehyde and subsequent ring opening with [TBA][OH].

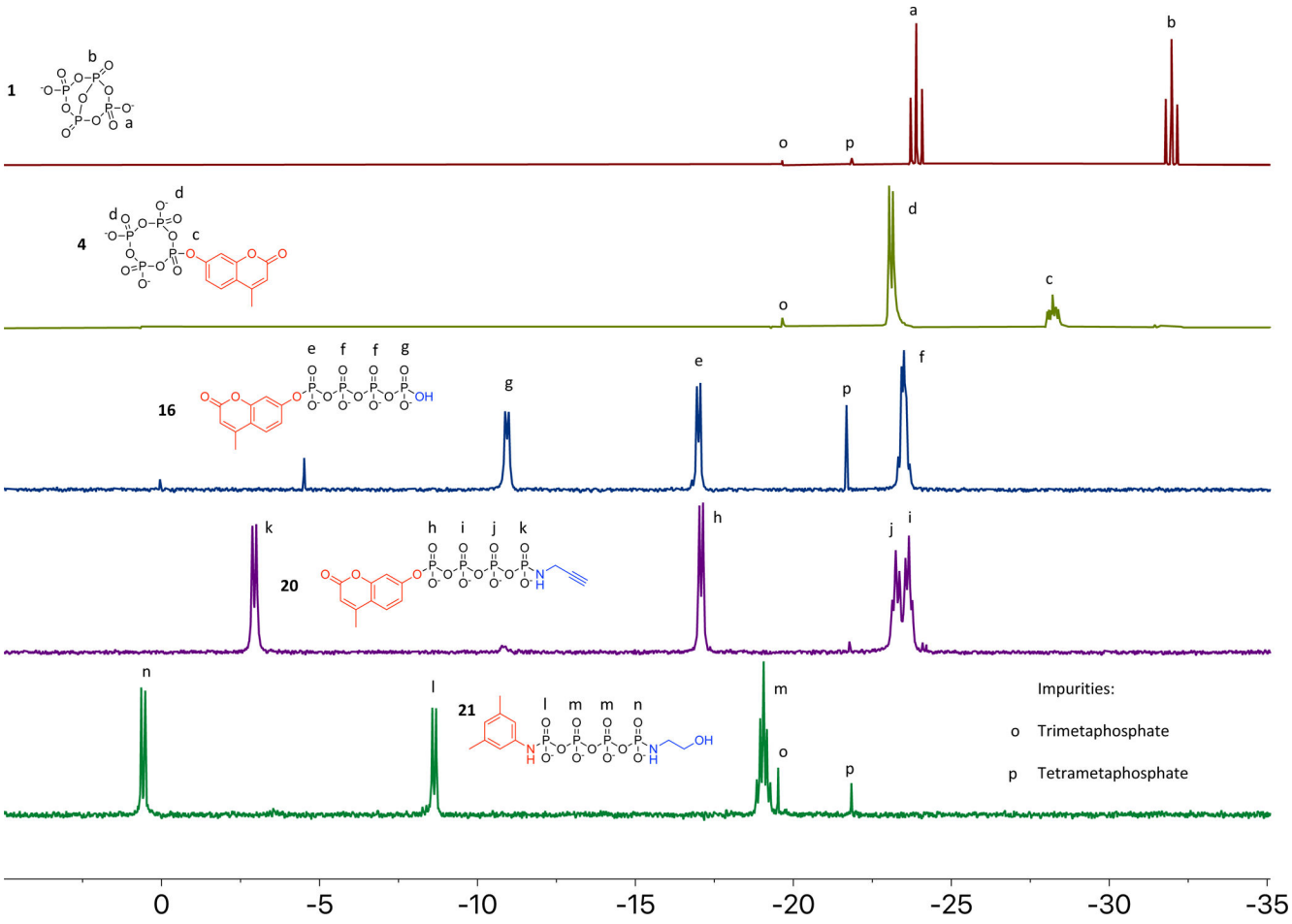

Figure 5.

$^{31}P\{^{1}H\}$ NMR spectra of selected compounds. Functionalized phosphates exhibit highly diagnostic chemical shifts according to their substituents. In conjunction with 2D NMR experiments and MS data, this affords definitive assignment of the isolated compounds. The chemical shifts of unfunctionalized terminal phosphates are, however, highly pH dependent.

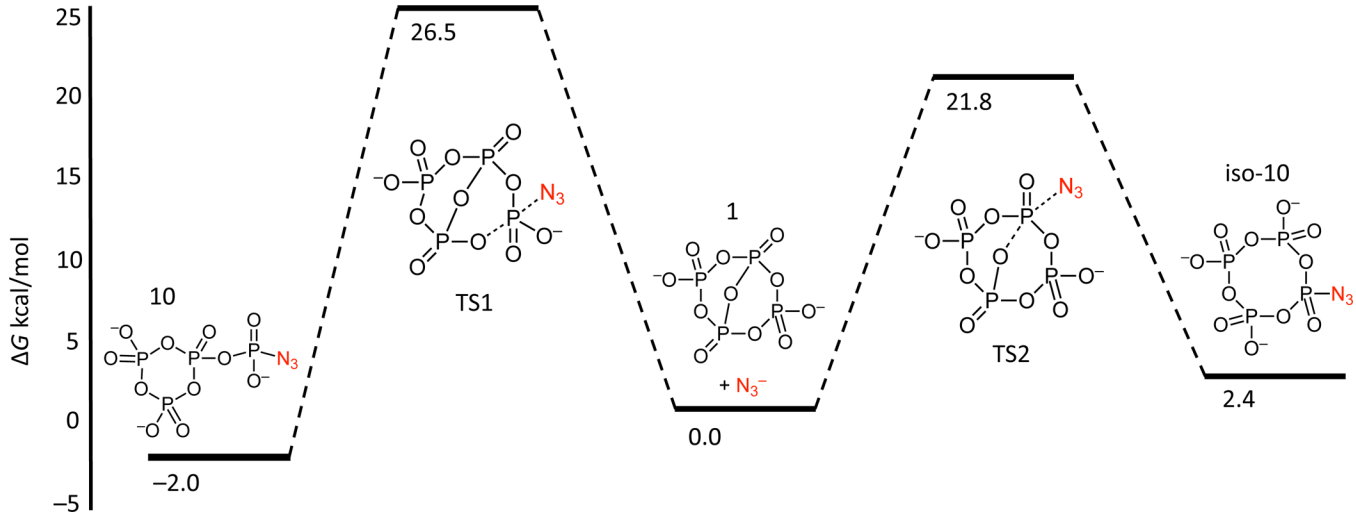

Figure 6.

Reaction coordinate for treatment of **1** with inorganic azide at 298.15 K. Geometries and energies were calculated at the B3LYP-D3BJ/ma-def2-TZVP(-f) level of theory[41–43] with an acetonitrile CPCM solvation model in Orca 4.2.[44]