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Chemoselective Dimerization of Phosphates

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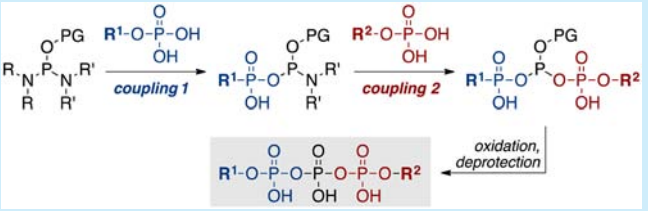
Supporting Information

ABSTRACT: A methodology for the synthesis of oligophosphate conjugates using phosphordiamidites is described. This strategy facilitates the straightforward preparation of C_2-symmetric dinucleoside tri-, penta-, and heptaphosphates. Moreover, unsymmetric compounds such as thiamine adenosine triphosphate and thiamine cytidine triphosphate can be prepared. The material is used to study the inhibitory activity of thiaminylated nucleotides against adenosine diphosphate ribosyltransferases.



Dinucleoside polyphosphates (Np_xNs) and thiaminylated nucleotides are terminally modified oligophosphates. In this paper, a rapid, general, and convenient synthesis of C_2-symmetric Np_xNs with odd numbers of phosphate units and thiaminylated nucleotides is presented, which is based on chemoselective homologative homo- and heterodimerizations of two phosphate monoesters with phosphordiamidites (P-diamidites, Figure 1). Radioactivity-based and colorimetric assays with Thp_3A call into question the reported inhibitory activity of this compound against adenosine diphosphate (ADP) ribosyltransferases (formerly poly-ADP-ribose polymerases; PARPs).

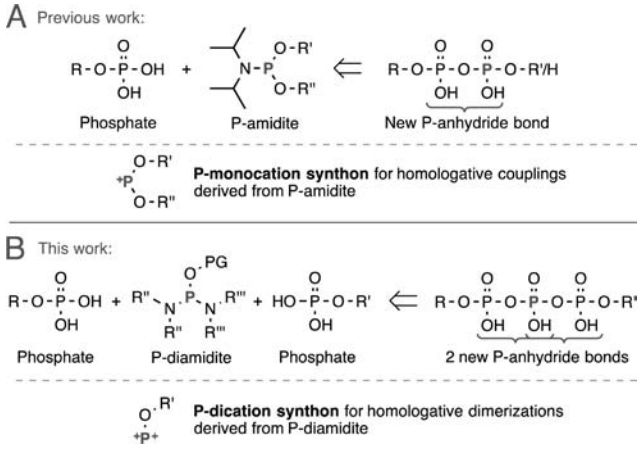


Figure 1. (A) Chemoselective couplings of P-amidites with phosphates for the iterative homologation of oligophosphate esters. (B) Proposed block coupling strategy for the synthesis of oligophosphate-bridged P-esters by homologative dimerization of two phosphate esters using P-diamidites.

Conjugates of nucleotides containing phosphoric anhydrides as bridging units have important and diverse functions in biology. Dinucleoside polyphosphates, for example, are signaling molecules involved in the regulation of blood pressure[1,2] or insulin and glucose levels.[3,4] ADP-ribose polymers are involved in DNA repair mechanisms, transcriptional regulations, apoptosis, and necrosis.[5,6] Adenosine thiamine triphosphate (Thp_3A) is the only known thiaminylated nucleotide to date[7] and is accumulated in *E. coli* under metabolic stress conditions.[8] Its inhibitory potential on ADP ribosyltransferase diphtheria toxin-like 1 (ARTD1, formerly PARP-1) activity has been studied.[9] However, its roles in a broader biological context remain to be elucidated.

Accumulation of negative charge on the phosphoric anhydride (P-anhydride) bond and the instability of the target compounds impose significant challenges on their synthesis and isolation. Even so, different strategies have been developed and summarized.[10−13]

Chemoselective phosphate condensations to minimize protecting group manipulations have been reported,[14−24] but such couplings often require long reaction times, tedious purification procedures, and give variable yields.[13,20]

Application of P^{III} chemistry to synthesize P-anhydrides enables very fast reactions.[25−29] Recently, it was demonstrated that phosphoramidites (P-amidites, Figure 1A) can be coupled to the terminal phosphates of nucleotides,[30,31] enabling the application of P^{III} chemistry on unprotected substrates.[32] A

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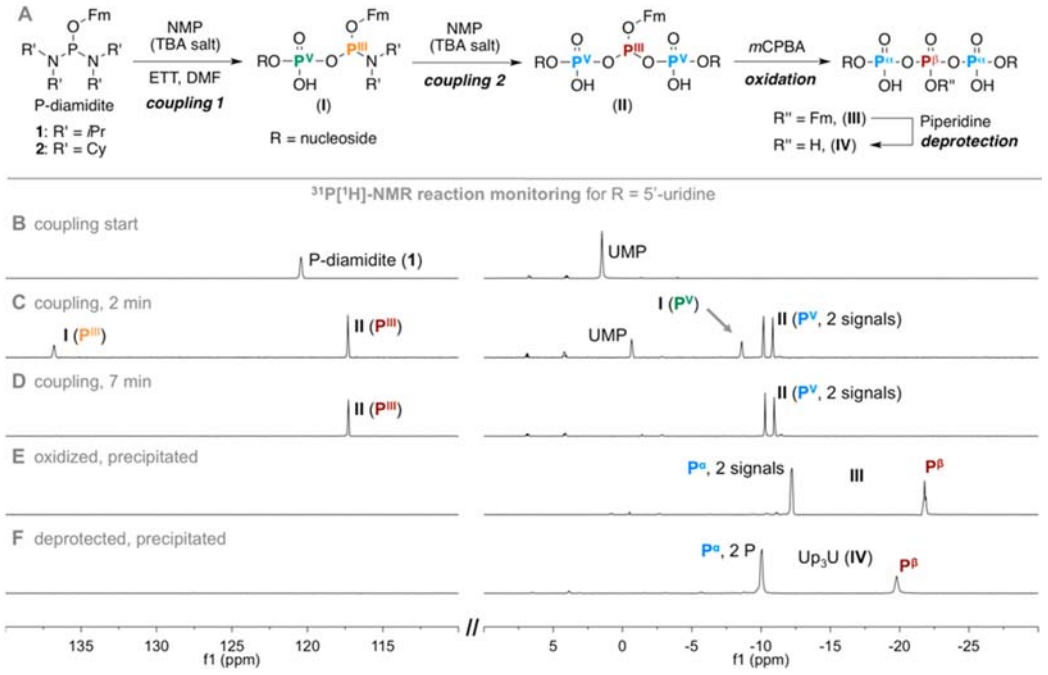

Figure 2. (A) Reaction sequence for the synthesis of C_2-symmetric dinucleoside triphosphates via homologative dimerization of two NMPs. (B−F) Monitoring of the reaction sequence for the synthesis of diuridine triphosphate (Up_3U) by $^{31}P[^1H]$ NMR with the proposed assignment of the signals after each step. *i*Pr, isopropyl; Cy, cyclohexyl; Fm, 9-fluorenylmethyl; NMP, nucleoside monophosphate; TBA, tetrabutylammonium; ETT, 5-ethylthio-1*H*-tetrazole; DMF, dimethylformamide; *m*CPBA, *meta*-chloroperbenzoic acid; UMP, uridine monophosphate.

major advance in the synthesis of modified condensed phosphates would allow using a similar approach to simultaneously homologate and bridge two unprotected nonactivated nucleotides (Figure 1B).[16,33−35]

It is known that P-diamidites can be reacted with alcohols to yield P-amidites.[36,37] In analogy, coupling to a phosphate would yield a terminal mixed phosphoric anhydride P-amidite (Figure 2A, **I**) as an unstable intermediate. Reaction with another phosphate would result in a $P^V-P^{III}-P^V$ (**II**) bridge, which could then be oxidized to the triphosphate (**III**). Deprotection would then yield product **IV**.

Herein, it is shown that this strategy enables a highly convenient and rapid synthesis of various symmetric Np_xNs by homologative homodimerization. Additionally, homologative heterodimerization allowed the convenient synthesis of Thp_3A and facilitated the first synthesis of an analogue, Thp_3C. The obtained highly pure thiamine derivatives showed no inhibition of ARDT1, neither in colorimetric nor radioactivity-based assays, thus calling into question the previously reported inhibitory effects of Thp_3A on ARDT1.

Initially, homodimerization of unprotected UMP was studied. Two equivalents of UMP TBA salt was treated in DMF with a substoichiometric amount of 9-fluorenylmethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl P-diamidite (Figure 2A, **1**) in the presence of excess ETT. ^{31}P NMR of the reaction mixture (Figure 2C) showed that **1** ($\delta(^{31}P)$: 120 ppm) was rapidly consumed, giving rise to two new P^{III} species. Three new signals attributed to P^V nuclei appeared in addition to the remaining UMP ($\delta(^{31}P)$: −1 ppm). Based on the chemical shifts and integrals observed, the products of a single coupling (**I**, ($\delta(^{31}P)$: 137 and −9 ppm) and of a subsequent coupling (**II**, ($\delta(^{31}P)$: 117, −10, and −11 ppm) are postulated. Interestingly, peak splitting due to homonuclear P−P coupling was not observed for the $P^{III}-P^V$ anhydride bonds. This is consistent with the observations made for the coupling of P-monoamidites with phosphates.[30,32]

After a few minutes, the signals assigned to **I** and UMP disappeared in the ^{31}P NMR (Figure 2D). The remaining set of signals can be assigned to the $P^V-P^{III}-P^V$ anhydride intermediate **II**. Prochirality of the P^{III} atom results in two distinct signals of the diastereotopic P^V nuclei. Oxidation with *m*CPBA gave Fm-protected diuridine triphosphate (**III**) after precipitation. ^{31}P NMR of the precipitated crude material confirmed the presence of a single species (Figure 2E). The intermediate was then deprotected with piperidine and precipitated. The precipitate was confirmed to be a piperidinium salt of diuridine triphosphate (Up_3U) with a purity of 85% (see Figure 2F for a ^{31}P NMR spectrum of the crude precipitate) and in 87% yield, obtained in less than 15 min reaction time under ambient conditions. Purification by strong anion exchange chromatography (SAX) gave pure Up_3U in 60% yield. A slightly modified protocol was then applied to the synthesis of symmetric dinucleoside triphosphates from three other unprotected NMPs. The results were comparable (see Table 1, entries 1−4). For the synthesis of dicytidine triphosphate (Cp_3C) and diadenosine triphosphate (Ap_3A), better results were obtained using P-diamidite **2** (Figure 2A).

Two examples of dinucleoside triphosphate analogues with a β-thiophosphate moiety were also synthesized. In the oxidation step, *m*CPBA was replaced by S_8, giving sulfurized β-P(S) triphosphates after deprotection and precipitation (Table 1, entries 5 and 6). These results demonstrate that P-diamidite-based dimerizations are a powerful new synthetic concept for the generation of modified condensed phosphates.

In comparison with previously developed procedures,[35,38−40] the presented methodology offers several advantages. The reactions can be run under ambient conditions and are very fast. Good-quality C_2-symmetric dinucleoside triphosphates can be isolated in high yields by simple precipitation. The methodology is applicable to all canonical NMPs, demonstrating its robustness, and without the need to protect or activate the

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Table 1. Synthesized Dinucleoside Triphosphates and Analogues

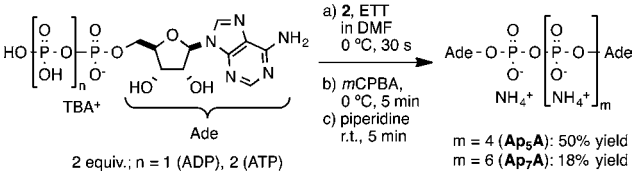
		coupling		precipitated		purified[c]
entry	product	P-diamidite[a]	time (min)	yield (%)	purity[b] (%)	yield (%)
1	Ap_3A	**2**	5	71	70	60
2	Gp_3G	**1**	5	64	65	45
3	Cp_3C	**2**	3	70	85	60
4	Up_3U	**1**	7	87	85	68
5	Ap-p(S)-pA	**1**	3	70	85	
6	Up-p(S)-pU	**1**	4	60	80	

[a]P-diamidite used for the homologative dimerization. [b]Purity according to ^{31}P and ^{1}H NMR. [c]Yield after purification.

nucleotides. Finally, synthesis of thiophosphate analogues is feasible by using S_8 in the oxidation step.

Different methods have been used to synthesize Ap_5A. They involve either the opening of trimetaphosphate intermediates[15,41] or the coupling of activated phosphates[42] and are sluggish. In the case of Ap_7A, treatment of a mixture of ATP and adenosine tetraphosphate with a carbodiimide for 24 h resulted in formation of the product that was isolated after three chromatographic steps in unspecified yield.[43,44] Therefore, homologative dimerization of ADP to give Ap_5A and ATP to Ap_7A is an interesting alternative. However, the intermediates were much more water-sensitive, and no products were formed with the developed method under ambient conditions. Running the coupling reaction under exclusion of water with predried solvents and nucleotide salts for only 30 s (Scheme 1) and with

Scheme 1. Synthesis of Ap_5A and Ap_7A[a]



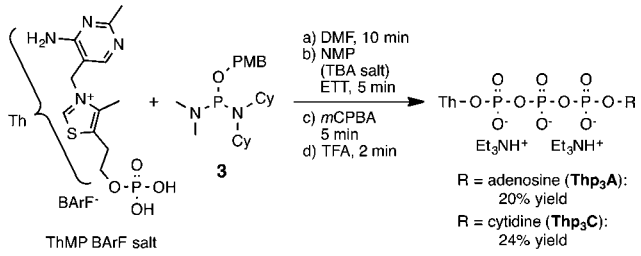
[a]TBA, tetrabutylammonium; ETT, 5-ethylthio-1*H*-tetrazole; DMF, dimethylformamide; *m*CPBA, *meta*-chloroperbenzoic acid.

P-amidite **2** solved this issue. After oxidation and deprotection followed by SAX, Ap_5A and Ap_7A were isolated in 50 and 18% yield (Scheme 1), respectively. Homologative dimerization strategy with P-diamidites enabled the preparation of oligophosphates with five and even seven phosphate units by direct bridging of ADP or ATP.

^{31}P NMR monitoring of the reaction sequence suggested that the P-diamidite was generally converted very rapidly to intermediate **I**. Rapid consumption of the P-diamidite and accumulation of **I** (Figure 2C) indicated that the first coupling is faster than the second one. Therefore, it should be possible to couple two different phosphate esters sequentially to a P-diamidite to synthesize asymmetric oligophosphate esters such as Thp_3A or any dinucleoside polyphosphate with regards to chain length and nucleotide composition.

Synthesis of thiamine triphosphate derivatives is a challenging task, underlined by the low yield (2.7%) and difficult purification procedure in the previously reported synthesis of Thp_3A.[7,45] Nevertheless, recent results indicated that Thp_3A is an inhibitor of PARP1, and it was therefore perceived as an interesting target for the extension of the methodology presented herein.

Scheme 2. Synthesis of Thp_3A and Thp_3C[a]



[a]BArF, tetrakis[(3,5-trifluoromethyl)phenyl]borate; DMF, dimethylformamide; NMP, nucleoside monophosphate; TBA, tetrabutylammonium; ETT, 5-ethylthio-1*H*-tetrazole; *m*CPBA, *meta*-chloroperbenzoic acid; TFA, trifluoroacetic acid.

To facilitate sequential couplings, a new P-diamidite (Scheme 2, **3**) featuring two different amine moieties was developed. The different reactivity toward acidic activation of the amine substituents should improve the selectivity of the first reaction, as the dimethylamine moiety would be replaced rapidly. To synthesize thiamine derivatives, the P-diamidite protecting strategy had to be changed, as decomposition under basic conditions was observed, likely due to deprotonation of the thiamine moiety. The Fm group was replaced with a *p*-methoxybenzyl (PMB) group, which can be cleaved under acidic conditions. Finally, thiamine monophosphate (ThMP) had to be solubilized in DMF, which was eventually achieved by conversion into its tetrakis[(3,5-trifluoromethyl)phenyl]borate (BArF) salt.

The ThMP BArF salt was treated with the asymmetric P-diamidite **3** (Scheme 2). The doubly protonated phosphate ester of ThMP was expected to both activate the P-diamidite and form the dimethylammonium salt by protonation of the amine. Two equivalents of UMP TBA salts were then added for the second coupling, followed by ETT to complete the reaction, as the TBA salt does not have the two protons required for conversion. After oxidation and acidic cleavage of the PMB group, the crude product was precipitated. Formation of the two symmetric triphosphates Ap_3A and Thp_3Th could not be prevented completely, and thus, a purification step was required. Pure product in 20% yield was obtained after only a single semipreparative ion pair HPLC. The same procedure was applied to synthesize the first described structural analogue featuring a pyrimidine nucleoside, namely, thiamine cytidine triphosphate (Thp_3C), in 24% yield.

Both Thp_3A and Thp_3C were tested for their inhibitory activity on human ARTD1/PARP-1, as a complete inhibition of the enzyme had been reported at 10 μM Thp_3A concentration with a commercial colorimetric assay.[9] Our radioactivity-based assay did not confirm any inhibitory activity on ARTD1 or ARTD2 (formerly PARP-2) at concentrations up to 20 μM of either Thp_3A or Thp_3C (see Figures S1–S4). To confirm these results, the commercial colorimetric assay that had been used in the initial report was repeated. Again, no inhibition of Thp_3A (or Thp_3C) on ARTD1 could be detected up to 20 μM (see Figure S5). It is likely that the inhibition was caused by an impurity, as unfortunately no analytical data were provided.[9]

In summary, these results show that a homologative dimerization of phosphate esters using P-diamidites is feasible. The strategy allows for homo- and heterodimerizations and facilitated the synthesis of highly challenging zwitterionic thiaminylated nucleotides, thus demonstrating the generality

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and robustness of these reactions. For all the examples, including the Thp_3A structural analogue Thp_3C, reaction times are short and pure products can be obtained easily. In addition, the method offers the possibility to prepare thiophosphate analogues and corresponding pentaphosphates and heptaphosphates. Thus, this strategy represents a novel and broadly applicable approach in the synthesis of condensed natural oligophosphate conjugates and their analogues with a huge potential to simplify current synthetic approaches.[46]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01466.

Supplementary figures, experimental procedures, and NMR spectra (^{31}P reaction monitoring, ^{1}H, ^{31}P, and ^{13}C NMR) (PDF)

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Notes

The authors declare no competing financial interest.

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