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2,4-Dinitrophenol as an activating reagent in a facile preparation of cyclic phosphate triesters

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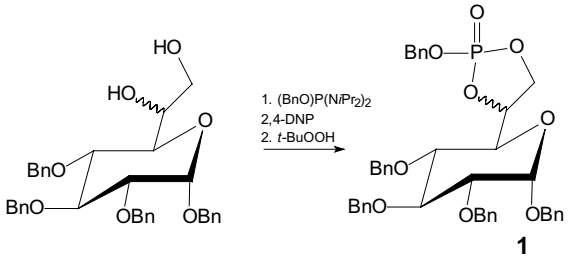
Abstract—2,4-Dinitrophenol was employed with benzyloxy-bis-(diisopropylamino)phosphine to synthesise the cyclic phosphate derivatives of a series of alkane diols (HO–$(CH_2)_n$–OH, $n = 2$–6) in good isolated yields. Tetrazole and DNP were compared by ^{31}P NMR spectroscopy for their ability to catalyse the cyclisation at the P(III) stage. Investigation of the phosphate triester stability under various oxidation and chromatographic conditions resulted in the optimisation of the isolation procedures of the chemically unstable cyclic compounds. Conditions for debenzylation were developed to yield the corresponding cyclic phosphodiesters quantitatively. The methodology was further applied to the preparation and isolation of the cyclic phosphate derivative of a carbohydrate.

When compared to the vast number of biologically active phosphate-containing compounds, cyclic phosphates are not so ubiquitous. Yet, two cyclic phosphate diesters, derivatives of nucleosides, are secondary messengers regulating numerous intracellular signalling events; cAMP and cGMP. Other cyclic phosphate diesters of carbohydrates are known to be either reaction intermediates during enzymatic catalysis or important reaction products such as 2′,3′-cyclic phosphate terminated RNAs.[1] In order to facilitate the preparation of cyclic phosphate diesters of carbohydrates, we aimed at developing a method, which allowed large-scale reaction, facile work-up and did not involve chromatography on fully deprotected species, in particular, anion exchange-based purification techniques. Consequently, we developed a method, which gave us access to hydrolytically labile cyclic phosphate triesters, which could be purified by chromatography and quantitatively converted to pure cyclic phosphate diesters by hydrogenolysis.

The combination of phosphoramidites and azole-type activators is amongst the most commonly used methods in the synthesis of phosphates by the 'phosphite' approach. Our interest in optimising a 'phosphite' methodology on solid support to access cyclic phosphotriesters forced us to look for reaction conditions that were suitable for solid-phase chemistry. A polymer-supported reagent that incorporated immobilised *p*-hydroxybenzyl alcohol and that was used for the phosphorylation of carbohydrates has recently been reported by Parang.[2] Based on this precedent, we decided to investigate (4-methoxy)benzyloxy- and benzyloxy-bis-(diisopropylamino)phosphine (**2**) as phosphitylating agents.



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The syntheses of very few terminal cyclic phosphate esters of sugars have been described in the literature.[3–5] Two of these reported sugar phosphates were formed from group migration due to an intramolecular transesterification of an α-hydroxy-phosphate triester and therefore were obtained in low yields. Isolation of pure compounds required extensive anion exchange chromatography, a technique known to offer very variable results depending on the nature of the compounds being

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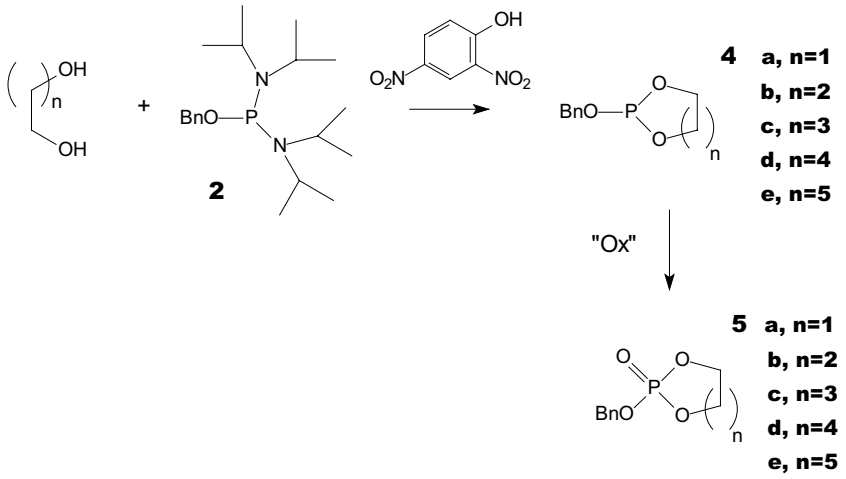


Scheme 1. 2,4-Dinitrophenol-activated cyclic phosphate synthesis.

isolated. Furthermore, the purified compounds are often isolated with high salt (i.e., NaCl) content, in concentrations sometimes unsuitable for biological evaluations. Although numerous syntheses of carbohydrate and nucleoside cyclic phosphates using tetrazole and bis-aminophosphites have been reported,[6–9] we were unable to synthesise cyclic phosphate triester **1** in reasonable yields with these reagents. Under standard conditions, an excess of highly pure tetrazole was required and contamination by such excess required extensive chromatography, leading to poor recovery due to decomposition of the cyclic triester. In addition, excess of phosphitylating reagent was required due to extended reaction times and reagent degradation. We therefore decided to investigate an alternative activator, 2,4-dinitrophenol (2,4-DNP), which had been reported for the preparation of nucleoside phosphites[10] and to employ it in combination with benzyloxy-bis-(diisopropylamino)phosphine (Scheme 1) as alternative reagents to the tri-(4-nitrophenol)phosphite that had been developed for the preparation of five-membered ring cyclic phosphorothioates.[11]

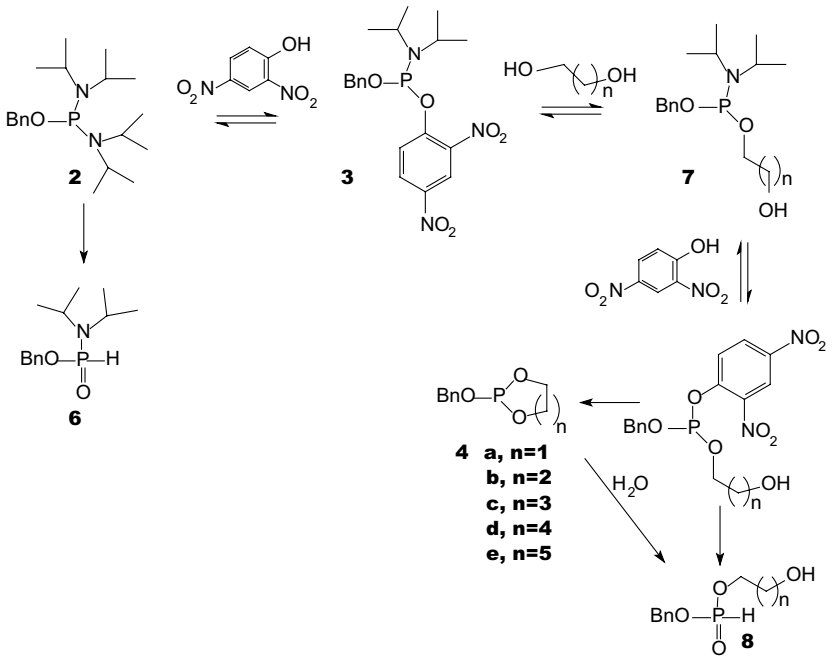
A systematic comparison of the ability of 2,4-DNP and tetrazole to promote the formation of small to medium size phosphate triester rings from diols and carbohydrates is reported here. The chemical sequence establishing the cyclisation mechanism and the nature of the reaction intermediates is described. Conditions for quantitative removal of the benzyl-protecting group to yield the cyclic diesters are also discussed.

We first considered 2,4-DNP for its availability and ease of use and purification. Unlike tetrazole, which involves hazardous purification conditions and is only available in solution, 2,4-DNP is purchased as an inexpensive solid that can easily be dried[†] and is easily removed from the reaction mixture by aqueous extraction. As a consequence, phosphitylation solvents could be selected according to the diol solubility.

The mechanism of activation by 2,4-DNP and cyclisation is described in Scheme 2. The P(III) phosphoradiamidite **2** first reacted with 2,4-DNP to offer **3** in a similar manner to that accepted for the activation of a P(III) amidite by 2,4-DNP or tetrazole.[10] Benzyloxy-2,4-dinitrophenyloxy-(diisopropylamino)-phosphine ($\delta = 152.5$ ppm) **3** was the only intermediate accumulating detectable by ^{31}P NMR and was detected for all diols, thus indicating that the subsequent reaction is the rate-limiting step for the overall conversion. The activated reagent **3** then reacted with one hydroxy group of the diol to yield **7** (identified by ^{31}P NMR ($n = 2$; P(III) $\delta = 148.0$ ppm; $n = 3$; P(III) $\delta = 147.7$ ppm), which subsequently cyclised to **4** after a second activation of the P(III) amidite with 2,4-DNP had taken place.

Over time, reagent **2** hydrolysed to the hydrogen phosphonamidate **6** ($\delta = 13.8$ ppm) at an identical rate whether 2,4-DNP or tetrazole was used. Hydrolysis of **7** to **6** was also expected to take place and was one of the reactions competing with the cyclisation of **7** to **4** as the first steps of the conversion appeared to be reversible. In the case of the medium size cyclic phosphites **4c**–**e**, the cyclisation failed to proceed to completion for either activators, but much higher yields were obtained for the 2,4-DNP-catalysed-reactions (Table 1) for which the ratio of cyclic phosphites **4** and therefore cyclic phosphates **5** to side product **6** was dramatically enhanced. For the medium size ring formation reaction catalysed by tetrazole, a large proportion of the hydrogen phosphonate **8** (Scheme 2) in addition to **6** was detected by ^{31}P NMR and identified by ^{31}P ^{1}H coupled NMR. In the case of the cyclisation reaction activated by 2,4-DNP, little of **8** could be detected. Finally, once formed the cyclic phosphites **4** did not hydrolyse to **8** in the presence of 2,4-DNP. The enhanced rate of the second alkoxylation via the use of 2,4-DNP was therefore of primary importance for the formation of the cyclic phosphite **4**.

[†] 2,4-DNP was purchased from Aldrich. Typically, 2,4-DNP (5–10 g) was dried under high vacuum in the presence of P_2O_5 overnight after azeotroping water with toluene (3x). Thus dried 2,4-DNP remained suitable as an activating reagent for 3–4 weeks when stored at rt under argon.



Scheme 2. Proposed chemical sequence yielding to cyclic phosphites **4**.

Table 1. Activator catalysed cyclisation reactions of diol **a–e** with reagent **2**

Entry	Activator	^{31}P(III) **4**	Reaction time	Ratio **4**/**3**[a]	^{31}P(V) **5**	Isolated yield **5** silica (%)	Isolated yield **5** HPLC (%)
a	DNP	135.7	2 h	10/1	18.3	21	56
	Tetrazole			2.5/1			
b	DNP	131.2	2 h	3.5/1	−6.7	50	62
	Tetrazole			3.5/1			
c	DNP	134.8	2 h	4.0/1	3.9	43	52
	Tetrazole			1.5/1			
d	DNP	133.9	18 h		−0.2	36	44
	Tetrazole			1/1			
e	DNP	140.3	18 h	3/1	−0.2	—	54
	Tetrazole			1/1			

[a] Based on ^{31}P NMR and ^{1}H NMR.

The ratio of cyclic phosphates **5** to hydrogen phosphonamidate **6** remained constant after oxidation with *t*-BuOOH in decane to that of the cyclic phosphites **4**–**6**. However, this ratio decreased when H_2O_2 or *t*-BuOOH in water was used instead of *t*-BuOOH in decane. Hydrolysis of cyclic phosphites and phosphates could take place at −78 °C and as expected, the rate was dependent on the ring size. Reversed-phase chromatography (C18-RP Supelcosyl HPLC column; acetonitrile–H_2O) was the only suitable method of purification of most cyclic phosphates, especially **5a** as less than 50% recovery could be obtained from silica chromatography columns (Table 1).

Having optimised the reaction and isolation conditions for simple diols, we turned our attention to the preparation of **1**. Attempts had been made to prepare a cyclic phosphate of a manno-heptoside using reagent **2** with tetrazole, however this strategy was reported to be unsuitable.[3–5] While the synthesis of the diol precursor to **1** will be reported in due course, we wish to report the first high-yielding conversion of a carbohydrate terminal diol to a cyclic phosphate triester, using that same reagent but with 2,4-DNP as activator. The conversion of the diol to the cyclic phosphite with **2** in the presence of 2,4-DNP was quantitative, while similar results to those observed by Schweda[3] were obtained when tetrazole was used. Subsequent oxidation with *t*-BuOOH in decane yielded **1** quantitatively.[12]

Since the ultimate aim of this work is to access cyclic phosphate diesters in high yields and high degrees of purity, we investigated means to remove selectively the benzyl group from the highly labile cyclic phosphate of triesters **5** without formation of any ring-opened side-products. While the cyclic phosphate triesters **5b–e** could be easily deprotected quantitatively using hydrogenolysis conditions (H_2, Pd/C), **5a** was rapidly hydrolysed and subsequently deprotected to yield 2-hydroxyethyl phosphate monoester (structure confirmed by ^{1}H, ^{13}C and ^{31}P NMR). It was postulated that a catalytic amount of acid generated in the early stages of the hydrogenolysis

is enough to catalyse the ring opening of the remaining cyclic phosphate triesters present in solution. Consequently, half an equivalent amount of solid K_2CO_3 was added to the THF solution containing **5a**. Under such conditions, **5a** was deprotected to yield the cyclic phosphate diester in quantitative yields. The purity of the samples thus obtained was confirmed by ^{1}H, ^{13}C and ^{31}P NMR and no purification by chromatography was required on the cyclic phosphate diesters.

Acknowledgements

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12. A dry DCM solution of the diol, **2** and 2,4-DNP (equimolar amounts) was stirred at rt until only one peak corresponding to the cyclic phosphite was observed by ^{31}P NMR. The oxidation, carried out by addition of *t*-BuOOH at −78 °C, was stopped by addition of sodium thiosulfate to afford **1**. ^{1}H NMR ($CDCl_3$) δ 7.38–7.26 (m, 25H), 5.12 (d, $J_{POC} = 4.9$ Hz, 2H), 5.04 (d, $J = 10.9$ Hz, 1H), 5.00 (d, $J = 8.1$ Hz, 1H), 4.84 (d, $J = 12.0$ Hz, 1H), 4.81 (d, $J = 11$ Hz, 1H), 4.74 (d, $J = 12.1$ Hz, 1H), 4.70 (s, 1H), 4.61 (d, $J = 13.6$ Hz, 1H), 4.70–4.50 (m, 1H), 4.58 (d, $J = 11.7$ Hz, 1H), 4.50 (d, $J = 11.9$ Hz, 1H), 4.12 (dd, $J = 7.2$, 14.3 Hz, 1H), 4.07 (dd, $J = 8.7$, 9.4 Hz, 1H), 3.98 (dd, $J = 1.7$, 10.5 Hz, 1H), 3.50 (ddd, $J = 7.6$, 8, 15.6 Hz, 1H), 4.47 (dd, $J = 3.7$, 9.6 Hz, 1H), 3.20 (dd, $J = 8.6$, 10.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 138.5, 137.8, 137.3, 136.7, 135.8, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.0, 94.2, 82.4, 79.7, 76.3, 75.8, 75.5, 74.4, 72.7, 70.1, 69.5, 69.3, 68.8. ^{31}P NMR ($CDCl_3$) δ 17.4. HRMS (FAB^+) Calcd for $C_{42}H_{44}O_9P$ ($M+H^+$) 723.2723, found 723.2702.