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BIS(ALLYLOXY)(DIISOPROPYLAMINO)PHOSPHINE AS A NEW PHOSPHINYLATION REAGENT FOR THE PHOSPHORYLATION OF HYDROXY FUNCTIONS

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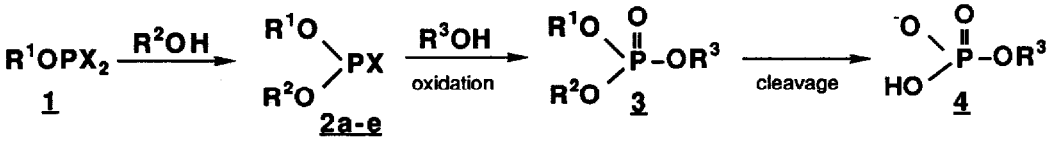
Abstract: Bis(allyloxy)(diisopropylamino)phosphine is a new phosphinylating agent which can be employed for an effective phosphorylation of hydroxy functions after activation by tetrazole followed by an oxidation step.The allyl protecting groups are removed afterwards with Pd (0)$P(C_6H_5)_3)_4$ leading to the corresponding phosphorylated substrate.

Recently we have described a mild and effective phosphorylation procedure which we have applied to the phosphorylation of oligonucleotides directly in the course of their synthesis on a solid support and the O-phosphorylation of serine, threonine, and tyrosine as well as for a serine-containing peptide[1]. The method is based on P(III) chemistry according to *scheme 1* and allows the adaptation of the protecting groups to the substrate to be phosphorylated. Reagents **2a** and **2b** can be used for the phosphorylation of DNA fragments (R^3OH) and the deprotection was achieved with NH_3 when **2a** was employed or by a combination of thiophenol and NH_3 when **2b** had been used [2-4]. After phosphorylation especially of amino acids and peptides with **2c** the benzyl protecting groups were removed by hydrogenation. Recently the system was extended by Perich and Johns[5] by synthesizing reagent **2d** of which after phosphorylation the tert. butyl protecting groups can be cleaved under acidic conditions.



	R^1	R^2	X
2a	$NC-CH_2-CH_2-$	$NC-CH_2-CH_2-$	$N(iPr)_2$
2b	$NC-CH_2-CH_2-$	$Cl(C_6H_4)_4CH_2-$	$N(iPr)_2$
2c	$C_6H_5-CH_2-$	$C_6H_5-CH_2-$	$N(iPr)_2$
2d	$(CH_3)_3-$	$(CH_3)_3-$	$N(Et)_2$
2e	$H_2C=CH-CH_2-$	$H_2C=CH-CH_2-$	$N(iPr)_2$

R^1,R^2: Protecting groups

R^3OH: Substrate to be phosphorylated

Scheme 1

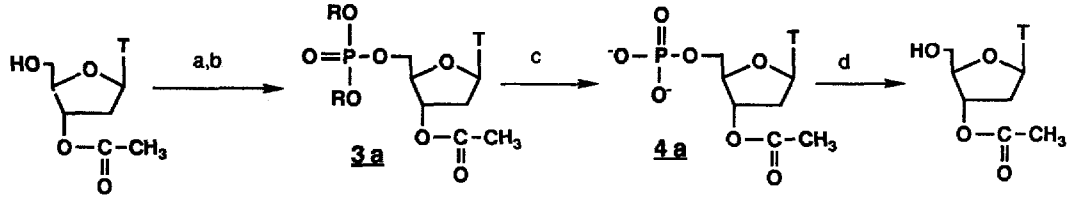
In order to give this phosphorylation system even more flexibility we have synthesized and used bis(allyloxy)(diisopropylamino)phosphine **2e** as a new phosphinylation/phosphorylation reagent.

Allyl or substituted allyl protecting groups are attracting more and more attention in synthetic chemistry especially in the nucleotide and the peptide field due to their effective removal under mild conditions with tetrakis(triphenylphosphine) Pd (0) [6-9]. Thus **2e** could be useful for the phosphorylation of substrates bearing already allyl protection in order to cleave off all protecting groups at the same time or in combination with other protecting groups allowing an orthogonal cleavage procedure.

For the synthesis of **2e**[10] PCl_3 was treated with one equivalent of allyl alcohol. The allyl phosphorodichloridite obtained in this way was transferred directly to **1** without isolation since allyl phosphorodichloridite was reported to be able to polymerize spontaneously undergoing explosions during distillations [11]. Distillation of **1** afforded a yield of 52 %.

The reaction of **1** with another equivalent of allyl alcohol proceeded smoothly in the presence of diisopropyl ammonium tetrazolide and gave **2e** as a colourless liquid which was purified by short column chromatography over silica (84 %).

In order to test the suitability of **2e** for phosphorylation reactions it was reacted with 3'-O-acetyl thymidine according to *scheme 2* which yielded after oxidation with *m*-chloroperbenzoic acid the corresponding 5'-phosphate **3a** (R^1;R^2=allyl) in the protected form in a yield of 90% after short column chromatography.

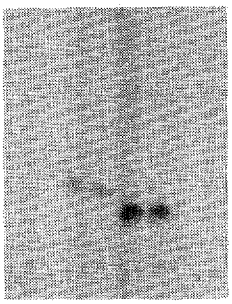


Scheme 2

The allyl protecting groups were then removed from **3a** by Pd $(P(C_6H_5)_3)_4$ according to ref. 6. Treatment of the resulting product **4a** with alkaline phosphatase produced again the starting material for the phosphorylation namely 3'-O-acetyl thymidine as checked by TLC and HPLC.

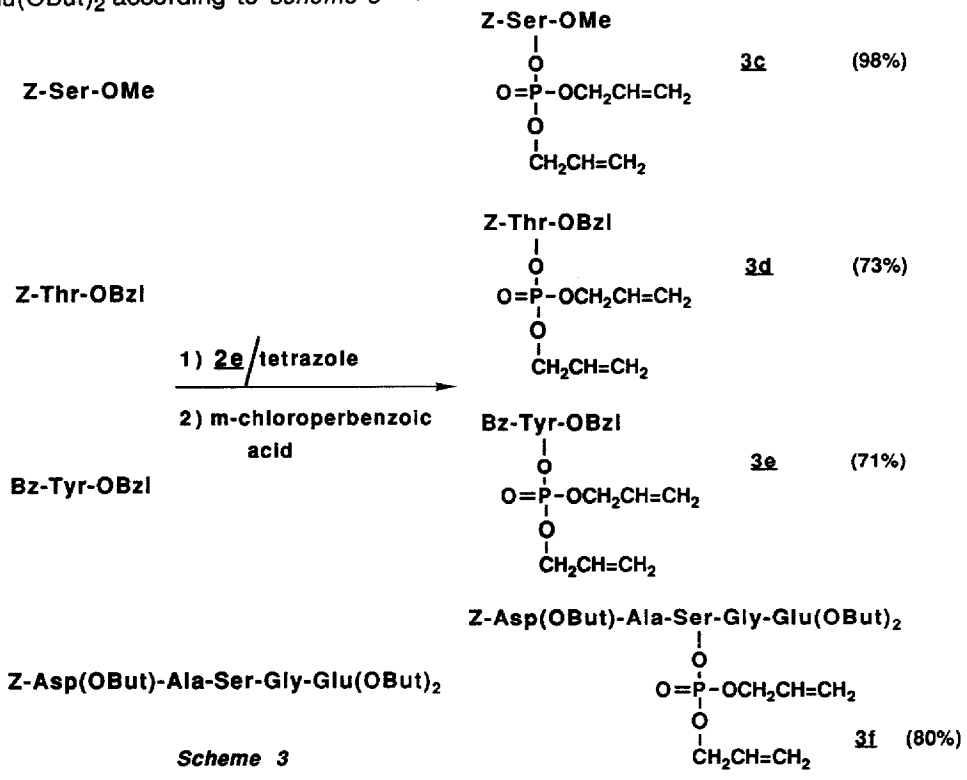
In another experiment **2e** was employed in a standard cycle for the phosphinylation of the oligomer $d(T_9)$ still attached to the solid support but lacking the 5'-dimethoxytrityl protecting group.

After oxidation with iodine the corresponding protected 5'-phosphate **3b** (R^1;R^2=allyl) could be obtained in good yield as can be judged from the UV-shadowing gel (*fig. 1*) which shows the final unprotected 5'-phosphate of $d(T_9)$ (**4b**) after removal from the support and cleavage of the protecting groups by Pd $(P(C_6H_5)_3)_4$ and ammonia. As a proof treatment of **4b** with alkaline phosphatase yielded again the starting material $d(T_9)$.



lane 1: p d(T_9) (**4b**) prepared with **2e** after treatment with alkaline phosphatase
lane 2: d(T_9) as comparison
lane 3: p d(T_9) prepared with **2a**
lane 4: p d(T_9) prepared with **2e**

In a further set of experiments **2e** was applied to the O-phosphorylation of properly protected serine, tyrosine and threonine as well as for the pentapeptide Z-Asp(OBut)-Ala-Ser-Gly-Glu(OBut)$_2$ according to *scheme 3* [12].



Scheme 3

In these cases the oxidation after the phosphinylation was performed with *m*-chloroperbenzoic acid leading to reasonably high yields of the products **3c-f**. which were characterized by ^{1}H-NMR and gave satisfactory elementary analysis.

In summary we have demonstrated that the bis (allyloxy) (diisopropylamino) phosphine **2e** is another alternative phosphinylating agent which can be easily prepared and used with high efficiency for the phosphorylation of suitable substrates under very mild conditions. It can be applied in combination with allyl-protected substrates in order to cleave all allyl protecting groups at the same time or as an orthogonal alternative in combination with other protecting groups.

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References and Notes

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10) Synthesis of **2e**: To a mixture of 1 mol of PCl_3 (137.3 g; 85.5 ml) and 1 mol of pyridine (81.0 ml) in 200 ml of diethyl ether was added at -78 °C during 90 min. 1 mol of 2-propen-1-ol (68.2 ml). After complete addition cooling was removed and stirring continued overnight. The precipitate was filtered off under Ar. The solution was cooled down to -10 °C and 7.1 mol (970 ml) of diisopropylamine were added with stirring during 60 min. and stirring was continued overnight. The precipitate was filtered off and the solution was evaporated. After addition of 2 g of CaH_2 it was distilled. Fractions were collected at 120 °C (0.8 mbar) and the distillation was stopped after we had obtained 150 g of **1**.
For the preparation of **2e** 200 mmol (57.7 g) of this material and 92 mmol (16 g) of diisopropylammonium tetrazolide were taken up in 500 ml of CH_2Cl_2 and 185 mmol (10.7 g) of 2-propen-1-ol were added during 1 h with stirring. Stirring was continued for 2 h. The mixture was poured into 500 ml of sat. $NaHCO_3$ solution. The organic layer was separated and the aqeous layer was extracted 3 times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated. Short column chromatography over 150 g of silica with diethylether as solvent afforded 38 g (84 %) of **2e** as a colourless liquid characterized by elementary analysis and ^{1}H-NMR.
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12) Phosphorylations with **2e** were carried out in the same way as described in ref. 1.

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