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Protecting of a Thermolabile Protecting Group: "Click-Clack" Approach

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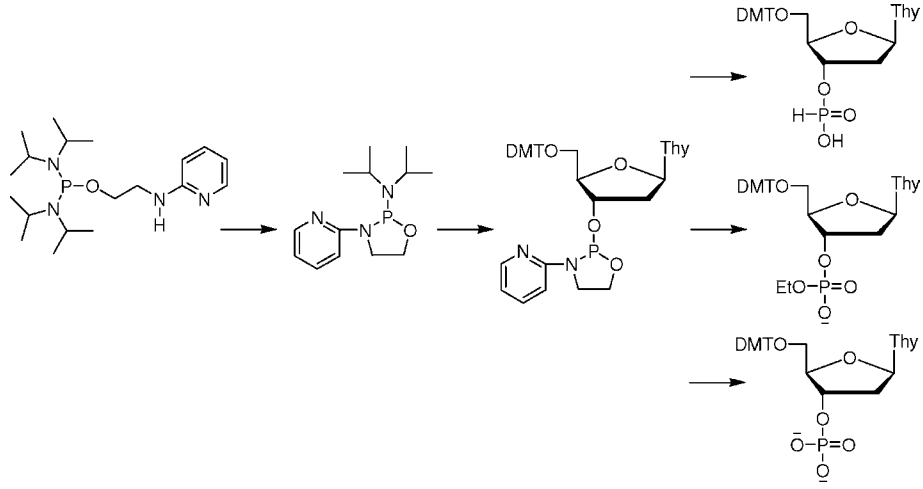
ABSTRACT



A new method for attaining higher stability of thermolabile protecting groups (TPG) using an intramolecular cyclization through a "click-clack" approach was demonstrated. It was found that during intramolecular cyclization of 2-pyridyl type of TPG the thermally stable 3-pyridyl-[1,3,2]oxazaphospholidine ring was formed and thermolabile properties were declined. Thermolability could be recovered upon hydrolytic ring-opening of a 3-pyridyl-[1,3,2]oxazaphospholidine. "Click-clack" chemistry was applied to the synthesis of biologically important phosphate esters and their analogues and some H-phosphonate derivatives.

Intramolecular cyclization is an important synthetic element of drug design and stereoselective preparation of various natural products.[1] Compounds with five-membered rings are also widely used in synthetic organic chemistry as suitable synthons for diverse chemical transformations.[2]

Recently, thermolabile protecting groups (TPG),[3] whose removal involves five-membered ring formation, have emerged as a new strategy for manipulation of protecting groups in organic synthesis.[4] Although deprotection of TPG takes place at elevated temperatures, these groups are not completely stable at ambient conditions. Due to this, the duration of synthesis and, thus, a number of synthetic steps that can be carried out at room temperature on compounds bearing TPG are limited.[5]

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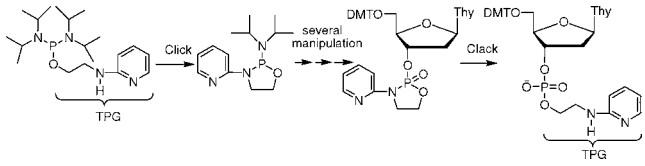
Some thermolabile protecting groups are based on *N*-(2-pyridyl)aminoethanol (**1**) as a precursor and are referred to as 2-pyridyl TPG.[6] Compound **1** was first synthesized in 1949 by Kaye[7] and only recently has it appeared as a promising candidate for designing thermolytic protecting groups for solid-phase oligonucleotides synthesis.[8] Unfortunately, its rather high instability at room temperature[9] limits the number of synthetic steps that can be carried out. A mechanism for the removal of 2-pyridyl TPG from a phosphate center is based on an intramolecular cyclization initiated by a nucleophilic attack of the pyridine moiety and the departure of a phosphate anion. Temperature highly influences the kinetics of this reaction but the elimination proceeds at a moderate rate also at room temperature.[6]

To eliminate this drawback, I have investigated a method to increase thermostability of a 2-pyridyl TPG via temporary engagement of a phosphate center in a cyclic structure. Transformation of a thermolabile protecting group into a cyclic form of an oxaphospholidine type would mean that the nucleophilic attack of N-pyridyl nitrogen into the α carbon should be impeded, making the group thermostable. On the other hand, the known susceptibility of oxazaphospholidines to hydrolytic ring-opening, could regenerate the original thermolabile properties of TPG. This led me to the idea of temporary protection of thermolabile properties of TPG through a process I call "click-clack", because it has several qualities of "click chemistry".[10] The "click" step is nucleophilic substitution, modular in wide scope, gives very high yields, and generates a stable product in water. Purification of the final product is possible through crystallization.

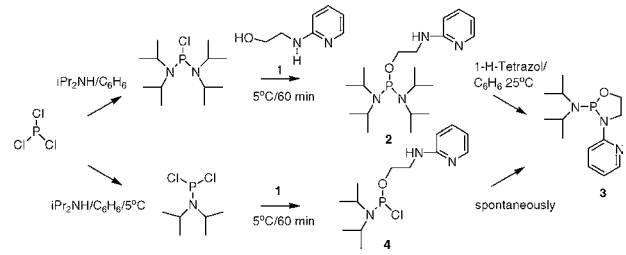
Scheme 1. Idea of a "Click-Clack" Approach Using 3-Pyridyl-[1,3,2]oxazaphospholidine as Temporary Protection



In this paper, I demonstrate a "click-clack" approach to the modulation of thermolabile properties of TPG. In the first step ("click"), a linear form of a 2-pyridyl TPG is converted into a cyclic form which lacks the thermolabile properties.

A hint for this "click" step came from the observation that the *N*-(2-pyridyl)aminoethyl group can undergo intramolecular cyclization to form a 3-pyridyl-[1,3,2]oxazaphospholidine derivative that is stable under conditions used for the removal of standard 2-pyridyl TPG. After performing all the desired transformations, in a "clack" step, thermolabile properties of the protecting group are recovered via the opening of the oxazaphospholidine ring under mild acidic conditions. This new "click-clack" approach allowed me to control thermolabile properties of TPG groups via intramolecular cyclization.

Scheme 2. "Click" Cyclization

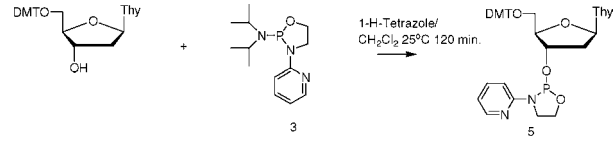


In practice, it was found that treatment of *O*-[*N*-(2-pyridyl)aminoethyl] *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**2**) with an excess of a weak acid under anhydrous conditions for 3 h provided a cyclic 2-isopropyl-3-pyridyl-[1,3,2]oxazaphospholidine (**3**). A mechanism of "click" cyclization of the 2-pyridyl TPG involves a nucleophilic attack on the phosphorus by the exocyclic nitrogen atom. The preference for a cyclization is due to the presence of a good leaving group and the formation of a five-membered ring. Such a cyclization does not always require protonation and it can also take place as a spontaneous path reaction whereby the anion of chloride spontaneously leaves the phosphorus center. The product of the cyclization, 2-*N*-isopropyl-3-pyridyl-[1,3,2]oxazaphospholidine (**3**), can be purified and isolated by a silica gel column or crystallization.[11]

Since nucleotides derivatives are good models to study and develop "click-clack" manipulation of TPG and control their thermolabile properties, 5′-*O*-DMT-thymidine reacted with **3** under standard phosphitylation conditions to produce the 3-pyridyl-[1,3,2]oxazaphospholidine derivative **5** (mixture of diastereoisomers).

Compound **5** and its precursor **3** were stable for months at room temperature. To check the thermostability of **5**, the compound was dissolved in a mixture of acetonitrile/aqueous phosphate buffer (pH 7) and heated at 90 °C for 30 min.

Scheme 3. Synthesis of Nucleotides Derivatives



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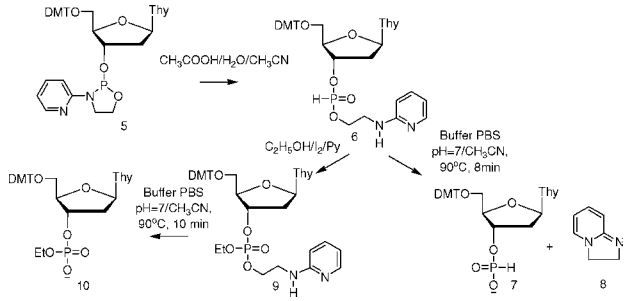
The **5** was completely stable under this condition and no changes were observed. This opens a possibility of using this group in a multistep chemical synthesis without a danger of partial degradation, so that ways of recovery of TPG are still possible. To demonstrate the usefulness of this approach, compound **5** was subjected to various transformations.

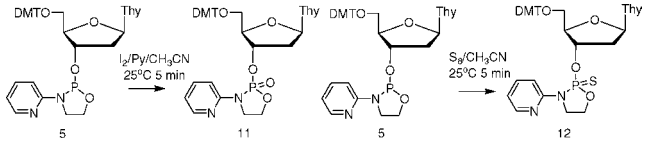
In the first example, the valency number of phosphorus did not change before the "clack" step, and thus a linear form of TPG, with full thermolabile properties, was recovered.

Scheme 4. "Clack" Ring-Opening and Transformation without Changes the Valency Numer of Phosphorus Atom



The best result for the ring-opening of **5** was obtained by acid hydrolysis that resulted in the formation of a 2-pyridyl-aminoethyl 5′-*O*-DMT-thymin-1-yl H-phosphonate (**6**).[12] Heating of **6** in solution in neutral buffer for 8 min at 90 °C transformed it completely into a 5′-*O*-DMT-thymin-1-yl H-phosphonate (**7**) and 2,3 dihydroimidazo[1,2-α]pyridine (**8**). The presence of the latter compound in a reaction mixture is convincing evidence for a cyclic mechanism of the removal of the 2-pyridyl TPG. Compound **6** could also be oxidized with iodine in the presence of ethanol[13] to produce the phosphotriester **9**, from which after the removal of the TPG under thermolitic conditions, thymidine phosphodiester (**10**) can be easily generated.

Scheme 5. Reaction of Oxidation and Sulfurylation



This "click-clack" transformation opens a way to the synthesis of H-phosphonate monoesters and phosphodiesters using thermolabile protecting groups. All the processes of the ring-opening and the conversion into the phosphate esters were studied by ^{31}P NMR spectroscopy (Figures 1 and 2).

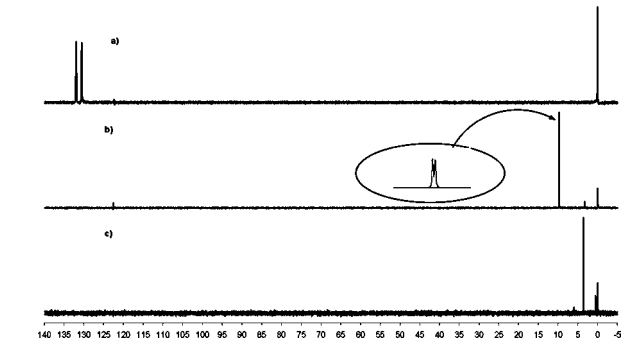


Figure 1. ^{31}P NMR analysis of formation an *H*-phosphonate diester and removal of a TPG. (a) 3′-Pyridyl-[1,3,2]oxazaphospholidine 5′-*O*-DMT-thymin-1-yl **5**; (b) 2-pyridylaminoethyl 5′-*O*-DMT-thymin-1-yl *H*-phosphonate **6**; (c) 5′-*O*-DMT-thymin-1-yl *H*-phosphonate **7**.

Another example of the transformation of the 3-pyridyl-[1,3,2]oxazaphosphopholidine ring,[14] which does not have thermolabile properties, is based on a change in the oxidation stage of phoshoprus. The experiments proved and demonstrated that this change does not affect the ability of the cyclic form to recover thermolabile properties as a result of the ring-opening under hydrolysis. The other transformation involved a change in oxidation stage of phosphorus in **5** prior to the oxazaphospholidine ring-opening to recover thermolabile properties. The oxidation of **5** with iodine in pyridine to produce **11** or sulfurization with elementary sulfur to phosphorothioate **12** occurred easily.

The ring-opening in **11** occurred rapidly to produce phosphodiester **13**. Thermal removal of the *N*-(2-pyridyl)-aminoethyl protecting group from phosphate diester **13** after the oxidations and "clack" process was not possible under aqueous conditions, apparently due to the presence of a negative charge in the phosphate moiety.

However, upon in situ silylation of **13** with trimethylsilyl chloride to produce nonionic phosphate triesters,[15] a smooth thermal deprotection occurred, producing phosphate monoester **14** (Scheme 6). The recovery of TPG is possible despite the fact that **11** easily hydrolyses under acidic conditions. In contrast, 3′-[3-pyridyl-[1,3,2]thioazaphospholidin-2-thio] 5′-*O*-DMT-thymin-1-yl (**12**) very resistant to acid hydrolysis, and thus thermolabile properties could not be recovered.

(11) In the presence of water and acid, the 3-pyridyl-[1,3,2]oxazaphospholidine **3** was easily transformed into an 3-pyridyl-[1,3,2]oxaza-*H*-phospholidin-2-one.

(12) The hydrolysis under alkaline conditions it led to cleavage of the P−O bonds without thermolabile properties of this group. Treatment of **5** with strong bases like DBU leads to several products with thymidin-3′-yl *N*-(2-pyridyl-2-hydroxyethyl) H-phosphonamidate being a main product.

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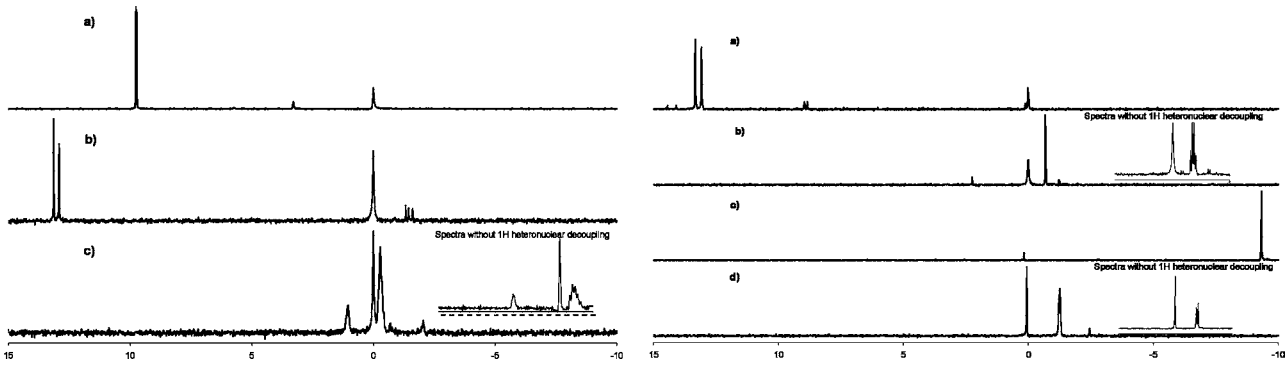
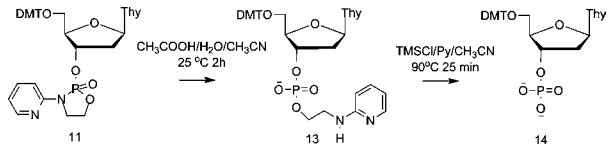


Figure 2. (Left) ^{31}P NMR analysis of reaction oxidative condensation *H*-phosphonate diester **6** and removal TPG from phosphate triester **7**. (a) 2-Pyridylaminoethyl 5′-*O*-DMT-thymin-1-yl *H*-phosphonate **6**; (b) 2-pyridylaminoethyl 5′-*O*-DMT-thymin-1-yl ethoxy phosphate **9**; (c) 5′-*O*-DMT-thymin-1-yl ethoxy phosphate **10**. (Right) ^{31}P NMR analysis of reaction removed thermolability group from phosphorodiester via silylation derivatives. (a) 3′-[3-Pyridyl-[1,3,2]oxazaphospholidin-2-one]-5′-*O*-DMT-thymin-1-yl (**11**); (b) 2-pyridylaminoethyl 5′-*O*-DMT-thymin-1-yl phosphate (**13**); (c) O-silyl derivatives; (d) 5′-*O*-DMT-thymin-1-yl phosphate (**14**).

In summary, an intramolecular cyclization was demonstrated to be helpful in the protection of thermolabile properties of TPG bearing an *N*-(2-pyridyl)aminoethyl moiety.

The 3-pyridyl-[1,3,2]oxazaphospholidine ring was found to be very susceptible to acid hydrolysis and thus enabling the recovery of a thermolabile form of TPG. This "click-clack" approach (cyclization followed by hydrolysis) offers a simple and efficient way to control thermolabile properties of protecting groups and can be used in the synthesis of biologically important phosphate esters. Studies on the development of click-clack procedures for other thermolabile groups are in progress in this laboratory.

Scheme 6. Reaction of Transformation to Phosphate



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Supporting Information Available: General remarks, experimental procedures and NMR, mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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