JOC The Journal of Organic Chemistry

Article

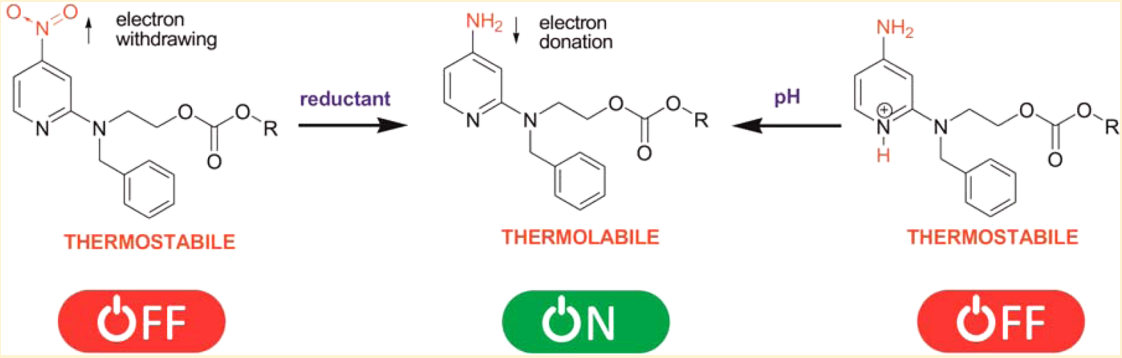
pubs.acs.org/joc

Modulating the Stability of 2-Pyridinyl Thermolabile Hydroxyl Protecting Groups via the "Chemical Switch" Approach

Agnieszka Witkowska, Dominika Krygier, Jolanta Brzezinska, and Marcin K. Chmielewski*

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

Supporting Information



ABSTRACT: A novel and effective method is presented for modulating the stability of 2-Pyridinyl Thermolabile Protecting Groups (2-Py TPGs) in the "chemical switch" approach. The main advantage of the discussed approach is the possibility of changing the nucleophilic character of pyridine nitrogen using different switchable factors, which results in an increase or decrease in the thermal deprotection rate. One of the factors is transformation of a nitro into an amine group via reduction with a low-valent titanium in mild conditions. The usefulness of our approach is corroborated using 3′-*O*-acetyl nucleosides as model compounds. Their stability in various solvents and temperatures before and after reduction is also examined. Pyridine *N*-oxide and pH are other factors responsible for the nucleophilicity and stability of 2-Pyridinyl Thermolabile Protecting Groups in thermal deprotection. Protonation of 4-amino 2-Pyridinyl Thermolabile Protecting Groups is demonstrated by $^{1}H—^{15}N$ HMBC and HSQC NMR analysis.

INTRODUCTION

Over the past decade, increased interest has been observed in a novel range of chemical methods which are useful in synthetic biology (defined as chemistry applied in biology).[1] The use of Thermolabile Protecting Groups (TPGs) in the protection of functional groups during chemical synthesis of nucleic acids can serve as a good example of this tendency.[2,3] The main advantage of TPGs is their capability to be removed by thermal deprotection, which occurs only as a result of increased temperature in neutral conditions. Such a unique approach eliminates the need for hazardous reagents used to deprotect other protecting groups, and complies with the principles of green chemistry.[4] Unlike oligonucleotides carrying the conventional 2-cyanoethyl phosphate/thiophosphate protecting group, deprotection of TPGs from oligonucleotides does not generate potent mutagens, such as acrylonitrile, which can alkylate DNA.[5] Thanks to this observation, TPGs have also been applied in the chemical synthesis of other nucleic acids,[6] especially for phosphate,[7] hydroxyl,[8] and amine groups[9] in nucleotides or DNA microarrays.[10] A certain disadvantage of TPGs lies in their partial instability at ambient temperature. This poses a serious problem when a TPG is intended to be used as protection for key functional groups in multistage chemical transformations. Partial loss of a TPG during synthesis reduces efficiency and results in the formation of byproducts. However, increasing stability at ambient temperature leads to an extension of deprotection time and prolonged exposure of the molecule to heating.

Useful TPGs are characterized by high stability at ambient temperature, and fast deprotection after raising the temperature by approximately 70 °C. This can be attained if TPGs have high activation energy in the thermal deprotection process. In contrast, those TPGs that are unstable at room temperature are marked by lower activation energy. An example of manipulating the stability of TPGs is the "click-clack" approach.[11] This is based on forming a 3-pyridinyl-[1,3,2]-oxazaphospholidine ring, which temporarily "freezes" thermolytic properties that are recovered after opening the oxazaphospholidine under mild acidic conditions. This method has been used to protect the phosphate function and for intramolecular oxidation of H-phosphonate.[12]

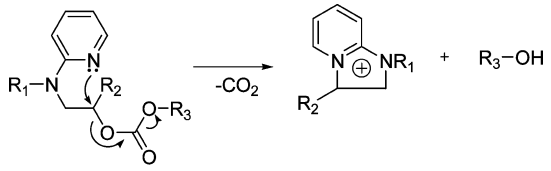
In practice, however, hydroxyl protecting groups are more significant, and some new protecting groups have been designed for this function.[13] Devising an efficient system of controlling the stability of TPGs for these functional groups

Received: September 4, 2015
Published: November 6, 2015

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129−12136

may significantly increase the popularity of TPGs in modern chemistry. Protection of the hydroxyl function through a TPG containing a nucleophilic 2-pyridinyl moiety is very promising,[14] and the TPG is likely to become a comprehensive protecting group. Application of these groups in the protection of the phosphate or hydroxyl function has revealed a persistent problem with attaining high stability at ambient temperature. The use of a 2-pyridinyl moiety as a nucleophilic agent in TPGs allows for control of stability by using substituents which change the electron density of the pyridine ring, and consequently the control of thermocyclization. This is based on a nucleophilic attack by the pyridine nitrogen on a carbon atom directly linked to a carbonate group (Scheme 1). This

Scheme 1. Mechanism of Thermal Deprotection



R_1=Bn, -CH_3; R_2=H, Ph, R_3= rest of moiety

process demonstrates the removal mechanism of 2-Py-TPG that occurs via intramolecular cyclization. The main advantage of 2-Py TPGs is that the deprotection restores the free state of the protected function which can be triggered in a controlled manner without using external chemical agents.

Currently, developed methods place particular emphasis on controlling molecular properties via chemical modification. "Chemical switch" is a term used to denote manipulations and transformations of molecules between two extreme chemical properties. One example of reversible off–on switching of the electron spin has been achieved by nitric oxide (NO) functioning as an axial ligand of cobalt(II)tetraphenylporphyrin (CoTPP).[15] The reversible off–on switching zinc-dependent factor in a mutant F1-ATPase motor containing a metal-binding site has been shown to be a functional and promising mechanical component in motor proteins.[16] Also, a chemical switch has been used in an optical sensor developed for sensing nitrite based on a monotonous decrease in absorbance of Rhodamine 6G at 525 nm (the absorbance maximum of dye) with increasing concentrations of nitrite.[17] This reduction is one very convenient chemical transformation; in particular, the reduction of aryl nitro compounds has found numerous applications in organic chemistry and remains an attractive method for changing the properties of molecules. Chemical reduction of anthraquinone has been applied in modulated switching between two forms of 90 bp DNA in Y-branched junctions.[18]

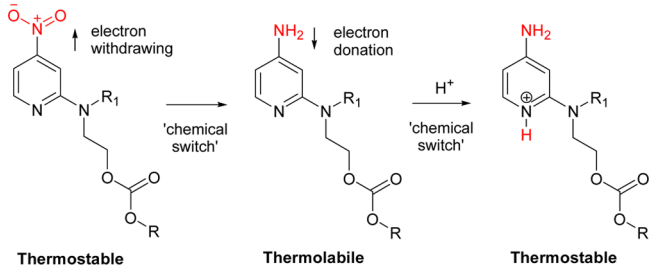
Selecting the proper reductant for nitro groups that would work efficiently in complex biological systems constitutes a great challenge. Such reductants as palladium-on-carbon,[19] or Raney nickel,[20] sodium hydrosulfite,[21] tin(II) chloride,[22] iron in acidic and alkaline media,[23] titanium(III) chloride,[24] zinc,[25] and samarium[26] are used in laboratory organic synthesis to obtain aromatic amines. However, it appears that the choice of a proper reductant that might be applied in biological systems may be based on metal complexes which can act as artificial enzymes.[27]

Here, we report on an efficient method for increasing or decreasing the stability of 2-pyridinyl TPGs (2-Py TPGs) at ambient temperature by switching a nitro into an amine group by reduction via titanium complexes.

RESULTS AND DISCUSSION

The rate of 2-Py TPG deprotection that occurs by thermocyclization mainly depends on the nucleophilic strength of pyridine nitrogen. The presence of the nitro group at position 4 of the pyridine ring results in lowered nucleophilicity (electron density) of pyridine nitrogen, which stops deprotection at ambient temperature and considerably contributes to their usefulness. However, switching the property may be possible by modulating the character of TPG using reduction of a nitro group (electron withdrawing group) into an amine group (electron donating group) (Scheme 2).

Scheme 2. "Chemical switch"—general idea



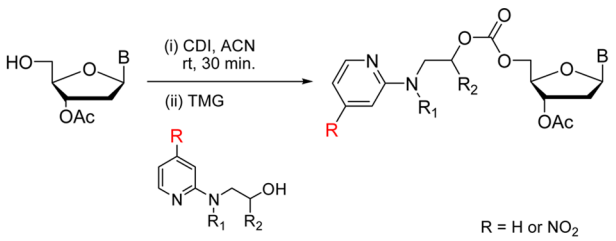
This "chemical switch" results in an increased nucleophilicity of the pyridine nitrogen and significantly accelerates thermocyclization. This transformation requires the application of a precise agent to reduce the nitro group into the amine group. Malinowski and co-workers[28] showed that low-valent titanium (LVT) is an efficient reagent to reduce the nitro-substituted pyridine and benzene derivatives with over 90% yield. Starting with this observation, we successfully applied a mixture of $TiCl_4$ with $LiAlH_4$ to selectively reduce the nitro group at the 2-pyridinyl ring. Moreover, under these conditions, the carbonate remains unchanged, allowing the TPG to retain its functionality.

A higher stability of TPGs with a nitro group across a wide range of temperatures was presented on the model compounds, 3′-*O*-acetyl nucleosides. Selection of nucleosides was dictated by two factors: (i) large potential application, (ii) multifunctionality which gives an opportunity to study the coexistence of various protecting groups in one molecule. Therefore, the 3′-*O*-acetyl thymidine with a free 5′-hydroxyl group was blocked by different TPGs: one with a nitro substituent **1**, and the second without a nitro substituent group **11**, which is known as unstable TPG (Scheme 3).[8a] The protected nucleosides were dissolved in anhydrous acetonitrile and kept at two different temperatures: 25 and 90 °C for 360 h and 120 min, respectively.[29]

As shown in Chart 1, incorporation of a nitro group into a 2-Py TPG increases its stability at both analyzed temperatures. The amount of blocked nucleosides **1** and **11** in the reaction mixture was calculated based on the RP-HPLC data obtained over the respective time periods. Nucleosides with a free hydroxyl group and bicyclic byproduct were observed as thermocyclization products. This observation suggests that the nitro group plays a key role as a stabilizing agent in a 2-PyTPG and almost completely blocks intramolecular cyclization[30] at

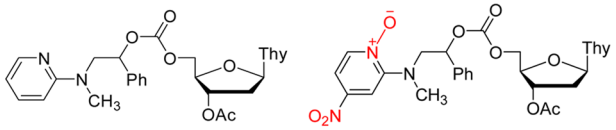
DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129–12136

Scheme 3. General Method of Synthesis and Structure of 3′-*O*-acetyl 5′-TPG-Protected Nucleosides



	B	R_1	R_2
1	Thy	CH_3	Ph
2	Cyt^{Bz}	CH_3	Ph
3	Ade^{Bz}	CH_3	Ph
4	Gua^{ibut}	CH_3	Ph
5	Thy	Bn	H

	B	R_1	R_2
6	Thy	CH_3	Ph
7	Cyt^{Bz}	CH_3	Ph
8	Ade^{Bz}	CH_3	Ph
9	Gua^{ibut}	CH_3	Ph
10	Thy	Bn	H



11 **12**

room temperature within a five-day measurement period. This increased stability is also noticeable at higher temperatures, but not as distinctly as at room temperature.

Increasing the stability of TPG by introducing a nitro group allows for easier manipulation at room temperature of the blocked TPG molecules, but its removal at higher temperatures requires prolonged heating.

The nitro group increases TPG stability, while the amine group has the opposite effect. Therefore, connecting these groups into an interdependent system (a quick switch of one into the other) enables control of the properties of the protecting group. The switch from a nitro into an amine group is possible in mild reduction conditions, such as titanium complexes. An effective reduction of the nitro group can be carried out with the use of black slurry produced from titanium(IV) chloride and $LiAlH_4$ in anhydrous tetrahydrofuran, in inert gas atmosphere

Under these conditions, due to slower decomposition and high activity of $LiAlH_4$, reduction is quantitatively completed within 15 min. Moreover, the reaction analysis has revealed that pH of the reduction mixture is very low (measured as 2), which has an impact on higher stability of carbonates (**6**–**10**). Malinowski and co-workers have observed that the LVT obtained from $TiCl_4/SnCl_2$ is a highly selective and mild reducing agent for nitro compounds, and it does not reduce carbonyl groups, unlike LVT attained from $TiCl_4/LiAlH_4$.

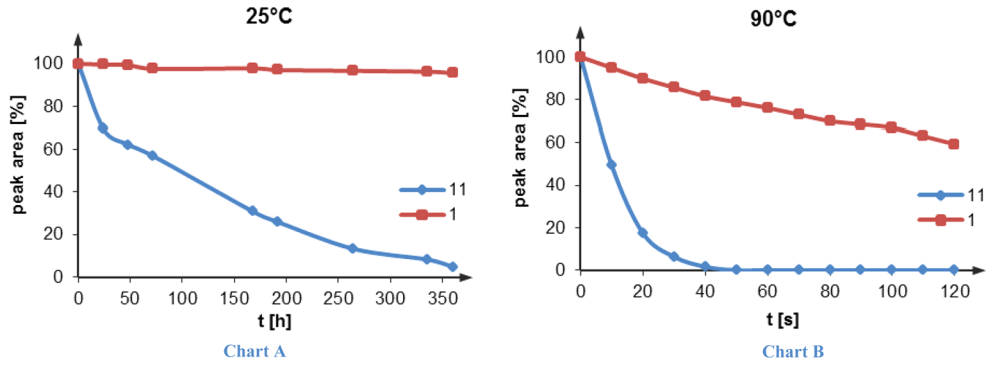
However, our research has proven that the reducing agent obtained from $TiCl_4/LiAlH_4$ (mole ratio: 1/0.8) effectively reduces a nitro group to a primary amine, even in the presence of functional groups such as a carbonate or ester (Chart 2).

During the formation of the amine group, charge distribution in the pyridine ring is changing, which enhances the nucleophilicity of the pyridine nitrogen. A change in electron distribution affects the susceptibility of TPG to thermal deprotection, which is more evident at higher temperatures. An example of using the switch is depicted in Chart 2. The effect of reduction is observed as the disappearance of **1** (chromatogram A) and the occurrence of a new mixture of diastereoisomers **6** (chromatogram B). After heating at 90 °C, within only 10 min, the TPG is completely removed from **6** and no traces of the blocked nucleoside are observed (chromatogram C). The presented HPLC data from subsequent transformations indicate that the deprotection mechanism is based on temperature-induced intramolecular cyclization whose rate is accelerated by changing the chemical nature of substituents.

Moreover, this mechanism is confirmed by the identification of thermal deprotection products, i.e., cyclic compound **14** and 3′-*O*-acetyl thymidine with a free hydroxyl group in 5′ position.

We have shown that the "chemical switch" on TPGs provides efficient hydroxyl protection for four common DNA-series nucleosides. The 4-nitro 2-Py TPG carbonates (**1**-**4**) were prepared from the new aminoalcohols obtained according to the literature procedure.[8a] Table 1 presents the results of removing the 2-Py TPG to reveal factors influencing thermal

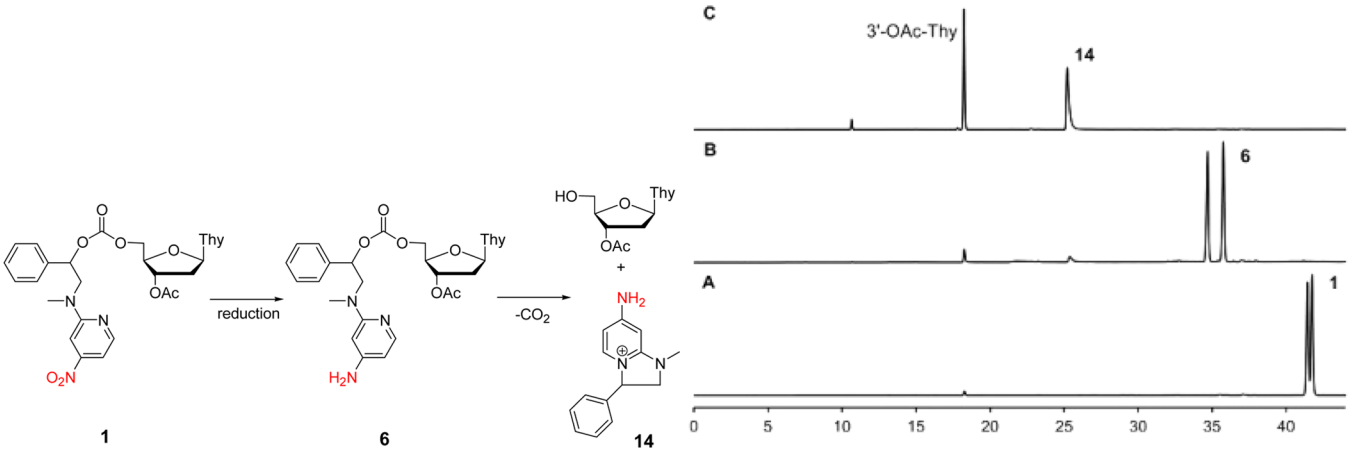
Chart 1. Comparison of the Thermal Stability of Two 2-Py TPGs, with (1) and without (11) Nitro Substituent[a]



[a]The HPLC data calculated as decreasing the amount of protected nucleosides **1** and **11**. A – The analysis was performed at room temperature (25°C) for 2 weeks. B – The analysis was performed at 90°C for 2 h. All measurements were taken in anhydrous acetonitrile.

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129–12136

Chart 2. HPLC Analysis of the "Chemical Switch" Approach in Nucleoside Protection[a]



[a](A) The nucleoside protected by the TPG with a nitro substituent. (B) After reduction with a titanium complex. (C) Thermolytic deprotection at 90 °C (8 min, buffer pH = 7).

Table 1. Percentage of TPG-Protected Nucleosides after Deprotection in Differ Solvents[a]

BEFORE REDUCTION										
	90°C, t = 45 minutes[b]					25°C, t = 240 hours[c]				
	1	2	3	4	5	1	2	3	4	5
CH_3CN	40.4%	50.8%	54.7%	44.3%	78.7%	79.5%	83.4%	42%	54.1%	87.5%
CH_3CN/H_2O	4.2%	1.3 %	0.9%	4.2%	2.2%	34.9%	42.9%	10.1%	42.7%	77.5%
CH_3CN/buffer, pH=7	6.8%	3.1%	0.7%	6.5%	0%	44.2%	30.9%	7.7%	51.4%	74.5%
AFTER REDUCTION										
	90°C, t = 5 minutes[b]									
	6	7	8	9	10					
CH_3CN/buffer, pH=7	6.0%	0.7%	0%	2.8%	8.3%					

[a]Analysis was performed both before and after reduction. [b]The deprotection time is showing the state when one of the nucleosides is fully deprotected. [c]In the case of 4-nitro derivatives at 25 °C, we have not observed total deprotection for any of nucleosides.

deprotection other than temperature or deprotection time. Deprotection times were selected to demonstrate complete deprotection of at least one of the presented blocked nucleosides.

We compared (Table 1) stability for **1**−**4** before reduction and **6**−**9** after reduction in various solvents at 90 °C. Our results indicate that 4-nitro-substituted 2-PyTPG in anhydrous acetonitrile is marked by high stability in the heating process independent of the used nucleosides. Moreover, we observed that stability in aqueous solutions is significantly lowered and also connected with the type of nucleosides. In the case of Cyt (**2**) and Gua (**4**) derivatives, faster deprotection can be explained by their higher basicity, which is particularly significant in aqueous solutions. The same relationship was also observed where the nitro group is switched into the amine group (Table 1). Even though using buffer (pH = 7) instead of water does not considerably affect the deprotection rate (Table 1), it can reduce the probability of unwanted alkaline hydrolysis. Our experimental data show that the deprotection rate is influenced not only by the nucleophilicity of the 2-pyridine nitrogen but also by the composition of the solvents in which heating occurs, as well as the character of the nucleoside used.

Introduction of the 4-nitro 2-Py TPG in compounds **1**−**4** and **6**−**9** (after reduction) leads to considerable disadvantages: the presence of a mixture of diastereoisomers and possible elimination of the benzyl proton during deprotection which could reveal a competitive way of removing the TPGs. In order to prevent this inconvenience, the phenyl group should be replaced, e.g., by hydrogen. Therefore, we prepared a 2-pyridinyl-2-(*N*-benzylamino)ethanol and its corresponding carbonate **5** following a standard procedure.[31] In the case of **5**, using a nitro group enhances the stability of TPGs, but not as significantly as in **1**−**4**.[32] This results in increased influence of the benzyl group on the nucleophilicity of the pyridine nitrogen and the steric effect that has been described earlier for substituents of this type.[30]

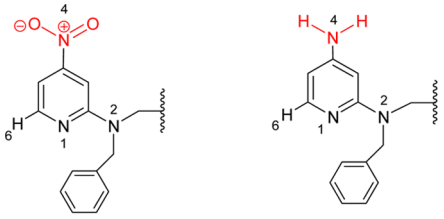
Reduction with $TiCl_4/LiAlH_4$ results in a decrease in the pH of the reaction mixture to 2. We noticed that nucleosides **6**−**10** obtained during reduction remain stable in thermal deprotection conditions in this pH value. On the basis of these two observations, we postulate protonation in the pyridine ring which can be another factor to stop thermal deprotection. However, changing the pH value to neutral and then basic leads to deprotonation and consequently initiates TPG thermal deprotection.[33]

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129−12136

These relations have proven that the change of pH is also important in modulation of the thermal deprotection process and is presumably related to protonation of a pyridine nitrogen. In other words, possible protonation of the nucleophilic center may hamper thermocyclization, which allows one to manipulate stability and control thermocyclization in certain pH values.

In order to determine how protonation occurs, we used two-dimensional $^{1}H—^{15}N$ NMR analysis of the 2-PyTPG before and after reduction Scheme 4. Proper assignments of nitrogen

Scheme 4. PKa Values for Precursor of 2PyTPG (Measurment Was Performed in Acetonitryle/H_2O)[a]



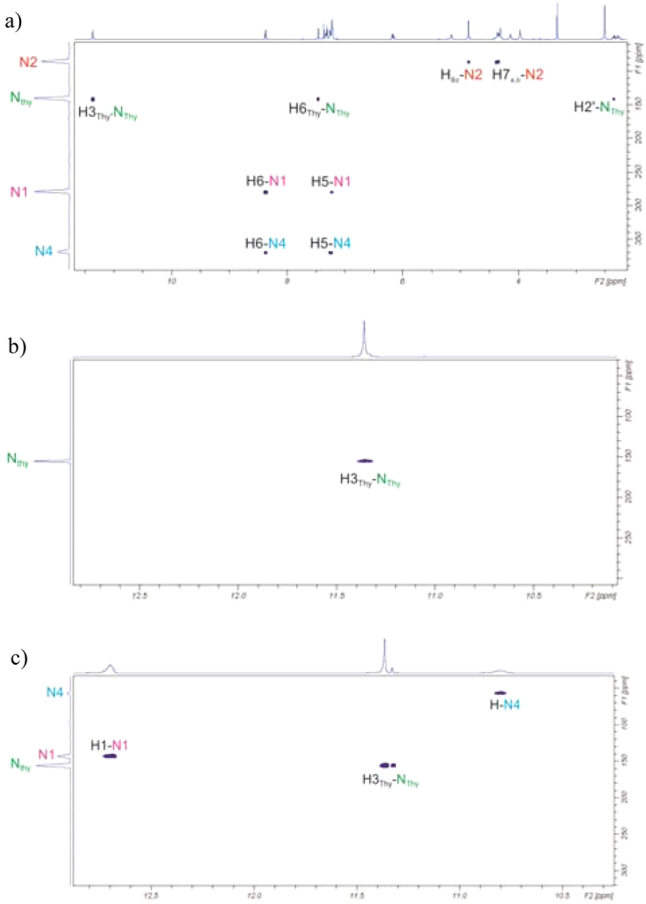
[a]Number of atoms for two-dimensional $^{1}H—^{15}N$ NMR descripted in Scheme 5.

resonances before reduction were performed following the analysis of the $^{1}H—^{15}N$ HMBC spectrum of **5** which is a model to present the usefulness of "chemical switch" and protonation (Scheme 5a). When **5** was completely reduced to **10**, aqueous HCl solution was added and the mixture was evaporated. Comparative analysis of the $^{1}H—^{15}N$ HMBC and HSQC NMR spectra (Scheme 5) for compounds **5** and **10** shows three observable changes in the chemical shifts of TPG protons: (1) signal of N1 is shifted downfield, (2) additional resonance of new hydrogen (δ = 12.5 ppm) and its corresponding cross-peak to the N1 (pyridine nitrogen) is observed, (3) appearance of signals attributed to the amino hydrogen correlated to N4. The correlations between H—N1 in HSQC spectra, as well as chemical shift differences of N1 (about 140 ppm) provided evidence for protonation.

This experimental proof of pyridine nitrogen protonation simultaneously decreases its nucleophilicity, which results in stabilization of the TPG. Since deprotection of the 4-amino 2-Py TPG does not occur in low pH, it should be triggered in increased pH. Consistent with this postulate, pH 4 is still too acidic to run thermal deprotection at higher temperatures, while increasing the pH to 6.5 is sufficient to totally eliminate protonation (heated for 7 min) and carry out thermal deprotection of 2-PyTPG (Schemes 3, 4, and S5 of the SI).[34]

On the basis of the discussion presented above, thermocyclization of a 2-pyridinyl TPG largely depends on the nucleophilicity of a nitrogen atom in a pyridine ring. However, electron distribution in a molecule does not always explain the rate of intramolecular deprotection.[35] Introduction of the 2-pyridinyl *N*-oxide (2-PNO) in the structure of a thermolabile protecting group might be an additional factor influencing thermocyclization because, according to the dipole-moment value, the oxygen atom in pyridine *N*-oxide is more nucleophilic than the pyridine nitrogen atom.[36] Therefore, the presence of *N*-oxide in TPGs should significantly accelerate thermocyclization, but no such relation was observed.[37] Impeding the formation of a 6-membered cyclic product which requires the high level of activation energy to be exceeded could have an

Scheme 5. 2D $^{1}H—^{15}N$ HMBC and HSQC NMR Analysis of Pyridine Nitrogen before and after Reduction



[a]$^{1}H—^{15}N$ HMBC spectra of compound **5**, depicts all correlations between nitrogen atoms and protons in molecule. [b]Enlarged region of $^{1}H—^{15}N$ HSQC spectra of **5**—before reduction. This spectrum shows one correlation between proton from thymidine and directly attached nitrogen from nucleobase. [c]Enlarged region of $^{1}H—^{15}N$ HSQC spectra of **10**—after reduction. This spectrum shows an additional correlation peaks between H1—N1 and H4—N4. This observation confirm a protonation of N1 and present of amine group NH_2 (N4) a result of reduction process.

influence on the high thermal stability of the 2-PNO TPG. This is noticeable in comparison with **11**, where a 5-membered product is generated more easily (90 °C, 15 min, aqueous conditions). Analysis of TPG thermolytic properties shows that nucleophilicity is required but not always decisive during thermal deprotection. Ring size has a considerable influence on the temperature necessary for thermocyclization, which has previously been shown in the case of thermal isomerization of cyclodienynes leading to corresponding benzocycloalkenes.[38]

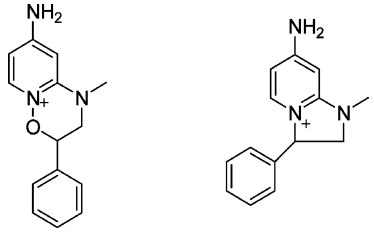
In the case of 2-PNO TPGs, thermocyclization is possible but needs additional support. Introduction of a nitro group to **11** and its "chemical switch" into an amine group reverses electron distribution in the molecule, which can result in thermal deprotection. Consequently, increasing the nucleophilicity of the *N*-oxide by means of an amine group has allowed the 6-membered ring to be obtained. Thus, it is assumed that the cyclic product was formed by a nucleophilic attack of an oxygen atom on carbon located at the carbonate. Such a reaction sequence has been deduced from the analysis of the

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129−12136

reaction mixture and the postulated structure of the byproduct (**13**).

The isolated cyclic byproducts **13** and **14** (Scheme 6) of thermocyclization were confirmed by UV−Vis and mass

Scheme 6. Thermocyclization Products—Possible Structures



13 **14**

analyses for **13** and MS, UV−Vis analysis, and NMR spectra for **14**. The presence of these compounds in the reaction mixture is a proof of the removal of TPGs by the intramolecular mechanism.

CONCLUSIONS

TPGs in the form of carbonates are easily incorporated into a molecule and thus protect hydroxyl groups with variable thermolability. Incorporation of a nitro group stabilizes TPGs across a wide range of temperatures and opens the way to modulate TPG properties, which also improves their potential application in organic chemistry. Thermolability steering is based on the "chemical switch" approach, i.e., selective reduction of a nitro group to an amine group by means of titanium complexes, which results in accelerated thermocyclization. The LVT was chosen as a reductant for the nitro group to generate low pH and preserve the carbonyl group in reduction conditions. Moreover, the highest importance for further applications is the fact that the pH manipulation provides the steering for TPG thermolability by protonation of a pyridine nitrogen.

The strong dependence between thermocyclization and pH values after reduction may have a very practical biological application. Since stabilizing 2-PyTPG by protonation is a very promising method for thermocyclization control, we are currently searching for cellular and molecular applications for the discussed approach, also using synthetic nucleic acids. The results of our future research will be the subject of subsequent reports.

EXPERIMENTAL SECTION

General Procedure (A) for Preparation of *N*-Protected-3′-*O*-acetyl-5′-(*N*-methyl-(4-nitropyridin-2-yl)]amino-1-phenylethyloxycarbonyl Nucleosides (1−4). 3′-*O*-Acetyldeoxynucleosides (0.1 mmol) were dissolved in dry acetonitrile (2 mL) and placed into the flask with a magnetic stirrer with 1′1′-carbonyldiimidazol (0.36 mM, 58.32 mg). The reaction was carried out for 30 min at room temperature and analyzed by TLC (ethyl acetate/*n*-hexane 9/1). Then *N*-methyl-(4-nitropyridin-2yl)-1-phenylaminoethyl-1-ol (0.1 mmol, 29.3 mg) and 1,1,3,3 -tetramethylguanidine (0.0008 mmol, 100 μL) were added. The resulting solution was allowed to stir for 2 h at room temperature. The reaction was analyzed by TLC analysis (dichloromethane/methanol 95:5). The solvent was evaporated under reduced pressure, and the resulting residue was purified by column chromatography over silica gel (DCM/MeOH) to obtain pure product (**1**-**4**, **11**).

*3′-O-acetyl-5′-O-[2-N-methyl-N-(4-nitropyridin-2-yl)]amino-1-phenylethyloxycarbonylthymidine (**1**).* Compound **1** was obtained from 3′-*O*-Acetyldeoxythymidine (0.1 mmol) with 82% (52 mg) yield following general procedure A. **^{1}H NMR** (400 MHz, DMSO-*d6*) δ (ppm) 11.37 (d, *J* = 6.3, 1H), 8.41−8.37 (m, 1H), 7.42−7.20 (m, 8H), 6.17−6.13 (m, 1H), 5.91−5.85 (m, 1H), 5.11−5.05 (m, 1H), 4.3−3.97 (m, 5H), 3.05 (s, 3H), 2.34−2.21 (m, 2H), 2.04 (s, 3H), 1.72 (s, 3H). **^{13}C NMR** (125 MHz, DMSO-*d6*) δ (ppm) 20.7, 35.3, 35.4, 37.4, 37.5, 54.3, 54.4, 67.2, 77.6, 80.7, 80.8, 83.9, 98.4, 103.7, 109.9, 126.3, 126.4, 128.3, 135.5, 137.4, 150.3, 153.6, 155.2, 159.3, 163.6, 169.9. **HRMS (ESI-q-TOF)** *m*/*z*: [M + H]$^+$ calcd. for $C_{27}H_{30}N_5O_{10}$ 584.1993; found 584.1988.

*N^4-Benzoyl-3′-O-acetyl--5′-O-[2-N-methyl-N-(4-nitropyridin-2-yl)]amino-1-phenylethyloxycarbonyl-2′-deoksycytidine (**2**).* Compound **2** was obtained from 3′-*O*-Acetyldeoxycytidine (0.1 mmol) with 79% (57 mg) yield following general procedure A. **^{1}H NMR** (400 MHz; DMSO-*d6*) δ (ppm) 11.28 (s, 1H), 8.38 (t, *J* = 5.63, 1H), 8.0 (m, 3H), 7.63 (m, 1H), 7.52 (m, 2H), 7.37 (m, 7H), 7.21 (m, 2H), 6.12 (t, *J* = 6.76, 1H), 5.88 (m, 1H), 5.14 (m, 1H), 4.26 (m, 3H), 4.02 (m, 2H), 3.05 (s, 3H), 2.27 (m, 1H), 2.05 (s, 3H). **^{13}C NMR** (100 MHz; DMSO-*d6*) δ 169.9, 167.3, 163.3, 159.3, 155.2, 154.2, 153.6, 150.3, 144.8, 137.4, 133.1, 132.7, 128.6, 128.4, 128.3, 126.3, 118.1, 103.6, 98.4, 96.3, 86.8, 81.8, 77.6, 73.7, 67.2, 55.9, 54.4, 40.0, 38.9, 37.6, 25.1, 20.7, 18.5. **HRMS (ESI-q-TOF)** *m*/*z*: [M + H]$^+$ calcd. for $C_{33}H_{33}N_6O_{10}$ 673.2258; found 673.2254

*N^6-Benzoyl-3′-O-acetyl--5′-O-[2-N-methyl-N-(4-nitropyridin-2-yl)]amino-1-phenylethyloxycarbonyl-2′-deoxyadenosine (**3**).* Compound **3** was obtained from 3′-*O*-Acetyldeoxyadenosine with 75% (56 mg) yield following general procedure A. **^{1}H NMR** (500 MHz, Cd_2Cl$_2$) δ (ppm) 9.03 (s, 1H), 8.74 (s, 1H), 8.37−8.34 (dd, *J* = 7.9, 5.8 Hz, 1H), 8.01 (d, *J* = 7.7, 2H), 7.65−7.62 (m, 1H), 7.56 (td, *J* = 7.6, 4.5, 2H), 7.43−7.31 (m, 5H), 7.20−7.16 (m, 2H), 6.56−6.52 (m, 1H), 5.94−5.91 (m, 1H), 5.41−5.38 (m, 1H), 4.43−4.31 (m, 3H), 4.09−3.92 (m, 2H), 3.06 (d, *J* = 7.3, 3H), 2.94−2.87 (m, 1H), 2.67−2.62 (m, 1H), 2.11 (s, 3H). **^{13}C NMR** (100 MHz; DMSO-*d6*) δ (ppm) 169.9, 169.6, 159.2, 155.2, 153.5, 151.92, 151.67, 150.50, 150.25, 142.9, 137.4, 130.3, 132.4, 128.5, 128.3, 126.3, 125.9, 103.6, 98.4, 83.7, 81.4, 77.5, 74.0, 67.2, 54.5, 37.5, 35.3, 20.7. **HRMS (ESI-q-TOF)** *m*/*z*: [M + H]$^+$ calcd. for $C_{34}H_{33}N_8O_9$ 697.2371; found 697.2363.

*N^2-isobutyryl-3′-O-acetyl-5′-O-[2-N-methyl-N-(4-nitropyridin-2-yl)]amino-1-phenylethyloxycarbonyl-2′-deoxyguanosine (**4**).* Compound **4** was obtained from 3′-*O*-Acetyldeoxyguanosine with 86% (56 mg) yield following general procedure A. **^{1}H NMR** (400 MHz, DMSO-*d6*) δ (ppm) 8.37 (m, 1H), 8.16 (s, 1H), 7.36 (m, 6H), 7.19 (m, 2H), 6.20 (t, *J* = 6.90, 1H), 5.81 (m, 1H), 5.25 (t, *J* = 7.64, 1H), 4.21 (m, 3H), 4.03 (m, 2H), 3.03 (d, *J* = 2.29, 3H), 2.84 (m, 1H), 2.05 (d, *J* = 3.21, 3H), 1.11 (m, 7H). **^{13}C NMR** (100 MHz, DMSO-*d6*) δ (ppm) 180.1, 169.8, 159.3, 155.2, 154.8, 153.7, 150.3, 148.55, 148.25, 137.4, 137.2, 128.3,126.8, 126.3, 120.3, 103.6, 98.4, 81.5, 77.6, 74.1, 67.1, 56.0, 54.5, 45.7,, 38.2, 37.5, 35.6, 34.8, 20.7, 18.8, 18.5. **HRMS (ESI-q-TOF)** *m*/*z*: [M + H]$^+$ calcd. for $C_{31}H_{35}N_8O_{10}$ 679.2476; found 679.2474.

*3′-O-acetyl-5′-O-[2-N-methyl-N-(pyridin-2-yl)]amino-1-phenylethyloxycarbonyl-thymidine (**11**).* Compound **11**[39] was obtained from 3′-*O*-acetylthymidine (0.1 mmol) and (±)-2-[*N*-Methyl-*N*-(pyridin-2-yl)amino]-1-phenylethanol (0.1 mmol, 28.9 mg) with 80% (54 mg) yield following general procedure A. **^{1}H NMR** (400 MHz, DMSO-*d6*) δ (ppm) 11.33 (bs, 1H), 8.11−8.08 (m, 1H), 7.53−7.32 (m, 7H), 6.62−6.55 (m, 2H), 6.21−6.13 (m, 2H), 5.88−5.83 (m, 1H), 5.12−5.08 (m, 1H), 4.35−3.9 (m, 5H), 2.92 (s, 3H), 2.33−2.2 (m, 2H), 1.73−17.2 (dd, *J* = 5.5, 0.9, 3H). **HRMS (ESI-q-TOF)** *m*/*z*: [M + H]$^+$calcd. for $C_{27}H_{31}N_4O_8$ 539.2142; found 539.2136.

*3′-O-acetyl-5′-O-[2-N-benzyl-N-(4-nitropyridin-2-yl)]-aminoethyloxycarbonylthymidine (**5**).* *N*-benzylethanolamine (81.9 mg, 0.30 mmol) and 1,1′-carbonyldiimidazole (58.3 mg, 0.36 mmol) were added to a anhydrous acetonitrile (3 mL) in a dried round-bottom flask. The reaction mixture was stirred at room temperature (60 min), in inert atmosphere, until starting material was no observed by thin layer chromatography. Then 3′-*O*-acetylthymidine (78.9 mg, 0.3 mmol) and 1,1,3,3-tertramethylguanidine (0.0008 mmol, 150 μL) were added. Reaction was carried out at room temperature for 2 h.

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129−12136

Afterward the solution was concentrated, under reduced pressure, and applied to the top of a silica gel (15 g) chromatography column, equilibrated in CH_2Cl_2. The column was eluted with a linear gradient of CH_2Cl_2 (100%) to CH_2Cl_2: MeOH (95:5) to afford pure **5** (129 mg, 80%). **^{1}H NMR** (500 MHz, DMSO-*d6*) δ (ppm) 11.36 (s, 1H), 8.37 (d, J = 5.4 Hz, 1H), 7.46 (s, 1H), 7.36 (s, 1H), 7.31 (t, J = 7.4 Hz, 2H), 7.26−7.21 (m, 5H), 6.16 (dd, J = 8.0, 6.5 Hz, 1H), 5.16−5.15 (m, 1H), 4.86 (s, 2H), 4.38−4.30 (m, 2H), 4.13 (dd, J = 7.6, 4.6 Hz, 1H), 3.96 (t, J = 5.3 Hz, 2H), 2.36−2.31 (m, 1H), 2.26 (ddd, J = 14.2, 6.1, 2.4 Hz, 1H), 2.06 (s, 3H), 1.73 (s, 3H). **^{13}C NMR** (125 MHz, DMSO-*d6*) δ (ppm) 169.9, 163.5, 159.2, 155.3, 154.2, 150.3, 137.6, 135.6, 128.6, 128.3, 127.0, 126.5, 109.9, 104.1, 98.7, 84.0, 80.8, 73.6, 67.2, 65.5, 51.5, 47.0, 35.4, 20.7, 12.0. **HRMS (ESI-q-TOF)** m/z: $[M + H]^+$ calcd. for $C_{27}H_{30}N_5O_{10}$ 584.1993; found 584.1984.

General Procedure (B) for Generating of the Reducing Agent. Anhydrous tetrahydrofuran was placed in a two-neck round-bottom flask and cooled to 0 °C in water/ice bath. Then the titanium tetrachloride (0.5 mmol, 55 μL) was added portionwise and kept for 10 min at room temperature. Lithium aluminum hydride (0.35 mmol, 13 mg) was added portionwise, and the resulting black slurry was stirred in inert atmosphere, at room temperature for 15 min ($TiCl_4$/$LiAlH_4$ − 1/0.8).

General Procedure for *N*-Protected-3′-*O*-acetyl-5′-(*N*-methyl-(4-aminopyridin-2-yl)]aminoethyloxycarbonyl Nucleosides (6−10). Compounds (**1**−**4**) (0.1 mmol), diluted in a anhydrous THF, was added portionwise at 0 °C to the stirred black slurry of titanium reagent. The reaction mixture was stirred at room temperature for 15−30 min, until the starting material was not observed by TLC. Traces of $LiAlH_4$ were decomposed with water, the black slurry was centrifuged, and the solution was flushed through thin layer of silica gel (DCM/MeOH 95:5).

*3′-O-acetyl-5′-O-[2-N-methyl-N-(4-aminopyridin-2-yl)]amino-1-phenylethyloxycarbonyl-thymidine (**6**).* Compound **1** was transformed into compound **6** with 68% (38 mg) yield following general procedure B. **^{1}H NMR** (400 MHz, DMSO) δ (ppm) 11.34 (d, J = 6.8, 1H), 11.03 (bs, 2H), 8.92 (d, J = 5.4, 1H), 8.13−8.09 (m, 1H), 7.53 (d, J = 3, 1H), 7.36−7.28 (m, 6H), 6.08 (dd, J = 12.3, 6.1, 1H), 5.84−5.79 (m, 1H), 5.02 (dd, J = 16.1, 6.5, 1H), 4.27−4.04 (m, 5H), 2.41−2.29 (m, 1H), 2.16−2.14 (m, 1H), 1.98 (d, J = 1.8, 3H), 1.66 (s, 3H), 1.22 (d, J = 6.4, 3H). **^{13}C NMR** (100 MHz, DMSO-*d6*) δ (ppm) 12.5, 21.2, 35.8, 37.8, 54.7, 67.6, 73.8, 78.1, 81.3, 84.5, 99.0, 104.2, 110.4, 126.9, 18.1, 129.0, 137.8, 136.4, 141.7, 146.3, 150.8, 155.6, 159.6, 164.1, 170.4. **HRMS (ESI-q-TOF)** m/z: $[M + H]^+$ calcd. for $C_{27}H_{32}N_5O_8$; 554.2251, found 554.2250.

*N^4-Benzoyl-3′-O-acetyl-5′-O-[2-N-methyl-N-(4-aminopyridin-2-yl)]amino-1-phenylethyloxycarbonyl-2′-deoksycytidine (**7**).* Compound **2** was transformed into compound **7** following general procedure B. **HRMS (ESI-q-TOF)** m/z: $[M + H]^+$ calcd. for $C_{33}H_{35}N_6O_8$; 643.2516; found 643.2517.

*3′-O-acetyl-5′-O-[2-N-benzyl-N-(4-aminopyridin-2-yl)]-aminoethyloxycarbonyl-thymidine (**10**).* Compound **5** (84 mg, 0.14 mmol) was reduced following the general procedure. To the obtained precipitate free solution aq 12 M HCl (1 equiv) was added, and the solvent was evaporated under reduced pressure. Crude product was diluted in methanol, applied on the PLC and eluted with iPrOH: H_2O (7:3) at 4 °C to afford product **10** (58 mg, 74%). **^{1}H NMR** (500 MHz, MeO*d*) δ (ppm) 7.45−7.44 (m, 1H), 7.32 (t, J = 7.5 Hz, 2H), 7.24(t, J = 7.4 Hz, 1H), 7.17 (d, J = 7.2 Hz, 2H), 6.25 (dd, J = 7.2, 2.0 Hz, 1H), 6.18 (dd, J = 8.4, 5.9 Hz, 1H), 5.95 (d, J = 2.0 Hz, 1H), 5.17 (dt, J = 6.5, 2.3 Hz, 1H), 4.72 (s, 2H), 4.42−4.35 (m, 2H), 4.32 (dd, J = 11.8, 3.3 Hz, 2H), 4.20−4.18 (m, 1H), 3.85 (dd, J = 10.7, 5.7 Hz, 2H), 2.37 (ddd, J = 14.3, 5.9, 2.2 Hz, 1H), 2.29−2.26 (m, 1H), 2.06 (s, 3H), 2.00 (s, 3H). **^{13}C NMR** (100 MHz, MeO*d*) δ (ppm) 172.1, 166.2, 161.2, 156.0, 153.7, 152.2, 137.9, 137.3, 136.5, 130.1, 128.9, 127.4, 111.9, 104.3, 89.8, 86.7, 83.5, 75.6, 68.7, 62.9, 56.3, 54.2, 30.7, 20.9, 12.9. **HRMS (ESI-q-TOF)** m/z: $[M + H]^+$ calcd. for $C_{27}H_{32}N_5O_8$ 554.2251, found 554.2245.

*O-5′-(3′-O-acetylthymidinylcarbonyloxy-1-phenylethyl)(methyl)amino)-4-nitropyridine 1-Oxide (**12**).* Compound **12** was obtained from 3′-*O*-acetylthymidine (0.3 mmol, 87.9 mg) and (±)-2-[*N*-methyl-*N*-(4-nitro-2-pyridyl-*N*-oxide)amino]-1-phenylethanol (0.3 mmol, 86.73 mg) with 77% (139 mg) yield following general procedure B. **^{1}H NMR** (400 MHz, DMSO-*d6*) δ (ppm) 8.26 (d, J = 7.2, 1H), 7.64 (m, J = 7.2, 1H), 7.56 (dd, 1H), 7.35 (m, 6H), 6.16 (m, 1H), 5.93 (m, 1H), 5.09 (m, 1H), 4.35 (m, 1H), 4.23 (m, 2H), 4.05 (m, 1H), 3.9 (m, 1H), 3.08 (s, 3H), 2.30 (m, 2H), 2.1 (m, 3H), 1.74 (dd, J = 1.2, 7.0, 3H). **^{13}C NMR** (100 MHz, DMSO-*d6*) δ (ppm) 170.0, 163. 6, 159.3, 155.2, 153.6, 150.4, 142.6, 140.7, 137.3, 135.7, 128.5, 128.3, 126.1, 110.9, 109.9, 108.7, 84.0, 83.6, 80.8, 78.2, 74.7, 73.4, 67.2, 53.9, 35.4, 20.7, 12.0. **HRMS (ESI-q-TOF)** m/z: $[M + H]^+$ calcd. for $C_{27}H_{30}N_5O_{11}$ 600.1942; found 600.1939.

*8-Amino-1-methyl-3-phenyl-2,3-dihydro-1H-pyrido[1,2-b][1,2,4]-oxadiazin-5-ium Chloride (**13**).* **HRMS (ESI-q-TOF)** m/z: $[M]^+$ calcd. for $C_{14}H_{16}N_3O^+$ 242.1288; found 242.1309.

*7-Amino-1-methyl-3-phenyl-2,3-dihydro-1H-imidazo[1,2-a]-pyridin-4-ium Chloride (**14**).* **^{1}H NMR** (400 MHz, DMSO) δ (ppm) 7.71 (s, 2H), 7.47−7.35 (m, 5H), 6.18 (d, J = 7.2 Hz, 1H), 5.91 (s, 1H), 5.72 (dd, J = 9.3, 6.8, 1H), 4.17 (t = , J = 9.7 Hz, 1H), 3.67 (dd, J = 9.8, 6.7 Hz, 2H), 2.96 (s, 3H). **HRMS (ESI-q-TOF)** m/z: $[M]^+$ calcd. for $C_{14}H_{16}N_3^+$ 226.1339; found 226.1339.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02033.

Materials, general methods, characterization of deprotection rates and NMR and HRMS data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +48618528503; e-mail: maro@ibch.poznan.pl (M.K.C.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank Professor Jacek Stawiński (IChB PAS) for reading this manuscript and for his helpful suggestions. This research was supported by the European Regional Development Fund within the Innovative Economy Program (Grant No. POIG.01.03.01-30-045). This publication was supported by the Polish Ministry of Science and Higher Education, under the KNOW program.

■ REFERENCES

(1) Keasling, J. D. *ACS Chem. Biol.* **2008**, *3*, 64−76.

(2) Grajkowski, A.; Pedras-Vasconcelos, J.; Wang, V. V.; Ausin, C.; Hess, S.; Verthelyi, D.; Beaucage, S. L. *Nucleic Acids Res.* **2005**, *33*, 3550−3560.

(3) Lebedev, A. V.; Paul, N.; Yee, J.; Timoshchuk, V. A.; Shum, J.; Miyagi, K.; Kellum, J.; Hogrefe, R. I.; Zon, G. *Nucleic Acids Res.* **2008**, *36*, 131.

(4) Anastas, P. T.; Kirchhoff, M. M. *Acc. Chem. Res.* **2002**, *35*, 686−694.

(5) (a) Wilk, A.; Grajkowski, A.; Philips, L. R.; Beaucage, S. L. *J. Org. Chem.* **1999**, *64*, 7515−7522. (b) Prokopczyk, B.; Bertinato, P.; Hoffman, D. *Carcinogenesis* **1988**, *9*, 2125−2128.

(6) (a) Wilk, A.; Chmielewski, M. K.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *Tetrahedron Lett.* **2001**, *42*, 5635−5639. (b) Grajkowski, A.; Wilk, A.; Chmielewski, M. K.; Phillips, L. R.; Beaucage, S. L. *Org. Lett.* **2001**, *3*, 1287−1290. (c) Wilk, A.; Chmielewski, M. K.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **2002**, *67*, 6430−6438.

(7) (a) Cieślak, J.; Grajkowski, A.; Livengood, V.; Beaucage, S. L. *J. Org. Chem.* **2004**, *69*, 2509−2515. (b) Kiuru, E.; Ahmed, Z.; Lönnberg, H.; Beigelman, L.; Ora, M. *J. Org. Chem.* **2013**, *78*, 950−959.

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129−12136

(8) (a) Chmielewski, M. K.; Marchan, V.; Cieslak, J.; Grajkowski, A.; Livengood, V.; Munch, U.; Wilk, A.; Beaucage, S. L. *J. Org. Chem.* **2003**, *68*, 10003−10012. (b) Marchán, V.; Cieślak, J.; Livengood, V.; Beaucage, S. L. *J. Am. Chem. Soc.* **2004**, *126*, 9601−9610.
(9) Ohkubo, A.; Kasuya, R.; Miyata, K.; Tsunoda, H.; Seio, K.; Sekine, M. *Org. Biomol. Chem.* **2009**, *7*, 687−694.
(10) Grajkowski, A.; Cieslak, J.; Chmielewski, M. K.; Marchan, V.; Phillips, L. R.; Wilk, A.; Beaucage, S. L. *Ann. N. Y. Acad. Sci.* **2003**, *1002*, 1−22.
(11) Chmielewski, M. K. *Org. Lett.* **2009**, *11*, 3742−3745.
(12) Ratajczak, T.; Chmielewski, M. K. *J. Org. Chem.* **2012**, *77*, 7866−7872.
(13) Kocienski, P. J. *Protecting Groups*, 3rd ed.; Georg Thieme Verlag: New York, 2005.
(14) Beaucage, S. L.; Chmielewski, M. K. Patent No US 7,612,197 B2 Nov 3, 2009.
(15) Wackerlin, C.; Chylarecka, D.; Kleibert, A.; Muller, K.; Nolting, F.; Jung, T. A.; Ballav, N. *Nat. Commun.* **2010**, *1*, 1−7.
(16) Liu, H.; Schmidt, J. J.; Bachand, G. D.; Rizk, S. S.; Looger, L. L.; Hellinga, H. W.; Montemagno, C. D. *Nat. Mater.* **2002**, *1*, 173−177.
(17) Vishnuvardhan, V.; Kala, R.; Rao, T. P. *Anal. Chim. Acta* **2008**, *623*, 53−58.
(18) Wettig, S. D.; Bare, G. A.; Skinner, R. J. S.; Lee, J. S. *Nano Lett.* **2003**, *3*, 617−622.
(19) Bavin, P. M. G. *Org. Synth.* **1960**, *40*, 5.
(20) Allen, C. F. H.; VanAllan, J. *Org. Synth.* **1942**, *22*, 9.
(21) Scheuerman, R. A.; Tumelty, D. *Tetrahedron Lett.* **2000**, *41*, 6531−6535.
(22) Jiang, Q.; Jiang, D.; Jiang, Y.; Fu, H.; Zhao, Y. *Synlett* **2007**, *12*, 1836−1842.
(23) Awad, W. I.; Hassan, S. S. M; Zaki, M. T. M. *Anal. Chem.* **1972**, *44*, 911−915.
(24) Ho, T. L.; Wong, C. M. *Synthesis* **1974**, *1*, 45−45.
(25) Khan, F. A.; Dash, J.; Sudheer, C.; Gupta, R. K. *Tetrahedron Lett.* **2003**, *44*, 7783−7787.
(26) Basu, M. K. *Tetrahedron Lett.* **2000**, *41*, 5603−5606.
(27) Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075−1077.
(28) Malinowski, M.; Kaczmarek, Ł.; Rozpłoch, F. *J. Chem. Soc., Perkin Trans. 2* **1991**, *6*, 879−883.
(29) In the SI, the full data was presented (Tables S1−S3; page S3)
(30) Preliminary laboratory tests show increased stability in ambient temperature TPG former from *N*-oxide derivatives.
(31) Chmielewski, M. K. *Tetrahedron Lett.* **2012**, *53* (6), 666−669.
(32) The charts with kinetic in different temperature and different solvent mixture was placed in the SI on page S6.
(33) In the SI, pH influence on the stability of TGO is presented (Scheme S3; page S5).
(34) In the SI, pH influence on stability of TGO is presented (Scheme S4; page S9).
(35) Chmielewski, M. K.; Tykarska, E.; Markiewicz, W. T.; Rypniewski, W. R. Engineering N-(2-pyridyl)aminoethyl alcohols as potential precursor of thermolabile protection groups. *New J. Chem.* **2012**, *36* (3), 603−612.
(36) Higher dipole-moment [4.37 D (PNO) vs2.03 D (pyridine)]-much weaker base[pK_a= 0.79 (PNO) vs pK_a= 5.2 (pyridine)].
(37) Initial experiments based mainly on the HPLC analysis. The obtained results suggested reinforcing nucleophilicity and introducing the "chemical switch" discussed herein.
(38) Hopf, H.; Krüger, A. *Chem. - Eur. J.* **2001**, *7*, 4378−4385.
(39) Chmielewski, M. K.; Marchan, V.; Cieslak, J.; Grajkowski, A.; Livengood, V.; Munch, U.; Wilk, A.; Beaucage, S. L. *J. Org. Chem.* **2003**, *68*, 10003−10012.

DOI: 10.1021/acs.joc.5b02033
J. Org. Chem. 2015, 80, 12129−12136