JOC The Journal of Organic Chemistry

Article

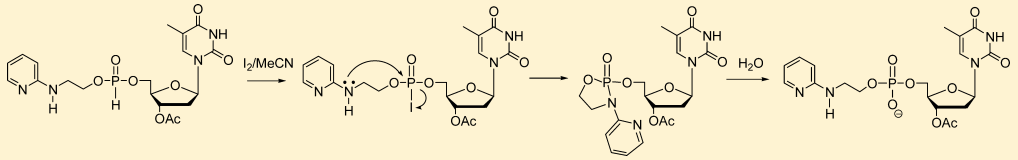
pubs.acs.org/joc

Oxidation of H-Phosphonates with Iodine by Intramolecular Support of a 2-Pyridyl Thermolabile Protecting Group

Tomasz Ratajczak and Marcin K. Chmielewski*

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

Supporting Information



ABSTRACT: Acceleration of H-phosphonate diester oxidation with iodine accompanied by a thermolabile protecting group (TPG) is presented. It is shown that the intermediate product of this reaction is an oxazaphospholidine oxide which forms a phosphate diester only when a 2-pyridyl TPG is applied. The intermediate product is formed with exocyclic nitrogen. The absolute configurations of phosphorothioate diesters, H-phosphonate diesters, and oxazaphospholidine oxides were determined. ^{31}P NMR spectroscopy was used to evaluate the relationship between chemical shift and absolute configuration at the phosphorus center of H-phosphonate diesters and oxazaphospholidine oxides.

INTRODUCTION

Protecting groups used in organic chemistry more and more frequently find multifunctional applications. Apart from protecting reactive sites, they may function as fluorophores,[1] as markers for monitoring the reaction progress[2,3] and changes in solubility, or as influencing molecule conformation changes.[4] Moreover, the protecting groups can affect the stereospecific character of chemical reactions. What is significant in nucleic acid chemistry is the stereospecific reactions on the phosphorus center to produce phosphorothioates.[5] It has been found recently that five-membered phospholidine rings are useful in controling this stereospecificity of the phosphorus center.[6,7]

Another tendency in developing protecting groups is applying mild and simple physicochemical processes, such as oxidation,[8] protonation,[9] reduction,[10,11] photosensitivity,[12] and temperature change. The last process has recently led to the introduction of thermolabile protecting groups (TPGs) in order to enhance effective protection of a phosphate,[13,14] hydroxyl,[15] or amine center.[16] Removal of these groups is based on intramolecular cyclization depending on temperature.[14]

Examples of TPGs presented in the literature show that introducing a 2-pyridyl moiety in a TPG significantly accelerates thermocyclization at temperatures over 50 °C[15] but at the same time increases its stability at ambient temperature.[17] So far these groups have been applied to protect a phosphate or hydroxyl center.[18]

2-Pyridyl TPGs are characterized by various thermocyclization times depending on their structure, and the times are shorter when the same structure is applied to protect phosphate, as compared to hydroxyl. However, it has been found that phosphate triesters with some TPGs are very unstable.[19]

To enhance their stability on a phosphate center, a "click-clack" approach has been applied.[20] The approach increases 2-pyridyl TPG stability by temporarily forming a five-membered oxazaphospholidine ring. A linear form of the TPG may be easily recovered by acid hydrolysis, during which also an H-phosphonate diester is formed (Figure 1). The removal of a TPG is now very simple in the thermocyclization process and takes about 10 min at 90 °C to form an H-phosphonate monoester.

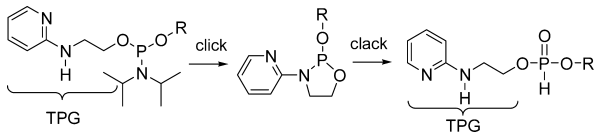
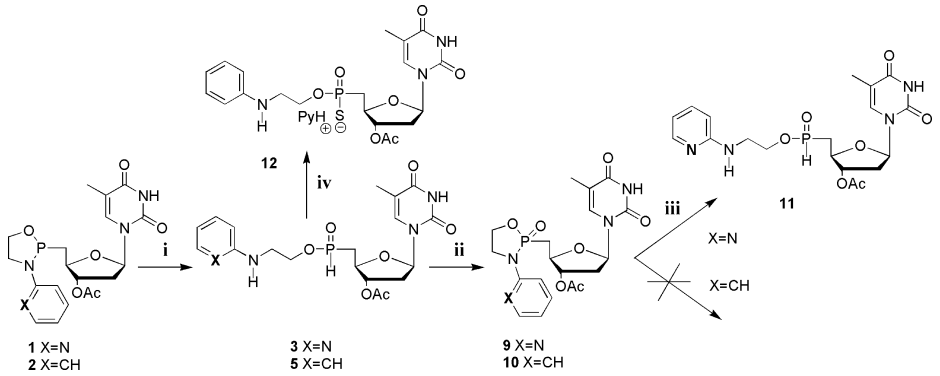


Figure 1. Strategy of the "click-clack" approach: closing and opening the oxazaphospholidine ring.

However, H-phosphonate diesters also are easily oxidized to the corresponding phosphates by iodine in the presence of a base[21,22] such as pyridine or lutidine[25] or an acid[23] but acid-catalyzed oxidation is complicated by some diester hydrolysis.[21] Oxidation of an H-phosphonate diester in the presence of a base such as pyridine involves a multistep mechanism: first, a phosphoroiodidate derivative is formed quickly[24] and then the transformation of phosphoroiodidate into a pyridinium cation[25,26] that is easily hydrolyzed is postulated.

Received: May 21, 2012
Published: August 21, 2012

Scheme 1. Conversion of Cyclic Rings in the Linear Form[a]



[a]Legend: (i) benzylthiotetrazole (BTT)/acetonitrile; (ii) I_2/acetonirile; (iii) H_2O.

RESULTS AND DISCUSSION

The present paper shows the possibility of oxidizing H-phosphonate diesters which contain a 2-pyridyl TPG to phosphate by using only an iodine solution. In this case we are taking advantage of the nucleophilic and basic properties of the TPG to perform intramolecular catalysis of the oxidation reaction. We have also shown that this oxidation mechanism forms an intermediate product (oxazaphospholidine oxide) which can be easily opened by means of water when a 2-pyridyl TPG is used (Scheme 1).

We have observed that adding iodine in acetonitrile to the present H-phosphonate diesters (see Figure 2) accelerates

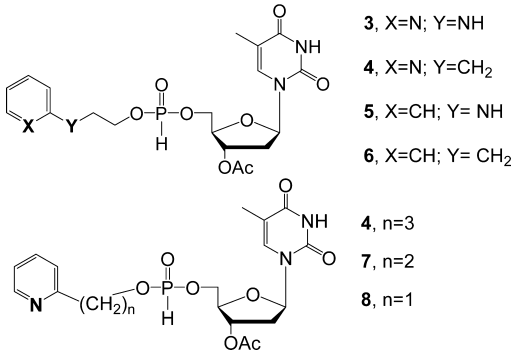


Figure 2. Structure of H-phosphonate diesters used in oxidation without pyridine.

oxidation from 160 min for **5** to 15 min for **3**. However, applying the same reaction conditions in the case of **6** (without nitrogen atoms) gives a phosphate diester within 5 h.[27] Differences in oxidation times (Table 1) depend on the nucleophility of the nitrogens and their distance from the phosphate center. The fastest oxidation process (15 min) occurs in the case of H-phosphonate **3** when a 2-pyridyl TPG is applied. Decreasing the nucleophilicity slows down the oxidation (Table 1).

Table 1. ^{31}P NMR Analysis of H-Phosphonate Diesters under Oxidation Conditions without an Additional Base

	3	4	5	6	7	8
time required for complete conversn to phosphate diester, min	<15[a]	150	160[a]	300	90	50

[a]The reaction stops in the form of phospholidine oxide (**10**).

On the basis of these results we have also postulated that the 2-pyridyl moiety from a TPG catalyzes oxidation with the iodine solution. It has been observed that a cyclic intermediate product is formed in oxidation only when the exocyclic nitrogen atom is present (**3**, **5**).[28] Our study shows that the 2-pyridyl TPG, and particularly the exocyclic nitrogen atom, is involved in a five-membered ring with a phosphate center after substituting the hydrogen atom by iodine. The oxazaphospholidine oxide **9** formed from the 2-pyridyl TPG opens easily in the presence of water. The rate of this process is about 1 order of magnitude faster than intramolecular ring closing. The formation of the oxazaphospholidine ring in the intermediate product is proof of the catalytic character of oxidation with iodine. In order to study the mechanism further, we have used model compound **5**, in which the pyridyl ring is replaced by phenyl. This mechanism is similar to that for **3**, and the formed oxazaphospholidine oxide **10** does not open as easily as does **9** under the same conditions. The oxazaphospholidine oxide **10** is stable,[29] and its opening requires conditions such as strong bases or acids.[30] Thus, the intermediate product **10** is isolated and characterized by NMR analysis. Oxidation of the mixture of **2** (oxazaphospholidine ring) and **5** (H-phosphonate diester) using iodine and pyridine gives the same product: oxazaphospholidine oxide **10** (see Figure 3).

These results confirm that the presence of the exocyclic nitrogen atom supports the formation of a five-membered ring. However, in **4** (only with the pyridyl ring) the formation of an intermediate cyclic ring is not observed in oxidation because it is limited by two factors:

(i) The only nitrogen atom of the pyridyl ring is too distant from the phosphate center (a seven-membered ring must be formed). However, shortening the distance will cause conformation restrictions in a potential cyclic ring.

(ii) The pK_a of pyridine is lower than that of 2-aminopyridine.[31]

It has been observed that the oxidation process accelerates when the distance between pyridine and phosphate is smaller. Reducing this distance (**7**, **8**) shortens the time of total oxidation (Table 1), but it is not substantial enough to indicate that it follows the intramolecular process. What we are probably observing here is an intramolecular interaction or increasing electron influence caused by pyridine. Even if the chain is only one carbon atom **8**, the acceleration is not as rapid as in **3**.

dx.doi.org/10.1021/jo300937k | J. Org. Chem. 2012, 77, 7866−7872

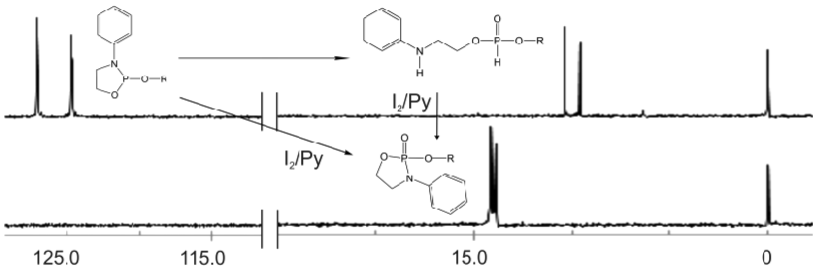


Figure 3. Application of the traditional oxidation method (iodine/pyridine) to produce an oxazaphospholidine oxide ring and the structure confirmation thereof. Oxidation was performed on the mixture of **2** and **5**. The upper ^{31}P NMR profile presents a mixture of **2** and **5**, and the lower ^{31}P NMR profile presents the product after oxidation with a traditional iodine/pyridine solution.

While studying the phosphate cyclic system mechanisms, it is important to assign absolute configuration to the H-phosphonate center. It is known that transforming H-phosphonate diesters into phosphorothioate diesters is a stereospecific reaction and occurs with retention of configuration. Thanks to this property as well as the fact that phosphodiesterase from snake venom stereospecifically digests only one diester (isomer R_P in the case of 5′-*O*-phosphorothioate-*O*-*p*-nitrophenyl ester[32] or phosphorothioate dinucleosides[33]), an absolute configuration may be assigned to the phosphate center. The transformation of H-phosphonate diesters into phosphorothioate diesters and the result of the digestion thereof (after removing the 3′-acetyl group) have been observed by ^{31}P NMR. As shown in Figure 4, one P-

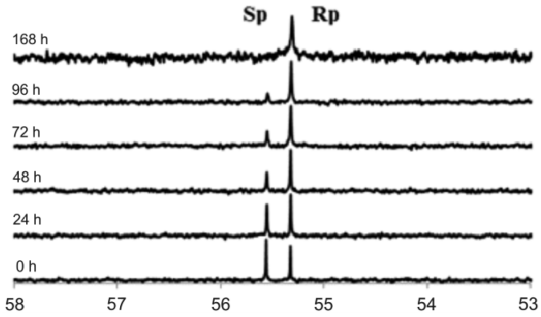
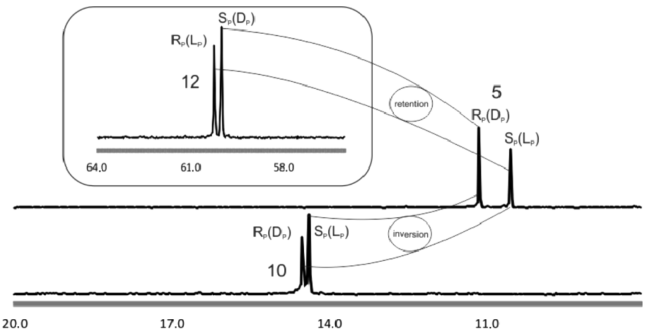


Figure 4. ^{31}P NMR analysis of thiophosphate **13** digestion by snake venom phosphodiesterase from *Crotalus atrox* (EC 3.1.4.1). To increase the solubility in buffer, the 3′-acetyl group has been removed. A shift of mutual positions of the major and minor isomers in relation to **12** is observed.

diastereoisomer (55.6 ppm, major isomer) has been digested by enzyme in a buffer solution. However, in this case, the absolute configuration of the digested diester changes from R_P to S_P because of another priority of substitutes in the Cahn–Ingold–Prelog convention. Because sometimes there are such discrepancies between absolute configurations in P-nucleotide analogues and physicochemical or biological properties, the precise names of phosphorus configurations designated as D_P and L_P have been introduced recently.[34] Thus, for the sake of clarity, the absolute configuration in phosphorothioate diesters **12** has been assigned: hence, the S_P (D_P) configuration of the major isomer to the signal at 59.99 ppm and the R_P (L_P) configuration of the minor isomer at 60.24 ppm. As shown in Scheme 2, the H-phosphonate diesters are disproportional, which helps to determine the absolute configuration in subsequent transformations.

Scheme 2. Assignment of Configuration on the Basis of ^{31}P NMR Spectra[a]



[a]Transformations were performed using the 3′-acetylated derivative of deoxythymidine.

Analyzing the transformation of minor and major thiophosphate isomers, the conformation of H-phosphonate diesters **5** can be defined as R_P (D_P) configuration of the major isomer at 11.11 ppm and S_P (L_P) configuration of the minor isomer at 10.51 ppm (Scheme 3).

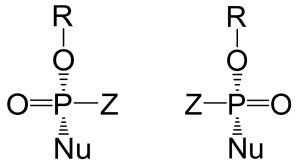
The correlation method has been also used to assign the configuration in oxazaphospholidine oxide derivatives **10** (S_P (L_P) minor isomer 14.12 ppm and R_P (D_P) major isomer 13.89 ppm).

CONCLUSION

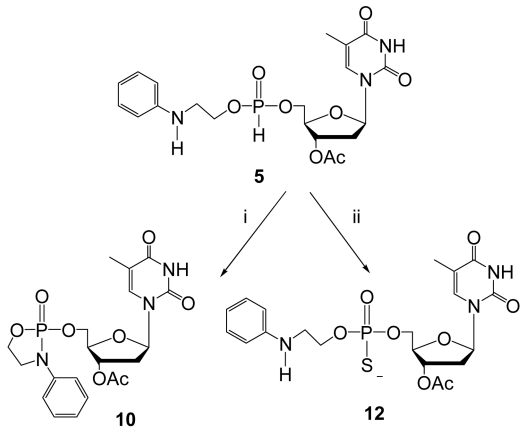
The paper presents the application of a 2-pyridyl TPG which intramolecularly catalyzes oxidation of H-phosphonate diesters with iodine only. We have shown that the nitrogen atom from the TPG may substitute iodine, forming an oxazaphospholidine oxide ring. However, forming a phosphate diester in this reaction is possible only when a 2-pyridyl TPG is applied. Moreover, the process is very fast and is quantitatively complete within 15 min. This method is an example of the multifunctional character of TPGs where deprotection occurs simultaneously with oxidation. It may be applied in subtle transformations where in one molecule of e.g. synthesized nucleic acid only one phosphate center is oxidized. Replacing the 2-pyridyl moiety from a TPG with an aryl-alkyl moiety practically slows down the oxidation of H-phosphonate diesters using only iodine. Still, applying only pyridine linked by an alkyl chain does not accelerate the reaction as much as using a 2-pyridyl moiety. The absolute configurations assigned to H-phosphonate diesters and cyclic oxazaphospholidines will facilitate further research when this method might be used in stereospecific transformations of phosphate centers.

dx.doi.org/10.1021/jo300937k | J. Org. Chem. 2012, 77, 7866–7872

Scheme 3. Assignment of the Absolute Configuration of the Phosphorus Center on the Basis of Enzymatic Digestion



		D_P	L_P
3	Z=H	R_P 10.49 ppm	S_P 9.79 ppm
5	Z=H	R_P 11.11 ppm	S_P 10.51 ppm
12	Z=S	S_P 59.99 ppm	R_P 60.24 ppm
10	Z=NR_2	R_P 13.89 ppm	S_P 14.12 ppm



(i) I_2/Acetonitrile (ii) S_8/Acetonitrile/Pyridine

EXPERIMENTAL SECTION

General Methods. All reagents (analytical grade) were obtained from commercial suppliers and used without further purification. Anhydrous benzene, pyridine, and CH_2Cl_2 were freshly distilled from CaH_2 and P_2O_5, respectively, and were kept over activated 4 Å molecular sieves. All other anhydrous solvents and liquid reagents were dried through storage over activated 3 Å (2-pyridinepropanol, 2-pyridineethanol, 2-pyridinemethanol, MeCN) molecular sieves. The progress of the reactions was monitored by thin-layer chromatography and ^{31}P NMR spectra, while purification was effected by column chromatography using silica gel (60−120 mesh).

^1H, ^{13}C NMR, ^1H−^1H COSY, ^1H−^{13}C HSQC, Mass Spectrometer Parameters. The NMR spectra were recorded at 298 K on a 400 MHz spectrometer operating at the frequencies 400 MHz (^1H) and 100 MHz (^{13}C). ^{31}P NMR spectra were recorded on 300 MHz spectrometer operating at the frequency 121 MHz with 5% H_3PO_4 in D_2O as an external reference and on a 500 MHz spectrometer operating at the frequency 202.5 MHz with 100% D_2O as an external reference. The MicroTofQ mass spectrometer was equipped with electrospray ionization (ESI) sources. Its source parameters are as follows: ESI source voltage of 3.2 kV, nebulization with nitrogen at 0.4 bar, dry gas flow of 4.0 L/min at temperature 220 °C. The instrument operated under EsquireControl version 5.1, and data were analyzed using the Data Analysis version 3.1 package.

Procedure for Chromatographic Analysis. HPLC analyses were performed using a UFLC system with LC-20AD pump and 3 μm C(18)2 100 Å column (15 cm × 4.6 mm) according to the following conditions: starting from 0.01 M triethylammonium acetate (pH 7.0), a linear gradient of 2.5% MeCN/min is pumped at a flow rate of 0.9 mL/min for 20 min, and then the concentration is increased to 10% MeCN/min and then held isocratically for 2 min.

Preparation of 3′-*O*-Acetyl-5′-[3-pyridyl[1,3,2]oxazaphospholidine]thymidine (1). 3′-Acetylthymidine (177 mg, 047 mmol) and 2-*N*-diisopropyl-3-pyridyl[1,3,2]oxazaphospholidine (0.10 g, 0.47 mmol) were dissolved in 2 mL of dry acetonitrile (MeCN). Then a solution of benzylthiotetrazole (BTT) in dry MeCN (2.37 mL, 0.2 M, 0.47 mmol) was added dropwise. After 2 h the reaction was complete and 1 mL of diisoproylamine was added. The mixture was evaporated, and the residue was dissolved in dichloromethane (DCM) and extracted three times with a saturated solution of $NaHCO_3$. The organic layer was dried (anhydrous Na_2SO_4) and subsequently evaporated to dryness. From 291 mg of the solid reaction product 145 mg (part A) was used for further purification. Part A was then placed on a PLC plate and developed in dichloromethane (DCM)/methanol (MeOH)/triethylamine (89/6/5). Pure product **1** was obtained (23 mg, 0.051 mmol); yield 21.7%.

^1H NMR (400 MHz, $CDCl_3$): δ 8.9 (d, J = 4.95 Hz, 1H); 7.62−7.58 (m, 2H); 7.34 (s, 1H); 6.80−6.77 (m, 1H); 6.20−6.19 (m, 1H); 5.30−5.28 (m, 1H); 4.57−4.48 (m, 2H); 4.14−4.07 (m, 1H); 4.01−3.97 (m, 1H); 3.59−3.52 (m, 2H); 3.47−3.39 (m, 1H); 2.29−2.26 (m, 1H); 2.20−2.18 (m, 1H); 2.01 (s, 3H); 1.78 (d, J = 1.12 Hz, 3H). ^{13}C NMR (75 MHz, CD_3CN): δ 170.6; 163.0; 156.2; 150.6; 138.3; 128.2; 115.4; 110.4; 107.6; 84.3; 83.6; 74.8; 69.3; 63.8; 45.8; 43.5; 36.9; 20.2; 11.6. ^{31}P NMR: δ (ppm) 129.8 (m, 1P), 125.5 (m, 1P). HRMS (ESI +): calcd for $C_{19}H_{23}N_4O_7PNa$, 473.1304; found, 473.1216. Mp: 86−88 °C.

Preparation of 3′-*O*-Acetyl-5′-[3-phenyl-[1,3,2]oxazaphospholidine]thymidine (2). A 300 mg portion of 2-*N*-diisopropylo-3-phenyl[1,3,2]oxazaphospholidine (1.13 mmol) and 500 mg of 3′-*O*-acetylthymidine (1.34 mmol) were dissolved in 5 mL of MeCN. Then a solution of BTT in MeCN (3 mL, 0.2 M, 0.6 mmol) was added dropwise. After 2 h the reaction was complete; 1 mL of triethylamine was added and the mixture evaporated to dryness afterward. The product mixture was dissolved in DCM, moved to a separating funnel, and extracted three times with saturated $NaHCO_3$ solution. The organic layer was poured into and dried with anhydrous Na_2SO_4 and evaporated. The crude product (550 mg) was chromatographically purified with the eluent DCM/MeOH/triethylamine (89/6/5). Compound **2** (0.45 mmol, 203 mg) was obtained in 80.2% yield.

^1H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, J =1.24 Hz, 1H); 7.20−7.27 (q, J_1 = 7.4 Hz, J_2 = 16.2 Hz, 2H); 7.00 (t, J = 7.36 Hz, 2H); 6.21−6.18 (m, 1H); 5.18−5.16 (m, 1H); 4.49−4.56 (m, 1H); 4.46−4.49 (m, 1H); 4.1−4.05 (m, 1H); 3.99−4.10 (m, 1H); 3.56 (m, 1H); 3.48 (t, J_1 = 8.8 Hz, 1H); 2.25−2.29 (m, 1H); 2.20−2.23 (m, 1H); 2.13 (m, 1H); 2.01 (s, 3H); 1.84 (d, J = 1.24 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.3; 163.4; 150.4; 143.8; 135.5.6; 129.3; 128.3; 120.0; 115.0; 110.5; 84.0; 83.3; 74.4; 69.3; 63.3; 44.8; 36.5; 20.1; 11.6. ^{31}P NMR: δ (ppm) 127.72, 124.47. HRMS (ESI): calcd for $C_{21}H_{28}N_3O_8P$, 482.1352; found, 482.1714. Mp: 68−70 °C.

Preparation of 3′-*O*-Acetylthymidine-5-yl *N*-(2-Pyridyl)-aminoethyl Phosphonate (3). The thus obtained **1** (23 mg, 0.051 mmol) was dissolved in 500 μL of MeCN with 5 μL of water. A 200 μL portion of BTT in MeCN (0.2 M, 0.04 mmol) was added dropwise, and the reaction mixture was kept in a refrigerator at 4 °C for 1 h until complete opening of the oxazaphospholidine ring (according to ^{31}P NMR). The thus prepared product **3** was ready without further purification for the oxidation reaction. The residue (146 mg, part B) of the crude product was dissolved in MeCN. A 200 μL portion of BTT solution (0.2 M, 0.04 mmol) and 50 μL of water were added. After 1 h the reaction was complete (according to ^{31}P NMR) and the reaction mixture was dried and purified on a PLC plate with the eluent DCM/MeOH (9/1) at 4 °C. A 20 mg amount of **3** was obtained (21% yield).

^1H NMR (400 MHz, $CDCl_3$): δ 8.76 (s, 1H); 7.50 (d, J = 16.7 Hz, 1H); 7.22−7.33 (m, 2H); 7.19 (t, J = 5.0 Hz, 1H); 6.32−6.36 (q, J_1 = 5.3 Hz, J_2 = 9.2 Hz, 1H); 5.23−5.28 (dd, J_1 = 10.9 Hz, J_2 = 6.6 Hz, 1H); 4.31−4.38 (m, 2H); 4.18−4.26 (m, 3H); 3.62−3.70 (m, 1H); 2.40−2.44 (m, 1H); 2.12−2.17 (m, 1H); 2.12 (s, 3H); 1.97 (d, J = 3.9

dx.doi.org/10.1021/jo300937k | J. Org. Chem. 2012, 77, 7866−7872

Hz, 3H); 1.36−1.4 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7; 163.4; 150.2; 134.9; 129.8; 119.9; 111.9; 84.2; 82.6; 74.2; 64.9; 62.6; 36.9; 34.4; 29.6; 23.4; 20.9; 16.4; 12.5. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 10.49; 9.79, ($^1J_{PH}$ = 706.1 Hz; $^3J_{PH}$ = 7.55 Hz), ($^1J_{PH}$ = 711.4 Hz; $^3J_{PH}$ = 7.98 Hz).

Preparation of 3′-*O*-Acetylthymidine-5-yl (2-Pyridyl)propyl Phosphonate (4). A 93 mg amount of 3′-acetylthymidine 5′-H-phosphonate monoester (0.21 mmol) was dissolved in 1.8 mL of DCM and 0.2 mL of pyridine. 2-Pyridinepropanol (28.3 mg, 0.21 mmol) and 60 μL of diphenyl chlorophosphate (78 mg, 0.33 mmol, ρ = 1.296 g/L) were added. After 1 h the reaction was over (according to ^{31}P NMR). The reaction mixture was moved to a separating funnel and extracted three times with water. The organic layer was poured and dried with anhydrous Na_2SO_4. The DCM was evaporated. The thus obtained oily product was purified on preparative thin-layer chromatography with the eluent MeCN/DCM (7/3). The solution was evaporated and lyophilized. A 42 mg amount of **4** was obtained (42.8% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, J_1 = 4.60 Hz, 1H); 7.61 (t, J_1 = 7.58 Hz, 1H); 7.46 (d, J = 6.78 Hz, 1H); 7.35 (s, 2H); 7.12−7.17 (q, 2H); 6.33−6.40 (m, 1H); 5.22−5.27 (dd, J_1 = 12.6 Hz, J_2 = 6.6 Hz 1H); 4.34 (d, J = 6.8 Hz, 1H); 4.30−4.38 (m, 2H); 4.16−4.2 1(m, 3H); 2.90 (t, J = 7.57 Hz, 2H); 2.38−2.43 (m, 1H); 2.13−2.17 (m, 1H); 2.11 (s, 3H); 1.92 (d, J = 3.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6; 163.8; 150.6; 149.2; 136.6; 135.2; 128.2; 122.9; 121.4; 111.9; 84.4; 82.5; 74.2; 65.7; 65.0; 36.8; 33.7; 30.1; 20.9; 12.6. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 9.02; 7.99 ppm, $^1J_{PH}$ = 702.98 Hz; $^3J_{PH}$ = 8.37 Hz, $^1J_{PH}$ = 702.88 Hz; $^3J_{PH}$ = 7.77 Hz; HRMS (ESI): calcd for $C_{20}H_{27}N_3O_8P^+$, 468.1458; found, 468.1550.

Preparation of 3′-*O*-Acetylthymidine-5-yl (2-Phenyl)-aminoethyl Phosphonate (5). Half of the previously obtained crude **2** (part B; 550 mg) was dissolved in DCM with addition of water (100 μL) and 2.9 mL of dichloroacetic acid (0.2 M, 0.5 mmol). After 30 min the reaction was complete. The aqueous layer was poured off while the organic layer was concentrated and purified on a chromatographic column. The eluent was DCM/MeOH. Product **5** ran out at 4% of methanol, giving 245 mg (80.7% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88−8.86 (m, 1H); 7.42 (d, J = 12.4 Hz, 1H); 7.15−7.19 (m, 2H); 6.60−6.63 (q, 2H); 6.32−6.36 (q, 1H); 5.19−5.24 (dd, J_1 = 12.6 Hz, J_2 = 6.8 Hz, 1H); 4.34 (d, J = 6.8 Hz, 1H); 4.33 (d, J = 8.1 Hz, 1H); 4.29−4.32 (m, 2H); 4.12−4.14 (m, 1H); 3.44−3.47 (q, J_1 = 5.04 Hz, J_2 = 9.09 Hz, 2H) 2.33−2.40 (m, 1H); 2.13−2.18 (m, 1H); 2.10 (s, 3H); 1.93 (d, J = 3.9 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.5; 163.4.; 150.3; 147.1; 134.9; 129.2 (d, J = 3 Hz); 118.2; 112.9; 111.8; 84.5; 82.6; 73.9; 65.2; 44.1; 37.0; 20.8; 12.5. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 11.11; 10.51 ppm, $^1J_{PH}$ = 719.22 Hz; $^3J_{PH}$ = 8.23 Hz, $^1J_{PH}$ = 722.0 Hz; $^3J_{PH}$ = 7.38 Hz. MS (ESI): calcd for $C_{20}H_{26}N_3O_8PNa$, 490.1458; found, 490.1370.

Preparation of 3′-*O*-Acetylthymidine-5-yl (2-Phenyl)propyl Phosphonate (6). A 200 mg amount of 3′-acetylthymidine 5′-H-phosphonate monoester (0.44 mmol) was dissolved in 4.5 mL of DCM and 0.5 mL of pyridine. 3-Phenylpropanol (60 mg, 0.44 mmol) and 101 μL of diphenyl chlorophosphate (129.8 mg, 0.55 mmol, ρ = 1.296 g/L) were added. After 1 h the reaction was complete (according to ^{31}P NMR). Subsequent purification was carried out as in compound **4**. A 101 mg amount of **6** was obtained (49.2% general yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (s, 1H); 7.45−7.48 (dd, J_1 = 1.05 Hz, J_2 = 11.12 Hz, 1H); 7.25−7.27 (m, 1H); 7.15−7.19 (m, 2H); 6.60−6.63 (q, 2H); 6.35−6.39 (m, 1H); 5.22−5.27 (dd, J_1 = 12.6 Hz, J_2 = 6.8 Hz 1H); 4.34 (d, J = 6.8 Hz, 1H); 4.33 (d, J = 8.1 Hz, 1H); 4.13−4.17 (m, 2H); 4.12−4.14 (m, 1H); 2.68−2.73 (m, 2H); 2.38−2.44 (m, 1H); 2.13−2.20 (m, 1H); 2.10 (s, 3H); 1.99−2.06 (m, 2H); 1.93 (d, J = 1.9 Hz, 3H). ^{13}C NMR (100.6 MHz MHz, $CDCl_3$): δ 170.5; 163.6.; 150.5; 134.7; 128.5; 128.3; 126.2; 111.9; 111.8; 84.5; 82.6; 74.1; 73.9; 65.2; 65.0; 37.0; 31.8; 31.7; 31.5; 20.8; 12.4. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 9.60, 8.62 ppm; $^1J_{PH}$ = 711.96 Hz; $^3J_{PH}$ = 8.23 Hz, $^1J_{PH}$ = 713.78 Hz; $^3J_{PH}$ = 9.20 Hz. HRMS (ESI): calcd for $C_{21}H_{27}N_2O_8PNa$, 489.1505; found, m/z 489.1420.

Preparation of 3′-*O*-Acetylthymidine-5-yl (2-Pyridyl)ethyl Phosphonate (7). An 85 mg amount of 3′-acetylthymidine 5′-H-phosphonate monoester (0.19 mmol) was dissolved in 1.8 mL of DCM and 0.2 mL of pyridine. A 24 μL portion of 2-pyridineethanol (25.6 mg, 0.21 mmol) and 55 μL of diphenyl chlorophosphate (70.8 mg, 0.3 mmol, ρ = 1.296 g/L) were added. After 1 h the reaction was over (according to ^{31}P NMR). Subsequent purification was carried out as in **4**, which gave 101 mg of **7** with 68.5% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53−8.52 (m, 1H); 7.61 (t, J_1 = 7.58 Hz, 1H); 7.61−7.56 (m, 1H); 7.40−7.38 (dd, J =1.03 Hz, 1H); 7.13−7.18 (m, 2H); 6.32−6.29 (q, J_1 = 5.46 Hz, J_2 = 9.01 Hz, 1H); 5.26 (s, 1H); 5.18 (t, J_1 = 6.87 Hz, 1H); 4.57−4.48 (m, 2H); 4.26−4.19 (m, 1H); 3.17 (t, J = 6.31 Hz, 2H); 2.37−2.32 (m, 1H); 2.16−2.08 (m, 1H); 2.06 (s, 3H); 1.84 (d, J = 8.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9; 164.3; 157.2; 151.1; 149.7; 137.5; 135.3; 124.2; 122.4; 112.2; 84.9; 83.1; 74.6; 65.7; 65.4; 38.9; 37.4; 21.2; 12.8. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 8.99; 7.98 ppm ($^1J_{PH}$ = 703.0 Hz, $^3J_{PH}$ = 8.32 Hz) ($^1J_{PH}$ = 707.29 Hz, $^3J_{PH}$ = 8.61 Hz). HRMS (ESI): calcd for $C_{19}H_{24}N_3O_8PNa$, 476.1301; found, m/z 476.1196.

Preparation of 3′-*O*-Acetylthymidine-5-yl (2-Pyridyl)methyl Phosphonate (8). An 85 mg amount of 3′-acetylthymidine 5′-H-phosphonate monoester (0.19 mmol) was dissolved in 1.8 mL of DCM and 0.2 mL of pyridine. A 21 μL portion of 2-pyridinemethanol (23.7 mg, 0.21 mmol) and 50 μL of diphenyl chlorophosphate (63.7 mg, 0.27 mmol, ρ = 1.296 g/L) were added. After 1 h the reaction was complete (according to ^{31}P NMR). Subsequent purification was carried out as for compound **4**. A 37 mg amount of compound **8** was obtained (44.2% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.89 (s, 1H); 8.53−8.57 (m, 2H); 7.39−7.36 (dd, J = 8.2 Hz, 1H); 7.22−7.19 (m, 2H); 6.30−6.25 (m, 1H); 5.11−5.21 (q, J_1 = 13.2 Hz, J_2 = 24.3 Hz, 1H); 4.67 (s, 1H); 4.25−4.33 (m, 2H); 4.06−4.10 (m, 1H); 3.82 (t, J_1 = 2.2 Hz, 1H); 3.72 (t, J_1 = 10.9 Hz, 1H) 2.26−2.34 (m, 1H); 2.06−2.14 (m, 1H); 2.0 (d, J = 3.9 Hz, 3H); 1.78 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.1; 164.5; 151.1; 150.0; 136.6; 128.7; 124.2; 121.9; 111.7; 85.9; 75.3; 64.7; 62.7; 37.7; 37.4; 21.4; 21.2; 12.9. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 9.41; 8.38 ($^1J_{PH}$ = 713.68 Hz; $^3J_{PH}$ = 8.64 Hz) ($^1J_{PH}$ = 716.36 Hz; $^3J_{PH}$ = 9.12 Hz). HRMS (ESI): calcd for $C_{18}H_{22}N_3O_8PNa$, 462.1145; found, m/z 462.1053.

Preparation of 3′-*O*-Acetyl-5′-[3-pyridyl[1,3,2]oxaza-phospholidine oxide]thymidine-5-yl (10), 3′-*O*-Acetyl-5′-[3-phenyl[1,3,2]oxazaphospholidine oxide]thymidine-5-yl (11), and 3′-*O*-Acetylthymidine-5-yl 2-*N*-(2-Pyridyl)ethyl Phosphonate (9). Portions of both compound **1** (12 mg, 0.026 mmol) and **2** (12 mg, 0.026 mmol) were taken and dissolved in dry MeCN. BTT (0.2 M, 65 μL, 0.013 mmol) in dry MeCN and 2 drops of water were added to each substrate. After 1 h the reactions were complete (according to ^{31}P NMR and TLC), giving products **3** and **5**, respectively. Then molecular sieves 4 Å were added to dry solutions. After 2.5 h 7 mg of iodine (0.026 mmol) was added to each solution. The course of reaction was followed with the use of ^{31}P NMR. The reaction was assumed as complete when peaks corresponding to H-phosphonate diesters totally disappeared. This way compounds **9** (from **3**) and **10** (from **5**) were obtained (100% yield according to ^{31}P NMR). Addition of BTT dissolved in acetonirile (0.2 M, 130 μL, 0.026 mmol) to each product **9** and **10** resulted in hydrolysis only in the first case, giving compound **11**. It was obtained after chromatographic purification (isocratic phase acetonirile/dichloromethane 1/1) as a white powder with 86% yield (11 mg).

Data for **9** are as follows. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) (R_P,S_P) 8.99; 7.98 ppm ($^1J_{PH}$ = 703.0 Hz, $^3J_{PH}$ = 8.32 Hz) ($^1J_{PH}$ = 707.29 Hz, $^3J_{PH}$ = 8.61 Hz).

Data for **10** are as follows. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, J =11.8 Hz, 1H); 7.29−7.34 (m, 2H); 7.12−7.14 (q, J_1 = 2.31 Hz, J_2 = 8.25 Hz, 2H); 7.05 (t, J = 7.35 Hz, 1H); 6.30−6.36 (m, 1H); 4.55−4.61 (m, 1H); 4.45−4.51 (m, 1H); 4.35−4.37 (dd, J = 2.6 Hz, 1H); 4.29−4.32 (m, 1H); 4.12−4.15 (m, 1H); 3.84−3.90 (m, 2H); 3.70−3.74 (q, J_1 = 7.01 Hz, J_2 = 14.02 Hz, 4H); 2.33−2.40 (m, 1H); 2.13−2.18 (m, 1H); 2.10 (s, 3H); 1.94 (s, 1H); 1.85 (s, 1H). ^{13}C NMR (75

dx.doi.org/10.1021/jo300937k | J. Org. Chem. 2012, 77, 7866−7872

MHz, $CDCl_3$): δ 170.5; 163.3; 150.2; 134.9; 129.6; 128.3; 122.7; 116.2; 115.9; 111.8; 111.6; 84.5; 82.6; 82.7; 74.4; 63.7; 46.1; 45.9; 37.1; 20.8; 12.4. ^{31}P NMR δ (ppm) (S_P,R_P) 14.12, 13.89. HRMS (ESI): calcd for $C_{20}H_{23}N_3O_8P$, 464.1301; found, m/z 464.1200.

Data for **11** are as follows. ^{1}H NMR (400 MHz, D_2O): δ 7.73 (t, J = 7.56 Hz, 1H); 7.66 (d, J = 5.76 Hz, 1H); 7.56 (s, 1H); 6.89 (d, J = 9.12 Hz, 1H); 6.75 (t, J = 6.56 Hz, 1H); 6.23−6.20 (q, J_1 = 6.32 Hz, J_2 = 8.24 Hz, 1H); 5.24 (d, J = 5.08 Hz, 1H); 4.01 (m, 2H); 3.99 (m, 1H); 3.58−3.54 (q, J_1 = 5.24 Hz, J_2 = 9.80 Hz, 2H); 2.38−2.33 (m, 1H); 2.27−2.20 (m, 1H); 2.12 (s, 3H); 1.79 (s, 3H); 1.76 (s, 3H). ^{13}C NMR (75 MHz, D_2O): δ 173.5; 166.1; 153.1; 151.4; 143.4; 137.0; 133.4; 112.6; 111.6; 84.7; 83.1; 75.1; 67.8; 65.3; 64.0; 42.1; 36.4; 24.1; 11.5. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) 0.66; $^3J_{PH}$ = 4.86 Hz. HRMS (ESI): calcd for $C_{19}H_{25}N_4O_9PNa$, 507.1359; found, 507.1274 $[M + Na]^+$.

Preparation of 3′-*O*-Acetylthymidine-5-yl (2-phenyl)-aminoethyl thiophosphonate (12). An 8.7 mg amount (0.27 mmol) of sublimed sulfur was added to 32 mg of **5** (0.0685 mmol) in a MeCN/pyridine (1/9) solvent mixture. The reaction mixture was stirred and kept at room temperature for 1 h. After that time the reaction proceeded quantitatively according to ^{31}P NMR. The mixture was evaporated and kept under reduced pressure until complete removal of pyridine. The product was purified on a PLC plate with the eluent toluene/MeCN/triethylamine (45/45/10). The product was washed out from silica gel with MeOH/DCM (7/3). A 32 mg amount of compound **12** was obtained (94.1% yield).

The thus obtained 32 mg (0.064 mmol) of compound **12** was treated with a mixture of methylamine and 32% ammonia (2 mL, 1/1) to remove the 3′-acetyl protecting group. The reaction mixture was kept for 10 min at 65 °C. The thus obtained compound **13** was chromatographically purified using the isocratic phase DCM/MeOH (9/1), evaporated to dryness, and lyophilized afterward. Full deprotection of **12** was confirmed with HPLC. A 26 mg amount of compound **13** was obtained (0.057 mmol, 89.1% yield).

^{31}P NMR (121 MHz, buffer A): δ (ppm) (R_P,S_P) 60.24, 59.99 ppm; $^3J_{PH}$ = 7.38 Hz, $^3J_{PH}$ = 6.41 Hz. Mp: 166−170 °C.

Data for **13** are as follows. ^{31}P NMR (121 MHz, buffer A): δ (ppm) (S_P,R_P) 55.65; 55.33 ppm. HRMS (ESI): calcd for $C_{18}H_{25}N_3O_7PS$, 458.1073; found, m/z 458.1134 $[M + H]^+$.

The preparation of ammonium fluorenylomethyl-H-phosphonate monoester **14** was conducted according to the method presented in the literature.[35]

Preparation of Fluorenylomethyl-3′-acetylthymidine 5′-H-Phosphonate Diester (15). An 816.3 mg amount (2.90 mmol) of oily ammonium fluorenylomethyl-H-phosphonate monoester **14**, prepared according to the method presented in the literature,[35] was dissolved in 10 mL of a pyridine/triethylamine mixture (4/1 v/v) and evaporated to dryness. Then 7.2 mL of DCM, 0.8 mL of pyridine, and 997 mg (2.67 mmol) of 3′-acetylthymidine were added. The mixture was stirred for 10 min, and then 141 μL of pivaloyl chloride was added dropwise. The reaction mixture was kept under inert gas, and after 30 min the reaction was complete (according to ^{31}P NMR). Subsequent extraction was carried out as in **4**, while further chromatographic purification was conducted with the eluent MeCN/DCM, giving solid product **15** (65.5% yield).

Data for **15** are as follows. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (s, 1H); 7.74 (d, J = 7.52 Hz, 2H); 7.58 (t, J = 7.48 Hz, 2H); 7.39 (t, J = 7.44 Hz, 2H); 7.32−7.30 (m, 2H); 7.28 (d, J = 1.16 Hz, 1H); 6.32−6.27 (m, 1H); 5.11 (d, J = 6.56 Hz, 1H); 4.58−4.53 (dd, J_1 = 6.04 Hz, J_2 = 7.72 Hz, 2H); 4.23−4.19 (q, J_1 = 5.52 Hz, J_2 = 9.12 Hz, 1H); 4.14−4.13 (dt, J_1 = 3.2 Hz, J_2 = 7.76 Hz, 1H); 4.05 (m, 1H); 2.37−2.29 (m, 1H); 2.08 (s, 3H); 2.07−1.96 (m, 1H); 1.80 (d, J = 2.04 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4; 163.6; 150.4; 142.6; 141.4; 134.7; 128.1; 127.2; 124.7; 120.1; 111.8; 84.4; 82.5; 75.0; 67.4; 64.8; 53.4; 48.1; 37.0; 27.1; 20.8; 12.3. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) 9.15, 8.09; $^1J_{PH}$ = 711.12 Hz; $^3J_{PH}$ = 6.4 Hz, $^1J_{PH}$ = 712.81 Hz; $^3J_{PH}$ = 7.38 Hz. HRMS (ESI): calcd for $C_{26}H_{27}N_2O_8PNa$, 549.1505; found, 549.1419. Mp: 63−66 °C.

Preparation of 3′-Acetylthymidine 5′-*H*-Phosphonate Monoester (16). A 200 mg amount (0.38 mmol) of **15** was dissolved in an acetonitrile/triethylamine mixture (2/1) and left until full deprotection occurred (confirmed by ^{31}P NMR), giving quantitatively the oily product **16**. Compound **16** was chromatographically purified using a DCM/methanol mixture as eluent. A 340 mg amount (0.75 mmol) of an oily product was obtained (98.8% yield).

Data for **16** are as follows. ^{31}P NMR (121 MHz, CH_3CN): δ (ppm) 6.25; $^1J_{PH}$ = 546.68 Hz; $^3J_{PH}$ = 16.3 Hz.

Oxidation with Iodine Solution. Each of substrates **4** (0.027 mmol, 13 mg), **6** (0.025 mmol, 12 mg), **7** (0.024, 11 mg), and **8** (0.03 mmol, 13 mg) was dissolved in 500 μL of dry acetonitrile separately. A 5 mg portion of iodine and a few drops (5 to 6) of water were added to each solution. The course of the oxidation reactions was followed with the use of ^{31}P NMR. The reaction was assumed as complete when peaks referred to as the substrate totally disappeared.

In the case of H-phosphonates **3** and **5** these compounds were prepared for oxidation in a different way. Compounds **1** (12 mg, 0.026 mmol) and **2** (12 mg, 0.026 mmol) were dissolved in dry MeCN. BTT (0.2 M, 65 μL, 0.013 mmol) in dry MeCN and 2 drops of water were added to each substrate. After 1 h the reactions were complete (according to ^{31}P NMR and TLC), giving products **3** and **5**, respectively. Then molecular sieves 4 Å were added to dry solutions. After 2.5 h 4 mg of iodine was added to each solution. The course of reaction was followed with the use of ^{31}P NMR. The reaction was assumed as complete when peaks referred to as H-phosphonates diesters totally disappeared. This way compound **9** (from **3**) and **10** (from **5**) were obtained (100% yield according to ^{31}P NMR).

The addition of 20 μL of water to both products **9** and **10** resulted in hydrolysis only in the first case (compound **11** was obtained), while product **10** remained stable. The hydrolysis time of **9** was shorter than 1 min when the first ^{31}P NMR spectrum was recorded. Product **10** was further purified on a PLC plate in the eluent DCM/MeOH/triethylamine (89/5/6) and washed out from the gel with DCM/MeCN (3/7). A 10 mg amount of pure compound **10** was obtained (82.7% yield).

Enzymatic Determination of the Correlation between Absolute Configuration at the Phosphorus Atom and Chemical Shifts in 13. A 10 mL amount of buffer solution containing 50 mM of Tris-HCl (78.8 mg, 0.5 mmol), 72 mM of NaCl (42.1 mg, 0.72 mmol), and 14 mM of $MgCl_2$ (13.3 mg, 0.14 mmol) and the proper quantity of Milli-Q water were prepared.[36] All salts were dissolved at room temperature. The buffer solution pH was adjusted to 8.5 with 0.1 M HCl solution and sterilized by membrane filtration (pore diameter 0.22 μm) at room temperature, giving buffer solution A. A 26 mg amount of compound **13** obtained as a white powder by the method previously mentioned was dissolved in 1 mL of solution A. The optical density of such a mixture was 80 OD. A 25 μL portion of the obtained mixture was taken (2 OD) and added to 500 μL of solution A. The whole mixture was added to 20 mg of lysate from *Crotalus atrox* containing phosphodiesterase I (0.026 unit/mg, EC 3.1.4.1) afterward. The thus obtained solution was incubated at 27 °C for 7 days. Every 24 h a one-dimensional ^{31}P NMR spectrum was recorded at 298.1 K with 100% D_2O as an external standard. The ^{31}P NMR parameters were as follows: sweep width 4045 Hz, pulse width 7.3 μs, acquisition time 8.1 s collected in 65.5 k, number of transients 2048, line broadening 1 Hz, and temperature of recorded spectra 298.1 K.

Determination of Intermolecular versus Intramolecular Oxidation Mechanism with the Use of Iodine Solution in the Case of Substances 4 and 6−8. A 10 mg portion of **4** (0.021 mmol) and 10 mg of **6** (0.021 mmol) were dissolved together in dry MeCN. Then 5 mg of iodine and 3 drops of water were added. The course of the reaction was followed with the use of ^{31}P NMR. The reaction was assumed as complete when peaks corresponding to H-phosphonate diesters totally disappeared.

Confirmation of the Structure of Oxazaphospholidine Oxide (10) by Oxidation with Iodine in the Presence of Pyridine and a Mixture of Oxazaphospholidine (2) and Diester H-phosphonate (5). A 10 mg amount of compound **2** and 10 mg of compound **5** were dissolved together in dry MeCN. Then 5 mg of

dx.doi.org/10.1021/jo300937k | J. Org. Chem. 2012, 77, 7866−7872

iodine was added. The reaction progress was followed by ^{31}P NMR (see Figure 3).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C NMR, $^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ HSQC, and MS spectra for **1**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: maro@ibch.poznan.pl.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the European Regional Development Fund within the Innovative Economy Programme (Grant No. POIG.01.03.01-30-045).

■ REFERENCES

(1) Jana, A.; Atta, S.; Sarkar, S. K.; Singh, N. D .P. *Tetrahedron* **2010**, *66*, 9798−9807.
(2) Leikauf, E.; Koster, H. *Tetrahedron* **1995**, *51*, 5557−5562.
(3) Sehaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.
(4) Isidro-Llobet, A.; Alvarez, M.; Albericio, F. *Chem. Rev.* **2009**, *109*, 2455−2504.
(5) Guga, P.; Koziołkiewicz, M. *Chem. Biodivers.* **2011**, *9*, 1642−81.
(6) Stec, W. J.; Karwowski, B.; Boczkowska, M.; Guga, P.; Koziołkiewicz, M.; Sochacki, M.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc,.* **1998**, *120*, 7156−7167.
(7) Wilk, A.; Grajkowski, A.; Philips, L. R.; Beaucage, S. L. *J. Am. Chem. Soc,.* **2000**, *122*, 2149−2156.
(8) Tlais, S. F.; Lam, H.; House, S. E.; Dudley, G. B. *J. Org. Chem.* **2009**, *74*, 1876−85.
(9) Reese, C. B.; Thompson, A. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2881−2885.
(10) Mairanovsky, V. G. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 281.
(11) Yus, M.; Martínez, P.; Guijarro, D. *Tetrahedron* **2001**, *57*, 10119.
(12) Givens, R. S.; Conrad, P. G. II; Yousef, A. L.; Lee, J.-I. Photoremovable Protecting Groups. In *CRC Handbook of Organic Photochemistry and Photobiology*, 2nd ed.; Horspool, W. M., Ed.; CRC Press: Boca Raton, FL, **2003**; Chapter 69, pp 69.1−69.46.
(13) Wilk, A.; Chmielewski, M. K.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **2002**, *67*, 6430−6438.
(14) Grajkowski, A.; Wilk, A.; Chmielewski, M. K.; Phillips, L. R.; Beaucage, S. L. *Org. Lett.* **2001**, *3*, 1287−1290.
(15) Chmielewski, M. K.; Marchan, V.; Cieślak, J.; Grajkowski, A.; Livengood, V.; Munch, U.; Wilk, A.; Beaucage, S. L. *J. Org. Chem.* **2003**, *68*, 10003−10012.
(16) Ohkubo, A.; Kasuya, R.; Miyata, K.; Tsunoda, H.; Seio, K.; Sekine, M. *Org. Biomol. Chem.* **2009**, *7*, 687−694.
(17) Chmielewski, M. K.; Tykarska, E.; Markiewicz, W. T.; Rypniewski, W. R. *New J. Chem.* **2012**, *36*, 603−612.
(18) Chmielewski, M. K. *Tetrahedron Lett.* **2012**, *6*, 666−669.
(19) Cieślak, J.; Beaucage, S. L. *J. Org. Chem.* **2003**, *68*, 10123−10129.
(20) Chmielewski, M. K. *Org. Lett.* **2009**, *11*, 3742−3745.
(21) Lewis, E. S.; Spears, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 3918−3921.
(22) Nylen, P. *Z. Anorg. Allg. Chem.* **1938**, *235*, 161−182.
(23) Silver, B.; Luz, Z. *J. Am. Chem. Soc.* **1962**, *84*, 1091−1095.
(24) (a) Stawiński, J.; Stromberg, R.; Zain, R. *Tetrahedron Lett.* **1992**, *33*, 3185−88. (b) Skowrońska, A.; Pakulski, M.; Michalski, J.; Cooper, D.; Trippett, S. *Tetrahedron Lett.* **1980**, *21*, 321−322.
(25) Garegg, P. J.; Regberg, T.; Stawiński, J.; Stromberg, R. *J. Chem. Soc., Perkin Trans. 1* **1987**, *6*, 1269−1274.
(26) (a) The pyridinium cation is postulated to be formed but it is not improved. The pyridinium adduct is described by: Stromberg.; et al. *J. Chem. Soc. Perkin Trans. 1* **1987**, *6*, 1269−1274. In this report, it is formed by attacking the silylated iodo monoester by further hydrolysis. They also oxidized H-phosphorodiesters where iodo derivatives were formed but the authors mentioned only hydrolysis in a pyridine/water mixture. Moreover, in ref 15 of this paper the authors talk about the addition of pyridine to a phosphoroiodidate solution, which results in immediate replacement of the original signal (−41 ppm; iodo derivative) by a new one at ca. −13 ppm. (b) Epimerization during oxidation in the presence of pyridine may occur, but according to Seela and Kretschmer, the reaction with iodine/pyridine/[^{18}O]H_2O preferentially leads to one of the diastereoisomers: Seela, F.; Kretschmer, U. *J. Org. Chem.* **1991**, *56*, 3861−3869.
(27) Oxidation with iodine may follow the iodine disproportionation mechanism.
(28) H-phosphonate diesters **3** and **5** have been obtained from a cyclic oxazaphospholidine by hydrolysis and oxidized only with the iodine solution without further isolation and purification. The reaction has occurred in the presence of a small amount of water (Scheme 1).
(29) Moerat, A.; Modro, T. A. *Phosphorous Sulfur Relat. Elem.* **1983**, *14*, 179−184.
(30) Brown, Ch.; Boudreau, J. A.; Hewitson, B.; Hudson, R. F. *J. Chem. Soc., Perkin Trans. 2* **1976**, *8*, 888−895.
(31) Brown, H. C. et al. In *Determination of Organic Structures by Physical Methods*; Braude, E. A., Nachod, F. C., Eds.; Academic Press: New York, 1955.
(32) Eckstein, F.; Burgers, P. J. M.; Hunneman, D. H. *J. Biol. Chem.* **1979**, *254*, 7476−7478.
(33) Almer, H.; Stawiński, J.; Stromberg, R.; Thelin, M. *J. Org. Chem.* **1992**, *57*, 6163−6169.
(34) (a) Sobkowski, M.; Stawiński, J.; Kraszewski, A. *Nucleosides, Nucleotides, and Nucleic Acids* **2005**, *24*, 1301−1307. (b) Sobkowski, M.; Stawiński, J.; Kraszewski, A. *Nucleosides, Nucleotides, and Nucleic Acids* **2006**, *25*, 1377−1389. (c) Sobkowski, M.; Stawiński, J.; Kraszewski, A. *Nucleosides, Nucleotides, and Nucleic Acids* **2006**, *25*, 1363−1375.
(35) Romanowska, J.; Szymańska-Michalak, A.; Pietkiewicz, M.; Sobkowski, M.; Boryski, J.; Stawiński, J.; Kraszewski, A. *Lett. Org. Chem.* **2009**, *6*, 496−499.
(36) Cummins, L. L.; Owens, S. R.; Risen, L. M.; Lesnik, E. A.; Freier, S. M.; McGee, D.; Guinosso, C. J.; Cook, P. D. *Nucleic Acids Res.* **1995**, *23*, 2019−2024.