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Selective Phosphorylation of Dihydroquercetin with Trivalent Phosphorus Reagents

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ABSTRACT: *Characteristic features of phosphorylation of dihydroquercetin with trivalent phosphorus reagents were studied. Preparative methods for regioselective phosphorylation were found. The most important chemical properties of dihydroquercetin phosphites and cyclophosphites were studied.* © 2003 Wiley Periodicals, Inc. Heteroatom Chem 14:399–403, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.10115

INTRODUCTION

The problem of phosphorylation of flavonoids with trivalent phosphorus compounds has not been studied previously, apparently, because of the structural complexity of these substrates. In view of the foregoing and taking into account our experience in targeted phosphorylation of polyhydric phenols with amides of trivalent phosphorus acids [1], we set ourselves the task to use these reagents for modification of dihydroquercetin **1**, which is among the most interesting flavonoids. This compound is readily available since a convenient method for isolating it from an inexpensive raw material has been developed [2].

The flavonoid molecule **1** contains five hydroxy groups, including four phenolic and one alcoholic groups. This brings about the problem of differentiating these hydroxyls during phosphorylation. Thus, we chose phosphorus hexaalkyltriamides as phosphorylating reagents because they usually phosphorylate phenols more readily than alcohols [1]. We further assumed that the hydroxy group at C(7) in ring *A* should be the most reactive among the phenolic hydroxyls, because the hydroxyl at C(5) in the same ring and two hydroxyls in ring *C* may be somewhat deactivated by intramolecular hydrogen bonds. This assumption is supported by the data obtained in a study of specific features of the hydroxy groups at C(7) in some flavonoids [3].

RESULTS AND DISCUSSION

An experiment showed that in the reaction between equimolar amounts of reagents **1** and phosphorus hexaalkyltriamides, phosphorylation occurs predominantly at position C(7); however, the hydroxy groups of ring C are also involved to some extent. The same outcome was noted when *N*,*N*-tetraalkylphosphorodiamidite chlorides were used. We attributed the absence of the expected selectivity to the high phosphorylating reactivity of the reagents. Hence, subsequently, we performed phosphorylation using *N*,*N*-tetraalkylphosphorodiamidites, which are less reactive than the reagents used initially. Our assumptions proved true, and the first representatives of dihydroquercetin 7-alkyl phosporamidites **2a–c** were prepared.

These products are labile compounds; therefore, they were stabilized by being introduced in the reaction with sulfur or selenium. This gave the corresponding phosphorothioates **3a–c** and phosphoroselenoates **4a** (see Scheme 1).

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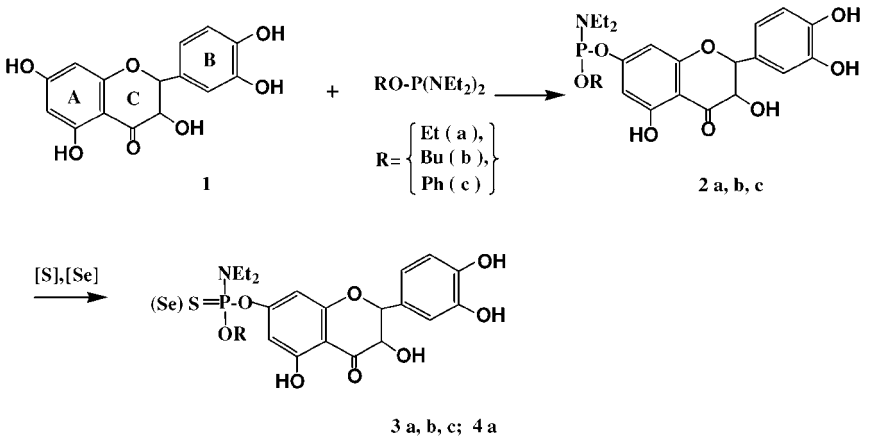


SCHEME 1

The method proposed for the phosphorylation of dihydroquercetin is general. This conclusion was based on the fact that we have successfully phosphorylated this flavonoid by phosphamides of various types, including oxazaphosphorinane and phosphormonoamidites (see Scheme 2).

The structures of all the obtained compounds were established using ^{1}H, ^{13}C, and ^{31}P NMR data.

The next stage of the study was devoted to further phosphorylation of dihydroquercetin 7-phosphoramidites. This was done using reactive phosphorylating reagents that belong to the phosphorus triamide type.

These reagents can be replaced by the corresponding phosphoramidite dichlorides. All the diphosphorus compounds prepared were stabilized by oxidation with sulfur (see Scheme 3).

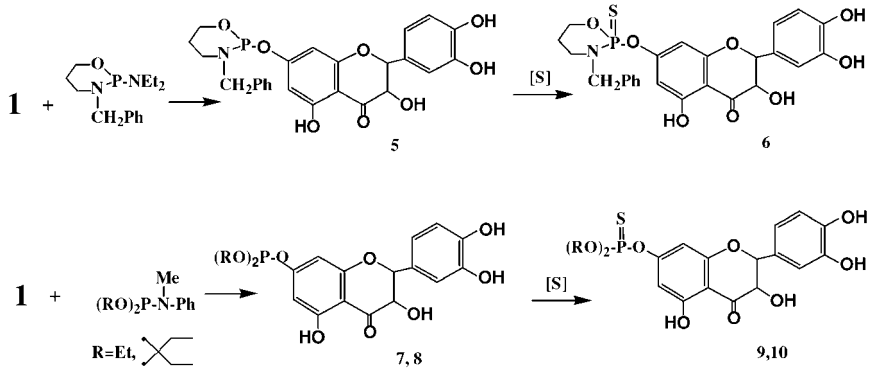
Thus, the results we obtained demonstrate the possibility of direct phosphorylation of flavonoid **1** and, hence, good prospects for the design of diverse phosphorus-containing systems based on this interesting bioregulator.

EXPERIMENTAL

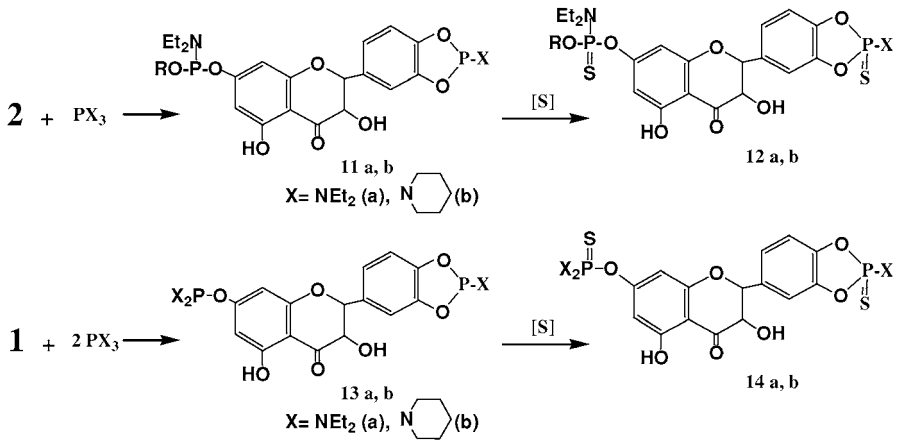
^{1}H NMR spectra of all compounds in $CDCl_3$ were recorded on a Bruker WM-250 instrument at 250 MHz; ^{31}P NMR spectra were recorded on a Bruker WP-80 SY instrument at 32.4 MHz against 85% H_3PO_4; ^{13}C NMR spectra were recorded on a Bruker AC-200 instrument. Column chromatography was performed on L 100/250 silica gel; TLC was performed on UV 250 Silufol plates, using (A) benzene–dioxane 3:1 and (B) benzene–dioxane 1:1 as eluants. The detection of compounds was achieved using iodine vapor treatment and calcination. All syntheses were performed in dry solvents in a dry nitrogen atmosphere.

*2,3-Dihydroquercetin 7-(Diethylamido)-ethylthionophosphate (***3a***)*

A solution of 0.36 g (1.64 mmol) of tetraethyldiamidoethylphosphite (in 2 ml of dioxane) was added dropwise to a solution of 0.5 g (1.64 mmol) of dihydroquercetin **1** (in 10 ml of dioxane) under cooling



SCHEME 2



SCHEME 3

to 10°C and vigorous stirring. The reaction mixture was stirred at room temperature for 15 min. A signal at 144 ppm (s) was observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.9$ (A). Then 0.053 g (1.64 mmol) of sulfur was added and the reaction mixture was stirred for the next 2 h. The product was isolated by column chromatography and eluted with system B. Yellow powder, yield 0.63 g (80%); m.p. 67–68°C. ^{1}H NMR: δ: 1.16 t (6H, CH_3, ^3J 7.14 Hz, CH_3—CH_2—N); 1.37 t (3H, CH_3, ^3J 6.60 Hz, CH_3—CH_2—O); 3.33 m (4H, CH_2, $^3J_{PH}$ 13.75 Hz, $^3J_{HH}$ 7.20 Hz, CH_3—CH_2—N—P); 4.13 m (2H, CH_2, $^3J_{PH}$ 9.35 Hz, $^3J_{HH}$ 6.72 Hz, CH_3—CH_2—O—P); 4.48 dd (H, $^3J_{3,2}$ 11.00 Hz, $^3J_{3,C3-OH}$ 6.05 Hz, H3); 4.97 d (H, $^3J_{2,3}$ 11.00 Hz, H2); 5.70 d (H, $^3J_{3,C3-OH}$ 6.05 Hz, C3—OH); 5.85 d (H, $^4J_{8,6}$ 2.20 Hz, H8); 5.90 d (H, $^4J_{6,8}$ 2.20 Hz, H6); 6.74 s (2H, H5′ + H6′); 6.87 s (H, H2′); 8.92 s (H, C4′—OH); 8.96 s (H, C3′—OH); 11.88 s (H, C5—OH). ^{13}C NMR: δ 83.1 s (C2); 71.7 s (C3); 197.1 s (C4); 163.3 s (C5); 96.1 s (C6); 166.8 d (C7, $^2J_{PC}$ 7.04 Hz); 95.1 s (C8); 162.5 s (C9); 100.6 s (C10); 128.1 s (C1′); 115.3 s (C2′); 144.9 s (C3′); 145.7 s (C4′); 115.4 s (C5′); 119.2 s (C6′); 13.96 s (CH_3, N(CH_2—CH_3)$_2$); 18.83 s (CH_3, O—CH_2—CH_3); 40.42 d (CH_2, N(CH_2—CH_3)$_2$, $^2J_{PC}$ 4.68 Hz); 63.65 d (CH_2, O—CH_2—CH_3, $^2J_{PC}$ 5.08 Hz). Anal. Calcd. for $C_{21}H_{26}O_8NSP$: C, 52.00; H, 5.40; N, 2.90. Found: C, 52.00; H, 5.65; N, 2.89.

*2,3-Dihydroquercetin 7-(Diethylamido)-butylthionophosphate (**3b**)*

A solution of 0.41 g (1.64 mmol) of tetraethyldiamidobutylphosphite (in 2 ml of dioxane) was added dropwise to a solution of 0.5 g (1.64 mmol) of dihydroquercetine **1** (in 10 ml of dioxane) under cooling to 10°C and vigorous stirring. The reaction mixture was stirred at room temperature for 20 min. A signal at 145 ppm (s) was observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.9$ (A). Then 0.053 g (1.64 mmol) of sulfur was added and the reaction mixture was stirred at 50°C for the next 2 h. The product was isolated by column chromatography and eluted with benzene and system B. Yellow powder; yield 0.84 g (65%); m.p. 76–78°C; $R_f = 0.6$ (A). ^{31}P NMR: δ 71 ppm (s). Anal. Calcd. for $C_{23}H_{30}O_8NSP$: C, 54.00; H, 5.80; N, 2.73. Found: C, 54.00; H, 5.62; N, 2.73.

*2,3-Dihydroquercetin 7-(Diethylamido)-ethylselenophosphate (**4a**)*

Selenium (0.13 g, 1.64 mmol) was added to product **2a** without its isolation under stirring. The reaction mixture was kept at room temperature for 24 h. The product was precipitated with hexane from the dioxane solution. Pink powder; yield 0.62 g (70%); m.p. 81–82°C; $R_f = 0.45$ (A). ^{31}P NMR: δ 74.2 ppm (s). Anal. Calcd. for $C_{21}H_{26}O_8NPSe$: C, 47.5; H, 4.9; N, 2.64. Found: C, 47.2; H, 5.1; N, 2.7.

*2-(7-O-2,3-Dihydroquercetin)-3-N-benzyl-1,3,2-oxazaphosphorinane (**6**)*

A solution of 0.3 g (1 mmol) of compound **1** (in 30 ml of dioxane) was added dropwise to a solution of 0.26 g (1 mmol) of 2-diethylamido-3-*N*-benzyl-1,3,2-oxazaphosphorinane (in 20 ml of dioxane) under stirring at room temperature. The reaction mixture was left to stand at room temperature for 24 h. A signal at 131 ppm (s) was observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.8$ (B). Then 0.03 g (1 mmol) of sulfur was added and the reaction mixture was left to stand for 24 h. The product was isolated by column chromatography, using system B as eluent. Light-yellow powder; yield 0.26 g (55%); m.p. 53–55°C; $R_f = 0.7$ (B). ^{31}P NMR: δ 66.5 ppm

(s). ^{1}H NMR: δ 1.78 m (CH_2); 3.1 m (CH_2); 4.11 dd (H, CH_2, N—CH_2Ph, $^2J_{HH}$ 15.36 Hz, $^3J_{HP}$ 7.26 Hz); 4.39 m (CH_2); 4.54 dd (H, $^3J_{HH}$ 12.38 Hz, $^3J_{3,C3-OH}$ 3.20 Hz, H3); 4.74 m (H, CH_2, $^2J_{HH}$ 15.36 Hz, $^3J_{PH}$ 15.36 Hz, N—CH_2Ph); 5.0 d (H, $^3J_{HH}$ 12.38 Hz, H2); 6.45 s (2H, H6 + H8); 6.90 d (H6′, $^3J_{5',6'}$ 8.54 Hz); 6.98 d (H5′, $^3J_{5',6'}$ 8.54 Hz); 7.06 s (H2′); 7.37 m (5H, Ph, $PhCH_2$—N); 11.08 s (H, C5—OH). Anal. Calcd. for $C_{25}H_{24}O_8NSP$: C, 56.71; H, 4.54; P, 5.86. Found: C, 56.77; H, 4.69; P, 5.84.

*2,3-Dihydroquercetin 7-Diethylthionophosphate (***9***)*

A solution of 0.24 g (1 mmol) of diethyl-*N*-metyl-*N*-phenylamidophosphite (in 5 ml of dioxane) was added dropwise to a solution of 0.3 g (1 mmol) of compound **1** (in 10 ml of dioxane) under stirring at room temperature. The reaction mixture was left to stand for 24 h. A signal at 133 ppm (s) was observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.9$ (A). Then 0.03 g (1 mmol) of sulfur was added and the reaction mixture was left to stand at room temperature for 24 h. The product was isolated by column chromatography, using system A as eluent. Yellow powder; yield 0.13 g (30%); m.p. 83–84°C; $R_f = 0.5$ (A). ^{31}P NMR: δ 61 ppm (s). ^{1}H NMR: δ 1.24 t (3H, CH_3, $^3J_{HH}$ 7.15 Hz, CH_3—CH_2—O), 1.35 t (3H, CH_3, $^3J_{HH}$ 7.15 CH_3—CH_2—O); 3.16 q (2H, CH_2, $^3J_{HH}$ 6.70 Hz, CH_3—CH_2—O); 4.22 q (2H, $^3J_{HH}$ 6.70 Hz, CH_3—CH_2—O); 4.52 d (H, $^3J_{2,3}$ 12.10 Hz, H3); 4.94 d (H, $^3J_{2,3}$ 12.10 Hz, H2); 5.40 s (H, C3—OH); 6.34 d (H, $^4J_{6,8}$ 2.75 Hz, H8); 6.38 d (H, $^4J_{6,8}$ 2.75 Hz, H6); 6.83 d (H, $^3J_{5',6'}$ 8.25 Hz, H6′); 6.86 d (H, $^3J_{5',6'}$ 8.25 Hz, H5′); 7.00 s (H, H2′); 8.21 s (2H, C3′—OH, C4′—OH); 11.1 c (H, C5—OH). Anal. Calcd. for $C_{19}H_{21}O_9SP$: C, 50.00; H, 4.60. Found: C, 50.10; H, 4.70.

*2,3-Dihydroquercetin 7-Neopentylenethionophosphate (***10***)*

Neopentylene-*N*-methyl-*N*-phenylamidophosphite (0.5 g, 1.64 mmol) (in 10 ml of dioxane) was added rapidly to a solution of 0.5 g (1.64 mmol) of compound **1** (in 10 ml of dioxane). The reaction mixture was left to stand at room temperature for 48 h. A signal at 115 ppm (s) was observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.6$ (A). Then 0.05 g (1.64 mmol) of sulfur was added and the reaction mixture was heated to 50°C for the next 2 h. The product was isolated by column chromatography, using system A as eluent. Yellow powder; yield 0.29 g (40%); m.p. 62–64°C; $R_f = 0.4$ (B). ^{31}P NMR: δ 51 ppm (s). ^{1}H NMR: δ 1.05 s (6H, CH_3); 3.96 d (4H, CH_2, $^3J_{PH}$ 11.32 Hz); 4.51 d (H, $^3J_{HH}$ 12.10 Hz, H3); 4.94 d (H, $^3J_{HH}$ 12.10 Hz, H2); 5.12 s (H, C3—OH); 5.89 d (H, $^3J_{6,8}$ 7.11 Hz, H8); 6.37 d (H, $^3J_{6,8}$ 7.11 Hz, H6); 6.77 d (H, $^3J_{5',6'}$ 8.24 Hz, H6′); 6.86 d (H, $^3J_{5',6'}$ 8.24 Hz, H5′); 6.93 s (H, H2′); 7.11 s (H, C4′—OH); 7.31 s (H, C3′—OH); 11.26 s (H, C5—OH). Anal. Calcd. for $C_{20}H_{21}O_9PS$: C, 51.28; H, 4.49; P, 6.62. Found: C, 51.32; H, 4.61; P, 6.56.

*2,3-Dihydroquercetine 7-(O-Ethyl-N,N-diethylamidothionophosphate)-3′,4′-diethylamidothionocyclophosphate (***12a***)*

A solution of 0.36 g (1.64 mmol) of tetraethyldiamidoethylphosphite (in 2 ml of dioxane) was added slowly to a solution of 0.5 g (1.64 mmol) of compound **1** (in 10 ml of dioxane) under cooling to 10°C and vigorous stirring. The reaction mixture was stirred at room temperature for 15 min. Then 0.29 g (1.64 mmol) of dichlorodiethylamidophosphite (in 5 ml of dioxane) and 0.33 g (3.28 mol) of triethylamine (in 5 ml of dioxane) were slowly added from two-drop funnels. The reaction mixture was stirred for 0.5 h, and the precipitate of triethylamine hydrochloride was filtered off in an inert gas atmosphere. Signals at 144 ppm (s) and 151 ppm (s) were observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.9$ (B). Sulfur 0.1 g, (3.28 mmol) was then added and the reaction mixture was stirred for the next 2 h. The product was isolated by column chromatography, using system B as eluent. Yellow powder; yield 0.54 g (52%); m.p. 70–72°C; $R_f = 0.7$ (B). ^{31}P NMR: δ 71 ppm (s), 88 ppm (s). ^{1}H NMR: δ 1.13 t (12H, CH_3, $^3J_{HH}$ 7.15 Hz, $(CH_3—CH_2)_2N$); 1.35 t (3H, CH_3, $^3J_{HH}$ 7.14 Hz, CH_3—CH_2—O); 3.30 m (8H, CH_2, $^3J_{HH}$ 7.20 Hz, $^3J_{PH}$ 13.70 Hz, $(CH_3—CH_2)_2N$); 4.12 m (2H, CH_2, $^3J_{PH}$ 9.40 Hz, $^3J_{HH}$ 6.80 Hz, CH_3—CH_2—O); 4.51 d (H, $^3J_{2,3}$ 11.90 Hz, H2); 5.06 d (H, $^3J_{2,3}$ 11.90 Hz, H3); 6.43 s (2H, H6 + H8); 7.10 s (2H, H5′ + H6′); 7.28 s (H, H2′); 11.04 s (H, C5—OH).

*2,3-Dihydroquercetine 7-(O-Ethyl-N,N-diethylamidothionophosphate)-3′,4′-piperidylcyclothionophosphate (***12b***)*

A solution of 0.36 g (1.64 mmol) of tetraethyldiamidoethylphosphite (in 2 ml of dioxane) was added slowly to a solution of 0.5 g (1.64 mmol) of compound **1** (in 10 ml of dioxane) under cooling to 10°C and vigorous stirring. The reaction mixture was stirred at room temperature for 15 min. Then 0.31 g (1.64 mmol) of piperidyldichlorophosphite (in 5 ml of dioxane) and 0.33 g (3.28 mol) of triethylamine (in 5 ml of dioxane) were added from two-drop funnels. The reaction mixture was stirred at room temperature for 1 h, and the precipitate of triethylamine hydrochloride was filtered off in an inert gas atmosphere. Signals at 144 ppm (s) and 147 ppm (s) were

observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.8$ (A). Sulfur 0.1 g, (3.28 mmol) was then added and the reaction mixture was stirred for 2 h. The product was isolated by column chromatography, using system B as eluent. Yellow powder; yield 0.62 g (60%); m.p. 82–84°C; $R_f = 0.8$ (B). ^{31}P NMR: δ 71 ppm (s), 86 ppm (s). ^{1}H NMR: δ 1.16 t (6H, CH_3, $^3J_{HH}$ 7.14 Hz, $(CH_3{-}CH_2)_2N$); 1.38 t (3H, CH_2, $^3J_{HH}$ 7.15 Hz, $CH_3{-}CH_2{-}O$); 1.53 s (H, C3–OH); 1.64 m (6H, CH_2, $^3J_{HH}$ 12.09 Hz, $(CH_2)_3(CH_2)_2N$); 3.32 m (4H, CH_2, $^3J_{PH}$ 13.75 Hz, $^3J_{HH}$ 7.15 Hz, $(CH_3{-}CH_2)_2N$); 3.36 t (4H, $^3J_{HH}$ 13.75 Hz, $(CH_3)_2(CH_2)_2N$); 4.15 m (2H, CH_2, $^3J_{PH}$ 9.90 Hz, $^3J_{HH}$ 6.73 Hz, $CH_3{-}CH_2{-}O$); 6.64 d (H, $^3J_{2,3}$ 11.00 Hz, H3); 6.66 d (H, $^3J_{2,3}$ 11.00 Hz, H2); 6.95 d (H, $^4J_{6,8}$ 2.20 Hz, H8); 7.19 d (H, $^3J_{5',6'}$ 8.25 Hz, H5′); 7.35 d (H, $^4J_{6,8}$ 2.20 Hz, H6); 7.93 d (H, $^3J_{5',6'}$ 8.25 Hz, H6′); 7.95 s (H, H2′); 11.60 s (H, C5–OH). Anal. Calcd. for $C_{23}H_{34}O_8N_2S_2P_2$: C, 46,62; H, 5.74; N, 4.73; P, 10.81. Found: C, 46.70; H, 5.80; N, 4.80; P, 10.92.

*2,3-Dihydroquercetine 7-(Tetraethyldiamidothionophosphate)-3′,4′-diethylamidocyclothionocyclophosphate (***14a***)*

Hexaethylphosphorous triamide (0.49 g, 2 mmol) (in 10 ml of dioxane) was added slowly to a solution of 0.3 g (1 mmol) of compound **1** (in 10 ml of dioxane). The reaction mixture was stirred for 2 h and left to stand for 24 h. Signals at 152 ppm (s) and 132 ppm (s) were observed in the ^{31}P NMR spectrum of the reaction mixture; $R_f = 0.8$ (B). Then 0.05 g (2 mmol) of sulfur was added and the reaction mixture was stirred for 1.5 h. The product was isolated by column chromatography, using system B as eluent. Yellow powder; yield 0.32 g (50%); m.p. 67–69°C; $R_f = 0.7$ (B). ^{31}P NMR: δ 75 ppm (s), 88 ppm (s). ^{1}H NMR: δ 1.19 t (18H, CH_3, $^3J_{HH}$ 13.23 Hz, $(CH_3{-}CH_2)_2N$); 3.26 m (12H, CH_2, $^3J_{PH}$ 13.75 Hz, $^3J_{HH}$ 7.20 Hz, $(CH_3{-}CH_2)_2N$); 6.57 s (H, H3); 6.65 s (H, C3–OH), 6.92 s (H, H2); 7.19 d (H, $^3J_{6',5'}$ 8.96 Hz, H6′); 7.35 s (2H, H6 + H8); 7.93 d (H, $^3J_{5',6'}$ 8.96 Hz, H5′); 7.95 s (H, H2′); 11.59 s (H, C5–OH). Anal. Calcd. for $C_{27}H_{39}O_7N_3S_2P_2$: C, 50.39; H, 6.07; N, 6.53; P, 9.64. Found: C, 51.00; H, 6.20; N, 6.60; P, 9.72.

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