Synthesis and biological activity of quaternary phosphonium salts based on 3-hydroxypyridine and 4-deoxypyridoxine*

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Methods for the synthesis of quaternary phosphonium salts based on 3-hydroxypyridine and 4-deoxypyridoxine were developed. Some of obtained compounds possess high antibacterial and antitumor activity *in vitro*.

Key words: 3-hydroxypyridine, 4-deoxypyridoxine, phosphonium salts, antibacterial activity, antitumor activity.

Nowadays, the area of application of quaternary phosphonium salts is very wide. In particular, they are used as catalysts in enantioselective synthesis,[1—3] phase-transfer catalysts,[4—6] ionic liquids,[7—10] as well as for the synthesis of polymers based on alkenes obtained by Wittig reaction.[11,12] It is also necessary to note a high biological activity of quaternary phosphonium salts: anticholinesterase,[13,14] antitumor,[15] antiviral,[16] analgesic,[17] antibacterial,[18—20] and antiparasitic.[21] Let us specially mention Vizomitin, an agent developed in our country, which contains a quaternary phosphonium salt SkQ1 (Skulachev ion) as an active component and is used as a keratoprotective agent for treatment of syndrome of "dry eye" and other eye diseases.[22,23]

Earlier,[24,25] we have obtained a number of quaternary phosphonium salts based on pyridoxine (vitamin B_6) derivatives, some of these salts possess high antibacterial activity against the strains *Staphylococcus aureus* and *Staphylococcus epidermidis* (the minimum inhibiting concentration values (MIC) <5 μg mL^{-1}). In continuation of the systematic studies of pyridoxine derivatives,[24—29] in the present work we synthesize quaternary phosphonium salts based on the closest pyridoxine structural analogues: 3-hydroxy-2-(hydroxymethyl)pyridine, 3-hydroxy-2,6-bis(hydroxymethyl)pyridine, as well as vitamin B_6 antagonist 4-deoxypyridoxine. The synthesized compounds were tested for antibacterial activity and antitumor activity *in vitro*.

Quaternary phosphonium salts based on 3-hydroxypyridine were synthesized in three steps, first, through the hydroxymethylation of 3-hydroxypyridine (**1**) with paraformaldehyde in an alkaline medium with the formation of the corresponding mono- and bishydroxymethyl derivatives **2** and **3**. Subsequent chlorination of these compounds was carried out by reflux in thionyl chloride for 12 h. The last step of the reflux of chloro derivatives **4** and **5** in acetonitrile with an excess of tertiary phosphines (triphenylphosphine, tributylphosphine, tris(*p*-tolyl)phosphine, tris(2-thienyl)phosphine, tris(4-fluorophenyl)-phosphine) led to the target phosphonium salts **6a—e** and **7a—e** (Scheme 1).

Pyridoxine hydrochloride (**8**) according to Scheme 2 was used to synthesize in four-five steps monophosphonium salts based on 4-deoxypyridoxine **13a—c** and **14a—c** *via* an initial reduction of the hydroxymethyl group at position 4 of the pyridine ring in pyridoxine with zinc in acetic acid and the formation of acetate **9**. Subsequent removal of the acetate protection in acidic medium with further chlorination of the hydroxymethyl group of 4-deoxypyridoxine **10** with thionyl chloride[30] led to chloro derivative **11**. Acylation of the aromatic hydroxy group in this compound and, in the last step, the reaction of chloro derivatives **11** and **12** with tertiary phosphines (triphenylphosphine, tris(*p*-tolyl)phosphine, tributylphosphine) in acetonitrile gave the corresponding quaternary phosphonium salts **13** and **14**.

Compound **13a** was chosen to demonstrate that phosphonium salts based on 4-deoxypyridoxine can be synthesized by much more simple method in two steps (Scheme 3).

In the first step, pyridoxine dichloro derivative **15** was obtained as hydrochloride according to the procedure described earlier.[31] In the second step, compound **15** was reacted with a four-fold molar excess of triphenylphosphine in DMF at 90 °C for 7 h. However, instead of expected bisderivative **16**, the monophosphonium salt **13a** was isolated. In our opinion, this fact can be explained as follows: when the reaction mixture is worked-

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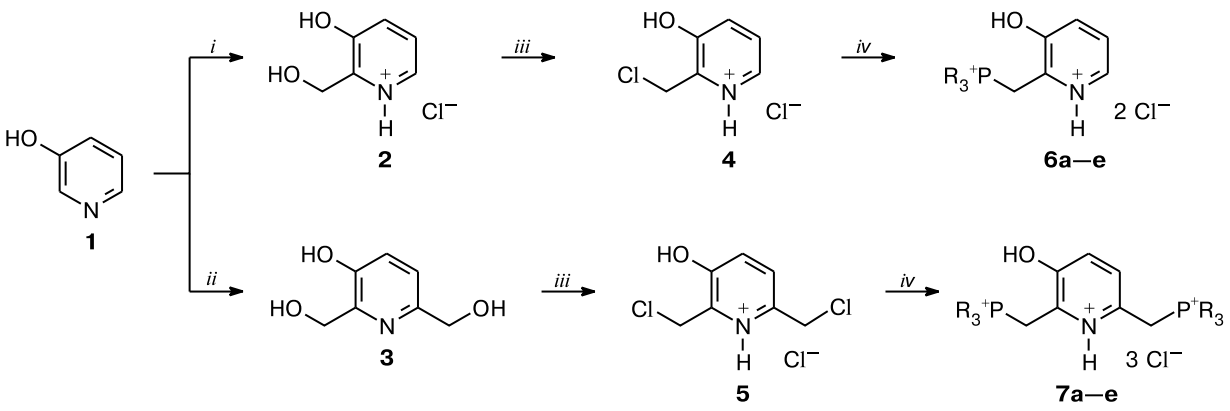
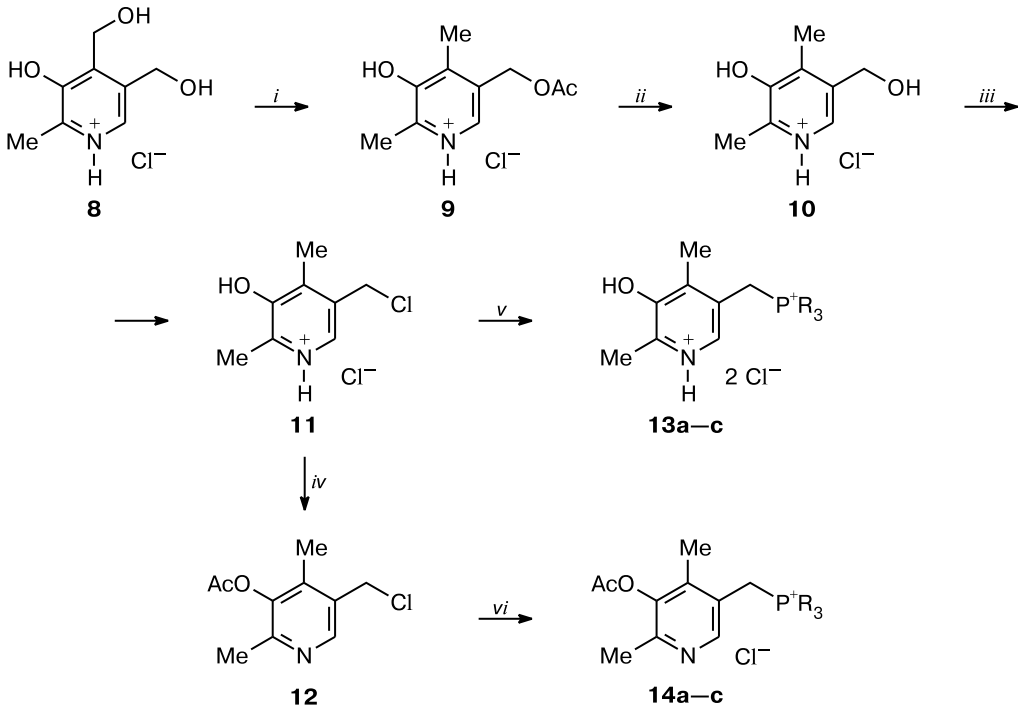
Scheme 1



6, **7**: R = Ph (**a**), Bu^n (**b**), *p*-tolyl (**c**), 2-thienyl (**d**), 4-FC_6H_4 (**e**)

Reagents and conditions: *i.* (*a*) CH_2O, NaOH, H_2O, 90 °C; (*b*) HCl (gas), MeC(O)Me; *ii.* CH_2O, NaOH, H_2O, 90 °C; *iii.* $SOCl_2$, reflux; *iv.* PR_3, MeCN, reflux.

Scheme 2



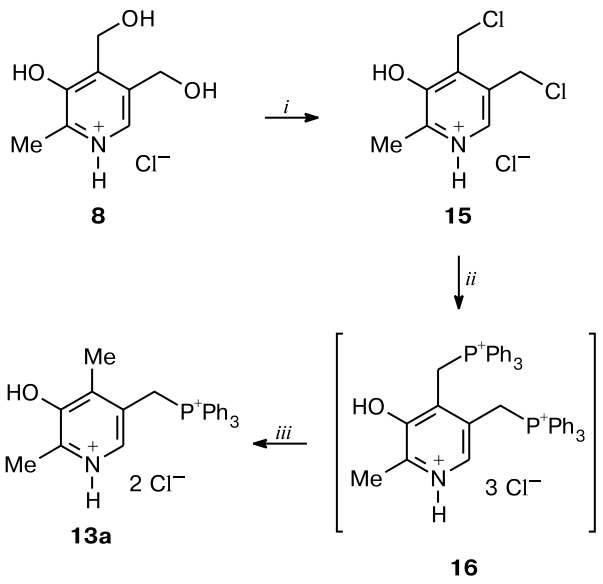
13, **14**: R = Ph (**a**), *p*-tolyl (**b**), Bu (**c**)

Reagents and conditions: *i.* Zn, AcOH; *ii.* HCl, EtOH—H_2O; *iii.* $SOCl_2$, $CHCl_3$, reflux; *iv.* MeC(O)Cl, NEt_3, CH_2Cl_2; *v.* PR_3, MeCN, reflux; *vi.* PR_3, MeCN, 55 °C.

up and the phosphonium salt is extracted into water, the phosphonium fragment at position 4 of pyridoxine undergoes selective hydrolysis with the formation of a methyl group.

Screening of antibacterial activity of phosphonium salts *in vitro* was carried out on the strains of conditionally-pathogenic microorganisms: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermis*, *Bacillus subtilis*, *Escheri-*

Scheme 3



Reagents and conditions: *i.* $SOCl_2$, CH_2Cl_2, reflux; *ii.* PPh_3, DMF, 90 °C; *iii.* H_2O.

chia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae*. Table 1 summarizes the minimum inhibiting concentration (MIC) values of the phosphonium salts in comparison with the known antibiotic of "last hope" vancomycin.

It is necessary to note a narrow range of the action of all the compounds synthesized, which, like vancomycin, as well as earlier obtained phosphonium salts based on pyridoxine,[24,25] exhibit antibacterial activity predominantly against the strains of gram-positive bacteria. Among the phosphonium salts based on 3-hydroxypyridine, the most active was bisphosphonium salt **7c** containing *p*-tolyl substituents at the phosphorus atom. The MIC values for this compound on the strains of gram-positive bacteria and *E. coli* varied within 0.5—4 μg mL^{-1}. Considerably less active was bisphosphonium salt **7a** containing phenyl substituents at the phosphorus atom. Other bisphosphonium salts virtually do not possess antibacterial activity. Among the monophosphonium salts, compounds **6c**, **13b**, and **14b** containing *p*-tolyl substituents at the phosphorus atom are characterized by weak antibacterial activity.

For compounds **6c** and **7c** exhibiting the highest antibacterial activity, we studied mutagenicity on the auxotrophic for histidine strains *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 (Table 2). It was found that these compounds did not possess mutagenic effect.

The studies of antitumor activity of synthesized phosphonium salts were carried out on the breast cancer cells MCF-7 (Table 3). Doxorubicin, one of the most widely used cytostatic agents, was used as a reference drug. Compounds **6c**, **7c**, and **14b** exhibited high antitumor activity (the value $IC_{50} \approx 2.8$—3.7 μmol L^{-1}) comparable with that in the case of doxorubicin. Somewhat lower activity was demonstrated by compounds **7a**, **6b**, and **13b** (the value $IC_{50} \approx 25$—32 μmol L^{-1}). Other compounds were considerably less active.

In conclusion, a number of new quaternary phosphonium salts based on 3-hydroxypyridine and 4-deoxypyrid-

Table 1. Antibacterial activity *in vitro of* mono- and bisphosphonium salts

Compound	MIC/μg mL^{-1}					
	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus ATCC 29213	*S. epidermidis*	*B. subtilis*	*E. coli* ATCC 25922	*K. pneumoniae*	*P. aeroginosa* ATCC 27853
6a	1000	500	1000	1000	1000	1000
6b	>1000	>1000	>1000	>1000	>1000	>1000
6c	100	16	62.5	62.5	>1000	>1000
6d	>1000	>1000	>1000	>1000	>1000	>1000
6e	1000	1000	>500	1000	>1000	>1000
7a	125	125	250	250	>1000	>1000
7b	>1000	>1000	>1000	>1000	>1000	>1000
7c	4	0.5	4	4	250	250
7d	500	250	>500	500	>1000	>1000
7e	500	125	>500	500	>1000	>1000
13a	500	250	250	500	250	250
13b	250	62.5	250	500	250	250
13c	500	500	250	500	250	250
14a	500	250	1000	500	>1000	>1000
14b	62.5	62.5	62.5	62.5	>1000	>1000
14c	>1000	1000	>1000	>1000	>1000	>1000
Vancomycin	2.5	2.5	2.5	1000	>1000	>1000

Table 2. Mutagenicity of some compounds* at the concentrations of 1, 10, and 100 μg mL^{-1}

Compound	*Saimonella typhimurium* TA98			*Saimonella typhimurium* TA100		
	1	10	100	1	10	100
6c	0.87	0.71	0.89	0.74	0.92	1.08
7c	0.73	0.66	0.65	1.40	1.33	0.91
4-Nitroquinoline *N*-oxide		8.20			—	
Sodium azide		—			6.53	

* The excess ratio of the average geometric number of revertants over control.

Table 3. Antitumor activity *in vitro of* mono- and bisphosphonium salts against the MCF-7 breast cancer cells

Compound	IC_{50} /μmol L^{-1}	Compound	IC_{50} /μmol L^{-1}
6a	339	**7e**	389.9
6b	31.1	**13a**	1139.0
6c	3.1	**13b**	32.2
6d	200.0	**13c**	>24000
6e	53.4	**14a**	205.5
7a	25.5	**14b**	2.8
7b	1241	**14c**	1532
7c	3.7	Doxorubicin	1.5
7d	265.5		

oxine were synthesized. Some of these compounds possess pronounced antibacterial and antitumor activity *in vitro*, which allows one to consider them as promising compounds in the development of new antibacterial and antitumor agents.

Experimental

Pyridoxine hydrochloride (DSM Nutritional Products), 3-hydroxypyridine (Acros), and phosphines (Acros) were used without preliminary purification. All the solvents and reagents were purified and dried according to the standard procedures.[32]

^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on a Bruker Avance 400 spectrometer (400.17, 100.62, 161.99, and 376.54 MHz, respectively). The signals of the residual nondeuterated chloroform (δ_H 7.26, δ_C 77.16) and DMSO (δ_H 2.50, δ_C 39.52) were used as references in the ^{1}H and ^{13}C NMR spectra, whereas H_3PO_4 (^{31}P) and CF_3Ph (^{19}F) were used as external standards in the ^{31}P and ^{19}F NMR spectra. HRMS experiment was carried out using a TripleTOF 5600 mass spectrometer (AB Sciex, Germany) for the solution in methanol by turboionic spray ionization (TIS) method with the collision energy with nitrogen molecules of 10 eV. Melting points of products were determined using a Stanford Research Systems MPA-100 OptiMelt appliance.

3-Hydroxy-2-(hydroxymethyl)pyridine hydrochloride (2). A mixture of 3-(hydroxymethyl)pyridine (3.00 g, 31.5 mmol), sodium hydroxide (1.26 g, 31.5 mmol), and 37% aqueous solution of formaldehyde (3.00 g, 37.0 mmol) in water (15 mL) was heated for 3 h at 90 °C. Then, the reaction mixture was cooled to ~20 °C and neutralized by the addition of glacial acetic acid. A solution obtained was dried *in vacuo*. The dry residue was extracted with boiling acetone (200 mL), the insoluble part was filtered off, whereas gaseous hydrogen chloride was passed through the filtrate. A precipitate formed was filtered off. The yield was 1.57 g (31%), a white crystalline compound, m.p. 212—216 °C (*cf.* Ref. 33: m.p. 213—216 °C). ^{1}H NMR (DMSO-d_6), δ: 4.77 (s, 2 H, CH_2OH); 6.45 (br.s, 1 H, $CH_2O\underline{H}$); 7.75 (dd, 1 H, 5-PyH, J_1 = 8.4 Hz, J_2 = 5.4 Hz); 8.12 (d, 1 H, 4-PyH, J = 8.4 Hz); 8.20 (d, 1 H, 6-PyH, J = 5.4 Hz); 12.36 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 56.22 (s, CH_2O); 125.84 (s, C_{Pyr}); 129.37 (s, C_{Pyr}); 131.16 (s, C_{Pyr}); 144.72 (s, C_{Pyr}); 153.22 (s, C_{Pyr}).

3-Hydroxy-2,6-bis(hydroxymethyl)pyridine (3). A mixture of 3-hydroxypyridine (10.0 g, 105.3 mmol), sodium hydroxide (4.2 g, 105.0 mmol), and 37% aqueous solution of formaldehyde (39.3 g, 484.7 mmol) in water (50 mL) was heated for 30 h at 90 °C. Then, the reaction mixture was cooled to ~20 °C and neutralized by the addition of glacial acetic acid. A solution obtained was dried *in vacuo*. The dry residue was extracted with boiling acetone (200 mL), the insoluble part was filtered off, whereas the filtrate was dried. The residue was recrystallized from methanol. The yield was 8.70 g (53%), a white crystalline compound, m.p. 137—139 °C (*cf.* Ref. 34: m.p. 137—138 °C). ^{1}H NMR (DMSO-d_6), δ: 4.76 (s, 2 H, $C\underline{H}_2OH$); 4.77 (s, 2 H, $C\underline{H}_2OH$); 7.77 (d, 1 H, 4-PyH, J = 8.4 Hz); 8.12 (d, 1 H, 6-PyH, J = 8.7 Hz); 12.11 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 55.57 (s, CH_2O); 58.63 (s, CH_2O); 123.94 (s, C_{Pyr}); 130.98 (s, C_{Pyr}); 142.87 (s, C_{Pyr}); 145.81 (s, C_{Pyr}); 152.17 (s, C_{Pyr}).

2-Chloromethyl-3-hydroxypyridine hydrochloride (4). A suspension of 3-hydroxy-2-(hydroxymethyl)pyridine hydrochloride (**2**) (5.0 g, 31.0 mmol) in thionyl chloride (30 mL, 646.0 mmol) was refluxed for 12 h. Then, the reaction mixture was cooled to ~20 °C. A precipitate was filtered off and washed with light petroleum ether. The yield was 4.60 g (83%), a white crystalline compound, m.p. 220 °C (decomp.) (*cf.* Ref. 35: m.p. 160 °C). ^{1}H NMR (DMSO-d_6), δ: 4.92 (s, 2 H, CH_2Cl); 7.74 (A<u>A</u>BX-system, 1 H, 5-PyH, J_1 = 8.4 Hz, J_2 = 5.2 Hz); 7.97 (AB<u>B</u>X-system, 1 H, 4-PyH, J = 8.4 Hz); 8.30 (ABX-system, 1 H, 6-PyH, J = = 5.2 Hz); 12.16 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 37.67 (s, CH_2Cl); 127.53 (s, C_{Pyr}); 130.09 (s, C_{Pyr}); 134.12 (s, C_{Pyr}); 139.18 (s, C_{Pyr}); 154.32 (s, C_{Pyr}).

2,6-Bis(chloromethyl)-3-hydroxypyridine hydrochloride (5). A suspension of 3-hydroxy-2,6-bis(hydroxymethyl)pyridine (**3**) (5.0 g, 26.0 mmol) in thionyl chloride (30 mL, 646.0 mmol) was refluxed for 12 h. Then, the reaction mixture was cooled to ~20 °C. A precipitate was filtered off and washed with light petroleum ether. The yield was 5.70 g (96%), a white crystalline compound, m.p. 240 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 4.73 (s, 2 H, $C\underline{H}_2Cl$); 4.74 (s, 2 H, $C\underline{H}_2Cl$); 7.46, 7.49 (AB-system, 2 H, 4-PyH, 5-PyH, J = 8.4 Hz); 11.15 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 41.76 (s, CH_2Cl); 45.82 (s, CH_2Cl); 124.97 (s, C_{Pyr}); 125.65 (s, C_{Pyr}); 142.44 (s, C_{Pyr}); 145.47 (s, C_{Pyr}); 152.30 (s, C_{Pyr}).

3-Hydroxy-2-(triphenylphosphoniomethyl)pyridinium dichloride (6a). A solution of triphenylphosphine (0.44 g, 1.67 mmol) and 2-chloromethyl-3-hydroxypyridine hydrochloride (**4**) (0.15 g, 0.83 mmol) in acetonitrile (20 mL) was refluxed for 4 h. Then, the solvent was evaporated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous layer was separated, dried *in vacuo*, and recrystallized from acetone.

The yield was 0.08 g (22%), a white crystalline compound, m.p. 260 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 5.28 (d, 2 H, CH_2P, J = 14.5 Hz); 7.10 (ABX-system, 1 H, 5-PyH, J_1 = 8.3 Hz, J_2 = = 4.7 Hz); 7.28 (ABX-system, 1 H, 4-PyH, J = 8.3 Hz); 7.67—7.84 (m, 16 H, 3 Ph + 6-PyH); 10.79 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 26.44 (d, CH_2P, J = 56 Hz); 119.81 (d, *i*-C_{Ar}, J = 87.8 Hz); 122.57 (s, C_{Pyr}); 124.25 (s, C_{Pyr}); 129.75 (d, *m*-C_{Ar}, J = 12.6 Hz); 133.78 (d, *o*-C_{Ar}, J = 10.2 Hz); 134.42 (d, *p*-C_{Ar}, J = 2.5 Hz); 137.83 (d, C_{Pyr}, J = 7.5 Hz); 138.86 (s, C_{Pyr}); 152.49 (d, C_{Pyr}, J = 7.2 Hz). ^{31}P NMR (DMSO-d_6), δ: 28.15. HRMS, found: m/z 370.1356 $[M - 2\,Cl - H]^+$. $C_{24}H_{22}NOPCl_2$. Calculated: $[M - 2\,Cl - H]$ = 370.1355.

3-Hydroxy-2-(tributylphosphoniomethyl)pyridinium dichloride (6b). A suspension of 2-chloromethyl-3-hydroxypyridine hydrochloride (**4**) (0.50 g, 2.78 mmol) and tri(*n*-butyl)phosphine (1.37 mL, 5.55 mmol) in acetonitrile (40 mL) was refluxed for 12 h. Then, the solvent was evaporated *in vacuo*, the dry residue was recrystallized from acetone. The yield was 0.25 g (24%), a white crystalline compound, m.p. 159—160 °C. ^{1}H NMR ($CDCl_3$), δ: 0.87—0.93 (m, 9 H, $P(CH_2CH_2CH_2C\underline{H}_3)_3$); 1.39—1.53 (m, 12 H, $P(CH_2C\underline{H}_2C\underline{H}_2CH_3)_3$); 2.34—2.41 (m, 6 H, $P(C\underline{H}_2CH_2CH_2CH_3)_3$); 4.24 (d, 2 H, CH_2P, J = 14.7 Hz); 7.35 (ABX-system, 1 H, 5-PyH, J_1 = 4.3 Hz, J_2 = 8.0 Hz); 7.99 (ABX-system, 1 H, 4-PyH, J = 4.3 Hz); 8.25 (ABX-system, 1 H, 6-PyH, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$), δ: 13.45 (s, $P(CH_2CH_2CH_2C\underline{H}_3)_3$); 19.82 (d, $P(C\underline{H}_2CH_2CH_2CH_3)_3$, J = 46.2 Hz); 22.36 (d, CH_2P, J = 47.5 Hz); 23.64 (d, $P(CH_2C\underline{H}_2CH_2CH_3)_3$, J = 4.7 Hz); 23.97 (d, $P(CH_2CH_2C\underline{H}_2CH_3)_3$, J = 15.6 Hz); 125.77 (s, C_{Pyr}); 128.75 (s, C_{Pyr}); 135.49 (s, C_{Pyr}); 136.25 (s, C_{Pyr}); 155.03 (s, C_{Pyr}). ^{31}P NMR ($CDCl_3$), δ: 35.01. HRMS, found: m/z 310.2294 $[M - 2\,Cl - H]^+$. $C_{19}H_{35}NOPCl_2$. Calculated: $[M - 2\,Cl - H]$ = 310.2294.

3-Hydroxy-2-[tris(*p*-tolyl)phosphoniomethyl]pyridinium dichloride (6c). 2-Chloromethyl-3-hydroxypyridine hydrochloride (**4**) (0.40 g, 2.22 mmol) was added to a solution of tris(*p*-tolyl)phosphine (1.01 g, 3.32 mmol) in acetonitrile (40 mL) and the mixture was refluxed for 12 h. Then, the solvent was evaporated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous layer was separated, dried *in vacuo*, and recrystallized from acetone. The yield was 0.07 g (7%), a white crystalline compound, m.p. 152—154 °C. ^{1}H NMR (DMSO-d_6), δ: 2.42 (s, 9 H, 3 *p*-Me); 5.13 (d, 2 H, CH_2P, J = = 14.6 Hz); 7.13 (ABX-system, 1 H, 5-PyH, J_1 = 8.2 Hz, J_2 = = 4.6 Hz); 7.29 (ABX-system, 1 H, 4-PyH, J = 8.2 Hz); 7.48—7.78 (m, 12 H, 3 C_6H_4); 7.77 (ABX-system, 1 H, 6-PyH, J = = 4.6 Hz); 10.78 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 21.20 (s, 3 *p*-Me); 26.37 (d, CH_2P, J = 55.7 Hz); 116.48 (d, *i*-C_{Ar}, J = 90.1 Hz); 121.81 (s, C_{Pyr}); 122.87 (s, C_{Pyr}); 130.31 (d, *m*-C_{Ar}, J = 13.0 Hz); 133.64 (d, *o*-C_{Ar}, J = 10.6 Hz); 137.53 (s, *p*-C_{Ar}); 138.77 (s, C_{Pyr}); 145.09 (d, C_{Pyr}, J = 2.8 Hz); 152.08 (s, C_{Pyr}). ^{31}P NMR (DMSO-d_6), δ: 22.35. HRMS, found: m/z 412.1825 $[M - 2\,Cl - H]^+$. $C_{27}H_{28}NOPCl_2$. Calculated: $[M - 2\,Cl - H]$ = 412.1825.

3-Hydroxy-2-tris[(2-thienyl)phosphoniomethyl]pyridinium dichloride (6d). 2-Chloromethyl-3-hydroxypyridine hydrochloride (**4**) (0.40 g, 2.22 mmol) was added to a solution of tris(2-thienyl)phosphine (0.93 g, 3.32 mmol) in acetonitrile (40 mL) and the mixture was refluxed for 12 h. Then, the solvent was evaporated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous layer was separated, dried *in vacuo*, and recrystallized from acetone. The yield was 0.24 g (24%), a white crystalline compound, m.p. 197—198 °C. ^{1}H NMR (DMSO-d_6), δ: 5.29 (d, 2 H, CH_2P, J = 14.4 Hz); 7.22 (ABX-system, 1 H, 5-PyH, J_1 = 8.0 Hz, J_2 = 4.6 Hz); 7.34 (ABX-system, 1 H, 4-PyH, J = 8.0 Hz); 7.49 (m, 3 H, $P(2\text{-thien})_3$ (thien means thienyl)); 7.91 (ABX-system, 1 H, 6-PyH, J = = 4.6 Hz); 7.97 (dd, 3 H, $P(2\text{-thien})_3$, J_1 = 8.3 Hz, J_2 = 3.4 Hz); 8.45 (t, 3 H, $P(2\text{-thien})_3$, J = 4.6 Hz); 10.83 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 32.92 (d, CH_2P, J = 64.3 Hz); 120.00 (d, C_{thien}, J = 112.2 Hz); 122.70 (s, C_{Pyr}); 124.62 (s, C_{Pyr}); 129.99 (d, C_{thien}, J = 16.2 Hz); 137.83 (d, C_{thien}, J = 3.9 Hz); 138.53 (s, C_{Pyr}); 140.51 (d, C_{thien}, J = 4.1 Hz); 141.98 (d, C_{Pyr}, J = 12.1 Hz); 152.36 (d, C_{Pyr}, J = 8.0 Hz). ^{31}P NMR (DMSO-d_6), δ: 10.68. HRMS, found: m/z 388.0048 $[M - 2\,Cl - H]^+$. $C_{18}H_{16}NOPS_3Cl_2$. Calculated: $[M - 2\,Cl - H]$ = 388.0048.

3-Hydroxy-2-[tris(4-fluorophenyl)phosphoniomethyl]pyridinium dichloride (6e). 2-Chloromethyl-3-hydroxypyridine hydrochloride (**4**) (0.40 g, 2.22 mmol) was added to a solution of tris(4-fluorophenyl)phosphine (1.05 g, 3.32 mmol) in acetonitrile (40 mL) and the mixture was refluxed for 12 h. Then, the solution was cooled to ~20 °C and a precipitate was filtered off. The filtrate was concentrated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous layer was separated, dried *in vacuo*, and recrystallized from acetone. The yield was 0.09 g (8%), a white crystalline compound, m.p. 232—234 °C. ^{1}H NMR ($CDCl_3$), δ: 5.20 (d, 2 H, CH_2P, J = = 14.1 Hz); 6.94 (ABX-system, 1 H, 5-PyH, J_1 = 8.2 Hz, J_2 = = 4.5 Hz); 7.26—7.34 (m, 6 H, C_6H_4); 7.55 (ABX-system, 1 H, 4-PyH, J = 8.2 Hz); 7.73 (ABX-system, 1 H, 6-PyH, J = 4.5 Hz); 7.75—7.81 (m, 6 H, C_6H_4); 10.94 (s, 1 H, OH). ^{13}C NMR ($CDCl_3$), δ: 29.67 (d, CH_2P, J = 53.3 Hz); 114.92 (dd, C_6H_4, J_1 = 92.1 Hz, J_2 = 3.2 Hz); 118.13 (dd, C_6H_4, J_1 = 22.1 Hz, J_2 = = 14.2 Hz); 124.82 (s, C_{Pyr}); 125.75 (s, C_{Pyr}); 136.98 (dd, C_6H_4, J_1 = 11.8 Hz, J_2 = 9.7 Hz); 139.93 (s, C_{Pyr}); 153.59 (d, C_{Pyr}, J = = 6.3 Hz); 166.75 (dd, C_6H_4, J_1 = 260.8 Hz, J_2 = 3.3 Hz). ^{31}P NMR ($CDCl_3$), δ: 23.32. ^{19}F NMR ($CDCl_3$), δ: –99.63. HRMS, found: m/z 424.1073 $[M - 2\,Cl - H]^+$. $C_{24}H_{19}F_3NOPCl_2$. Calculated: $[M - 2\,Cl - H]$ = 424.1073.

3-Hydroxy-2,6-bis(triphenylphosphoniomethyl)pyridinium trichloride (7a). 2,6-Bis(chloromethyl)-3-hydroxypyridine hydrochloride (**5**) (0.20 g, 0.87 mmol) was added to a solution of triphenylphosphine (1.15 g, 4.39 mmol) in acetonitrile (20 mL) and the mixture was refluxed for 6 h. Then, a precipitate formed was filtered off, dissolved in chloroform, and washed with water. The aqueous part (filtrate) was separated, dried *in vacuo*, and washed with acetone. The yield was 0.16 g (24%), a white crystalline compound, m.p. 280 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 4.93 (d, 2 H, CH_2P^+, J = 15.2 Hz); 5.16 (d, 2 H, CH_2P, J = = 15.7 Hz); 6.99, 7.11 (AB-system, 2 H, PyH, J = 8.4 Hz); 7.45—7.85 (m, 15 H, 3 Ph); 11.03 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 26.11 (d, CH_2P, J = 50.3 Hz); 29.66 (d, CH_2P, J = 48.8 Hz); 118.57 (d, *i*-C_{Ar}, J = 86.2 Hz); 118.71 (d, *i*-C_{Ar}, J = 86.1 Hz); 123.57 (s, C_{Pyr}); 126.96 (d, C_{Pyr}, J = 4.7 Hz); 129.92 (d, *m*-C_{Ar}, J = 12.6 Hz); 129.96 (d, *m*-C_{Ar}, J = 12.5 Hz); 133.68 (d, *o*-C_{Ar}, J = 10.2 Hz); 133.86 (d, *o*-C_{Ar}, J = 10.2 Hz); 134.76 (d, *p*-C_{Ar}, J = 3.5 Hz); 134.85 (d, *p*-C_{Ar}, J = 3.5 Hz); 137.44 (d, C_{Pyr}, J = 10.1 Hz); 139.47 (d, C_{Pyr}, J = 10.4 Hz); 152.26 (d, C_{Pyr}, J = 2.1 Hz). ^{31}P NMR (DMSO-d_6), δ: 22.62; 23.11. HRMS, found: m/z 322.6170 $[M - 3\,Cl - H]^{2+}$. $C_{43}H_{38}NOP_2Cl_2$. Calculated: $[M - 3\,Cl - H]^{2+}$ = 322.6170.

3-Hydroxy-2,6-bis(tributylphosphoniomethyl)pyridinium dichloride (7b). A suspension of 2,6-bis(chloromethyl)-3-hydroxypyridine hydrochloride (**5**) (0.50 g, 2.18 mmol) and tri(*n*-butyl)phosphine (2.16 mL, 8.75 mmol) in acetonitrile (40 mL) was refluxed for 12 h. Then, the solvent was evaporated *in vacuo*,

the dry residue was recrystallized from diethyl ether. The yield was 0.35 g (25%), a white crystalline compound, m.p. 161—162 °C. ^{1}H NMR ($CDCl_3$), δ: 0.83—0.88 (m, 18 H, 2 P($CH_2CH_2CH_2C\underline{H}_3$)$_3$); 1.33—1.54 (m, 24 H, 2 P($CH_2C\underline{H}_2C\underline{H}_2CH_3$)$_3$); 2.28—2.46 (m, 12 H, 2 P($C\underline{H}_2CH_2CH_2CH_3$)$_3$); 4.05—4.20 (m, 4 H, 2 CH_2P); 7.41 (A<u>B</u>XY-system, 1 H, 5-PyH, J_1 = 7.2 Hz, J_2 = 7.2 Hz); 7.99 (A<u>B</u>XY-system, 1 H, 4-PyH, J_1 = 7.2 Hz, J_2 = 8.2 Hz, J_3 = = 8.2 Hz); 11.27 (s, 1 H, OH). ^{13}C NMR ($CDCl_3$), δ: 13.46 (s, 2 P($CH_2CH_2CH_2C\underline{H}_3$)$_3$); 19.26 (d, P($C\underline{H}_2CH_2CH_2CH_3$)$_3$, J = 45.6 Hz); 19.38 (d, P($C\underline{H}_2CH_2CH_2CH_3$)$_3$, J = 46.3 Hz); 23.62—23.98 (m, 2 P($CH_2C\underline{H}_2CH_2CH_3$)$_3$ + 2 P($CH_2CH_2C\underline{H}_2CH_3$)$_3$ + + CH_2P); 27.86 (d, CH_2P, J = 48.3 Hz); 125.43 (s, C_{Pyr}); 126.03 (s, C_{Pyr}); 138.10 (s, C_{Pyr}); 140.38 (s, C_{Pyr}); 152.96 (s, C_{Pyr}). ^{31}P NMR ($CDCl_3$), δ: 32.89; 33.71. HRMS, found: m/z 262.7109 [M − 3 Cl − − H]$^{2+}$. $C_{31}H_{62}NOP_2Cl_2$. Calculated: [M − 3 Cl − H] = 262.7109.

3-Hydroxy-2,6-bis[tris(*p*-tolyl)phosphoniomethyl]pyridinium dichloride (7c). 2,6-Bis(chloromethyl)-3-hydroxypyridine (**5**) hydrochloride (0.40 g, 1.75 mmol) was added to a solution of tris(*p*-tolyl)phosphine (1.60 g, 5.26 mmol) in acetonitrile (50 mL) and the mixture was refluxed for 12 h. Then, the solvent was evaporated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous filtrate was dried *in vacuo*, and washed with acetone. The yield was 0.09 g (6%), a white crystalline compound, m.p. 162—164 °C. ^{1}H NMR (DMSO-d_6), δ: 2.41 (s, 18 H, 6 *p*-Me); 4.81 (d, 2 H, CH_2P, J = = 15.1 Hz); 5.00 (d, 2 H, CH_2P^+, J = 15.5 Hz); 6.99—7.01 (m, 1 H, 5-PyH); 7.11—7.13 (m, 1 H, 5-PyH); 7.28—7.46 (m, 24 H, 6 C_6H_4); 10.95 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 21.20 (s, 6 *p*-Me); 26.33 (d, CH_2P, J = 51.7 Hz); 29.94 (d, CH_2P, J = 49.8 Hz); 115.54 (d, 2 *i*-C_{Ar}, J = 88.9 Hz); 122.55 (s, C_{Pyr}); 126.87 (s, C_{Pyr}); 130.39 (d, *m*-C_{Ar}, J = 13.0 Hz); 130.44 (d, *m*-C_{Ar}, J = 12.9 Hz); 133.45 (d, *o*-C_{Ar}, J = 10.6 Hz); 133.64 (d, *o*-C_{Ar}, J = 10.6 Hz); 137.66 (d, *p*-C_{Ar}, J = 8.1 Hz); 139.60 (d, *p*-C_{Ar}, J = 8.3 Hz); 145.37 (m, C_{Pyr}); 152.13 (s, C_{Pyr}). ^{31}P NMR (DMSO-d_6), δ: 21.54; 22.08. HRMS, found: m/z 364.6639 [M − 3 Cl − H]$^{2+}$. $C_{49}H_{51}NOP_2Cl_2$. Calculated: [M − 3 Cl − H] = 364.6639.

3-Hydroxy-2,6-bis[tris(2-thienyl)phosphoniomethyl]pyridinium dichloride (7d). 2,6-Bis(chloromethyl)-3-hydroxypyridine hydrochloride (**5**) (0.20 g, 0.88 mmol) was added to a solution of tris(2-thienyl)phosphine (0.98 g, 3.50 mmol) in acetonitrile (40 mL) and the mixture was refluxed for 48 h. Then, the solvent was evaporated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous filtrate was dried *in vacuo*, and recrystallized from acetone. The yield was 0.10 g (15%), a white crystalline compound, m.p. 176—178 °C. ^{1}H NMR (DMSO-d_6), δ: 5.00 (d, 2 H, CH_2P, J = 15.3 Hz); 5.11 (d, 2 H, CH_2P, J = 15.7 Hz); 7.10, 7.34 (AB-system, 2 H, PyH, J = 8.4 Hz); 7.47—7.49 (m, 3 H, P(2-thien)$_3$); 7.51—7.53 (m, 3 H, P(2-thien)$_3$); 7.89 (dd, 3 H, P(2-thien)$_3$, J_1 = 8.3 Hz, J_2 = 3.6 Hz); 7.97 (dd, 3 H, P(2-thien)$_3$, J_1 = 8.3 Hz, J_2 = 3.6 Hz); 8.47—8.50 (m, 6 H, P(2-thien)$_3$); 11.33 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 20.92 (s, CH_2P); 25.93 (d, CH_2P, J = 54.4 Hz); 119.25 (d, C_{thien}, J = 86.5 Hz); 123.89 (s, C_{Pyr}); 124.71 (s, C_{Pyr}); 129.64 (d, C_{thien}, J = 13.4 Hz); 131.00 (d, C_{thien}, J = 10.1 Hz); 133.74 (d, C_{thien}, J = 10.2 Hz); 135.23 (d, C_{thien}, J = 2.1 Hz); 137.02 (s, C_{Pyr}); 138.01 (C_{Pyr}); 139.45 (d, C_{thien}, J = 12.7 Hz); 153.09 (t, C_{Pyr}, J = 5.1 Hz). ^{31}P NMR (DMSO-d_6), δ: 4.07; 4.31. HRMS, found: m/z 340.4863 [M − 3 Cl − H]$^{2+}$. $C_{31}H_{26}NOP_2S_6Cl_2$. Calculated: [M − 3 Cl − H] = 340.4863.

3-Hydroxy-2,6-bis[tris(4-fluorophenyl)phosphoniomethyl]-pyridinium dichloride (7e). 2,6-Bis(chloromethyl)-3-hydroxypyridine hydrochloride (**5**) (0.20 g, 0.88 mmol) was added to a solution of tris(4-fluorophenyl)phosphine (1.11 g, 3.48 mmol) in acetonitrile (40 mL) and the mixture was refluxed for 36 h. Then, the solution was cooled to ~20 °C and a precipitate was filtered off. The filtrate was concentrated *in vacuo*, the dry residue was dissolved in chloroform and washed with water. The aqueous filtrate was dried *in vacuo*, and recrystallized from acetone. The yield was 0.05 g (7%), a white crystalline compound, m.p. 248—250 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 4.95 (d, 2 H, CH_2P, J = 15.4 Hz); 5.12 (d, 2 H, CH_2P, J = 15.8 Hz); 7.00, 7.12 (AB-system, 2 H, PyH, J = 8.4 Hz); 7.52—7.78 (m, 24 H, 6 C_6H_4); 10.97 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 26.44 (d, CH_2P, J = 50.6 Hz); 30.04 (d, CH_2P, J = 48.1 Hz); 114.60 (d, C_6H_4, J = 91.0 Hz); 117.49 (d, C_6H_4, J = 23.0 Hz); 117.60 (dd, C_6H_4, J_1 = 22.1 Hz, J_2 = 3.3 Hz); 117.72 (d, C_6H_4, J = 23.0 Hz); 123.86 (s, C_{Pyr}); 127.21 (s, C_{Pyr}); 137.22 (dd, C_6H_4, J_1 = = 24.3 Hz, J_2 = 12.4 Hz); 139.04 (dd, C_{Pyr}, J_1 = 8.8 Hz, J_2 = = 1.7 Hz); 152.45 (m, C_{Pyr}); 166.94 (d, C_6H_4, J = 256.1 Hz). ^{31}P NMR (DMSO-d_6), δ: 21.54; 21.97. ^{19}F (DMSO-d_6), δ: −102.09. HRMS, found: m/z 376.5885 [M − 3 Cl − H]$^{2+}$. $C_{43}H_{32}F_6NOP_2Cl_2$. Calculated: [M − 3 Cl − H] = 376.5887.

5-Acetoxymethyl-3-hydroxy-2,4-dimethylpyridinium chloride (9). Pyridoxine hydrochloride (10.0 g, 48.7 mmol) and activated zinc dust (13.0 g, 198.8 mmol) were dissolved in glacial acetic acid (40 mL). The reaction mixture was refluxed for 3 days. A precipitate was filtered off, the filtrate was concentrated *in vacuo*. An oily residue was dried *in vacuo* and recrystallized from water. The yield was 4.20 g (36%), a white crystalline compound, m.p. 156—157 °C (*cf.* Ref. 36: m.p. 180—181 °C). ^{1}H NMR (DMSO-d_6), δ: 2.03 (s, 3 H, Me); 2.15 (s, 3 H, Me); 2.36 (s, 3 H, Me); 5.04 (s, 2 H, CH_2O); 7.89 (s, 1 H, CH_{Pyr}); 8.80 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 11.29 (s, Me); 19.82 (s, Me); 20.59 (s, Me); 61.98 (s, CH_2O); 128.57 (s, C_{pyr}); 132.24 (s, C_{pyr}); 140.50 (s, C_{pyr}); 146.64 (s, C_{pyr}); 149.34 (s, C_{pyr}); 170.19 (s, C=O).

3-Hydroxy-5-hydroxymethyl-2,4-dimethylpyridinium chloride (10). Concentrated hydrochloric acid (4.2 mL) was added to a solution of compound **9** (4.20 g, 20.0 mmol) in water (30 mL) and the mixture was stirred for 8 h at 50 °C. Then, the solution was dried *in vacuo*. The yield was 3.20 g (95%), a white crystalline compound, m.p. 252—255 °C (*cf.* Ref. 37: m.p. 273 °C). ^{1}H NMR (DMSO-d_6), δ: 2.32 (s, 3 H, Me); 2.62 (s, 3 H, Me); 4.60 (s, 2 H, CH_2); 8.07 (s, 1 H, CH_{Pyr}). ^{13}C NMR (DMSO-d_6), δ: 12.27 (s, Me); 14.92 (s, Me), 58.23 (s, CH_2O); 128.49 (s, C_{pyr}); 138.75 (s, C_{pyr}); 139.93 (s, C_{pyr}); 141.70 (s, C_{pyr}); 151.67 (s, C_{pyr}).

5-Chloromethyl-3-hydroxy-2,4-dimethylpyridinium chloride (11). 3-Hydroxy-5-hydroxymethyl-2,4-dimethylpyridinium chloride **10** (3.20 g, 16.9 mmol) and DMF (1 mL) was added to thionyl chloride (30 mL, 413.0 mmol). The reaction mixture was refluxed for 5 h. Then, the solution was concentrated *in vacuo*. Chloroform was poured to the residue. The undissolved precipitate was filtered off and dried. The yield was 1.85 g (53%), a brown crystalline compound, m.p. 210—215 °C (*cf.* Ref. 38: m.p. 220—225 °C). ^{1}H NMR (DMSO-d_6), δ: 2.45 (s, 3 H, Me); 2.64 (s, 3 H, Me); 4.96 (s, 2 H, CH_2Cl); 8.41 (s, 1 H, CH_{Pyr}), 10.93 (s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 12.90 (s, Me); 15.16 (s, Me); 40.53 (s, CH_2Cl); 131.40 (s, C_{pyr}); 134.13 (s, C_{pyr}); 142.06 (s, C_{pyr}); 143.46 (s, C_{pyr}); 152.52 (s, C_{pyr}).

3-Acetoxy-5-chloromethyl-2,4-dimethylpyridine (12). 5-Chloromethyl-3-hydroxy-2,4-dimethylpyridinium chloride (**11**) (1 g, 4.81 mmol) was added to anhydrous dichloromethane (30 mL) with stirring, followed by a sequential addition of triethylamine (1.54 mL, 11.1 mmol) and acetyl chloride (0.44 mL, 6.2 mmol). The reaction mixture was refluxed for 3 h. The solu-

tion was concentrated to 10 mL and washed with water. The aqueous filtrate was additionally washed with dichloromethane. The combined organic solutions were dried *in vacuo* and purified by column chromatography (eluent ethyl acetate—dichloromethane (1 : 2)). The yield was 0.93 g (90%), a red crystalline precipitate, m.p. 59 °C. ^{1}H ($CDCl_3$), δ: 2.22 (s, 3 H, Me); 2.37 (s, 3 H, Me); 2.39 (s, 3 H, Me); 4.62 (s, 2 H, CH_2); 8.29 (s, 1 H, CH_{Pyr}). ^{13}C NMR ($CDCl_3$), δ: 12.00 (s, Me); 19.65 (s, Me); 20.57 (s, Me); 41.56 (s, CH_2); 130.93 (s, C_{pyr}); 139.44 (s, C_{pyr}); 145.15 (s, C_{pyr}); 146.75 (s, C_{pyr}); 152.12 (s, C_{pyr}); 168.36 (s, C=O). HRMS, found: *m/z* 214.0629 [M + H]. $C_{10}H_{12}ClNO_2$. Calculated: *M* = 214.0635.

3-Hydroxy-2,4-dimethyl-5-(triphenylphosphoniomethyl)-pyridinium dichloride (13a). ***A.*** 5-Chloromethyl-3-hydroxy-2,4-dimethylpyridinium chloride (**11**) (0.35 g, 1.68 mmol) was added to a solution of triphenylphosphine (0.88 g, 3.36 mmol) in acetonitrile (20 mL) and the mixture was refluxed for 7 h. Then, a precipitate was filtered off and washed with acetonitrile. The yield was 0.45 g (57%), a white crystalline compound, m.p. 303—305 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 1.62 (s, 3 H, Me); 2.57 (s, 3 H, Me); 5.42 (d, 2 H, CH_2P, *J* = 15.1 Hz); 7.76—7.97 (m, 16 H, 3 Ph + CH_{Pyr}); 10.74 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 13.29 (s, Me); 15.52 (s, Me); 23.90 (d, CH_2P, *J* = 48.3 Hz); 116.78 (d, *i*-C_{Ar}, *J* = 85.7 Hz); 124.78 (d, C_{Pyr}, *J* = 7.8 Hz); 130.39 (d, *m*-C_{Ar}, *J* = 12.6 Hz); 132.83 (d, C_{Pyr}, *J* = 4.5 Hz); 134.29 (d, *o*-C_{Ar}, *J* = 10.2 Hz); 135.53 (d, *p*-C_{Ar}, *J* = = 2.0 Hz); 141.74 (br.s, C_{Pyr}); 143.56 (d, C_{Pyr}, *J* = 4.4 Hz); 152.55 (d, C_{Pyr}, *J* = 1.4 Hz). ^{31}P NMR (DMSO-d_6), δ: 23.14. HRMS, found: *m/z* 398.1668 [M − 2 Cl − H]$^+$. $C_{26}H_{26}Cl_2NOP$. Calculated: [M − 2 Cl − H] = 398.1668.

B. 4,5-Bis(chloromethyl)-3-hydroxy-2-methylpyridinium chloride (**15**) (0.50 g, 2.10 mmol) was added to a solution of triphenylphosphine (2.16 g, 8.25 mmol) in DMF (20 mL), the solution was refluxed for 7 h. Then, the solvent was removed *in vacuo*, the dry residue was dissolved in chloroform and washed with water, the aqueous filtrate was dried. The product was recrystallized from acetone. The yield was 0.60 g (62%).

3-Hydroxy-2,4-dimethyl-5-[tris(*p*-tolyl)phosphoniomethyl]-pyridinium dichloride (13b). 5-Chloromethyl-3-hydroxy-2,4-dimethylpyridinium chloride (**11**) (0.30 g, 1.44 mmol) was added to a solution of tris(*p*-tolyl)phosphine (0.88 g, 2.88 mmol) in acetonitrile (20 mL), the solution was refluxed for 7 h. Then, the solvent was evaporated *in vacuo*, the dry residue was dissolved in water, the insoluble precipitate was filtered off. The filtrate was concentrated to dryness. The product was recrystallized from a mixture of acetone and chloroform. The yield was 0.49 g (66%), a white crystalline compound, m.p. 289—291 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 1.64 (s, 3 H, Me); 2.46 (s, 9 H, 3 *p*-Me); 2.58 (d, 3 H, Me, *J* = 1.1 Hz); 5.31 (d, 2 H, CH_2P, *J* = 15.1 Hz); 7.55—7.65 (m, 12 H, 3 C_6H_4); 7.81 (d, 1 H, CH_{Pyr}, *J* = 2.3 Hz); 10.78 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 13.46 (s, Me); 15.44 (s, Me); 21.32 (s, 3 *p*-Me); 24.16 (d, CH_2P, *J* = 49.5 Hz); 113.68 (d, *i*-C_{Ar}, *J* = 88.4 Hz); 125.11 (d, C_{Pyr}, *J* = 7.5 Hz); 130.92 (d, *m*-C_{Ar}, *J* = 13.0 Hz); 132.73 (d, C_{Pyr}, *J* = 6.7 Hz); 134.10 (d, *o*-C_{Ar}, *J* = 10.6 Hz); 141.62 (s, C_{Pyr}); 143.66 (d, C_{Pyr}, *J* = 4.3 Hz); 146.36 (d, *p*-C_{Ar}, *J* = 2.7 Hz); 152.54 (s, C_{Pyr}). ^{31}P NMR (DMSO-d_6), δ: 22.51. HRMS, found: *m/z* 440.2138 [M − 2 Cl − H]$^+$. $C_{29}H_{32}Cl_2NOP$. Calculated: [M − 2 Cl − H] = 440.2138.

3-Hydroxy-2,4-dimethyl-5-(tributylphosphoniomethyl)pyridinium dichloride (13c). Tri(*n*-butyl)phosphine (1.26 mL, 5.05 mmol) and 5-chloromethyl-3-hydroxy-2,4-dimethylpyridinium chloride (**11**) (0.35 g, 1.68 mmol) were added to acetonitrile (20 mL), the solution was refluxed for 7 h. Then, the solvent was evaporated *in vacuo*, the dry residue was washed with light petroleum ether, the insoluble precipitate was filtered off and recrystallized from a mixture of chloroform and light petroleum ether. The yield was 0.46 g (67%), a white crystalline compound, m.p. 226—228 °C (decomp.). ^{1}H NMR (DMSO-d_6), δ: 0.89 (t, 9 H, $P(CH_2CH_2CH_2C\underline{H}_3)_3$, *J* = 6.2 Hz); 1.26—1.57 (m, 12 H, $P(CH_2C\underline{H}_2C\underline{H}_2CH_3)_3$); 2.22—2.39 (m, 6 H, $P(C\underline{H}_2CH_2CH_2CH_3)_3$); 2.44 (s, 3 H, Me); 2.66 (s, 3 H, Me); 4.17 (d, 2 H, CH_2P, *J* = = 15.5 Hz); 8.39 (s, 1 H, CH_{Pyr}); 11.05 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 13.27 (s, $PCH_2CH_2CH_2\underline{C}H_3$); 14.68 (s, Me); 15.05 (s, Me); 17.51 (d, $P\underline{C}H_2CH_2CH_2CH_3$, *J* = 45.9 Hz); 21.26 (d, CH_2P, *J* = 45.1 Hz); 22.67 (d, $PCH_2\underline{C}H_2CH_2CH_3$, *J* = 4.1 Hz); 23.39 (d, $PCH_2CH_2\underline{C}H_2CH_3$, *J* = 16.0 Hz); 127.04 (d, C_{Pyr}, *J* = = 7.5 Hz); 132.49 (s, C_{Pyr}); 140.35 (br.s, C_{Pyr}); 143.52 (br.s, C_{Pyr}); 152.86 (br.s, C_{Pyr}). ^{31}P NMR (DMSO-d_6), δ: 35.59. HRMS, found: *m/z* 338.2607 [M − 2 Cl − H]$^+$. $C_{20}H_{38}Cl_2NOP$. Calculated: [M − 2 Cl − H] = 338.2607.

3-Acetoxy-2,4-dimethyl-5-(triphenylphosphoniomethyl)pyridine chloride (14a). 3-Acetoxy-5-chloromethyl-2,4-dimethylpyridine (**12**) (0.25 g, 1.17 mmol) was added to a solution of triphenylphosphine (0.61 g, 2.34 mmol) in acetonitrile (20 mL), the solution was stirred for 10 h at 55 °C. Then, the solvent was evaporated *in vacuo*, the dry residue was washed with diethyl ether, the insoluble precipitate was filtered off and recrystallized from acetone. The yield was 0.26 g (46%), a white crystalline compound, m.p. 244—246 °C (decomp.). ^{1}H NMR ($CDCl_3$), δ: 1.47 (d, 3 H, Me, *J* = 0.7 Hz); 2.21 (s, 3 H, C(O)Me); 2.22 (d, 3 H, Me, *J* = 2.4 Hz); 5.55 (d, 2 H, CH_2P, *J* = 14.3 Hz); 7.55—7.78 (m, 15 H, 3 Ph); 7.98 (d, 1 H, CH_{Pyr}, *J* = 2.7 Hz). ^{13}C NMR ($CDCl_3$), δ: 12.76 (s, Me); 19.47 (s, Me); 20.41 (s, Me); 25.92 (d, CH_2P, *J* = 47.7 Hz); 117.35 (d, *i*-C_{Ar}, *J* = 85.5 Hz); 122.15 (d, C_{Pyr}, *J* = 8.3 Hz); 130.43 (d, *m*-C_{Ar}, *J* = 12.6 Hz); 134.32 (d, *o*-C_{Ar}, *J* = 10.0 Hz); 135.13 (d, *p*-C_{Ar}, *J* = 2.6 Hz); 141.31 (d, C_{Pyr}, *J* = 5.1 Hz); 144.97 (d, C_{Pyr}, *J* = 3.4 Hz); 148.29 (d, C_{Pyr}, *J* = 5.2 Hz); 151.38 (d, C_{Pyr}, *J* = 4.0 Hz); 167.98 (s, $\underline{C}$(O)Me). ^{31}P NMR ($CDCl_3$), δ: 22.62. HRMS, found: *m/z* 440.1774 [M − Cl]$^+$. $C_{28}H_{27}ClNO_2P$. Calculated: [M − Cl] = 440.1774.

3-Acetoxy-2,4-dimethyl-5-{tris(*p*-tolyl)phosphoniomethyl]-pyridine chloride (14b). 3-Acetoxy-5-chloromethyl-2,4-dimethylpyridine (**12**) (0.20 g, 0.94 mmol) was added to a solution of tris(*p*-tolyl)phosphine (0.57 g, 1.87 mmol) in acetonitrile (20 mL), the solution was stirred for 10 h at 55 °C. Then, the solvent was evaporated *in vacuo*, the dry residue was washed with diethyl ether, the insoluble precipitate was filtered off and recrystallized from acetone. The yield was 0.39 g (80%), a white crystalline compound, m.p. 253—255 °C (decomp.). ^{1}H NMR ($CDCl_3$), δ: 1.60 (br.s, 3 H, Me); 2.26 (s, 3 H, C(O)Me); 2.28 (d, 3 H, Me, *J* = 2.2 Hz); 2.43 (s, 9 H, 3 *p*-Me); 5.30 (d, 2 H, CH_2P, *J* = = −13.7 Hz); 7.39—7.56 (m, 12 H, 3 C_6H_4); 7.91 (d, 1 H, CH_{Pyr}, *J* = 2.5 Hz). ^{13}C NMR ($CDCl_3$), δ: 13.06 (s, Me); 19.52 (s, Me); 20.51 (s, Me); 21.95 (s, 3 *p*-Me); 26.56 (d, CH_2P, *J* = 49.3 Hz); 114.19 (d, *i*-C_{Ar}, *J* = 88.4 Hz); 122.70 (d, C_{Pyr}, *J* = 8.3 Hz); 131.24 (d, *m*-C_{Ar}, *J* = 13.0 Hz); 134.23 (d, *o*-C_{Ar}, *J* = 10.3 Hz); 141.67 (d, C_{Pyr}, *J* = 5.0 Hz); 145.20 (d, C_{Pyr}, *J* = 3.2 Hz); 146.53 (d, *p*-C_{Ar}, *J* = 2.8 Hz); 148.02 (d, C_{Pyr}, *J* = 5.0 Hz); 151.37 (d, C_{Pyr}, *J* = 3.7 Hz); 168.07 (s, $\underline{C}$(O)Me). ^{31}P NMR ($CDCl_3$), δ: 21.65. HRMS, found: *m/z* 482.2243 [M − Cl]$^+$. $C_{31}H_{33}ClNO_2P$. Calculated: [M − Cl] = 482.2243.

3-Acetoxy-2,4-dimethyl-5-(tributylphosphoniomethyl)pyridine chloride (14c). Tri(*n*-butyl)phosphine (0.58 mL, 2.34 mmol) and compound **12** (0.25 g, 1.17 mmol) were added to acetoni-

trile (20 mL), the solution was stirred for 10 h at 55 °C. Then, the solvent was evaporated *in vacuo*, the dry residue was washed with light petroleum ether. The yield was 0.41 g (84%), a white crystalline compound, m.p. 168—170 °C (decomp.). ^{1}H NMR ($CDCl_3$), δ: 0.89 (t, 9 H, $P(CH_2CH_2CH_2C\underline{H}_3)_3$, J = 6.8 Hz); 1.32—1.53 (m, 12 H, $P(CH_2C\underline{H}_2C\underline{H}_2CH_3)_3$); 2.33 (s, 3 H, $C(O)C\underline{H}_3$); 2.34 (d, 3 H, Me, J = 2.3 Hz); 2.36 (d, 3 H, Me, J = = 0.8 Hz); 2.41—2.48 (m, 6 H, $P(C\underline{H}_2CH_2CH_2CH_3)_3$); 4.40 (br.s, 2 H, CH_2P); 8.19 (d, 1 H, CH_{Pyr}, J = 2.4 Hz). ^{13}C NMR ($CDCl_3$), δ: 13.45 (s, $PCH_2CH_2CH_2\underline{C}H_3$); 14.29 (s, Me); 18.90 (d, $P\underline{C}H_2CH_2CH_2CH_3$, J = 46.0 Hz); 19.48 (s, Me); 20.52 (s, Me); 22.65 (d, CH_2P, J = 45.7 Hz); 23.80 (d, $PCH_2\underline{C}H_2CH_2CH_3$, J = = 4.9 Hz); 24.13 (d, $PCH_2CH_2\underline{C}H_2CH_3$, J = 15.4 Hz); 123.60 (d, C_{Pyr}, J = 8.2 Hz); 140.50 (d, C_{Pyr}, J = 4.7 Hz); 145.69 (d, C_{Pyr}, J = 2.8 Hz); 147.45 (d, C_{Pyr}, J = 4.6 Hz); 151.59 (d, C_{Pyr}, J = 3.5 Hz); 168.22 (s, $\underline{C}(O)CH_3$). ^{31}P NMR ($CDCl_3$), δ: 34.39. HRMS, found: m/z 380.2713 $[M - Cl]^+$. $C_{22}H_{39}ClNO_2P$. Calculated: $[M - Cl]$ = 380.2713.

4,5-Bis(dichloromethyl)-3-hydroxy-2-methylpyridinium chloride (15). Pyridoxine hydrochloride (20.0 g, 0.10 mol) and DMF (5 mL) were added to dichloromethane (200 mL). Then, thionyl chloride (24 mL, 0.33 mol) was added to the mixture with continuous stirring over 10 min. The reaction mixture was refluxed for 12 h. A precipitate was filtered off and washed with dichloromethane. The yield was 22.6 g (96%), a white crystalline compound, m.p. 203—204 °C (decomp.) (*cf.* Ref. 30: m.p. 203 °C). ^{1}H NMR (DMSO-d_6), δ: 2.66 (s, 3 H, Me); 4.98 (s, 2 H, CH_2Cl); 5.01 (s, 2 H, CH_2Cl); 8.44 (s, 1 H, CH_{Pyr}). ^{13}C NMR (DMSO-d_6), δ: 16.03 (s, Me); 35.27 (s, CH_2Cl); 39.31 (s, CH_2Cl); 133.14 (s, C_{pyr}); 133.69 (s, C_{pyr}); 138.39 (s, C_{pyr}); 144.84 (s, C_{pyr}); 152.48 (s, C_{pyr}).

Studies of antibacterial activity. The minimum inhibiting concentration was determined as described earlier.[39] The following gram-positive strains were used: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermis* (clinical isolate, Kazan Scientific Research Institute of Epidemiology and Microbiology, Kazan, Russian Federation), *Bacillus subtilis* (Museum of the Microbiology Department of the Kazan Federal University, Kazan, Russian Federation); as well as the gram-negative strains *Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853, *Klebsiella pneumoniae* (clinical isolate, Kazan Scientific Research Institute of Epidemiology and Microbiology, Kazan, Russian Federation).

Comparative evaluation of antibacterial action was carried out using a micromethod for the determination of minimum inhibiting concentration (MIC) by the serial dilution method in broth, using 96-well sterile plates. The starting solution of test compounds were prepared in a concentration of 2000 μg mL^{-1}.

The first lowest concentration of a test compound (from the series of sequential dilutions), where no bacterial growth was visually determined, was considered as the minimum inhibiting concentration. A broth and a bacterial culture growth control were present in each experiment.

A pure, daily culture of microorganisms grown on a solid nutrient medium was used for the preparation of inoculum. A suspension of microorganisms was prepared in a sterile isotonic solution of sodium chloride, bringing the inoculum density to 0.5 according to the McFarland standard ($1.5 \cdot 10^8$ CFU mL^{-1}). Then, the inoculate was diluted to the concentration of $1 \cdot 10^7$ CFU mL^{-1} with a nutrient broth. The inoculum was used during 15 min after the preparation. The purity of bacterial strains was monitored before each experiment.

The broth (100 μL) was placed in the wells of each plate. In the first well, 100 μL of a test compound in a concentration of 2000 μg mL^{-1} were placed and by the sequential two-fold dilution its concentration was brought to 0.5 μg mL^{-1}. Then, a a prepared inoculum was added to each well, thus decreasing two-fold the concentration of the test compounds. The wells containing no test compound were included as controls (culture growth control). The purity of nutrient media and solvents was controlled in each experiment. The plates were incubated in a thermostat for 24 h at 36 °C. The culture growth was evaluated using a Multiskan FC photometer, comparing the growth of microorganisms in the presence of a test compound with the culture growth without them.

Determination of mutagenicity. The auxotrophic for histidine strains *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 were used as the indicator microorganisms. The presence of the mutagenic action in the agent was inferred from the induction of reverse mutations from the auxotrophy for histidine to the prototrophy. The testing was carried out according to a traditional scheme without a microsomal fraction. Distilled water was used as a negative control, 4-nitroquinoline *N*-oxide in a concentration of 10 μg dish^{-1} and sodium azide in a concentration of 1.5 μg dish^{-1} were used as a positive control for the strain *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100, respectively. The concentration of a compound under study in the test system was 100, 10, and 1 μg mL^{-1}. The results were assessed according to the algorithm described earlier.[39] For each variant, an average geometric number of revertants was calculated and the excess ratio of the average geometric number of revertants in the experiment over control was found. The presence of mutagenicity was registered at the excess ratio of 1.5 for the strains *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100).[40]

Determination of cytotoxicity. The breast cancer cells MCF-7 (ATCC® HTB-22™) were cultured in the medium α-MeM (PanEko, Russia) with the addition of 10% fetal calf serum (PAA, Australia), L-glutamine, and 1% penicillin—streptomycin in the CO_2 atmosphere (5%) at 37 °C to the formation of a monolayer. To prepare the cell suspension, the cell monolayer was trypsinized with subsequent inactivation of the trypsin by the addition of the α-MeM medium with the serum. Then, the cells were precipitated by centrifugation at 500 *g*. The precipitate was resuspended in the phosphate buffered saline. Evaluation of viability and calculation of density were performed after the cells were colored with 0.4% solution of trypan blue in a Neubauer haemocytometer chamber. Suspensions with the amount of viable cells ≥90% were used in the experiment.

The sensitivity of the tumor cells MCF-7 with respect to synthesized compounds was evaluated using a proliferative MTT-test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Promega, USA).

The cells (2000 cells in a well) were cultured in a nutrient medium (180 μL) according to the standard culturing conditions in a 96-well plate for 1 day. Then, a test agent (20 μL) was added and this was incubated for 72 h under standard conditions. Then, the medium with the agents was exchanged for the nutrient medium (80 μL), the MTT-reagent (20 μL, 5 mg mL^{-1}) was added, and this was incubated for 3.5 h. Then, the medium was removed and DMSO (100 μL) was added. After 10 min, optical density of the cell solutions was measured at 555 nm (the reference wavelength 650 nm) on a TECAN plate reader (Switzerland). The results were presented in the percent ratio to the control sample,

treated with no agents. The curve of the dose—effect dependence was plotted for the agents and the values IC_{50} ($P < 0.05$) were determined.

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