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Synthesis of the novel series of bispyridinium compounds bearing (*E*)-but-2-ene linker and evaluation of their reactivation activity against chlorpyrifos-inhibited acetylcholinesterase

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Abstract—Six potential AChE reactivators were synthesized using modification of currently known synthetic pathways. Their potency to reactivate AChE inhibited by insecticide chlorpyrifos was tested in vitro. According to the results, (*E*)-1-(2-hydroxyiminomethylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide seems to be the most potent AChE reactivator. The reactivation potency of these compounds depends on structural factors such as constitution of the linking chain between both pyridinium rings, position of the oxime moiety at the pyridinium ring and presence of quaternary nitrogens.

Organophosphates (OP) are used for military purposes as nerve agents (tabun, sarin, soman or VX) and also as pesticides in agriculture and for various purposes in the industry.[1] The threat of intoxications by these compounds rapidly increases in relationship with menace of terrorist attacks. The substances commonly used for agricultural purposes are, for example, parathion (*O*,*O*-diethyl-*O*-(4-nitrophenyl)thiophosphate), chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)thiophosphate) (Fig. 1).

These compounds inhibit irreversibly enzyme acetylcholinesterase (AChE, EC 3.1.1.7) in the same course as nerve agents do.[2] Their toxic effect is based on phosphorylation of the enzyme active site, where they are covalently bounded on serine hydroxyl group (Scheme 1).[2–5]

The inhibition of AChE leads to hyperstimulation of muscarinic and nicotinic receptors due to excess of acetylcholine.[6] The reactivator is able to cleave the OP moiety from inhibited AChE and thereby restore activity of the enzyme. The effective antidotes used among these intoxications are oxime reactivators usually in combination with atropine.[1] The often used reactivators of AChE are pralidoxime (**1**, 2-PAM, 2-hydroxyiminomethyl-1-methylpyridinium chloride), trimedoxime (**2**, TMB-4, 1,3-bis(4-hydroxyiminomethylpyridinium)-propane dibromide), obidoxime (**3**, 1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxa-propane dichloride), HI-6 (**4**, 1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride) and methoxime (**5**, MMC, bis(4-hydroxyiminomethylpyridinium)methane dichloride) (Fig. 2).[1,7–13] None of known reactivators is able to satisfactorily reactivate AChE inhibited by various types of OP due to the broad structural variability of OP.[14]

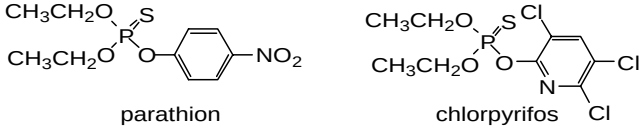

Figure 1. Organophosphate insecticides.

Keywords: Organophosphate; Acetylcholinesterase; Reactivation; Pesticide; Chlorpyrifos; Oxime.
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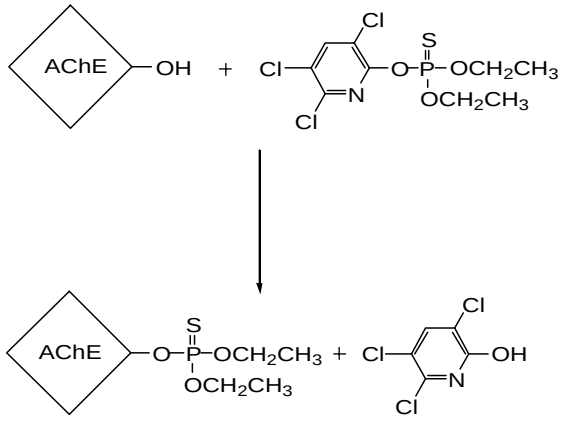


Scheme 1. Inhibition of AChE by chlorpyrifos.

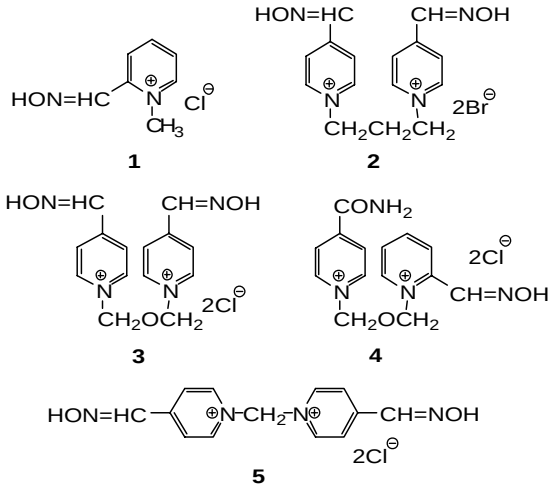


Figure 2. Currently used reactivators of AChE.

The development and selection of new effective reactivators of AChE like antidotes of OP are very important due to the extended usage of pesticides in agriculture and therefore eventual intoxications of human.[15]

Our work was focused on finding novel effective structures for the treatment of chlorpyrifos poisonings and testing their ability in vitro.

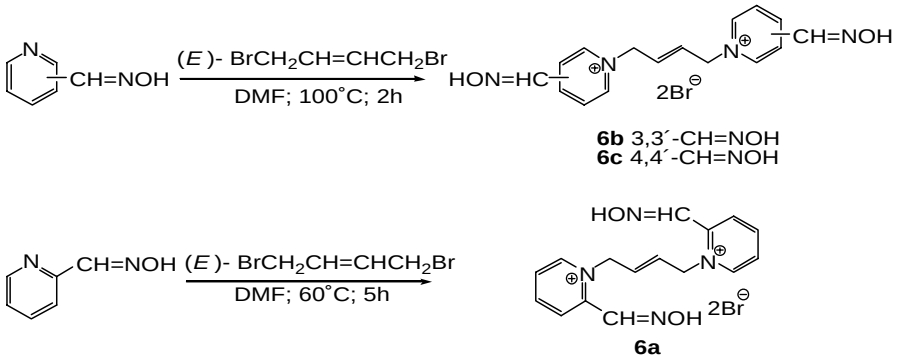
All reported compounds were prepared by alkylation of corresponding hydroxyiminomethylpyridine derivatives. In general, three chemical pathways were used. The symmetrical bispyridinium compounds **6b**,**c** were synthesized by modification of an older published system (Scheme 2).[16] A decomposition of the product in the reaction mixture owing to the powerful reaction conditions rendered it unable to apply the same procedure to the compound **6a**; therefore, the reaction temperature was decreased to 60 °C and time of reaction extended to 5 h (Scheme 2).

The asymmetric substances were prepared using a novel approach in two-step synthesis. At first the monoquaternary salts **8a**,**b** were obtained in acetone in various conditions depending on the position of the hydroxyiminomethyl group on the pyridinium ring (Scheme 3). An improvement used to increase the yields without excess bisquaternary product depends on the addition of the 5 equiv of alkylating agent to the reaction mixture. The monoquaternary salt was easily purified by the recrystallization from acetonitrile, where the bi-product is almost insoluble.

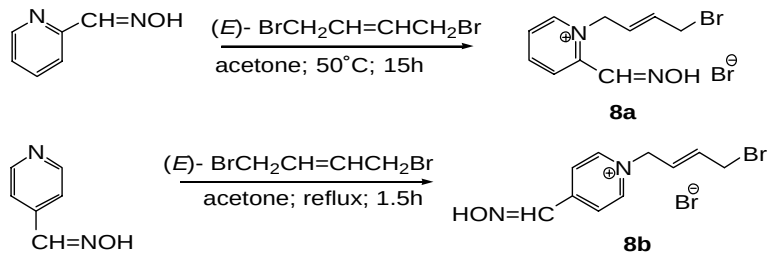
The second step consists in the completion of asymmetric bisquaternary compounds similar to the preparation of symmetrical substances (Scheme 4) in high yields (**7a**—82% and **7b**—85%) according to our unsuccessful attempts afford those from 3- or 4-monoquaternary salts and 2-hydroxyiminomethylpyridine under the same conditions.

In vitro testing of synthesized oximes involved a standard collection of experimental procedures. The 10% rat brain homogenate (source of AChE) in water was inhibited by chlorpyrifos to achieve 95% inhibition of AChE. After 30 min of incubation with chlorpyrifos, the reactivator was added to the solution for the next 10 min. Activities of intact AChE (a_0), inhibited AChE (a_i) and reactivated AChE (a_r) were deduced from the influence of consumption of NaOH solution (0.01 M) on time. The percentage of reactivation (%) was calculated from the measured data according to the formula:

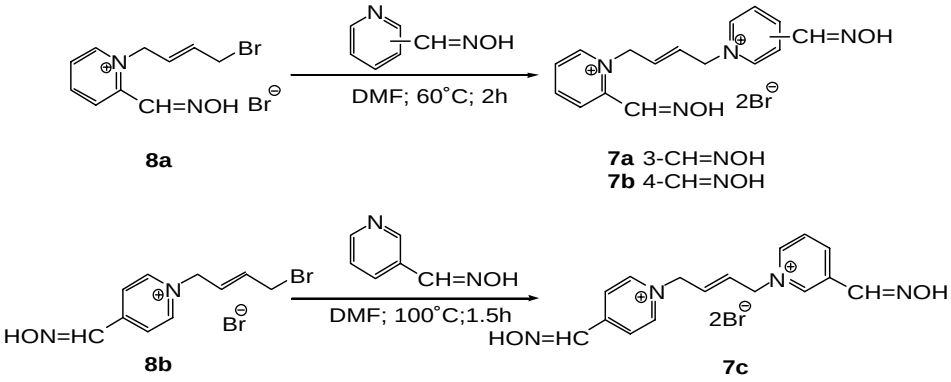
$$x = \left(1 - \frac{a_r - a_i}{a_0 - a_i}\right) \cdot 100[\%]$$



Scheme 2. The synthesis of symmetrical bispyridinium compounds.



Scheme 3. Preparation of monoquaternary salts in unsymmetrical synthesis.



Scheme 4. Completion of asymmetric bispyridinium compounds.

The whole method is described in detail in the work of Kuca and Kassa.[14] Pralidoxime (**1**), trimedoxime (**2**), obidoxime (**3**), HI-6 (**4**) and methoxime (**5**) previously synthesized in our laboratory were used as reference compounds. Their purity was estimated using HPLC. Obtained data are summarized in Table 1 and Figure 3.

The reactivation potency of tested compounds depends on the structure of the OP inhibitor.[14,17] The most potent reactivators of chlorpyrifos-inhibited AChE seem to be reference compounds obidoxime (**3**) and trimedoxime (**2**) in a concentration of 10^{-3} M. However, the newly synthesized oxime **7b** shows satisfactory reactivation results at this concentration too. Unfortunately, concentration of 10^{-3} M is not applicable for further in vivo testing.[14,17–19] The concentration of 10^{-5} M is more suitable from the point of view of reactivator's toxic effect on the patient.[14] It means that the oxime **7b** surpasses all other in vitro tested compounds (48% reactivation) and is the most promising among all the compounds tested. Oximes **6c**, **7a**,**c**, obidoxime (**3**) and trimedoxime (**2**) have also satisfactory reactivation ability at 10^{-5} M concentration.

Consequently, we can recommend structural factors appropriate for reactivation of chlorpyrifos-inhibited AChE.[14]

The oxime functional group breaks down the bond OP inhibitor enzyme and is essential for activity of the reactivator.[20,21] Our results confirm that position of hydroxyiminomethyl group influences the reactivation potency. While compound **6b** bearing both oxime groups in position 3 is practically ineffective, substances

Table 1. Reactivation potencies (%, mean value of three independent determinations) of tested oximes (time of inhibition by chlorpyrifos—30 min; time of reactivation by AChE reactivators—10 min; pH 8; temperature 25 °C)

	Compound	% (10^{-3} M)	% (10^{-5} M)
Reference compounds	Pralidoxime (**1**)	38	4
	Trimedoxime (**2**)	66	38
	Obidoxime (**3**)	63	35
	HI-6 (**4**)	20	11
	Methoxime (**5**)	45	10
Synthesized oximes	**6a**	4	9
	6b	13	2
	6c	25	38
	7a	30	22
	7b	63	48
	7c	34	24

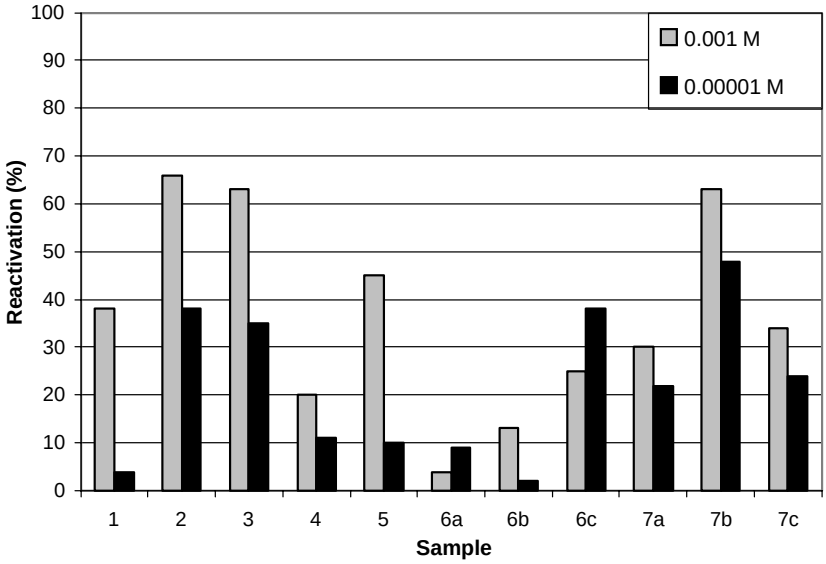


Figure 3. Efficacy of tested oximes in reactivation of chlorpyrifos-inhibited AChE.

6c and **7a**–**c** bearing at least one oxime group in position 2 or 4 show promising results with the surprising exception of compound **6a**.

The quaternary nitrogen is other important factor necessary for the ability of the reactivator to bind on the anionic sites of both intact and inhibited AChE.[20,21] It is generally known that bisquaternary substances have a higher affinity towards AChE in comparison with monoquaternary compounds due to the two cationic binding domains in the molecule of AChE.[22]

The length of the linking chain also influences the reactivation potency.[3] In our case, there is the same length of (*E*)-but-2-ene bridge for all compounds. This bridge is shorter than butane and longer than propane linker due to the known fact that a double bond is shorter than a single bond.[23] The optimal length of the linking chain lies between three and four atoms as was previously reported and synthesized compounds suit this purpose.[3]

On the other hand, there is a specific kind of 'rigidity' caused by the double bond in position (*E*-). The only bonds accessible for free rotation in these molecules are methylene junctions in contrast to free rotation in the molecules with propane **2** or 2-oxa-propane linking chains **3** and **4**. Therefore, the spatial orientation of pyridinium rings is limited.

The higher reactivation activity of some compounds (**6a**,**c**) at lower concentrations is caused by inhibition of the intact AChE by high affinity of some reactivators towards AChE (in vivo overdose can occur). The measurements were made for two concentrations and the whole concentration scale is generally bell-shaped (Fig. 4).[14] The reactivation process is characterized by the increasing part and the decreasing part shows both reactivation and subsequent inhibition of liberated intact AChE by the reactivator itself. Every reactivator varies with the optimal concentration for maximal reactivation potency and in the case of **6a** or **c** the optimum lies at lower concentrations.

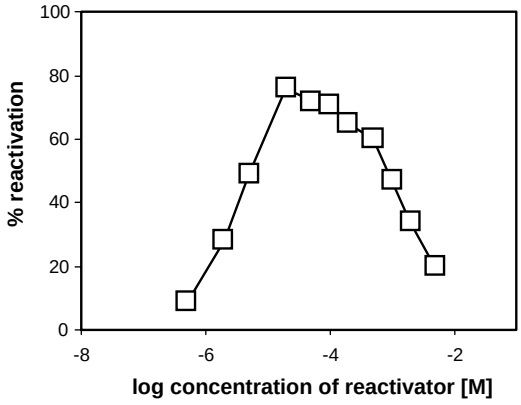


Figure 4. Reactivation curve of HI-6 (source of the enzyme—rat brain homogenate; AChE inhibitior—sarin; time of inhibition—30 min; time of reactivation—10 min; pH 8; temperature—25 °C).

Five novel reactivators **6a**,**b** and **7a**,**c** and one currently known reactivator **6c** were prepared in satisfactory yields and purity. Their ability to reactivate chlorpyrifos-inhibited AChE was measured in vitro. The most promising compound was (*E*)-1-(2-hydroxyiminomethylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide **7b**, which overlaps the effectiveness of trimedoxime **2** and obidoxime **3**. The reactivation potency of these compounds depends on structural factors such as position of the functional oxime group at the pyridinium ring, presence of quaternary nitrogens and the constitution of the linking chain.

Preparation of quaternary salts.[24,25] We had used three synthetic pathways for the preparation of bisquaternary aldoximes:

(A) A solution of the hydroxyiminomethylpyridine (1.0 g, 8.2 mmol) and (*E*)-1,4-dibromobut-2-ene (8.76 g, 40.9 mmol) in acetone (30 mL) was stirred (50 °C—reflux). The reaction mixture was cooled to room temperature and the crystalline crude product was collected by filtration, washed with acetone (2× 30 mL) and recrystallized from acetonitrile.
(B) A solution of the hydroxyiminomethylpyridine (1.0 g, 8.2 mmol) and (*E*)-1,4-dibromobut-2-ene (0.79 g, 3.7 mmol) in DMF (10 mL) was stirred in the range 60–100 °C depending on the substance synthesized. The reaction mixture was cooled to room temperature and portioned with acetone (30 mL); the crystalline crude product was collected by filtration, washed with acetone (2× 30 mL) and recrystallized from acetonitrile.
(C) A solution of the 1-(4-bromobut-2-enyl)-2-hydroxyiminomethylpyridinium bromide or 1-(4-bromobut-2-enyl)-4-hydroxyiminomethylpyridinium bromide (0.5 g, 1.5 mmol) and 3-hydroxyiminomethylpyridine or 4-hydroxyiminomethylpyridine (0.27 g, 2.2 mmol) in DMF (10 mL) was stirred (60–100 °C). The reaction mixture was cooled to room temperature and portioned with acetone (30 mL); the crystalline crude product was collected by filtration, washed with acetone (2× 30 mL) and recrystallized from acetonitrile.

Biochemistry. The 10% rat brain homogenate was used as a source of AChE. The brain homogenate (0.5 mL) was mixed with 20 μL of isopropanol solution of chlorpyrifos (99.2% analytical standard from Sigma–Aldrich) and incubated at 25 °C for 30 min. Three molar solution of sodium chloride (2.5 mL) was added to the mixture and filled to the volume of 23 mL with distilled water. Finally, 2 mL of solution of acetylcholine iodide (0.02 M) was added. The enzyme activity was measured at pH 8.0 and temperature 25 °C on autotitrator RTS 822 (Radiometer, Denmark). The same procedure was repeated with the reactivated enzyme subjected to further 10 min incubation with the reactivator.[14]

Acknowledgments

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24. Solvents (acetone and DMF) and reagents were purchased from Fluka and Sigma–Aldrich (Czech Republic), and used without further purification. Reactions were monitored by TLC using DC-Alufolien Cellulose F (Merck, Germany) and mobile-phase BuOH–CH_3COOH–H_2O, 5:1:2, detection by Dragendorff reagent (solution containing 10 mL CH_3COOH, 50 mL H_2O and 5 mL of basic solution prepared by mixing of two fractions—fraction I: 850 mg $Bi(NO_3)_3$, 40 mL H_2O, 10 mL CH_3COOH; fraction II: 8 g KI, 20 mL H_2O). Melting points were measured with a microheating stage PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany) and are uncorrected. NMR spectra were generally recorded at Varian Gemini 300 (^1H 300 MHz, ^{13}C 75 MHz, Palo Alto CA, USA). In all cases, the chemical shift values for ^1H spectra are reported in parts per million (δ) relative to residual $CHD_2SO_2CD_3$ (δ 2.50), shift values for ^{13}C spectra are reported in parts per million (δ) relative to solvent peak dimethylsulfoxide-d_6 δ 39.43. Signals are quoted as s (singlet), br s (broad singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded using a combination of high performance liquid chromatography and mass spectrometry. HP1100 HPLC system was obtained from Agilent Technologies (Waldbronn, Germany). It consisted of vacuum degasser G1322A, quaternary pump G1311A, autosampler G1313A and quadrupole mass spectrometer MSD1456 VL equipped with electrospray ionization source. Nitrogen for mass spectrometer was supplied by Whatman 75–720 nitrogen generator. Data were collected in positive ion mode with an ESI probe voltage of 4000 V. The pressure of nebulizer gas was set up to 35 psig. Drying gas temperature was operated at 335 °C and flowrate at 13 L/min.
25. (*E*)-1,4-Bis(2-hydroxyiminomethylpyridinium)-but-2-ene dibromide (**6a**). Prepared by method B. The reaction mixture was stirred at 60 °C and stopped after 5 h. Yield

0.78 g (46%), TLC R_f 0.2, mp decomp. 208 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.08 (d, 2H, J = 6.0 Hz, aryl), 8.67–8.54 (m, 4H, aryl + –*CH*=NOH), 8.39 (d, 2H, J = 8.0 Hz, aryl), 8.17–8.09 (m, 2H, aryl), 6.11–6.05 (m, 2H, –CH=), 5.57–5.53 (m, 4H, $-CH_2-$), −1.93 (br s, 2H, –CH*NOH*). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 147.05, 145.81, 145.69, 141.28, 129.09, 127.63, 125.68, 58.11. ESI-MS: *m*/*z* 149.1 $[M]^{2+}$ (calculated for $[C_8H_9N_2O]^{2+}$: 149.07). Anal. ($C_{16}H_{18}Br_2N_4O_2$): C, H, N. (*E*)-1,4-Bis(3-hydroxyiminomethylpyridinium)-but-2-ene dibromide (**6b**). Prepared by method B. The reaction mixture was stirred at 100 °C and stopped after 2 h. Yield 1.64 g (97%), TLC R_f 0.2, mp 249–250 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 12.26 (s, 2H, –CH=*NOH*), 9.33 (s, 2H, aryl), 9.10 (d, 2H, J = 6.0 Hz, aryl), 8.77 (d, 2H, J = 8.0 Hz, aryl), 8.40 (s, 2H, –*CH*=NOH), 8.26–8.15 (m, 2H, aryl), 6.32–6.23 (m, 2H, –CH=), 5.49–5.33 (m, 4H, $-CH_2-$). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 144.14, 143.89, 142.46, 141.96, 133.32, 129.64, 128.04, 61.58. ESI-MS: *m*/*z* 149.1 $[M]^{2+}$ (calculated for $[C_8H_9N_2O]^{2+}$: 149.07). Anal. ($C_{16}H_{18}Br_2N_4O_2$): C, H, N. (*E*)-1,4-Bis(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (**6c**). Prepared by method B. The reaction mixture was stirred at 100 °C and stopped after 1.5 h. Yield 1.58 g (93%), TLC R_f 0.2, mp 250–251 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.05 (d, 4H, J = 5.8 Hz, aryl), 8.46 (s, 2H, –*CH*=NOH), 8.26 (d, 4H, J = 6.0 Hz, aryl), 6.24-6.17 (m, 2H, –CH=), 5.34 (d, 4H, J = 3.3 Hz, $-CH_2-$), 3.41 (br s, 2H, –CH=*NOH*). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.66, 145.11, 145.06, 130.06, 124.04, 60.30. ESI MS: *m*/*z* 149.1 $[M]^{2+}$ (calculated for$[C_8H_9N_2O]^{2+}$: 149.07). Anal. ($C_{16}H_{18}Br_2N_4O_2$): C, H, N. (*E*)-1-(2-Hydroxyiminomethylpyridinium)-4-(3-hydroxyiminomethylpyridinium)-but-2-ene dibromide (**7a**). Prepared by method C. The reaction mixture was stirred at 60 °C and stopped after 2 h. Yield 0.56 g (82%), TLC R_f 0.2, mp 192–195 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 13.09 (s, 1H, –CH=*NOH*), 12.25 (s, 1H, –CH=*NOH*), 9.28 (s, 1H, aryl), 9.13 (d, 1H, J = 6.0 Hz, aryl), 9.01 (d, 1H, J = 6.0 Hz, aryl), 8.75 (d, 1H, J = 8.0 Hz, aryl), 8.69–8.55 (m, 2H, aryl + –*CH*=NOH), 8.48–8.33 (m, 2H, aryl + –*CH*=NOH), 8.23–8.11 (m, 2H, aryl), 6.40–6.24 (m, 1H, –CH=), 6.05–5.90 (m, 1H, –CH=), 5.56 (d, 2H, J = 4.7 Hz, $-CH_2-$), 5.36 (d, 2H, J = 6.3 Hz, $-CH_2-$). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 147.12, 146.03, 145.73, 144.24, 143.17, 142.41, 141.85, 141.42, 133.37, 131.51, 128.11, 127.70, 125.68, 61.01, 58.24, 35.76, 34.14. ESI-MS: *m*/*z* 149.1 $[M]^{2+}$ (calculated for $[C_8H_9N_2O]^{2+}$: 149.07). Anal. ($C_{16}H_{18}Br_2N_4O_2$): C, H, N. (*E*)-1-(2-hydroxyiminomethylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (**7b**). Prepared by method C. The reaction mixture was stirred at 60 °C and stopped after 2 h. Yield 0.58 g (85%), TLC R_f 0.2, mp 192–194 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 13.09 (s, 1H, –CH=*NOH*), 12.84 (s, 1H, –CH=*NOH*), 9.15 (d, 1H, J = 6.0 Hz, aryl), 9.02 (d, 2H, J = 6.0 Hz, aryl), 8.72–8.55 (m, 2H, aryl + –*CH*=NOH), 8.51–8.36 (m, 2H, aryl + –*CH*=NOH), 8.25 (d, 2H, J = 6.0 Hz, aryl), 8.20–8.10 (m, 1H, aryl), 6.37–6.22 (m, 1H, –CH=), 6.06–5.91 (m, 1H, –CH=), 5.57 (d, 2H, J = 4.7 Hz, $-CH_2-$), 5.32 (d, 2H, J = 6.3 Hz, $-CH_2-$). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.64, 147.10, 146.03, 145.73, 145.04, 144.90, 141.48, 131.20, 127.95, 127.70, 125.72, 123.98, 60.23, 58.23. ESI-MS: *m*/*z* 149.1 $[M]^{2+}$ (calculated for $[C_8H_9N_2O]^{2+}$:149.07). Anal. ($C_{16}H_{18}Br_2N_4O_2$): C, H, N. (*E*)-1-(3-Hydroxyiminomethylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (**7c**). Prepared by method C. The reaction mixture was stirred at 100 °C and stopped after 1.5 h. Yield 0.66 g (97%), TLC R_f 0.2, mp 228–230 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 12.85 (s, 1H, –CH=*NOH*), 12.25 (s, 1H, –CH=*NOH*), 9.33 (s, 1H, aryl), 9.12–9.04 (m, 3H, aryl), 8.77 (d, 1H, J = 8.0 Hz, aryl), 8.47 (s, 1H, –*CH*=NOH), 8.40 (s, 1H, –CH=NOH), 8.27 (d, 2H, J = 6.0 Hz, aryl), 8.24–8.16 (m, 1H, aryl), 6.33–6.15 (m, 2H, –CH=), 5.46–5.30 (m, 4H, $-CH_2-$). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.66, 145.13, 145.05, 144.48, 143.21, 142.54, 141.81, 133.38, 130.47, 129.70, 128.18, 124.04, 61.02, 60.28. ESI-MS: *m*/*z* 149.1 $[M]^{2+}$ (calculated for $[C_8H_9N_2O]^{2+}$: 149.07). Anal. ($C_{16}H_{18}Br_2N_4O_2$): C, H, N.

1-(4-Bromobut-2-enyl)-2-hydroxyiminomethylpyridinium bromide (**8a**). Prepared by method A. The reaction mixture was stirred at 50 °C and stopped after 15 h. Yield 1.04 g (38%), TLC R_f 0.2, mp 118–122 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.11 (d, 1H, J = 6.0 Hz, aryl), 8.78–8.54 (m, 2H, aryl + –*CH*=NOH), 8.42 (d, 1H, J = 6.6 Hz, aryl), 8.22–8.08 (m, 1H, aryl), 6.18–6.04 (m, 1H, –CH=), 6.03–5.82 (m, 1H, –CH=), 5.67–5.42 (m, 2H, $-CH_2-$), 4.77 (br s, 1H, –CH=*NOH*), 4.14 (d, 2H, J = 7.4 Hz, $-CH_2-$). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 147.06, 145.82, 145.68, 141.31, 132.05, 127.85, 125.66, 121.41, 60.13, 58.13. ESI-MS: *m*/*z* 254.9 $[M]^+$ (calculated for $[C_{10}H_{12}N_2O]^+$: 255.01). Anal. ($C_{10}H_{12}Br_2N_4O_2$): C, H, N.

1-(4-Bromobut-2-enyl)-4-hydroxyiminomethylpyridinium bromide (**8b**). Prepared by method A. The reaction mixture was stirred at reflux and stopped after 1.5 h. Yield 2.60 g (95%), TLC R_f 0.2, mp 187–191 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 12.85 (s, 1H, –CH=*NOH*), 9.02 (d, 2H, J = 6.0 Hz, aryl), 8.45 (s, 1H, –*CH*=NOH), 8.27 (d, 2H, J = 6.0 Hz, aryl), 6.26–6.09 (m, 1H, –CH=), 5.32 (d, 2H, J = 4.4 Hz, $-CH_2-$Br), 4.17 (d, 2H, J = 5.2 Hz, $-CH_2-$N=). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.67, 145.05, 144.85, 133.72, 127.69, 124.17, 60.25. ESI-MS: *m*/*z* 254.9 $[M]^+$ (calculated for $[C_{10}H_{12}N_2O]^+$: 255.01). Anal. ($C_{10}H_{12}Br_2N_4O_2$): C, H, N.