Brief Communications

Synthesis and antibacterial activity of new dimeric pyridinium chlorides based on 2,2-bis(hydroxymethyl)propane-1,3-diyl spacer*

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New bispyridinium dichlorides based on 2,2-bis(hydroxymethyl)propane-1,3-diyl spacer were synthesized from pentaerythritol, 4-aminopyridine, and carboxylic acids. The resulting biocides possess a high antibacterial effect. The minimal inhibitory concentration (MIC) of these compounds against pathogenic bacteria, namely, gram-positive *Methicillin-resistant Staphylococcus aureus* (strain ATCC 25923) and gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), was established. The influence of the alkyl substituent length in salts on their microbiological activity was studied.

Key words: bis-quaternary ammonium compounds, antibacterial agents, pyridinium salts, pentaerythritol, alkyl chlorides.

Quaternary ammonium compounds have been widely used as antiseptics and disinfectants since the middle of the 20th century.[1—3] Among them, quaternary bispyridinium salts are an important group of chemicals widely used as biocides due to their strong antimicrobial effect, even in very low concentrations, against a wide range of gram-positive and gram-negative bacteria, fungi, and some viruses.[4—9] Typically, these compounds contain two pyridine rings bound by a spacer (most often this is a long aliphatic chain). The rings have alkyl, alkenyl, or alkynyl substituent (chain) at *meta* or *para* positions from the spacer. Octenidine dihydrochloride **1** is one of the most efficient dimeric pyridinium salt. Octenidine-based antiseptic drugs have a damaging effect against a wide range

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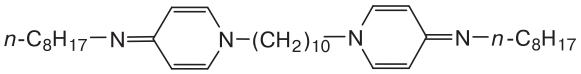
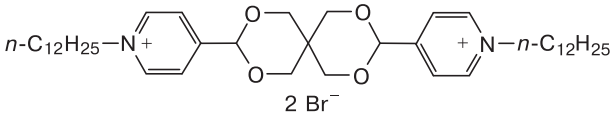
of multiresistant pathogens.[10–12] These pathogenic bacteria are the causative agents of a wide range of diseases, including nosocomial ones (tuberculosis, various types of pneumonia, gastroenteritis, *etc.*).

The study of the structure—biological activity relationship is one of the modern interdisciplinary approaches used in organic chemistry.[13] It is known that the nature of the spacer has a significant effect on the manifestation of biocidal action by dimeric quaternary ammonium compounds. The work[14] proposed an original approach to the synthesis of several new bispyridinium salts with oxygen-containing linear branched and cyclic spacers in the structure based on inexpensive pentaerythritol. It was found that type **2** compounds containing oxygen atoms in the cyclic spacer are less toxic than dimeric quaternary ammonium compounds without oxygen atoms, with their efficiency being not inferior to that of known antiseptics.[15]



1



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The present work reports the results of the synthesis and study of the microbiological activity of new dimeric quaternary pyridinium salts **3** based on 2,2-bis(hydroxymethyl)propane-1,3-diyl spacer. Compounds **3** were obtained from 2,2-bis(chloromethyl)propane-1,3-diol (**4**) and 4-alkylaminopyridines **5** (Scheme 1) by reflux in 4-methylpentan-2-one. The target bispyridinium salts **3a—f** were obtained in 65—75% yields. 2,2-Bis(chloromethyl)propane-1,3-diol (**4**) was obtained in two steps by the reaction of pentaerythritol and thionyl chloride with subsequent reflux of the resulting cyclic sulfite in an alcoholic solution of potassium hydroxide according to the known procedure.[16] 4-Alkylaminopyridines **5a—f** were synthesized from 4-aminopyridine and carboxylic acids in three steps using known procedures: 1) conversion of carboxylic acid into chloride;[17] 2) reaction of the acid chloride with 4-aminopyridine;[18] 3) reduction of the resulting *N*-(4-pyridyl)alkanamide.[18]

4-Methylpentan-2-one (methyl isobutyl ketone) was chosen as a solvent due to the fact that it was successfully used at the stage of *N*-alkylation of pyridine derivatives with alkyl halides in the synthesis of new dimeric quaternary ammonium compounds.[8,9] The resulting salts precipitated from the reaction mixture and were isolated by simple filtration.

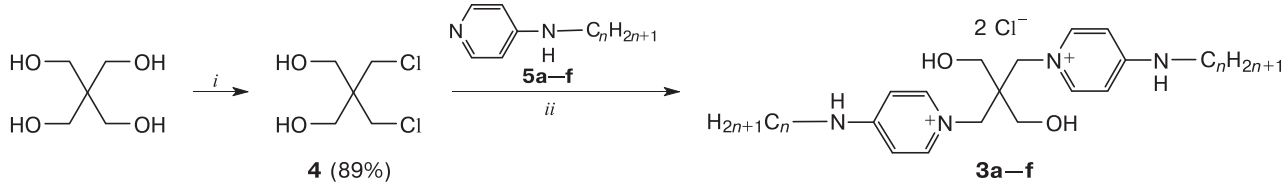
New compounds **3a—f** were tested for bacteriostatic action against resistant pathogenic bacteria: gram-positive *Methicillin-resistant Staphylococcus aureus* (*MRSA*, strain ATCC 25923) and gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) (Table 1). Octenidine dihydrochloride (**1**) and pentaerythritol-based compound **2** were used as reference compounds.

The relationship between the length of the alkyl chain in new dimeric salts **3** and their inhibitory concentration was established. The high activity (similar to that of the reference samples) against gram-positive *Staphylococcus aureus* was observed for compounds **3b—d** with the

Table 1. Minimum inhibitory concentration (MIC) of compounds **1**, **2**, and **3a—f**

Compound	MIC, ppm		
	MRSA	*E. coli*	*P. aeruginosa*
1	2	4	8
2	2	4	32
3a	64	128	>256
3b	2	8	64
3c	2	4	16
3d	2	4	64
3e	4	16	256
3f	8	64	>256

Scheme 1



4 (89%) **3a—f**

i. $SOCl_2$, then KOH/EtOH; ***ii***. 4-methylpentan-2-one, 118 °C, 24 h.

3	*n*	Yield (%)	**3**	*n*	Yield (%)
a	7	65	**d**	10	74
b	8	71	**e**	11	70
c	9	71	**f**	12	75

C(8)—C(10) alkyl substituents. Salts **3c** and **3d** are also not inferior to octenidine **1** and compound **2** against gram-negative *E.coli*. The synthesized compounds **3** are less efficient against *P.aeruginosa* than the reference samples. The best bacteriostatic effect among the new compounds is possessed by compound **3c** (with the *n*-nonyl group). An increase or decrease in the length of the alkyl fragment leads to a decrease in microbiological activity. Thus, compounds **3a** (C_7H_{15}), **3e** ($C_{11}H_{21}$), and **3f** ($C_{12}H_{25}$) are noticeably inferior to salts **3b—d** in the activity against *MRSA* and *E.coli* and they are inactive against *Pseudomonas aeruginosa*.

In conclusion, we synthesized new bis-quaternary ammonium compounds of the pyridinium series based on 2,2-bis(hydroxymethyl)propane-1,3-diyl spacer, which have pronounced antibacterial properties. Six salts with the length of the alkyl substituent from C_7H_{15} to $C_{12}H_{25}$ were obtained in 65—75% yields. The best MIC values against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* were shown by the compound with an alkyl substituent C_9H_{19}. This compound is comparable in microbiological activity with the known antibacterial agent octenidine dihydrochloride. An increase or decrease in the length of the alkyl substituent leads to a decrease in the microbiological effect.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer (300.13 MHz and 75.47 MHz, respectively). Electrospray ionization (ESI) high-resolution mass spectra were recorded on a Bruker micrOTOF II instrument. IR spectra were recorded in KBr tablets on a Specord M82 Fourier-transform spectrometer with Soft Spectra software. Melting points were obtained on a Gallenkamp apparatus. Thin layer chromatography was performed on precoated DC Kieselgel 60 F_{254} silica gel on aluminum plates.

2,2-Bis(chloromethyl)propane-1,3-diol (**4**) and 4-alkylaminopyridines **5a—f** were obtained using known procedures.[16—18]

Microbiological test. Test samples were prepared at a concentration of 20,000 ppm with ethanol, an aliquot (150 μL) was transferred using an Eppendorf tube and diluted in two steps with a liquid nutrient medium (to a final concentration of 256—0.25 ppm). An aliquot (150 μL) was added into a 96-well plate. Then a 10^6 CFU mL^{-1} aliquot of bacteria (150 μL) was added. The MICs were determined visually based on turbidity after incubation for 24 h at 37 °C.

Synthesis of bispyridinium salts 3a—f (general procedure). A mixture of 2,2-bis(chloromethyl)propane-1,3-diol (**4**) (0.346 g, 2 mmol) and 4-alkylaminopyridine **5a—f** (4 mmol) was refluxed in 4-methylpentan-2-one (4 mL) for 24 h. The reaction completion was determined by TLC (hexane—ethyl acetate (3 : 1)) based on the disappearance of spots of 4-alkylaminopyridines **5a—f**. The mixture was cooled and allowed to stand at 0 °C for 30 min. The precipitate was collected by filtration and dried to obtain pure products **3a—f**.

1,1′-[2,2-Bis(hydroxymethyl)propane-1,3-diyl]bis(4-heptylaminopyridinium) dichloride (3a). The yield was 0.72 g (65%), a white powder, m.p. 168—170 °C. ^{1}H NMR (DMSO-d_6, 300.13 MHz), δ: 0.85 (t, 6 H, 2 CH_3, J = 7.3 Hz); 1.18—1.40 (m, 16 H, 8 CH_2); 1.52—1.64 (m, 4 H, 2 CH_2); 3.04 (s, 4 H, 2 OCH_2); 3.23 (d, 4 H, 2 NCH_2, J = 7.0 Hz); 4.18 (s, 4 H, 2 N^+CH_2); 5.66 (s, 2 H, 2 OH); 6.84—7.00 (m, 4 H, 4 CH, Py), 8.06 (d, 2 H, Py, J = 6.6 Hz); 8.16 (d, 2 H, Py, J = 6.6 Hz); 9.00 (s, 2 H, 2 NH). ^{13}C NMR (DMSO-d_6, 75.47 MHz), δ: 13.9 (s, 2 C, CH_3); 21.9 (s, 2 C, CH_2); 26.2 (s, 2 C, CH_2); 27.9 (s, 2 C, CH_2); 28.5 (s, 2 C, CH_2); 31.0 (s, 2 C, CH_2); 42.3 (s, 2 C, NCH_2); 47.0 (s, 1 C); 55.6 (s, 2 C, OCH_2); 57.5 (s, 2 C, N^+CH_2); 104.9 (s, 2 C, Py); 109.9 (s, 2 C, Py); 143.0 (s, 2 C, Py); 144.4 (s, 2 C, Py); 156.4 (s, 2 C, Py). IR (KBr), ν/cm^{-1}: 3428, 3208, 2968, 1656, 1554, 1466, 1360, 1224, 1192, 848. MS (ESI): found m/z 243.1980 $[M - 2\,Cl]^{2+}$; calculated for $C_{29}H_{50}Cl_2N_4O_2$ 243.1967.

1,1′-[2,2-Bis(hydroxymethyl)propane-1,3-diyl]bis(4-octylaminopyridinium) dichloride (3b). The yield was 0.83 g (71%), a white powder, m.p. 158—160 °C. ^{1}H NMR (DMSO-d_6, 300.13 MHz), δ: 0.85 (t, 6 H, 2 CH_3, J = 7.3 Hz); 1.18—1.40 (m, 20 H, 10 CH_2); 1.50—1.62 (m, 4 H, 2 CH_2); 3.02 (s, 4 H, 2 OCH_2); 3.25 (d, 4 H, 2 NCH_2, J = 7.0 Hz); 4.18 (s, 4 H, 2 N^+CH_2); 5.70 (s, 2 H, 2 OH); 6.88—7.00 (m, 4 H, 4 CH, Py); 8.05 (d, 2 H, Py, J = 6.6 Hz); 8.18 (d, 2 H, Py, J = 6.6 Hz); 8.98 (s, 2 H, 2 NH). ^{13}C NMR (DMSO-d_6, 75.47 MHz), δ: 13.9 (s, 2 C, CH_3); 22.0 (s, 2 C, CH_2); 26.3 (s, 2 C, CH_2); 27.9 (s, 2 C, CH_2); 28.5 (s, 2 C, CH_2); 28.6 (s, 2 C, CH_2); 31.1 (s, 2 C, CH_2); 42.2 (s, 2 C, NCH_2); 46.8 (s, 1 C); 55.8 (s, 2 C, OCH_2); 57.3 (s, 2 C, N^+CH_2); 104.8 (s, 2 C, Py); 109.9 (s, 2 C, Py); 142.5 (s, 2 C, Py); 144.9 (s, 2 C, Py); 156.7 (s, 2 C, Py). IR (KBr), ν/cm^{-1}: 3423, 3210, 2968, 1656, 1560, 1463, 1362, 1224, 1192, 850. MS (ESI): found m/z 257.2131 $[M - 2\,Cl]^{2+}$; calculated for $C_{31}H_{54}Cl_2N_4O_2$ 257.2123.

1,1′-[2,2-Bis(hydroxymethyl)propane-1,3-diyl]bis(4-nonylaminopyridinium) dichloride (3c). The yield was 0.87 g (71%), a white powder, m.p. 152—155 °C. ^{1}H NMR (DMSO-d_6, 300.13 MHz), δ: 0.85 (t, 6 H, 2 CH_3, J = 7.3 Hz); 1.18—1.40 (m, 24 H, 12 CH_2); 1.48—1.64 (m, 4 H, 2 CH_2); 3.00 (s, 4 H, 2 OCH_2); 3.28 (d, 4 H, 2 NCH_2, J = 7.0 Hz); 4.20 (s, 4 H, 2 N^+CH_2); 5.68 (s, 2 H, 2 OH); 6.86—7.02 (m, 4 H, 4 CH, Py); 8.02 (d, 2 H, Py, J = 6.6 Hz); 8.21 (d, 2 H, Py, J = 6.6 Hz); 9.02 (s, 2 H, 2 NH). ^{13}C NMR (DMSO-d_6, 75.47 MHz), δ: 13.8 (s, 2 C, CH_3); 21.9 (s, 2 C, CH_2); 26.3 (s, 2 C, CH_2); 27.9 (s, 2 C, CH_2); 28.1 (s, 2 C, CH_2); 28.5 (s, 2 C, CH_2); 28.7 (s, 2 C, CH_2); 31.0 (s, 2 C, CH_2); 42.2 (s, 2 C, NCH_2); 46.7 (s, 1 C); 55.8 (s, 2 C, OCH_2); 57.3 (s, 2 C, N^+CH_2); 104.7 (s, 2 C, Py); 110.0 (s, 2 C, Py); 142.6 (s, 2 C, Py); 144.7 (s, 2 C, Py); 157.0 (s, 2 C, Py). IR (KBr), ν/cm^{-1}: 3434, 3200, 2964, 1656, 1560, 1468, 1362, 1224, 1192, 850. MS (ESI): found m/z 271.2291 $[M - 2\,Cl]^{2+}$; calculated for $C_{33}H_{58}Cl_2N_4O_2$ 271.2280.

1,1′-[2,2-Bis(hydroxymethyl)propane-1,3-diyl]bis(4-decylaminopyridinium) dichloride (3d). The yield was 0.95 g (74%), a white powder, m.p. 165—168 °C. ^{1}H NMR (DMSO-d_6, 300.13 MHz), δ: 0.85 (t, 6 H, 2 CH_3, J = 7.3 Hz); 1.18—1.40 (m, 24 H, 12 CH_2); 1.48—1.64 (m, 4 H, 2 CH_2); 3.00 (s, 4 H, 2 OCH_2); 3.28 (d, 4 H, 2 NCH_2, J = 7.0 Hz); 4.20 (s, 4 H, 2 N^+CH_2); 5.68 (s, 2 H, 2 OH); 6.86—7.02 (m, 4 H, 4 CH, Py); 8.02 (d, 2 H, Py, J = 6.6 Hz); 8.21 (d, 2 H, Py, J = 6.6 Hz); 9.02 (s, 2 H, 2 NH). ^{13}C NMR (DMSO-d_6, 75.47 MHz), δ: 13.8 (s, 2 C, CH_3); 21.9 (s, 2 C, CH_2); 26.3 (s, 2 C, CH_2); 27.9 (s, 2 C, CH_2); 28.1 (s, 2 C, CH_2); 28.5 (s, 2 C, CH_2); 28.7 (s, 2 C, CH_2); 28.9 (s, 2 C, CH_2); 31.2 (s, 2 C, CH_2); 42.2 (s, 2 C, NCH_2); 46.7 (s, 1 C); 55.8 (s, 2 C, OCH_2); 57.3 (s, 2 C, N^+CH_2); 104.7 (s, 2 C, Py); 110.0 (s, 2 C, Py); 142.6 (s, 2 C, Py); 144.7 (s, 2 C, Py);

157.0 (s, 2 C, Py). IR (KBr), ν/cm^{-1}: 3438, 3204, 2968, 1656, 1560, 1468, 1360, 1224, 1190, 848. MS (ESI): found *m/z* 285.2424 [M – 2 Cl]$^{2+}$; calculated for $C_{35}H_{62}Cl_2N_4O_2$ 285.2436.

1,1′-[2,2-Bis(hydroxymethyl)propane-1,3-diyl]bis(4-undecylaminopyridinium) dichloride (3e). The yield was 0.94 g (70%), a white powder, m.p. 144—147 °C. ^{1}H NMR (DMSO-d_6, 300.13 MHz), δ: 0.84 (t, 6 H, 2 CH_3, J = 7.3 Hz); 1.16—1.42 (m, 28 H, 14 CH_2); 1.48—1.62 (m, 4 H, 2 CH_2); 2.97 (s, 4 H, 2 OCH_2); 3.30 (d, 4 H, 2 NCH_2, J = 7.0 Hz); 4.18 (s, 4 H, 2 N^+CH_2); 5.70 (s, 2 H, 2 OH); 6.86—7.04 (m, 4 H, 4 CH, Py); 8.00 (d, 2 H, Py, J = 6.6 Hz); 8.18 (d, 2 H, Py, J = 6.6 Hz); 9.06 (s, 2 H, 2 NH). ^{13}C NMR (DMSO-d_6, 75.47 MHz), δ: 13.6 (s, 2 C, CH_3); 21.9 (s, 2 C, CH_2); 26.2 (s, 2 C, CH_2); 27.8 (s, 2 C, CH_2); 28.1 (s, 2 C, CH_2); 28.5 (s, 2 C, CH_2); 28.7 (s, 4 C, CH_2); 28.9 (s, 2 C, CH_2); 31.4 (s, 2 C, CH_2); 42.0 (s, 2 C, NCH_2); 46.5 (s, 1 C); 55.6 (s, 2 C, OCH_2); 57.1 (s, 2 C, N^+CH_2); 104.8 (s, 2 C, Py); 109.9 (s, 2 C, Py); 142.5 (s, 2 C, Py); 144.6 (s, 2 C, Py); 157.2 (s, 2 C, Py). IR (KBr), ν/cm^{-1}: 3444, 3198, 2968, 1660, 1564, 1468, 1360, 1224, 1192, 848. MS (ESI): found *m/z* 299.2600 [M – 2 Cl]$^{2+}$; calculated for $C_{37}H_{66}Cl_2N_4O_2$ 299.2593.

1,1′-[2,2-Bis(hydroxymethyl)propane-1,3-diyl]bis(4-dodecylaminopyridinium) dichloride (3f). The yield was 1.04 g (75%), a white powder, m.p. 150—153 °C. ^{1}H NMR (DMSO-d_6, 300.13 MHz), δ: 0.84 (t, 6 H, 2 CH_3, J = 7.3 Hz); 1.16—1.46 (m, 32 H, 16 CH_2); 1.50—1.64 (m, 4 H, 2 CH_2); 2.97 (s, 4 H, 2 OCH_2); 3.28 (d, 4 H, 2 NCH_2, J = 7.0 Hz); 4.14 (s, 4 H, 2 N^+CH_2); 5.74 (s, 2 H, 2 OH); 6.88—7.04 (m, 4 H, 4 CH, Py); 7.96 (d, 2 H, Py, J = 6.6 Hz); 8.14 (d, 2 H, Py, J = 6.6 Hz); 9.02 (s, 2 H, 2 NH). ^{13}C NMR (DMSO-d_6, 75.47 MHz), δ: 13.5 (s, 2 C, CH_3); 22.0 (s, 2 C, CH_2); 26.3 (s, 2 C, CH_2); 27.8 (s, 2 C, CH_2); 28.1 (s, 2 C, CH_2); 28.5 (s, 2 C, CH_2); 28.6 (s, 2 C, CH_2); 28.7 (s, 4 C, CH_2); 28.9 (s, 2 C, CH_2); 31.3 (s, 2 C, CH_2); 42.1 (s, 2 C, N—CH_2); 46.3 (s, 1 C); 55.4 (s, 2 C, OCH_2); 57.0 (s, 2 C, N^+CH_2); 104.6 (s, 2 C, Py); 109.7 (s, 2 C, Py); 142.3 (s, 2 C, Py); 144.4 (s, 2 C, Py); 157.0 (s, 2 C, Py). IR (KBr), ν/cm^{-1}: 3440, 3210, 2972, 1664, 1560, 1468, 1360, 1224, 1190, 850. MS (ESI): found *m/z* 313.2756 [M – 2 Cl]$^{2+}$; calculated for $C_{39}H_{70}Cl_2N_4O_2$ 313.2749.

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