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Biological activity of new amino phospha betaines with C_{10}–C_{18} alkyl groups

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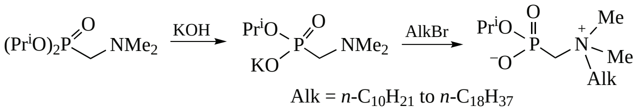
New amino phospha betaines were obtained by quaternization of potassium salt of amino phosphonate and higher alkyl bromides. The structure of isopropyl (*N*-dodecyl-*N*,*N*-dimethylammoniomethyl)phosphonate was confirmed by single crystal X-ray diffraction. All synthesized compounds demonstrate high antibacterial and antifungal activities.



Keywords: betaines, organophosphorus compounds, quaternary ammonium salts, amino phosphonates, antimicrobial activity, antifungal activity, X-ray diffraction.

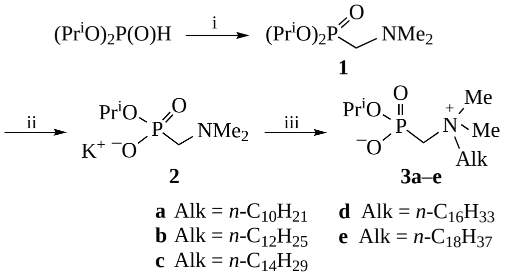
The ability of pathogenic microorganisms to inhabit in water, soil and air and therefore threaten human health makes the control of microbial infections an important task. The various types of antimicrobial agents, antibiotics, disinfectants and antiseptics have been developed to combat microbial pathogens.[1–4] Nowadays, quaternary ammonium salt (QAS) derivatives are the most common commercial antiseptics.[5–8] QAS chemistry has attracted the attention since early 1900s when bactericidal properties of compounds containing a quaternary nitrogen atom were discovered.[9–14] Currently, QASs and relative zwitterionic compounds have found wide use as hair shampoo components,[15] in antifouling applications,[16] biological and medical fields,[17,18] as surfactants in oil recovery,[19,20] and corrosion inhibitors.[21,22] Among electroneutral zwitterionic compounds, of practical interest are representatives bearing cationic moiety as quaternary ammonium or phosphonium group,[23,24] and the anionic moiety including carboxylate,[25,26] sulfonate,[27–29] sulfuric or phosphoric semiesters.[30–34]

In recent years, our interest has been focused on amino phospha betaines, analogues of QASs, in which one alkyl group contains phosphonate anionic substituent. This study is devoted to the synthesis of new amino phospha betaines which seem to be potential antimicrobial agents. To study their biological activity, water-soluble compounds with short-chain fragments at nitrogen and phosphorus atoms were regarded. However, to increase the antimicrobial activity, one substituent at the quaternary nitrogen atom should be higher normal alkyl. Therefore, amino phospha betaines of the $^{-}OP(O)(Pr^{i}O)CH_2N^{+}Me_2C_nH_{2n+1}$ (n = 10–18) formula were obtained.

The Kabachnik–Fields reaction of diisopropyl phosphite/paraformaldehyde/dimethylamine afforded diisopropyl (dimethylaminomethyl)phosphonate **1** in almost quantitative yield (Scheme 1). Next step required alkaline hydrolysis of only one ester group. For this purpose, the process was carried out in 1,4-dioxane with a 10% excess of aqueous alkali; however, the reaction was not complete even on heating and using more alkali (for ^{31}P NMR monitoring, see Online Supplementary Materials, Figure S1). To remove unreacted amino phosphonate **1**, we used liquid–liquid extraction in the water–benzene system (*cf*. Figure S2). The final quaternization of amino group in potassium salt **2** resulted in target amino phospha betaines **3a–e** (see Scheme 1).[35]

The herein performed transformation of the phosphoryl framework was also monitored by IR spectroscopy, which indicated a shift of the P–O–C band. For patterns of the FTIR spectra of diisopropyl phosphite, diisopropyl (dimethylaminomethyl)phosphonate **1** and the corresponding potassium salt **2** as well as amino phospha betaine **3a**, see Figure S4.

Compounds **3a–e** were purified by rinsing with petroleum ether and appeared as powders at room temperature. The yields of the reaction products were about 80%. Their structures were determined using FTIR, ^{1}H, ^{13}C, ^{31}P NMR spectroscopy and mass spectrometry. The structure of dodecyl derivative **3b** was ultimately established by single crystal X-ray study (Figure 1). Molecule of **3b** crystallizes as a zwitterion in $P\bar{1}$ space group. Only one symmetry independent molecule and water molecule exist in the unit cell.[†] In the crystal, intermolecular O–H···O hydrogen bonds between oxygen atom of phosphonate group and the water molecule link the molecules into chains propagating along the *0a* axis. The crystal packing is stabilized by multiple C–H···O interactions. Overall charge delocalization around P



Scheme 1 *Reagents and conditions*: i, $(CH_2O)_x$, Me_2NH, TsOH (cat.), benzene, reflux (50 °C), 4 h; ii, KOH (1.1 equiv.), 1,4-dioxane, reflux, 5 h; iii, AlkBr, Pr^iOH, reflux ($T \leqslant 80$ °C), 2 h.

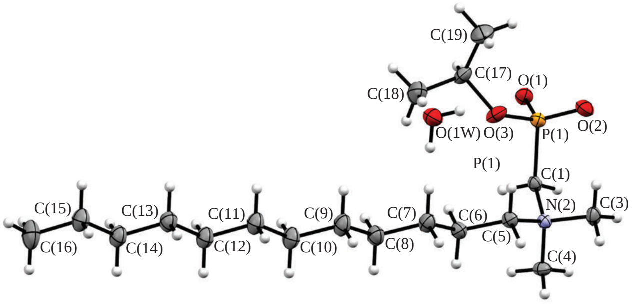


Figure 1 Geometry of compound **3b**. Thermal ellipsoids are drawn at 50% probability.

causes equating two P–O bond lengths [P(1)–O(1) = 1.489(2) Å and P(1)–O(2) = 1.487(2) Å].

The *in vitro* antibacterial and antifungal activities of the isopropyl (*N*-alkyl-*N*,*N*-dimethylammoniomethyl)phosphonates against the Gram-positive bacterial strains *Bacillus cereus* and *Staphylococcus aureus*; Gram-negative bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa* and fungi strains *Candida albicans* were evaluated (Table 1). Chlorhexidine and Clotrimazole were examined as the control substances. All new amino phospha betaines were active against Gram-positive bacteria and *Candida Albicans*. Inside the series, compounds **3c** and **3d** showed both highly significant antibacterial and antifungal activity against Gram-positive *Bacillus cereus*, *Staphylococcus aureus* and *Candida albicans*. An antimicrobial activity of betaines **3a–e** is a dome-shaped dependence and achieves a maximum at alkyl chain length C_{16} at nitrogen atom (*Bacillus cereus* and *Candida albicans*). Compound **3e** (C_{18}) exhibited less activity against Gram-positive *Bacillus cereus*, *Staphylococcus aureus* and *Candida albicans*, which is comparable with those of Chlorhexidine and Clotrimazole.

Compounds **3c** and **3d** showed significant activity against *Bacillus cereus* and *Candida albicans*, which is comparable or higher then that of reference compounds. All tested betaines were inactive against Gram-negative bacterial strains. Compounds **3c** and **3d** can be considered as leaders and are recommended for further study.

Table 1 Antibacterial and antifungal activity of isopropyl (*N*-alkyl-*N*,*N*-dimethylammoniomethyl)phosphonates **3a–e** and reference compounds (c = 10 mg ml^{-1}).[a]

Compound	Zone of inhibition, d/mm				
	Escherichia coli (–)	*Bacillus cereus* (+)	*Pseudomonas aeruginosa* (–)	*Staphylococcus aureus* (+)	*Candida albicans*
3a	–	9	–	7	8
3b	–	18	–	13	15
3c	–	23	–	13	20
3d	–	20	–	14	20
3e	–	14	–	15	15
Chlorhexidine	15	14	13	16	16
Clotrimazole	–	–	–	–	16

[a] Zone of inhibition 22 to 33: highly significant, between 15 to 21 mm: less significant, below 14 mm: poor activity.

In conclusion, we have developed a new efficient synthesis of amino phospha betaines bearing a diisopropyl moiety and with long alkyl chains at nitrogen atom. The synthetic strategy is based on the preparation of α-aminophosphonate **1** *via* the Kabachnik–Fields reaction followed by partial alkaline hydrolysis and N-quaternization. In the crystal of representative **3b**, intermolecular O–H···O hydrogen bonds between oxygen atom of phosphonate group and the water molecule linked into chains were discovered. The crystal packing is stabilized by multiple C–H···O interactions. All new compounds demonstrate a high antibacterial and antifungal activity against Gram-positive bacterial strains, which is comparable to or higher than that of Chlorhexidine. All compounds exhibit antifungal activity against *Candida albicans*.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.03.009.

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† *Crystal data for* **3b**. $C_{18}H_{42}NO_4P$ (M = 367.49 g mol^{-1}), triclinic, space group $P\bar{1}$ at 150(2) K: a = 6.1127(3), b = 7.7980(4) and c = 23.0293(10) Å, α = 88.408(2)°, β = 83.298(2)°, γ = 83.322(2)°, V = 1082.72(9) Å^3, Z = 2, d_{calc} = 1.127 g cm^{-3}, μ(MoKα) = 0.146 mm^{-1}, F(000) = 408. A total of 16126 reflections were collected [4930 independent reflections and 3339 independent reflections with $I \geqslant 2(\sigma)$, R_{int} = 0.0540], GOOF 1.071, final R indices [$I \geqslant 2(\sigma)$]: R_1 = 0.0506, wR_2 = 0.1212, R indices (all data): R_1 = 0.0895, wR_2 = 0.1575.

The X-ray diffraction data for the single crystal of compound **3b** were collected on a Bruker AXS Kappa diffractometer at 150 K using MoKα radiation (λ = 0.71073 Å). The data reduction package APEX3 v2019.1-0 was used for data processing.[36] Images were integrated and the final cell constants were determined by global refinement of reflections from the complete data using the SAINT v8.40A integration software.[37] Data were then scaled and corrected for systematic errors; the multi-scan absorption correction was applied by means of SADABS-2016/2.[38] The structure was solved by the direct methods using SHELXT-2018/2[39] and refined by the full matrix least-squares using SHELXL-2018/3[39] against F^2. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms at carbon atoms were placed into calculated positions and refined as riding atoms. The hydrogen atoms at the water molecule were revealed from difference Fourier series and refined isotropically. The details of hydrogen bonds and selected geometric parameters are given in Online Supplementary Materials, Tables S1,S2 and Figure S5.

CCDC 2093976 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

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