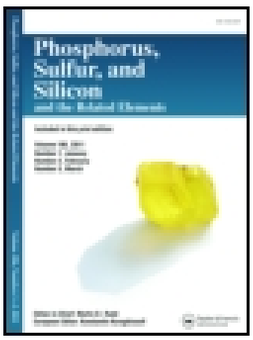

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ISSN: 1042-6507 (Print) 1563-5325 (Online) Journal homepage: http://www.tandfonline.com/loi/gpss20

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To cite this article: Irina V. Galkina, Alfiya F. Aksunova, Dmitriy I. Bakhtiyarov, Marina P. Shulaeva, Oskar K. Pozdeev, Svetlana N. Egorova, Yuliya V. Bakhtiyarova & Vladimir I. Galkin (2016): Synthesis and Biological Evaluation of Novel Carboxylate Phosphabetaines Derivatives with Long Alkyl Chains, Phosphorus, Sulfur, and Silicon and the Related Elements, DOI: 10.1080/10426507.2016.1227821

To link to this article: http://dx.doi.org/10.1080/10426507.2016.1227821

Accepted author version posted online: 29 Aug 2016.
Published online: 29 Aug 2016.

Synthesis and Biological Evaluation of Novel Carboxylate Phosphabetaines Derivatives with Long Alkyl Chains

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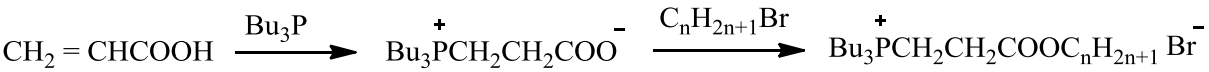
GRAPHICAL ABSTRACT



n = 10, 12, 14, 16, 18

Abstract *The purpose of the present study was to investigate the antibacterial activity of novel alkyl esters of carboxylate phosphabetaine: β-(carboxyalkyl)ethyltributylphosphonium bromides 4-8. The in vitro microbiological activity of the synthesized phosphonium bromides against gram-positive, gram-negative bacteria and the yeast Candida albicans was determined in comparison to standard agents. Microbiological results indicate the synthesized phosphonium salts possess a broad spectrum of activity against the tested microorganisms. Every newly synthesized compound was characterized by elemental analyses, IR, ^{1}H NMR, ^{31}P NMR spectral studies.*

Keywords Phosphabetaines; long alkyl chain*;* antimicrobial activity

INTRODUCTION

Demand for new antimicrobial agents is high because more microorganisms develop resistance against drugs currently available on the market. Resistance of pathogenic bacteria to antibiotics is rapidly becoming a major problem in the medical community and hospital-based healthcare settings. The search for novel agents to combat resistant bacteria has become one of the most important areas of antibacterial research today[1,2]. Pharmaceutical and organic chemists are trying to synthesize new drugs with better pharmacokinetic and dynamic properties. We have earlier described the methods of preparation various phosphabetaines based on tertiary phosphines and unsaturated carboxylic acids and reported on their structure and reactivity[3-7].

In this study, we prepared tributyl-substituted phosphonium salts 4-8 on the basis of phosphabetaines (1) containing alkyl chains of various lengths. The synthesis of such phosphonium salts is more difficult in comparison with ammonium analogs[8,9]. Betaines easily react with alkyl halogenides with short alkyl chains to form the corresponding phosphonium salts without biological activity[10,11].

RESULTS AND DISCUSSION

Chemistry

In this paper we present the synthesis and biological activity of a series of nanosized (30 nm) quaternary phosphonium salts 4-8 with long alkyl chains (R = C_nH_{2n+1}; n = 10, 12, 14, 16 18; here *n* is the number of carbon atoms in alkyl groups) on the basis of phosphabetaine 1 and

higher alkyl halogenides. All the synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, and ^{31}P NMR spectroscopy. The synthetic routes are given in Scheme 1.

Scheme 1 Depicts the synthesis of β-(carboxyalkyl)ethyltributylphosphonium bromides (4-8). Treatment of acrylic acid with tributylphosphine at room temperature in chloroform during 6 hours yielded (87%) phosphabetaine 1. Alkylation of the starting phosphabetaine 1 – *β*-tributylphosphonium ethylcarboxylate with alkyl halogenides (reflux for 14 hours in CH_3Cl) gave the corresponding phosphonium bromides 4-8 with long alkyl chains. The yield was 52%-72%.

Biological results

The synthesized compounds – a new class of bioactive nanomolecules – were screened for antibacterial and antifungal activity at 50μ/0.1mL concentration by using the cup-plate agar diffusion method, and standard drugs used were Chlorhexidine 9, Penicillin 10, and Griseofulvin 11. The novel synthesized compounds 4-8 with long alkyl chains (n = 10, 12, 14, 16, 18) show maximal activity against pathogenic microorganisms. Starting phosphabetaine 1 and all phosphonium salts with shot alkyl chains, synthesized earlier[10,11], were not active at all. Compound 6 and 7 were highly significant against tested bacteria as well as fungi. Our results are reported in Table 1.

Such a high biological activity of cationic biocides 4-8 we explain by their ability to be integrated into the lipid layers of biomembranes of pathogenic microflora eventually leading to the destruction of this last. To confirm this idea we studied the interaction mechanism of *β-(carboxyalkyl)ethyltriphenylphosphonium bromides* – analogs of compounds 4-8 and phosphorus

analogs synthetic of biomembranes – with natural biological membranes (lecithin) using the model of Langmuir monolayers[11]. It was discovered that alkylated phosphabetaines interact with lecithin, by forming a pores, and thus deteriorating the membrane functions.

Many years we thought that it is impossible to grow single crystals of oil products with long alkyl chains suitable for X-ray diffraction, but after five years we have a real chance to obtain the crystalline structure of the *β*-(carboxyhexadecyl)ethyltriphenylphosphonium bromide, which gave good quality crystals[3] (Figure 1).

CONCLUSIONS

In conclusion, carboxylate phosphabetaines derivatives with alkyl chains of various lengths were synthesized in good yield, characterized by different spectral studies, and their antimicrobial activity has been evaluated. Compounds 6 and 7 demonstrated good inhibitions against all the strains tested comparable to Chlorhexidine, Penicillin and Griseofulvin as positive standard. So, it may be concluded from our results that the synthesized compounds are potent nanoantimicrobial agents against pathogenic bacteria and fungi.

EXPERIMENTAL

Chemistry

Materials and Methods

All chemicals purchased from Sigma-Aldrich were reagent grade and used without purification. Analytical data were obtained from Perkin Elmer 2400 LS. And were found within

± 0.4% of the theoretical values. IR spectra were taken on a spectrophotometer Specord 251R in the range 400-3700 cm^{-1} from mulls in mineral oil between KBr plates. ^{1}H NMR (D_2O) and ^{31}P NMR (DMSO-d_6) spectra were determined on a Bruker Avance digital spectrometer 400.13 MHz. Chemical shifts were determined with respect to external reference, 85% H_3PO_4. The purity and thermal stability of crystal compounds were determined by simultaneous TG/DSC analysis on a NETZSCH STA 449C instrument (temperature range 20-400^{o}C, heating rate 10 deg/min, argon atmosphere).

Synthesis of tributyl-(3-alkoxy-3-oxopropyl)-phosphonium bromides (4-8)

General Procedure. A mixture of equimolar quantities of 3-(tributylphosphonio) propanoate **1** (0.01 mol) and appropriate alkyl halogenides (0.01 mol) was refluxed in dry chloroform (75 ml) for 1 h. Excess of solvent was removed under reduced pressure. The resulting salts 4-8 were obtained as yellow oils and purified by diethyl ether from starting reagents.

Preparation of tributyl-(3-decyloxy-3-oxopropyl)-phosphonium bromide (4). Yield 67%. IR (cm^{-1}): 1720, 1125. ^{1}H NMR (D_2O 400.13 MHz): 0.78-0.93 (m, 12H, 4Me), 1.23-1.45 (m, 26H, 13CH_2), 1.58-1.65 (m, 2H, OCCH_2), 2.35-2.41 (m, 2H, PCCH_2), 2.48-2.87 (m, 8H, PCH_2), 4.13-4.23 (m, 2H, OCH_2). ^{31}P NMR (DMSO-d_6, 161.9 MHz): 35.4. Anal (%) Calc. for $C_{25}H_{52}BrO_2P$: C, 60.61; H 10.51. Found: C, 59.97; H, 10.73. (FW=494.9).

Preparation of tributyl-(3-dodecyloxy-3-oxopropyl)-phosphonium bromide (5). Yield 60%. IR (cm^{-1}): 1721, 1121. ^{1}H NMR (D_2O, 400.13 MHz): 0.75-0.97 (m, 12H, 4Me), 1.25-1.43 (m, 30H, 15CH_2), 1.60-1.69 (m, 2H, OCCH_2), 2.31-2.41 (m, 2H, PCCH_2), 2.47-2.85 (m, 8H, PCH_2), 4.11-4.25 (m, 2H, OCH_2); ^{31}P NMR (DMSO-d_6, 161.9 MHz): 35.2. Anal (%) Calc. for $C_{27}H_{56}BrO_2P$ (FW=522.9): C 61.95; H 10.71. Found: C, 60.35; H, 10.39.

Preparation of tributyl-(3-tetradecyloxy-3-oxopropyl)phosphonium bromide (6). Yield 52%. IR (cm^{-1}): 1720, 1123. ^{1}H NMR (D_2O, 400.13 MHz): 0.81-0.91 (m, 12H, 4Me), 1.20-1.44 (m, 34H, $17CH_2$), 1.57-1.67 (m, 2H, $OCCH_2$), 2.37-2.43 (m, 2H, $PCCH_2$), 2.45-2.97 (m, 8H, PCH_2), 4.13-4.23 (m, 2H, OCH_2); ^{31}P NMR (DMSO-d_6, 161.9 MHz): 35.5. Anal (%) Calc. for $C_{29}H_{60}BrO_2P$: C, 63.16, H; 10.89. Found: C, 63.70; H, 10.13. (FW=550.9).

Preparation of tributyl-(3-hexadecyloxy-3-oxopropyl)phosphonium bromide (7). Yield 47%. IR (cm^{-1}): 1719, 1120. ^{1}H NMR (D_2O, 400.13 MHz): 0.73-0.92 (m, 12H, 4Me), 1.13-1.45 (m, 38H, $19CH_2$), 1.52-1.61 (m, 2H, $OCCH_2$), 2.31-2.38 (m, 2H, $PCCH_2$), 2.43-2.93 (m, 8H, PCH_2), 4.13-4.23 (m, 2H, OCH_2); ^{31}P NMR (DMSO-d_6, 161.9 MHz): 35.0. Anal (%) Calc. for $C_{31}H_{64}BrO_2P$ (FW=579): C, 64.25; H, 11.05. Found: C, 65.11; H, 11.66.

Preparation of tributyl-(3-octadecyloxy-3-oxopropyl)phosphonium bromide (8). Yield 72%. IR (cm^{-1}): 1720, 1125. ^{1}H NMR (D_2O, 400.13 MHz): 0.77-0.97 (m, 12H, 4Me), 1.19-1.47 (m, 42H, $21CH_2$), 1.59-1.65 (m, 2H, $OCCH_2$), 2.33-2.39 (m, 2H, $PCCH_2$), 2.45-2.86 (m, 8H, PCH_2), 4.13-4.23 (m, 2H, OCH_2); ^{31}P NMR (DMSO-d_6, 161.9 MHz): 35.3. Anal (%) Calc. for $C_{33}H_{68}BrO_2P$ (FW=606.9): C, 65.24; H, 11.20; found C, 65.87; H, 11.11.

Biological results

The antimicrobial activity of the newly synthesized compounds was determined in vitro using the agar disk-diffusion method using Mueller-Hilton agar medium against a variety of pathogenic microorganisms: *Staphylococcus aureus* (ATCC 29213) (Gram-positive bacteria), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginoza* (ATCC 27853), *Bacillus cereus* (ATCC 11778) and *Proteus mirabilis* (ATCC 12453) (Gram-negative bacteria) and fungus *Candida albicans* (ATCC 885-653). The inhibition zones of the tested compounds were

measured after 24-48 h incubation at 37^{o}C for bacteria and after 5 days of incubation at 28^{o}C for fungi. Penicillin (Sigma-Aldrich) and Chlorhexidine (Sigma-Aldrich) were used as reference drug for bacteria, whereas Griseofulvin (Sigma-Aldrich) was used as reference drug for fungi. Every experiment in the antibacterial and antifungal assay was replicated twice. For the antibacterial and antifungal activity, the compounds were dissolved in dimethylsulfoxide (DMSO).

The antifungal activity was carried out by using cup-plate method using cup-plate method using Sabouraud's agar medium. Fungal strains used were *Candida albicans* (ATCC 885-653) with incubation period of 48 hours at temperature 28^{o}C. The standard drug used was Griseofulvin (50 µg/0.1 mL) and the test compounds at concentration of 50 µg/0.1 mL by using dimethyl sulfoxide (DMSO).

ACKNOWLEDGEMENTS

This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities.

REFERENCES

1. Aysel, G.; Taylan, I.; Nalan, T.; and Gulten, O. *Turkish Journal of Pharmaceutical Sciences*, **2005**, 2 (1), 134-136.
2. Costerton, J.W.; Cheng, K.-J. *Journal of Antimicrob Chemother*, **1975**, 1, 363-377.
3. Galkina, I.; Bakhtiyarova, Yu.; Shulaeva, M.; Pozdeev, O.; Egorova, S.; Myrzakhmetova, N.; Cherkasov, R.; Galkin, V. *Journal of Chemistry*, **2013**, ID 302937.
4. Galkin, V.I.; Bakhtiyarova, Yu.V.; Polezhaeva, N.A.; Shaikhutdinova, R.A.; Klochkov, V.V. *Zhurnal Obshchei Khimii (Russian Journal of General Chemistry)*, **1998**, 68, 1104-1108.
5. Galkin, V.I.; Bakhtiyarova, Yu.V.; Polezhaeva, N.A.; Cherkasov, R.A.; Krivolapov, D.B.; Gubaidullin, A.T.; Litvinov, I.A. *Phosphorus, Sulfur Silicon Relat. Elem.*, **1999**, 144-146, 918-920.
6. Galkin, V.I.; Bakhtiyarova, Yu.V.; Zaripova, A.N.; Galkina, I.V.; Cherkasov, R.A.; Krivolapov, D.B.; Gubaidullin, A.T.; Litvinov I.A.; Pudovik, A.N. *Phosphorus, Sulfur, and Silicon and Related Elements*, 2002, 177, 2063-2065.
7. Galkin, V.I.; Bakhtiyarova, Yu.V.; Sagdieva, R.I.; Galkina, I.V.; Cherkasov, R.A. *Heteroatom Chemistry*, **2006**, 17(6), 557-565.
8. Kourai, H.; Horie, T.;Takeichi, K. *Journal of Antibacterial and Antifungal Agents*, **1980**, 8, 9-17.
9. Kanazava, A.; Ikeda, T.; Endo, T. *Antimicrobial Agents and Chemotherapy*, **1994**, 38 (5), 945-952.

10. Galkin, V.I.; Bakhtiyarova, Yu.V.; Polezhaeva, N.A.; Galkina, I.V.; Cherkasov, R.A.; Krivolapov, D.B;. Gubaidullin, A.T.; Litvinov. I.A. *Zhurnal Obshchei Khimii (Russian Journal of General Chemistry),* **2002**, 72, 404-411.
11. Galkina, I.V.; Melnikova, N.B.; Bakhtiyarova, Yu.V.; Stakheev, V.V.; Galkin, V.I.; Zhiltsova, O.Ye.; Zhukova, O.V.; Egorova. S.N. *Pharmaciya (Russian Journal of Farmacy),* **2010**, 3, 47-49.

Table 1. Antimicrobial activity of the newly synthesized compounds and the control drugs (50 μg/0.1 mL)

Zone of inhibition (mm)							
n	Compound	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Proteus mirabilis*	*Candida albicans*
4	$Bu_3\overset{\oplus}{P}CH_2CH_2COOC_{10}H_{21}\overset{\ominus}{Br}$	16	17	9	14	13	25
5	$Bu_3\overset{\oplus}{P}CH_2CH_2COOC_{12}H_{25}\overset{\ominus}{Br}$	21	11.5	14	11	15	23.5
6	$Bu_3\overset{\oplus}{P}CH_2CH_2COOC_{14}H_{29}\overset{\ominus}{Br}$	26	20	15	15	17.5	31
7	$Bu_3\overset{\oplus}{P}CH_2CH_2COOC_{16}H_{33}\overset{\ominus}{Br}$	27.5	21	17	17.5	21	37
8	$Bu_3\overset{\oplus}{P}CH_2CH_2COOC_{18}H_{37}\overset{\ominus}{Br}$	14	13	11	8	14	20.5
9	Chlorhexidine	16	15	13	13	15	14
10	Penicillin	20	17	11	7	10	-
11	Griseofulvin	-	-	-	-	-	20

n: Compound number

All tests were performed in triplicate. Zone of inhibition 22 to 31: highly significant, between 15 to 21 mm: less significant, below 14 mm: poor active.

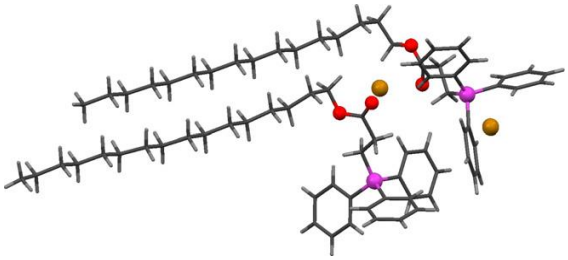
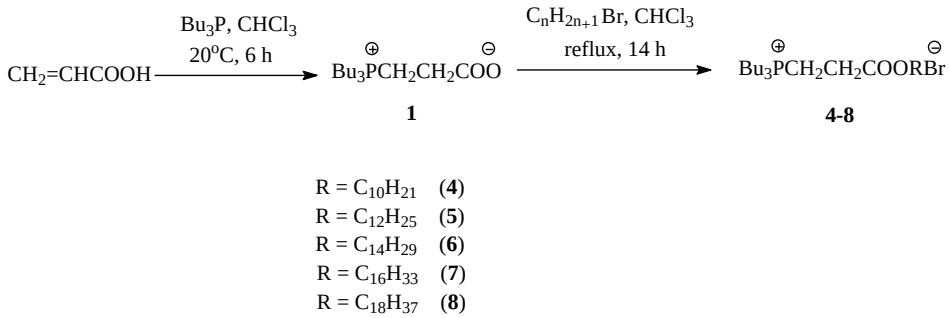


Figura 1. Molecular structure of the asymmetric unit of the $Ph_3PCH_2CH_2C(O)OC_{16}H_{33}$ in crystal.



Scheme 1. Synthetic routes of *β*-(carboxyalkyl)ethyltributylphosphonium bromides 4-8; reagents and conditions.