JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1273-1280

Synthesis, Characterization and Antimicrobial Activities of Dicationic Triphenylphosphonium Organic Salts with a *para* xylene Bridge and Fluorinated Counter Anions

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(Received: 12 February 2015; accepted: 18 March 2015)

Four fluorinated triphenylphosphine (PPh_3)-based organic salts were synthesised from PPh_3 and á,á'–dibromo-*p*-xylene by first producing quaternised salts, followed by anionic exchange with fluorinated anions, $NaBF_4$, KPF_6, CF_3SO_3Li and CF_3COONa. This is the first reported synthesis of the trifluoromethyl sulphonate and the trifluoro acetate ionic salts with the 1,4-phenylenebis(methylene)bis(triphenylphosphonium) dication. The compounds were synthesised in yields of between 80-98% using simple techniques. The salts were characterized by ^1H, ^{13}C, ^{19}F and ^{31}P NMR, and IR spectroscopy. The organic salts showed significant activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Candida albicans*, and *Streptococcus pyogenes* when compared with the activities of the standard antimicrobial chloramphenicol and antifungal nystatin.

Key words: Fluorinated phosphonium organic salts, Antimicrobial activity, Triphenylphosphine organic salts.

Phosphonium cations possess a tetrahedral geometry about the positively charged phosphorus atom, which is similar in structure to the ammonium cations commonly used as disinfectants and detergents[1]. The ammonium salts combine the features of a positive charge and a long hydrophobic alkyl chain which has been shown to be important in bactericidal action. The alkyl tails seem to improve interaction with the cell membrane and longer alkyl chains favour insertion into the hydrophobic region of the membranes and cause cell disruption[1]. While there is much literature on the antibacterial activity of ammonium salts, the literature on the antibacterial activity of phosphonium salts is rather limited with most of the reports published in Japanese patents. While both quaternary ammonium and phosphonium salts have shown high antibacterial activities with broad spectrums[2], the phosphonium salts were shown to have higher activities than their ammonium counterparts and are expected to be the next generation of antibacterial agents.

Trimethyl and dimethyl quaternary phosphonium salts functionalised with alkyl chains of varying lengths or aromatic groups ($^+P(CH_3)_2R_2$ or $^+P(CH_3)_3R$ where R = alkyl or Ph) were shown to be active against several strains of *S. aureus* including methicilin-resistant *S. aureus* (MRSA) and *E. coli*[3-4]. The trimethyl phosphonium salts

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with long alkyl chains had particularly good antimicrobial activity. In contrast, the activity of the dimethyl phosphonium salts was found to decrease on increasing the length of the alkyl chain[3]. Of particular importance is the fact that the phosphonium salts containing double decyl groups displayed the broadest spectrum of activity against the microorganisms tested and the greatest bacteriostatic properties. Further to this, the phosphonium salts showed a greater level of bactericidal activity and higher potency as compared to their ammonium counterparts[1]. Tetradecyl tributyl phosphonium bromide intercalated with clay minerals also showed activity against *S. aureus* and *E. coli*[5].

Antibacterial copolyesters prepared from tributylhexadecylphosphonium salts or tributyldodecylphosphonium salts with polyethylene terephthalate, dimethyl terephthalate and dimethyl-5-sulfoisophthalate were incorporated into laminates, which showed good antibacterial activity against *S. aureus* and *E. coli* and was used for packaging food and medical products[6-11]. A quaternary phosphonium salt was also used as part of a bactericide in an apparatus used in the treatment of waste gas discharged from a wastewater treatment plant[12].

The salts reported to have antibacterial activity were mainly prepared with either the chloride or bromide anion. A range of phosphonium ionic liquids functionalized with alkyl groups of varying chain lengths ranging from C2 to C16 on a trihexyl(alkyl)phosphonium salt was seen to have both antibacterial and antifungal activity[13]. The trihexyl(tetradecyl) phosphonium salts were studied with counter anions other than chloride or bromide which included BF_4^- and PF_6^- anions and the results showed a decrease in activity with their incorporation.

There are a few methods involved in the synthesis of tertiary phosphonium salts, but the simplest remains the mixing of a neutral organic compound with an alkyl or aryl halide followed by subsequent metathesis reaction with either organic or inorganic salts[14]. In this study we have substituted the conventional long chain alkylated phosphonium salts with a bis (triphenylphosphonium) salt bridged by a *para* xylene moiety to which four fluorinated anions, $CF_3SO_3^-$, BF_4^-, PF_6^- and CF_3COO^- were exchanged for the bromide in the initial reaction. All the anions contain fluorine bound to carbon, boron or phosphorus. To the best of our knowledge, the anions are commonly encountered in the preparation of ionic liquids, but in conjunction with the quaternary phosphonium cation their antimicrobial effect has not yet been studied. Hence, in this study we report on the synthesis and characterisation of two novel fluorinated organic salts with the phenylene *bis* (methylene)*bis* (triphenylphosphine) cation and the $CF_3SO_3^-$ and CF_3COO^- anions in addition to the cation's combination with the BF_4^- and PF_6^- anions, which had been reported previously[15-17]. In this study, we have studied their antimicrobial activity with five bacterial and one fungal strain.

MATERIALS AND METHODS

General experimental procedures

NMR spectra of all compounds were measured in DMSO-d_6 at room temperature on either a Bruker 400 or 600 MHz NMR spectrometer. The chemical shifts were referenced to tetramethylsilane for ^1H and ^{13}C NMR, triphenyl phosphate for ^{31}P NMR and trifluorotoluene for ^{19}F NMR and the operating frequencies on the 400 MHz NMR spectrometer for the ^{13}C, ^{31}P and ^{19}F NMR spectra were 100, 162 and 376.5 MHz respectively. The ^1H and ^{13}C NMR spectrum of compound **1** was acquired on the 600 MHz NMR instrument at operating frequencies of 600 MHz and 150 MHz respectively at a temperature of 50 °C. ESI and FAB Mass Spectrometry was carried out on a UPLC-DAD-MS with a Waters SYNAPT HDMS system (4KDA) connected in series to a SYNAPT G1 QTOF mass spectrometer and equipped with an Acquity HSS T3 Waters column (1.8 µm, 150 x 2.1 mm). IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR Spectrometer with a universal ATR sampling accessory. Melting points were recorded on an Ernst Leitz Wetziar micro-hot stage melting point apparatus. CHN analysis was carried out on a LECO CHNS Model 932 instrument and the analysis done in duplicate on 2.0 mg of sample.

Synthesis of 1,4-phenylenebis (methylene) bis (triphenylphosphonium) dibromide, $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}[2Br^-]$ (1)

In distilled dichloromethane, α,α'-

dibromo-*p*-xylene (0.528 g, 0.002 moles) was dissolved, to which triphenylphosphine (1.049 g, 0.004 moles) was added and stirred for 24 hrs at room temperature to afford the quaternised salt. The solvent was removed under pressure and the residue collected and washed with acetone to remove unreacted material. The residue was filtered and dried, yielding white precipitates of dicationic phosphonium salt, $C_{44}H_{38}Br_2P_2$ (90.0% yield), mp. 320 °C (decomposed). ^{1}H-NMR (600 MHz, DMSO-d$_{6,}$ 323 K), (δ_H, ppm): 5.12 (d, *J* = 14.6 Hz, 4H, Ar-$\underline{CH_2}$), 6.81 (s, 4H, Ar-H), 7.67 (m, 24H, Ar-H), and 7.90 (m, 6H, Ar-H). ^{13}C-NMR (δ_c, ppm): 28.49 (d, *J* = 46.3 Hz, (methylene)), 118.12 (d, *J* = 86.4 Hz, C-1'), 128.67 (d, *J* = 4.0 Hz, C-1/4), 130.52 (d, *J* = 5.9 Hz, C-3'/5')*, 130.56 (d, *J* = 6.4 Hz, C-3'/5')*, 131.63 (s, C-2/3/5/6/), 134.39 (d, *J* = 5.0 Hz, C-2'/6')*, 134.42 (d, *J* = 4.8 Hz, C-2'/6')*, 135.60 (s, C-4'). ^{31}P-NMR (δ_P, ppm): 23.06. IR (cm^{-1}): 3474, 3409, 2852, 1434, 1101. FAB-MS (*m/z*): 707.5 $[C_6H_4(CH_2)_2(PPh_3)_2Br]^+$; ESI-MS (*m/z*): 314 $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}$. Anal. C 66.72, H 4.88%, calcd for $C_{44}H_{38}Br_2P_2$, C 67.02, H 4.86%.

*The C-2'/6' and C-3'/5' resonances of the triphenylphosphine groups at each end are not equivalent with the other four phenyl groups and hence there are two separate resonances, very close to each other.

General procedure for the metathesis reaction

The salt prepared above (1) was dissolved in a mixture of water and ethanol (1:4) and the anionic source CF_3SO_3Li, $NaBF_4$, KPF_6, and CF_3COONa was added stoichiometrically in a ratio of 1:2 and the mixture was stirred respectively for 4 hrs at room temperature. It was concentrated under reduced pressure and $MgSO_4$ was added to remove the water. The solid material was extracted with ethanol to afford the corresponding anionic exchange salt. The solvent was evaporated and the samples dried to yield the fluorinated phosphonium organic salts (2-5).

The ^{31}P, ^{1}H and ^{13}C NMR resonances of the cations of compounds 2-5 are the same as for compound 1 and are not repeated below. The ^{19}F NMR resonances of the anions as well as any additional ^{13}C or ^{31}P resonances from the anions are however given below.

phenylenebis (methylene) bis (triphenylphosphonium) ditrifluoromethanesulphonate, $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}[2CF_3SO_3^-]$ (2)

$C_{46}H_{38}F_6O_6P_2S_2$, white powdery substance, yield 96.0%, mp. 258 °C, ^{13}C-NMR (100 MHz, δ_c, ppm): 120.63 (q, *J* = 320.1 Hz, $\underline{C}F_3SO_3^-$), ^{19}F NMR (400 MHz, DMSO-d$_6$, 298 K), (d$_F$, ppm): -77.74 (s, $C\underline{F}_3SO_3^-$), IR (cm^{-1}): 3065, 2955, 2916, 1439 and 1254. ESI MS (*m/z*): 777 $[C_6H_4(CH_2)_2(PPh_3)_2CF_3SO_3]^+$. Anal. C 58.03, H 4.74%, calcd for $C_{46}H_{38}F_6O_6P_2S_2$, C 59.61, H 4.13%.

phenylenebis(methylene) bis (triphenylphosphonium) ditetrafluoroborate, $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}[2BF_4^-]$ (3)

$C_{44}H_{38}B_2F_8P_2$, white crystalline substance, yield 79.8%, mp. 295 °C (decomposed), ^{19}F NMR (400 MHz, DMSO-d$_6$, 298 K), (δ_F, ppm): -148.22 ($^{10}BF_4^-$), -148.27 ($^{11}BF_4^-$), IR (cm^{-1}): 3062, 2977, 2935, 1438, 1111, 1046. ESI-MS (*m/z*): 716 $[C_6H_4(CH_2)_2(PPh_3)_2{}^{11}BF_4]^+$. Anal. C 64.68, H 4.81%, calcd for $C_{44}H_{38}B_2F_8P_2$, C 65.87, H 4.77%.

phenylenebis (methylene) bis (triphenylphosphonium) dihexafluorophosphate, $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}[2PF_6^-]$ (4)

$C_{44}H_{38}F_{12}P_4$, white powdery substance, yield 93.6%, mp. 274 °C, ^{31}P-NMR (δ, ppm): -144.20 (septet, *J* = 711.2 Hz, $\underline{P}F_6^-$), ^{19}F NMR (DMSO-d$_6$, 298 K), (d$_F$, ppm): -70.14 (d, $J_{P,F}$ = 711.2 Hz, $P\underline{F}_6^-$), IR (cm^{-1}): 3066, 1438, 1112. ESI-MS (*m/z*): 773 $[C_6H_4(CH_2)_2(PPh_3)_2PF_6]^+$. Anal. C 57.93, H 4.01%, calcd for $C_{44}H_{38}F_{12}P_4$, C 57.53, H 4.17%.

phenylenebis (methylene) bis (triphenylphosphonium) ditrifluoroacetate, $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}[2CF_3COO^-]$ (5)

$C_{48}H_{38}F_6O_4P_2$, white powdery substance, yield 98.0%, mp. 300 °C (decomposed), ^{13}C-NMR (100 MHz, δ_c, ppm): 157.68 (q, $J_{C,F}$ = 30.6 Hz, $CF_3\underline{C}OO^-$), 117.48 (q, $J_{C,F}$ = 296.2 Hz), ^{19}F NMR (DMSO-d$_6$, 298 K), (d$_F$, ppm): -73.44 (CF_3COO^-), IR (cm^{-1}): 3648, 3390, 3053, 3009, 2878, 2843, 2777, 1435 and 1109. ESI-MS (*m/z*): 314 $[C_6H_4(CH_2)_2(PPh_3)_2]^{2+}$, the monocation could not be detected. Due to impurities, the CHN analysis was not reported.

Antimicrobial assays

The antimicrobial activities (MIC's and MBC's) of compounds **1-5** were determined using standard American Type Culture Collection (ATCC), National Collection Type Cultures (NCTC) and clinical isolates from the department of Medical Microbiology, Ahmadu Bello University, Teaching Hospital, Zaria, Nigeria: *Staphylococcus aureus* ATCC 13709, *Escherichia coli* NCTC 10418, *Klebsiella pneumoniae* ATCC 10031, *Salmonella typhi* ATCC 9184, *Candida albicans* ATCC 10231 and *Streptococcus pyogenes* (clinical isolates). All the bacteria and fungi (*Candida albicans*) were checked for purity and maintained in slants of nutrient agar and sabouraud dextrose agar respectively. The "well diffusion" method was used to determine the antimicrobial activity of the compounds[18]. Chloramphenicol and nystatin were used as positive controls. A 250 mg capsule of chloramphenicol was diluted into 1 L of distilled water to obtain a concentration of 0.250 mg mL^{-1} (0.774 mM), which was used as a stock solution for further two-fold dilutions. For nystatin, 10 mL was dissolved in 100 mL of water to reach a concentration of 0.1 mL mL^{-1} (107.98 mM), which was used as the stock solution and diluted accordingly for the assays.

To calculate the zones of inhibition, blood agar medium was used as the growth medium for the microbes and was prepared according to the manufacturer's instruction; it was boiled to dissolve and sterilized at 121 °C for 15 minutes. The medium was dispensed into sterilized petri dishes and left to solidify, after which it was seeded with standard inoculums of the microbes by means of a sterile swab, spreading the inoculum evenly over the surface of the medium and allowing it to dry at 37 °C for 30 minutes. A standard cork borer of 6 mm in diameter was used to cut a well at the centre of each seeded medium. Compounds **1-5** (0.05 g) were weighed and dissolved in 10 mL of distilled water to a concentration of 5 mg mL^{-1} and 100 mL was introduced into each well and incubated at 37 °C for 24 hrs. The activity of the compound was determined by the presence of inhibition zones which were measured in millimeters using a transparent ruler.

The Minimum Inhibitory Concentration (MIC) of compounds **1-5** was determined against the microbes using the "broth dilution" method[18]. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. Nutrient broth was prepared according to the manufacturer's instruction and 10 mL of the broth dispensed into each test tube after which it was sterilized at 121 °C for 15 minutes and allowed to cool. The microbial innoculum was standardized using Mc-Farland's standard no. 0.5 to yield a microbial density of 1.5×10^8 cfu mL^{-1} or its optical equivalent. A two-fold serial dilution of the compound in the broth was done to obtain concentrations of 5 mg mL^{-1}, 2.5 mg mL^{-1}, 1.25 mg mL^{-1}, 0.625 mg mL^{-1}, 0.312 mg mL^{-1}, 0.1565 mg mL^{-1} and 0.0781 mg mL^{-1}. The stock solution was prepared by dissolving 0.05 g of the compounds in 10 mL of the sterilized broth. The micro-organisms (0.1 mL in normal saline) were then introduced into the test tubes with different concentrations and were incubated at 37 °C for 24 hrs, after which the test tubes were observed for turbidity. The lowest concentration of the compound in which the broth showed no turbidity was recorded as the MIC and the concentrations converted to mM.

Minimum bactericidal concentration (MBC) of the compounds **1-5** was determined in order to check whether the micro-organisms were killed as opposed to only their growth being inhibited. MBC is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture onto antibiotic free media[18]. Blood agar was prepared, sterilized at 121 °C for 15 minutes and dispensed into sterile petri dishes. The contents of the MIC in the serial dilution were then sub-cultured into the labelled plates. The plates were incubated at 37 °C for 24 hrs, after which the plates were observed for growth. The MBC was the plate with the lowest concentration of the compound without growth. All experiments were repeated in triplicate.

RESULTS AND DISCUSSION

The synthesis of dimeric ionic liquids using imidazole as the neutral organic compound was adopted from Ganesan and Alias[19] and modified by using dichloromethane in place of acetonitrile and changing the neutral organic compound to triphenylphosphine. The reaction of α,α'-dibromoxylene and triphenylphosphine resulted in the formation of the quaternised salt, 1,4-phenylenebis (methylene) bis (triphenylphosphonium) dibromide (**1**), which was followed by metathesis with four fluorinated anions ($CF_3SO_3^-$, BF_4^-, PF_6^- and CF_3COO^-) to afford the organic salts as anionic exchange products (**2-5**) (Figure 1). This is the first reported synthesis of the trifluoromethyl sulphonate (**2**) and the trifluoro acetate (**5**) ionic salts with the 1,4-phenylenebis (methylene) bis(triphenylphosphonium) dication, which has been achieved in excellent yields (80-

98%) using relatively simple techniques. They are all high melting organic salts (250 - 320 C) and therefore have the potential to be incorporated into packaging and medical products to confer their antimicrobial properties to them.

Characterisation

A full characterization of compound **1** was carried out and compared to **2-5** in order to understand the effect of changing the anion on the chemical and structural behaviour of the various organic salts. The salts (**1-5**) were characterized by ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR, and IR spectroscopy. The ^{1}H NMR spectrum of compound **1** in DMSO-d_6 showed a doublet resonance for the methylene group at d_H 5.12 (d, *J* = 14.58 Hz) since the protons are split by phosphorus of the PPh_3 group. The aromatic methine protons of the phenyl groups attached to the phosphorus are present as multiplet resonances at d_H 7.67 (*ortho* and *meta* protons) and 7.90 (*para* proton) in a 4:1 integral ratio. The four aromatic protons of the central aromatic ring are all equivalent and appear as a singlet at d_H 6.81.

The ^{13}C NMR spectrum shows the methylene carbon resonance as a doublet at d_c 28.49 ($J_{P,C}$ = 46.3 Hz) and the carbon methine resonances of the central aromatic group at d_c 131.63 (evidenced by a HSQC correlation with aromatic protons of the central aromatic group at d_H 6.8). The C-4' carbon resonance of all the phenyl groups attached to the phosphonium cation are equivalent and is located at d_c 135.59, which was confirmed by a HSQC correlation to H-4'. However, the C-2'/6' and C-3'/5' carbon resonances of the phenyl groups at each end of the central aromatic ring are not equivalent with the other four phenyl groups. They were assigned with the aid of data from the literature[20]. The two C-2'/6' resonances of the triphenylphosphine group appear as overlapping doublets at d_c 134.42 (*J* = 4.8 Hz) and d_c 134.39 (*J* = 5.0 Hz) respectively. The small coupling constant is due to $^{2}J_{P\text{-}C}$ coupling. The resonances appeared as a triplet resulting from coalescence of these resonances. The C-3'/5' resonances appeared at d_C 130.56 (*J* = 6.4 Hz) and d_C 130.52 (*J* = 5.9 Hz). This also appeared as a triplet as for C-2'.

The quaternary carbon resonance of the phosphine phenyl groups (C-1') can be seen at d 118.12 (*J* = 86.4 Hz), also consistent with literature[20] and C-1/4 of the central aromatic group could be detected as a doublet due to $^{2}J_{P\text{-}C}$ coupling at d

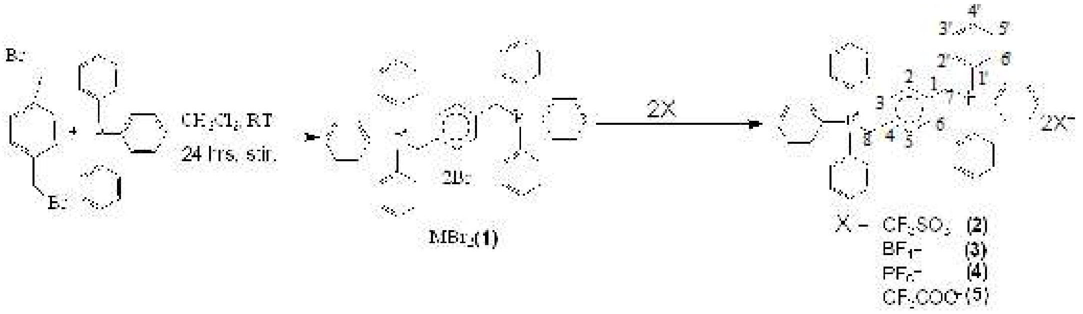

Fig. 1. Reaction scheme for the synthesis of the phosphonium organic salts 1-5

Table 1. Zones of inhibition of the phosphonium salts (**1-5**) against the microorganism (mm)

Sample/Test organisms	1	2	3	4	5	$6^{\#}$	$7^{\#}$
S. aureus	43	30	42	45	53	27	*
S. pyogenes	38	42	40	34	50	24	*
E. coli	45	45	20	45	42	31	*
S. typhi	45	40	38	42	42	38	*
K. pneumoniae	45	40	38	38	45	0	*
C. albicans	40	38	45	45	40	*	32

* test not performed.$^{\#}$ 6 and 7 are the controls, chloramphenicol and nystatin respectively

128.67 (J = 4.0 Hz), confirmed by an HMBC correlation to H-2/3/5/6 of the central aromatic group as well as with the methylene group. The C-1' carbon resonance also shows HMBC coupling to the methylene group further supporting its assignment. Coupling in the NOESY spectrum is evident between the methylene group and H-2'/6' of the phenyl groups as well as with the central aromatic protons. Also present in the NOESY spectrum is coupling between the central aromatic protons and that assigned to H-2'/3'/5'/6', putting the phenyl groups attached to phosphorus in close proximity to the central aromatic group. A crystal structure of **3** shows the interaction of the phenyl groups of the phosphine moieties with the central benzene group[17].

The ^{31}P NMR spectrum showed the phosphorus resonance of the phosphonium moieties at d 23.06. Compounds **2-5** all showed the same ^{1}H and ^{13}C NMR resonances for the cation when compared to **1** indicating the anion had no effect on the NMR resonances of the cation and that the anion was weakly co-ordinating. For the $CF_3SO_3^-$ anion **2**, an additional CF_3 carbon resonance was seen at d_C 120.63 (q, J = 320.1 Hz). This CF_3 carbon resonance was also present in the ^{13}C NMR spectrum of **5** at d_C 117.48 (q, J = 296.2 Hz) along with the carbonyl quartet at d 157.68 (J = 30.6 Hz). In the case of $M(PF_6)_2$, an additional phosphorus resonance for the PF_6^- anion could be seen at d -144.20, which is split into a septet with a coupling constant of 711.2 Hz. The fluorine NMR resonance for compound **4** (the PF_6^- anion) was detected at d -70.14 as a doublet resulting from P-F coupling with $J_{P,F}$ = 711.2 Hz. As the electronegativity of the central atom in the anion decreased from phosphorus through to boron, the chemical shift of the fluorine resonance decreased from d -70.14 in **4** ($M(PF_6^-)_2$) to d -73.44 in **5** ($M(CF_3COO^-)_2$), d -77.40 in **2** ($M(CF_3SO_3^-)_2$), and d -148.22 ($M(^{10}BF_4^-)_2$) and d -148.27 ($M(^{11}BF_4^-)_2$) in **3** ($M(BF_4^-)_2$). The ^{13}C, ^{31}P and ^{19}F NMR resonances of the anions compare well with that in the literature[21-22].

Antimicrobial Activity

All of the five samples synthesized were sensitive to the six microorganisms tested. Chloramphenicol (**6**), a broad spectrum antibiotic, effective against a wide variety of gram positive and gram negative bacteria, and nystatin (**7**), a

Table 2. The Minimum Inhibitory Concentrations (MIC) of compounds 1-5 in mM

Sample/Test organisms	1	2	3	4	5	6#	7#
S. aureus	0.496	0.674	0.400	0.340	0.365	0.387	*
S. pyogenes	0.994	0.337	0.400	0.680	0.365	0.387	*
E. coli	0.496	0.337	1.601	0.340	0.731	0.195	*
S. typhi	0.496	0.337	0.801	0.340	0.731	0.195	*
K. pneumoniae	0.496	0.337	0.801	0.680	0.731	R	*
C. albicans	0.496	0.674	0.400	0.340	0.731	*	0.027

R denotes resistance. * indicates experiment not carried out. # 6 and 7 are the controls, chloramphenicol and nystatin respectively

Table 3. The Minimum Bactericidal Concentration (MBC) of compounds 1-5 in mM

Sample/Test organisms	1	2	3	4	5	6#	7#
S. aureus	0.994	1.349	0.801	0.680	0.731	0.774	*
S. pyogenes	1.988	0.674	0.801	1.361	0.731	0.774	*
E. coli	0.994	0.674	1.601	0.680	1.462	0.387	*
S. typhi	0.994	0.674	1.601	0.680	1.462	0.387	*
K. pneumoniae	0.994	0.674	1.601	1.361	1.462	R	*
C. albicans	0.994	1.349	0.801	0.680	1.462	*	0.054

R denotes resistance. * indicates experiment not carried out. # 6 and 7 are the controls, chloramphenicol and nystatin respectively polyene antifungal drug to which *Candida* is sensitive, were used as antibacterial and antifungal controls respectively. Zones of inhibition of the samples and the controls (Table 1) indicate that in general, the samples **1-5** showed greater inhibition zones than the controls, with compound **5** having the highest inhibition zones of 53 and 50 mm for *S. aureus* and *S. pyogenes* respectively.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined for the five samples and the controls. In general, compounds **1-5** showed MIC values of between 0.340 to 0.994 mM (Table 2) and MBC values of between 0.680 to 1.988 mM (Table 3). Compound **3** was not as active as the other compounds against *E. Coli*, having a zone of inhibition of 20 mm and a MIC value of 1.601 mM. The greatest and broadest spectrum of activity was shown by **2** and **4**, with **1**, **3** and **5** showing slightly weaker activity. A decrease in activity on exchange of the halide by other anions was reported earlier[13]. However, a contrasting effect was seen on exchange with the fluorinated anions. In comparison to chloramphenicol, the synthesised compounds are in many cases comparable to its activity. No real trend could be observed between the various anions and the antimicrobial activity except that in general, compounds **2** ($CF_3SO_3^-$) and **4** (PF_6^-) showed better activity than **3** (BF_4^-) and **5** (CF_3COO^-). Thus, the SO_3^- group compares better than the COO^- group when attached to CF_3 and the expanded octet of phosphorus seems to be better than boron to co-ordinate to fluorine for antimicrobial activity.

CONCLUSION

The synthesis of two novel fluorinated phosphonium organic salts has been achieved in excellent yields using relatively simple techniques and their structures have been characterised by NMR analysis. Changing the anion had no effect on the NMR resonances of the cation. It is also shown that the dimeric phosphonium organic salts are potential sources of new antimicrobials with different characteristics to those of established drugs. Comparatively, the $CF_3SO_3^-$ anion is a better source of antimicrobial anion than CF_3COO^- and PF_6^- is better than BF_4^-.

ACKNOWLEDGEMENTS

We thank the NRF and University of KwaZulu-Natal for financial support. Appreciation also goes to Mal. Mikailu S. Abdullahi from the National Research Institute of Chemical Technology (NARICT) in Nigeria for assistance with the antimicrobial studies and the Mass Spectrometry Unit at the University of the Witwatersrand for carrying out the mass spectral data. This work is based upon research supported by the South African Research Chairs Initiative of the Department of Science and Technology.

REFERENCES

1. Kanazawa, A., Ikeda, T. Multifunctional tetracoordinate phosphorus species with high self-organizing ability. *Coord. Chem. Rev.* 2000; **198**: 117-131.
2. Chen, Y., Bengao, L., Xieging, W. Advances in organic antibacterial agents. *Shiyou Lianzhi Yu Huagong* 1997; **28**: 56-63.
3. Kanazawa, A. Ikeda, T., Endo, T. Synthesis and antimicrobial activity of dimethyl- and trimethyl-substituted phosphonium salts with alkyl chains of various lengths. *Antimicrob Agents Chemother.* 1994; **38**: 945-952.
4. Jin, D., Lu, X., Wang, H., Shen, X., Lin, L. Water-insoluble quaternary phosphonium salt antimicrobial agent and its preparation. Chinese Patent 101305733, 19 Nov 2008.
5. Wu, T., Xie, A-G., Tan, S-Z., Cai, X. Antimicrobial effects of quaternary phosphonium salt intercalated clay minerals on *Escherichia coli* and *Staphylococci aureus*. *Colloids Surf. B* 2011; **86**: 232-236.
6. Hayakawa, S., Ohashi, H., Kobase, S. Antimicrobial multilayer packaging materials, and packages therefrom. *Japanese Patent* 11034264, 9 Feb 1999.
7. Hayakawa, S., Ohashi, H., Kobase, S. Laminated thermoplastic resin sheets having matte coatings with high antibacterial activity. Japanese Patent 2000263709, 26 Sep 2000.
8. Hayakawa, S., Ohashi, H., Kobase, S. Laminated thermoplastic resin sheets having matte coatings with high antibacterial activity. Japanese Patent 2000263706, 26 Sep 2000.
9. Konagaya, S., Ohashi, H., Hamano, A., Seko, M., Tanaka, M. Antibacterial composition and antibacterial laminate. US Patent 6013275, 11 Jan 2000.

10. Konagaya, S., Ohashi, H., Hayakawa, S., Ishihara, H., Endo, T. Novel antibacterial acrylic acid-grafted copolyester with a phosphonium salt. *Abstracts of Papers, 227th ACS National Meeting*, Anaheim, CA, United States 2004; pp. 103.
11. Ohashi, H., Kohaze, S. Antimicrobial metal plates having hydrophilic substance layer containing microbicides. Japanese Patent 11010766, 19 Jan 1999.
12. Zhao, R., Liu, Z., Gao, Y., Du, P., Sun, B., Qian, J., Cai, X. Method and apparatus for treatment of waste gas discharged from wastewater treatment plant. Chinese Patent 102380294, 21 Mar 2012.
13. Cieniecka-Roslonkiewicz, A., Pernak, J., Kubis-Feder, J., Ramani, A., Robertson, A.J., Seddon, K.R. Synthesis, anti-microbial activities and anti-electrostatic properties of phosphonium-based ionic liquids. *Green Chem*. 2005; **7**: 855-862.
14. Wasserscheid, T., Welton, T. *Ionic Liquids in Synthesis*, 1st ed., Weinheim, Wiley-VCH, 2008.
15. Kyoyanagi, M., Yamamoto, J., Yamamura, S. Phosphonium compound and electrophotographic toner using it. Japanese Patent 06080681, 22 Mar 1994.
16. Ito, Y., Aoyagi, M., Oonishi, M. Electrochromic device with improved color density and durability. Japanese Patent 10253996, 25 Sep 1998.
17. Ibrahim, H., Koorbanally, N., Ramjugernath, D., Bala M.D., Nyamori, V.O [1,4-Phenylenebis (methylene)] bis (triphenylphosphonium) bis (tetrafluoroborate). *Acta Crystallogr., Sect. E: Struct. Rep. Online* 2011; **67**: o3391.
18. Andrews, J.M., Determination of inhibitory concentrations. *J. Antimicrob. Chemother*. 2001; **48**: 5-16.
19. Ganesan, K., Alias, Y. Synthesis and characterisation of novel dimeric ionic liquids by conventional approaches. *Int. J. Mol. Sci*. 2008; **9**: 1207-1213.
20. Weigert, F.J., Roberts, J.D. Nuclear magnetic resonance spectroscopy. Spin-spin coupling of carbon to phosphorus, mercury, nitrogen, and other elements, *Inorg. Chem*. 1973; **12**: 313-316.
21. Pampaloni, G, Peloso, R., Graiff, C., Tiripicchio, A. Olefin trifluoro derivatives of copper(I) and their olefin/CO exchange reactions. *Organometallics* 2005; **24**: 819-825.
22. Macchioni, A., Zuccaccia, C., Clot, E., Gruet, K., Crabtree, R.H. Selective ion pairing in $[Ir(bipy)H_2(PRPh_2)_2]A$ (A = PF_6, BF_4, CF_3SO_3, BPh_4, R = Me, Ph): Experimental identification and theoretical understanding. *Organometallics* 2001: **20**: 2367-2373.