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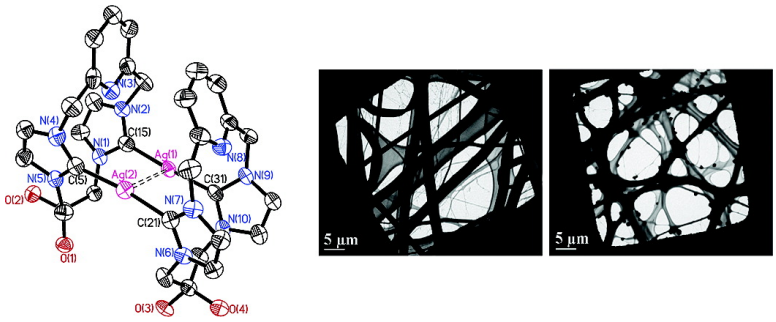

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Silver(I)−Imidazole Cyclophane *gem*-Diol Complexes Encapsulated by Electrospun Tecophilic Nanofibers: Formation of Nanosilver Particles and Antimicrobial Activity

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Abstract: Silver(I)−imidazole cyclophane *gem*-diol complex, **3** $[Ag_2C_{36}\ N_{10}O_4]^{2+}2(x)^-$, where x = OH^- or CO_3^{2-}, was synthesized and well characterized. The minimum inhibition concentration tests showed that the aqueous form of **3** is 2 times less effective as an antibiotic than 0.5% $AgNO_3$, with about the same amount of silver. The antimicrobial activity of **3** was enhanced when encapsulated into Tecophilic polymer by electrospinning to obtain mats made of nano-fibers. The fiber mats released nanosilver particles, which in turn sustained the antimicrobial activity of the mats over a long period of time. The rate of bactericidal activity of **3** was greatly improved by encapsulation, and the amount of silver used was much reduced. The amount of silver contained in the fiber mat of **3**, with 75% of **3** and 25% Tecophilic, was 8 times less than that in 0.5% $AgNO_3$ and 5 times lower than that in silver sulfadiazine cream 1%. The fiber mat was found to kill *S. aureus* at the same rate as 0.5% $AgNO_3$, with zero colonies on an agar plate, and about 6 times faster than silver sulfadiazine cream. The silver mats were found effective against *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, *A. niger*, and *S. cerevisiae*. Transmission electron microscopy and scanning electron microscopy were used to characterize the fiber mats. The acute toxicity of the ligand (imidazolium cyclophane *gem*-diol dichloride) was assessed by intravenous administration to rats, with an LD 50 of 100 mg/kg of rat.

Introduction

Elemental silver and silver salts have been used for decades as antimicrobial agents in curative and preventive health care. The application of silver and its salts in the treatment of burn wounds[1] is of interest. The advances in wound care management are not only focused on the antimicrobial effect of silver on chronic ulcers, extensive burns, and difficult-to-heal wounds,[2] but also on the convenience of application, patient comfort, and sustained release of silver ions with increased concentration at the wound surfaces.[3,4] These lead to the development of fabric impregnated with silver (FIS), a new silver technology, that has antimicrobial efficacy and provides a protective barrier against infection. In the treatment of burn wounds, good hydration is recognized as an important factor for wound healing and re-epithelialization.[5]

In the last two decades, cyclophane compounds received much attention in the development of supramolecular chemistry.[6] Works by our group[7] and others[8] describe the synthesis and structural analysis of imidazolium cyclophanes and their metal complexes. We previously reported the antimicrobial activity of silver(I)-*N*-pincer 2,6-bis(hydroxylethylimidazolemethyl)-pyridine hydroxide, a water-soluble silver(I) carbene complex **1**, on some clinically important bacteria.[9] Compound **1** is sparingly soluble in absolute ethanol but highly soluble in methanol. The solubility of type **1** silver(I) carbene complexes in ethanol was improved by varying the functionalized groups

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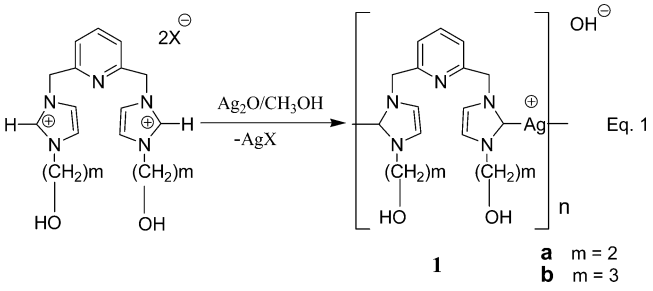
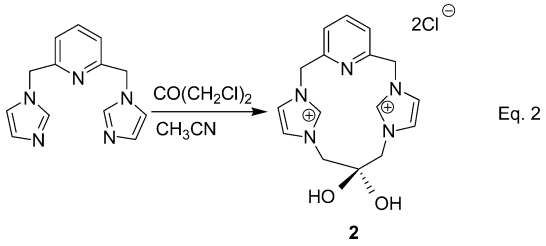
coupled to the nucleophilic end of the bis(imidazolemethyl) pyridine compound. There have been no other reports on the application of metal-*N*-heterocyclic carbene cyclophane compounds as antimicrobial agents.



Electrospinning[10] is a versatile method used to produce fibers with diameters ranging from a few nanometers to several micrometers by creating an electrically charged jet of polymer solution or polymer melt, which elongates and solidifies. The resulting fibers are being used, or have the potential to be used, in filters, coating templates, protective clothing, biomedical applications, wound dressing, drug delivery, solar sails, solar cells, catalyst carriers, and reinforcing agents for composites.[11−13] We report here the first electrospun fiber encapsulating silver(I) *N*-heterocyclic carbene complexes and the antimicrobial activity of the resulting fibers.

Result and Discussion

The imidazolium (NHC) cyclophane *gem*-diol salt **2** is easily prepared by reacting 2,6-bis(imidazolemethyl)pyridine with 1,3-dichloroacetone. The formation of **2** as a *gem*-diol in preference to the carbonyl form is rare but not unprecedented when electron-withdrawing groups are present. The formation of **2** as a *gem*-diol might have proceeded by an acid-catalyzed process. The solution was observed to be slightly acidic, having a pH range of 5−6.



The ^{1}H NMR spectra showed the presence of the *gem* hydroxyl group as a broad resonance at 7.65 ppm, and the absence of carbonyl in **2** was observed by both ^{13}C NMR and IR spectroscopy. The O−H stretching vibration was observed at 3387 cm^{-1}, whereas the C−O stretching vibration was at 1171 cm^{-1}. The carbon of the *gem* hydroxyl had a ^{13}C NMR chemical shift of 91 ppm. X-ray crystallography provided further evidence and the detailed structure of **2** (see Figure 1).

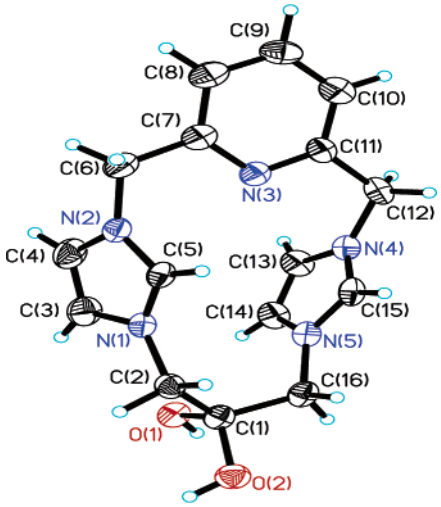
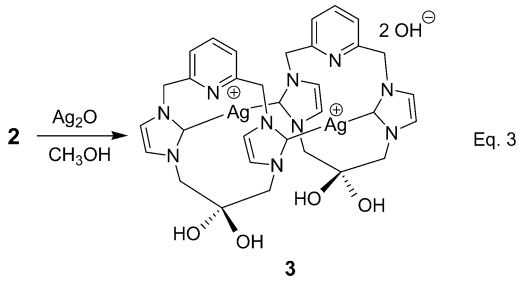


Figure 1. Thermal ellipsoid plot of **2** with the thermal ellipsoid drawn at the 50% probability level. The counteranions are omitted for clarity.

The combination of silver(I) oxide with **2** in methanol results in **3** as an air and light stable yellow solid in high yield, as confirmed by the loss of the imidazolium proton at 9.35 ppm in the ^{1}H NMR spectra. The ^{1}H NMR spectrum of **3** showed a broad complicated set of resonances that could not be assigned. This may be due to the fluxional behavior of the compound on the NMR time scale.[7b,14,15]



Upon reaction with Ag_2O, the resonance of the imidazole carbon of **2** at 138 ppm is replaced by a downfield resonance at 184 and 186 ppm for the carbene carbon of **3**. This resonance shows a ^{109}Ag−^{13}C coupling constant (J_{AgC} = 211 Hz) in the range of other such one-bond couplings (204−220 Hz).[7b,14,15] Couplings to ^{109}Ag are not often observed. Even more rare are couplings to ^{107}Ag,[14] which were not observed in **3**. X-ray crystallography confirms the structure of **3** (see Figure 2) with bond distances of Ag1−C15 = 2.085(5) Å, Ag1−C31 = 2.077-(5) Å, Ag2−C5 = 2.073(5) Å, and Ag2−C21 = 2.072 Å. A weak Ag1···Ag2 interaction was observed at a Ag−Ag distance of 3.3751(10) Å, longer than the commonly reported Ag−Ag bond range of 2.853−3.290 Å,[16] but only slightly shorter than

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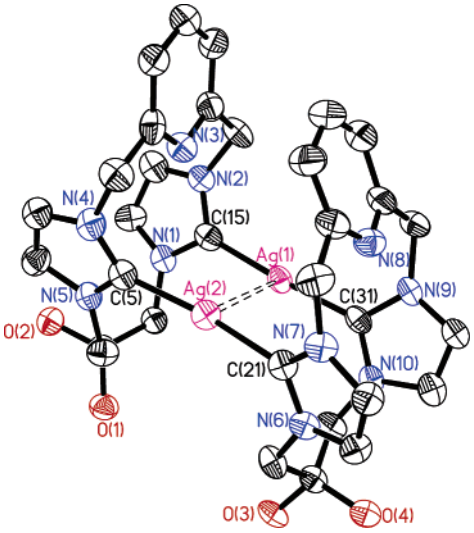

Figure 2. Thermal ellipsoid plot of **3** with the thermal ellipsoid drawn at the 50% probability level. The counteranions are omitted for clarity.

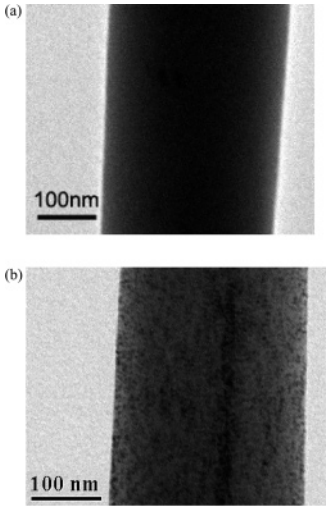

Figure 3. Electrospun fibers prepared from a mixture of **3** and Tecophilic at a weight ratio of 25 to 75. (a) As-spun fiber, and (b) silver particles formed by exposing the as-spun fiber to water.

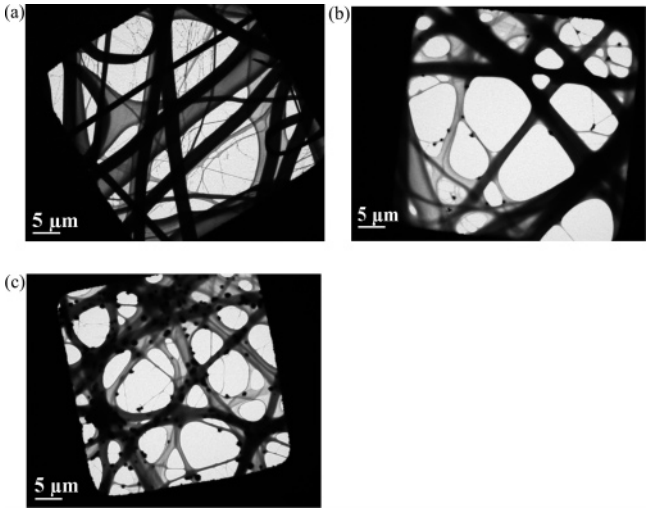

Figure 4. TEM images showing the release of silver particles by exposing fibers of **3** and Tecophilic (weight ratio 50:50) to water vapor environment. (a) As-spun fiber, (b) fibers in water vapor environment for 0.5 h, and (c) fibers in water vapor environment for 65 h.

the van der Waals radii (3.44 Å).[17] In silver metal, the Ag−Ag bond distance is 2.888 Å.[18] The C−Ag−C bond angles are almost linear with the C15−Ag1−C31 bond angle of 175.20-(18)° and C21−Ag2−C5 bond angle of 170.56(18)°.

Tecophilic is a family of hydrophilic polyether-based thermoplastic aliphatic polyurethanes. It is a medical grade polymer capable of absorbing water content up to 150% of the weight of its dry resin. Tecophilic was chosen as the polymer for encapsulating the silver complex **3** because it can be electrospun from ethanol and has excellent hydrophilic properties. Excellent hydrophilic properties are important because water is necessary to facilitate the release of silver ions from the encapsulated silver complex in the polymer matrix. It is also envisaged that Tecophilic will maintain a moist environment around the wound bed, as it is known that good hydration is essential for optimal wound healing.[5,19] Both the polymer and **3** are soluble in ethanol, a biocompatible solvent, and can easily be electrospun together to achieve a uniform blend of the as-spun fiber. The electrospun fibers from the Tecophilic polymer and silver complex **3** were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). No obvious phase separation was observed in the as-spun fibers (Figure 3a), which indicated a uniform mixing of Tecophilic and silver complex. The thickness of the fiber mat was measured by scanning electron microscopy (SEM) with pure Tecophilic (100 μm), 25:75 silver complex **3**/Tecophilic (30 μm), and 75:25 **3**/Tecophilic (60 μm), respectively. The encapsulation of **3** by polymer retards the quick decomposition of the silver complex into silver ions or particles in aqueous media. The formation of silver particles at nanometer scale has been observed in the polymer matrix, when the electrospun fiber is exposed to water. Transmission electron microscopy studies showed that the formation of nanosilver particles in the fiber is a process that occurs gradually over a period of time. By exposing the as-spun fibers to water, **3** decomposed and released silver ions, which aggregated into silver particles at nanoscale measurement. The formation of aggregates of silver particles has been observed within 30 min of exposure to water vapor (as shown in Figure 4). The aggregation of the silver ions in the presence of water, with the aggregate adsorbed on the surface of the fibers, is considered to be a simplified mechanism by which the fiber mat releases the active form(s) of the silver for its antimicrobial activity. The fiber mat of **3** is stable in light and dry air for months, but sensitive to an environment with very high humidity.

Bactericidal Effect. Using a modified Kirby Bauer technique, mats of electrospun Tecophilic fiber encapsulating **3** and pure electrospun Tecophilic fiber as control were placed on a lawn of organisms in an agar plate and incubated overnight at 35 °C. The inoculants used were both Gram positive and Gram negative prokaryotes (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) of clinical interest. The fungi used were *Candida albicans*, *Aspergillus niger*, and *Saccharomyces cerevisiae*. The bactericidal activity showed a clear zone of inhibition within and around the fiber mat after an overnight incubation of the agar plate at 35 °C. The fungicidal activity

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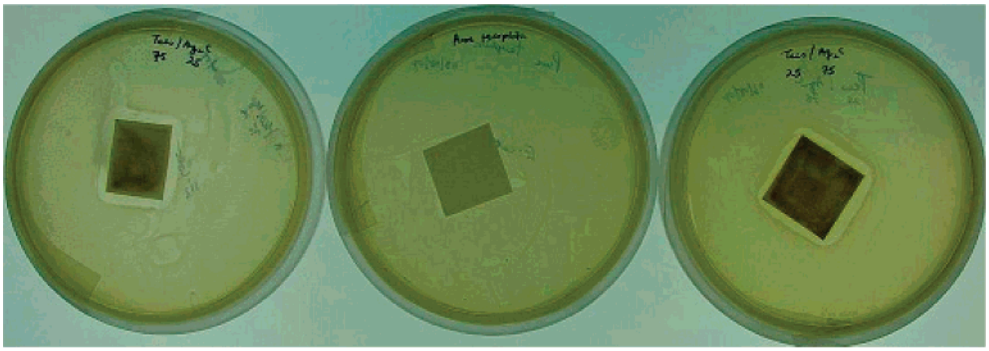

Figure 5. Susceptibility test of the fiber mat encapsulating **3**, with bactericidal activity as compared to pure Tecophilic fiber mat. (a) **3**/Tecophilic (25:75), (b) pure Tecophilic, and (c) **3**/Tecophilic (75:25).

was observed after 48 h of incubation at 25 °C. Pure Tecophilic fiber mat as control showed no growth of inhibition (see Figure 5). The diameter of the zone of inhibition for the 75% (**3**/Tecophilic) fiber mat is 4.00 mm, whereas that of 25% (**3**/Tecophilic) is 2.00 mm. The difference in the diameter of the zone of inhibition between the two types of fiber mat does not have a linear relationship with the amount of silver (3:1 ratio) present in the two fiber mats. These results further show the limitation of the Kirby Bauer technique as a quantitative tool to determine the antimicrobial activity of drugs. The diffusing ability of the silver ions might have been limited by the formation of secondary silver compounds. Ionic silver is known to undergo ligand exchange reactions with biological ligands such as nucleic acids, proteins, and cell membranes.[20]

Deposition of a few silver particles was observed at the bottom of a test tube when a piece of the fiber mat was placed in 5 mL of distilled water and exposed to light for 4 days. The leaching of the silver particles from the fiber mat surfaces to the solution occurred gradually over time. The release of nanosilver particles from the as-spun mats of **3** into an aqueous medium led to the investigation of the kinetics of kill (bactericidal activity) of the as-spun fiber mat of **3** with respect to time by comparing it to silver nitrate and silver sulfadiazine 1% cream or silvadene (SSD), a clinical drug widely in use. Both types of the fiber mat composition 75:25 ([Ag] = 424 μg/mL) and 25:75 ([Ag] = 140 μg/mL) used in this study showed a faster kill rate than SSD ([Ag] = 3020 μg/mL). Silver nitrate (0.5%) with 3176 μg/mL of Ag showed about the same kill rate as **3**/Tecophilic 75:25 ([Ag] = 424 μg/mL) at a silver concentration 8-fold lower than silver nitrate (see Figure 6). The silver compounds kill *P. aeruginosa* faster than *S. aureus*. The fiber mats killed bacteria faster than silvadene.

The time dependence of the bacteriostatic and bactericidal activities of the as-spun mat of **3** as a function of the volume of organism inoculated was examined. The fiber mats of **3** showed effective bactericidal activity on *P. aeruginosa*, *E.coli*, and *S. aureus* for over a week with daily inoculation (25 μL) of freshly grown organism. This is an indication that the as-spun fiber mat sustained the continuous release of active silver species over a long period of time. Pure Tecophilic mat as control showed no antimicrobial activity within 24 h of incubation. The as-spun mat of **3** with the 75% **3**/Tecophilic composition showed a better bactericidal effect on *P. aeruginosa*

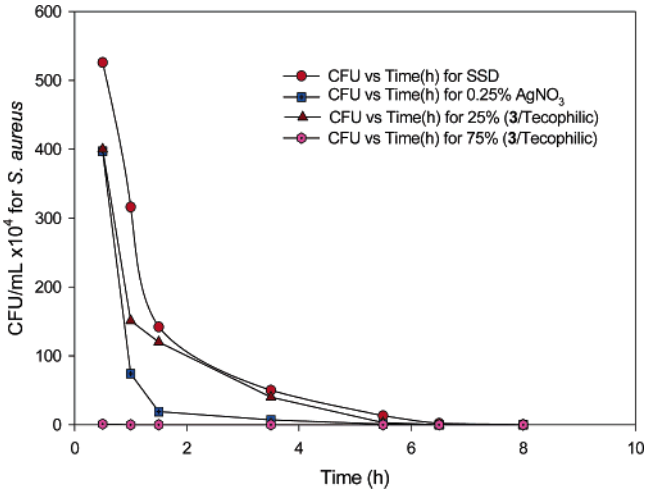

Figure 6. The plot of CFU (colony forming unit) versus time (h) of the silver compounds on *S. aureus*, which expresses the kinetics of the bactericidal activity for each of the silver compounds tested.

than the 25% **3**/Tecophilic for over 2 weeks after inoculating with over 200 μL (2×10^7) of freshly grown organism. Bacteriostatic activity was observed for *S. aureus* and *E. coli* after 10 days of the daily streaking of the LB broth solution on an agar plate. Visual inspection of the incubated solutions showed no growth of the organism.

The bactericidal activity of **2**, **3**, and $AgNO_3$ in aqueous LB broth was studied using the minimum inhibitory concentration (MIC) test. There was no difference in the bactericidal activity and MIC of **3** and $AgNO_3$ after 24 h of incubation as shown in Table 1. However, after 48 h of incubation, silver nitrate showed a better antimicrobial activity at a concentration 2-fold lower than **3** (838 μg/mL). The MIC value was not determined for silver sulfadiazine because a suspension rather than a solution was obtained, and the concentration of **2** used showed no antimicrobial activity in the MIC test. The dilutions with the least concentration of **3** (209 μg/mL) and $AgNO_3$ (216 μg/mL) showed growth of the same number of colonies of *S. aureus* on an agar plate after 24 h of incubation. The 25% **3**/Tecophilic fiber mat has the least concentration of silver, 140 μg/mL (see Table 2), and sustained the release of active silver species that were bio-available for days. No growth of the organism was observed with the daily increase in the volume of inocula. Thus, the antimicrobial activity of **3** was enhanced for a longer period, at a very low concentration of silver particles by encapsulation in a suitable polymeric fiber. Interestingly, the bactericidal

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Table 1. MIC Results Comparing the Activity of $AgNO_3$ and **3**, with Both Having about the Same Amount of Silver[a]

sample ID	concentration of sample (wt/V %)	concentration of sample (μg/mL)	vol. of bacteria (μL)	*E. coli* (day)		*P. aereginousa* (day)		*S. aureus* (day)	
				1	2	1	2	1	2
$AgNO_3$	0.50	3462	100	−	−	−	−	−	−
	1DF	1731	100	−	−	−	−	−	−
	2DF	866	100	−	−	−	−	−	−
	3DF	433	100	−	−	−	−	−	−
	4DF	216	100	−	+	−	−	−	+
3	1.38	3341	100	−	−	−	−	−	−
	1DF	1676	100	−	−	−	−	−	−
	2DF	838	100	−	−	−	−	−	−
	3DF	419	100	−	+	−	+	−	+
	4DF	209	100	−	+	−	+	−	+

[a] DF is the dilution factor (1 mL). + = growth, − = no growth. The amount of silver (μg) per mL for each compound was calculated as (molecular mass of Ag/formula wt of compound) × wt %.

Table 2. Details of Silver Compounds Used for the Kinetic Studies[a]

sample ID	wt of Ag compds. used (mg)	volume of LB broth (mL)	amount of Ag in sample (mg)	μg of Ag/mL
SSD	20.00	5.00	6.05	1210
$AgNO_3$	12.80	5.00	8.13	1626
$AgNO_3$	25.00	5.00	15.90	3176
3/Tecophilic (25:75)	11.30	5.00	0.73	146
3/Tecophilic (75:25)	11.40	5.00	2.21	441

[a] SSD: silver sulfadiazine 1% cream.

activity of the fiber mat 75% (**3**/Tecophilic) with 424 μg/mL of silver is 8-fold lower in the concentration of silver than $AgNO_3$ (3176 μg/mL) and showed not only a kill rate as fast as silver nitrate, but also retained the original color of the LB broth, a clear yellow solution unlike silver nitrate which stains and changed the LB broth color to dark brown (Figure 7). The silver sulfadiazine cream did not readily dissolve in the aqueous LB broth, thus affecting the rate of its bactericidal activity. Results obtained were consistent over the course of three experiments.

The antimicrobial activity of the fiber mat encapsulating **3** can be considered to be a combination of active silver species, which may include $AgCl_2^-$ ions, clusters of Ag^+ ions, AgCl, and free Ag^+ ions. Theoretically, the slow release of the active silver particles in the solution leads to the quick formation of silver chloride. The presence of more chloride anion as the major counterion will further result in the formation of negatively charged $[Ag_yCl_x]^{n-}$ ion species (where $y = 1, 2, 3$, etc.; $x = 2, 3, (y + 1)$; $n = x - 1$). Anionic silver complexes of the type $[AgI_3]^{2-}$, $[Ag_2I_4]^{2-}$, $[Ag_4I_8]^{4-}$, and $[Ag_4I_6]^{2-}$ have been reported.[21,22] The formation of anionic silver chloride species may not be limited to the leached aggregates of silver particles in the solution, but may also be found on the surface of the fiber mats as shown in the SEM images of Figure 8. Anionic silver dichloride is known to be soluble in aqueous media and thus will be bio-available.[23] Anionic silver halides are toxic to both sensitive and resistance strain bacteria.[24] The adsorbed active silver species on the network of fibers in the mat is an advantage the fiber mat has to increase the surface area of the active silver species over the conventional use of aqueous silver ions. This mechanism might have accounted for the effective bactericidal activity of the fiber mat in an aqueous media, even at such a low concentration of silver as compared to the unencapsulated form of **3**. Although **3** is sparingly soluble in water, it quickly decomposes in aqueous media. Thus, the bactericidal activity of **3** is reduced due to poor availability of the active silver species in the LB broth media, which might be due to the formation of secondary silver compounds, especially AgCl.

Acute Toxicity Assessment. The LD 50 assessment was done by intravenous administration of **2**, dissolved in a buffered saline solution, into the tail vein of the rats. Adult rats were used with an average weight of 500 g. Progressive administration of 0.3 mL of the dose (5 mg, 50 mg) was done weekly. The rats were carefully examined for the dose−response effect. Death occurred 10 min after administrating 50 mg of **2**, when 50% of the rats showed powerful convulsion before death. The autopsy report showed pulmonary hemorrhage and hemorrhage in the brain of the dead rats, a diagnosis of stroke. The surviving rats were observed to lose weight, with a drastic loss in appetite, and low urine output. The LD 50 assessment was found to be 100 mg/kg of rat.

Conclusion

In addition to their application as a metal transfer agent and their potential as industrial catalyst, we have been able to advance the chemistry of silver(I) *N*-heterocyclic carbene complexes to the medicinal relevant use, as effective antimicrobial agents. This class of compounds is effective as an antimicrobial agent on the bacteria and fungi mentioned. The synthesis of **2** with functionalized groups aids in tailoring the encapsulation of the silver(I)−imidazole cyclophane *gem*-diol into a nanofiber. The fiber mat has been shown to have improved the antimicrobial activity of the silver(I)−heterocyclic carbene complexes on the microorganisms, with a faster kill rate than silvadene in an LB broth medium at a concentration 8-fold lower than silvadene. The encapsulation of the silver heterocyclic carbene complexes increases the bio-availability of active silver species and also reduces the amount of silver used. Encapsulated silver(I) carbene complexes in nano-fibers have been demonstrated to be promising materials for sustained and effective delivery of silver ions with maximum bactericidal activity over a longer period of time than aqueous silver. The amount of silver used for antimicrobial activity has been greatly reduced with this technique of encapsulation as compared to the unencapsulated form, which often is related to the amount of silver in 0.5% silver nitrate. Furthermore, the ability of the fiber mat to

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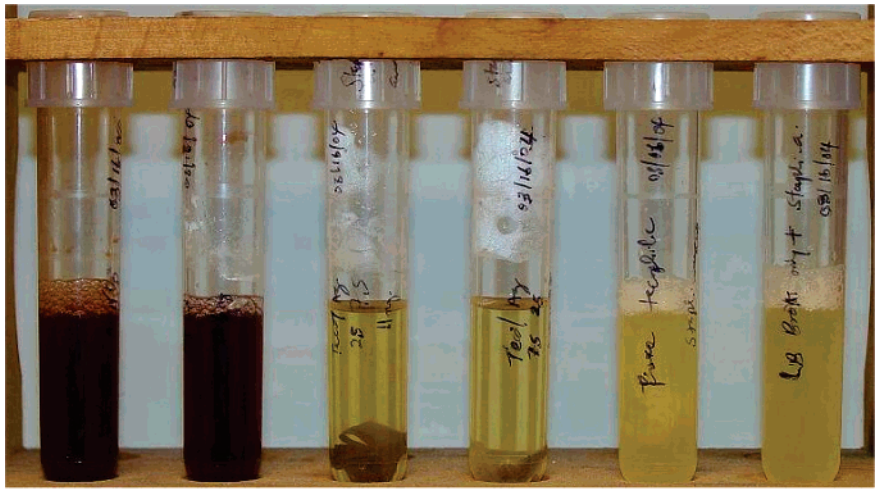

Figure 7. LB broth solution in the culture tubes after 1 week of bactericidal testing. From left to right: (a) Brown (0.25% $AgNO_3$); (b) brown (0.50% $AgNO_3$); (c) yellow (actual color of LB broth) 25% (3/Tecophilic); (d) yellow 75% (3/Tecophilic); (e) pure Tecophilic (100%), showing growth of organism; and (f) LB broth solution with organism only.

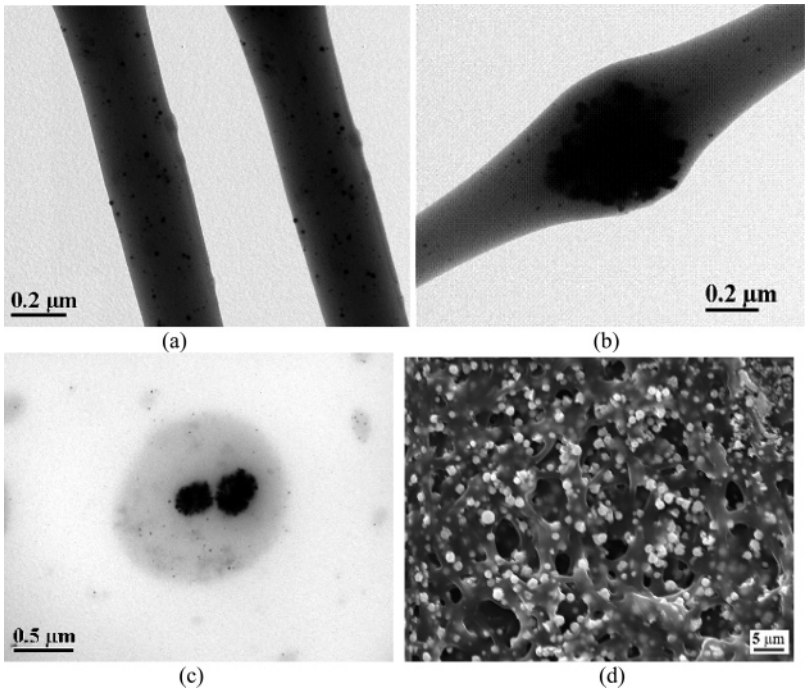

Figure 8. TEM images (a, b, c) of electrospun fibers from **3** and Tecophilic (75:25) after 2 weeks of antimicrobial activity in LB broth media. (a) Stereoimages of a segment of fiber; (b) a large aggregate (400 nm) of silver particles encapsulated in Tecophilic fiber; (c) silver aggregates (200−300 nm in diameter) and silver particles (10−20 nm in diameter) in Tecophilic matrix; and (d) SEM image showing the top view of the fiber mat with aggregates of silver particles.

retain the original color of the LB broth is a major cosmetic plus. The assessment of the acute toxicity of the ligand on rats showed an LD 50 of 100 mg/kg of rat, a value considered to be moderately toxic. We are currently exploring the use of less toxic ligands as carriers for silver.

Experimental Section

Silver(I) oxide, silver sulfadiazine, and 1,3-dichloroacetone were purchased from Aldrich, and silver sulphadiazine cream 1% from Rugby-Blue Ridge. Acetone, acetonitrile, methanol, ethanol, ammonium hexafluorophosphate, and organisms *S. cerevisiae* (ATCC 2601), *C. albicans* (ATCC 10231), *A. niger* (ATCC 16404), *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), and *S. aureus* (ATCC 6538) were purchased from Fisher. All reagents were used without further purification. Infrared spectra were recorded on a Nicolet Nexus 870 FT-IR spectrometer. The ^1H and ^{13}C NMR data were recorded on a Varian Gemini 300 MHz instrument, and the spectra obtained were referenced to the residual protons of the deuterated solvents. Mass spectroscopy data were recorded on an ESI-QIT Esquire-LC with a positive ion polarity. The TEM images were recorded on FEI TE CNAI-12 transmission electron microscope (TEM) at 120 kV.

Synthesis of the Imidazolium Cyclophane *gem*-Diol Dichloride (2). A solution containing 0.24 g (1.0 mmol) of 2,6-bis(imidazolemethyl)pyridine and 0.254 g (2.0 mmol) of 1,3-dichloroacetone in 60 mL of acetonitrile was stirred at 75 °C for 8 h to obtain **2** as a brown solid after filtration and air-drying. Yield: 0.9 mmol, 89.6%. Colorless crystals of the PF_6 salt of **2** were obtained by slow evaporation from acetonitrile/water, after anion exchange of the chloride of **2** with aqueous ammonium hexafluorophosphate solution. Mp: 175−178 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 4.68 (s, 4H, $CH_2C(OH)_2CH_2$), 5.67

(s, 4H, CH_2), 7.40, (s, 2H, NC(H)CH), 7.47 (d, 2H, $J = 7.8$ Hz, *m*-pyr), 7.65 (s, 2H, $C(OH)_2$), 7.89 (s, 2H, NCHC(H)), 7.94 (t, 1H, $J = 7.8$ Hz, *p*-pyr), 9.34 (s, 2H, NC(H)N). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.8, 55.2, 91.1, 120.5, 122.0, 123.9, 138.0, 138.8, 152.6. ESI-MS *m*/*z*: 384 [$M^{2+}2Cl^-$], 348 [$M^{2+}Cl^-$]. FT IR (Nujol, cm^{-1}): 3387, 3105, 1597, 1564, 1439, 1346, 1171, 1085, 996, 755. Anal. Calcd: C, 48.54; H, 4.41; N, 16.94; Cl, 17.13. Found: C, 48.33; H, 4.32; N, 16.71; Cl, 16.76.

Synthesis of the Dinuclear Silver Carbene Cyclophane *gem*-Diol Hydroxide (3). A solution of 0.232 g (1.0 mmol) of silver(I) oxide and 0.366 g (0.9 mmol) of **2** in 70 mL of methanol was stirred at room temperature for 50 min. The filtrate was concentrated to obtain **3** as a yellow solid. Single crystals of **3** were obtained from ethanol after anion exchange of the hydroxide with carbonate by slow evaporation of the solution.

Yield: 0.618 g, 0.738 mmol, 82%. Mp: 202−204 °C. ESI-MS *m*/*z*: 400 [$0.5M^{2+}$], 801 [$2M^+$], 837 [$2M^+2OH^-$]. FTIR (Nujol, cm^{-1}): 3415, 3105, 1596, 1564, 1439, 1344, 1169, 1084, 1028, 996, 758. ^{13}C NMR (75 MHz, DMSO-d_6): δ 48.6, 51.1, 53.8, 92.1, 119.9 ($J =$ 1.4 Hz), 121.6, 128.6, 137.8 ($J = 2.4$ Hz), 154.2, 184.9 ($J_{carbene-Ag} =$ 211 Hz). Anal. Calcd: Ag, 24.54; C, 43.79; H, 4.20; N, 15.24. Found: C, 43.15; H, 4.22; N, 14.89.

Electrospun Tecophilic/3 Fibers. Tecophilic (SP-80A-150 grade) was dissolved in 9:1 ethanol/tetrahydrofuran. A solution of **3** in ethanol was mixed with a premade solution of Tecophilic. Solutions with different weight ratios between **3** and Tecophilic were prepared. The ratios were 0/100, 25/75, and 75/25. The solutions of **3** and Tecophilic were held in a pipet. An electrical potential difference of 15 kV was applied between the surfaces of the solution drop to the grounded collector, a distance of about 20 cm. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to characterize the as-spun fibers and fibers exposed to water.

Antimicrobial Tests. Sterilized LB broth was measured (5 mL) into a sterile tube. A loopful of stationary phase cultured microorganism (*E. coli*, *P. aeruginosa*, *S. aureus*) was introduced into the tube containing the LB broth solution. The mixture was cultured overnight, at 35 °C in a shaking incubator. The same procedure was done with stationary phased cultured fungi (*C. albican*, *S. cerevisae*, *A. niger*) and incubated without shaking at room temperature for 72 h.

Fiber Mat Testing. A constant volume (25 μL) of the freshly grown organism was placed on an LB agar plate and grown to obtain a lawn of the organism. A fiber mat (2.0 cm × 2.0 cm) of **3** and pure Tecophilic was placed on a lawn of bacteria (*E. coli*, *P. aeruginosa*, *S. aureus*) of an LB agar plate and incubated overnight at 35 °C. The bactericidal activity was observed by visual inspection of growth and no growth in and around the area of the fiber mat. About the same dimension of the fiber mat was placed on a lawn of fungi (*C. albican*, *S. cerevisae*, *A. niger*) and incubated at room temperature for 48 h. The diameter of the clear zone was measured.

Minimum Inhibitory Concentration (MIC) Test. Serial dilutions were made to obtain a range of concentrations by transferring 1 mL of freshly prepared stock solution of the silver compounds (with the same amount of silver particles) into a sterile culture tube containing 2 mL of LB broth, marked A. A 1 mL aliquot of well-mixed solution of A was transferred to culture tube B containing LB broth. The same procedure was repeated to obtain the dilute solution for tubes C, D, and E. The MIC was determined by visual inspection of growth/no-growth of the above concentrations of the silver compounds marked A−E inoculated with 25 μL of the organisms. After incubation at 35 °C overnight with no growth of organism, an additional 80 μL of freshly grown organisms was added to each of the cultures on the second day and incubated at the same temperature.

Kinetic Test of Bactericidal Activity. Equal volumes (5 mL) of LB broth were measured into sterile culture tubes and inoculated with 100 μL of *S. aureus* to each tube containing silver nitrate (12.8 mg, 25 mg), silver sulfadiazine (20 mg), 11.3 mg **3**/Tecophilic (25:75), and 11.4 mg **3**/Tecophilic (75:25) fiber mats. The mixtures were incubated at 35 °C, and the bactericidal activity was checked over a range of predetermined time by streaking one loopful of each mixture on an agar plate. The agar plate was then incubated at 37 °C overnight, and the numbers of colonies of organism formed were counted. The same procedure was repeated using 100 μL of *P. aeruginosa*. A plot of the CFU versus time was made as shown in Figure 6.

Animal Studies. Male Sprague Dawley (Harlen Sprague Dawley, Indianapolis, IN) adult rats (500 g average body weight) were housed in the University of Akron animal facility. Temperature and humidity were held constant, and the light/dark cycle was 6 am to 6 pm, light; 6 pm to 6 am, dark. Food (Lab diet 5P00, Prolab, PMI nutrition, Intl., Bretwood, MO) and water were provided ad libitim. Animals were anesthetized with ether to inject the compound into the tail vein, using a 27 gauge syringe needle in a volume of 0.3 mL sterile saline. The dosages for the ligand were 5 mg and 50 mg. At the end dosages of the experiment, animals were terminated, and the liver, lung, kidney, and heart tissues were removed and frozen at −70 °C. Urine samples were collected daily for later examination of the compound distribution. These studies were approved by the University of Akron Institutional Animal Care and Use Committee (IACUC).

X-ray Crystallographic Structure Determination. Crystal data and structure refinement parameters are reported in the Supporting Information. Crystals of **2** and **3** were each coated in paraffin oil, mounted on a loop, and placed on a goniometer under a stream of nitrogen gas. X-ray data were collected at a temperature of 100 K on a Brucker Apex CCD diffractometer using Mo Kα radiation ($\lambda = 0.71073$ Å). Intensity data were integrated using SAINT software, and an empirical absorption correction was applied using SADABS. Structures **2** and **3** were solved by direct methods and refined using full-matrix least-squares procedures. All non-hydrogen atoms were refined with anisotropic displacement.

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Supporting Information Available: Complete listing of the crystallography data for **2** and **3** (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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