Synthesis, Stability, and Antimicrobial Studies of Electronically Tuned Silver Acetate *N*-Heterocyclic Carbenes

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A series of methylated imidazolium salts with varying substituents on the 4 and 5 positions of the imidazole ring were synthesized. These salts were reacted with silver acetate to afford their corresponding silver *N*-heterocyclic carbene (NHC) complexes. These complexes were then evaluated for their stability in water as well as for their antimicrobial efficacy against a variety of bacterial strains associated with cystic fibrosis and chronic lung infections.

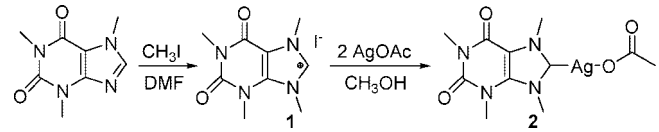
Introduction

Our group has a long standing interest in the synthesis of silver *N*-heterocyclic carbene (NHC) complexes for their antimicrobial activity, in particular for the treatment of cystic fibrosis (CF) and chronic lung infections. Silver has been used for centuries in a variety of forms for its antimicrobial properties. Medicinally, silver was introduced in the 1880s and used as a prophylactic 2% silver nitrate eye solution to prevent ophthalmia neonatorum in newborns. Silver nitrate was also used to treat a variety of other problems such as skin ulcers, postsurgical wounds, and suppurating wounds.[1] In the late 1960s, silver sulfadiazine (SSD) was introduced, and it remains one of the most effective topical burn treatments.[2] The activity of silver against Gram-positive and Gram-negative bacteria has long been established. In an inert atmosphere, silver displays no effect against bacterial organisms. In the presence of oxygen, however, Ag^+ ions are produced. It has been noted that silver cations are responsible for the bactericidal activity.[2–5] However, the mechanisms of its antimicrobial action are not completely understood. Fortunately, despite its wide use in both clinical and nonclinical applications, only a few accounts of silver resistance have been reported.[6–10]As an added advantage, silver has no effect upon the mammalian cell membrane and has limited toxicity to humans.

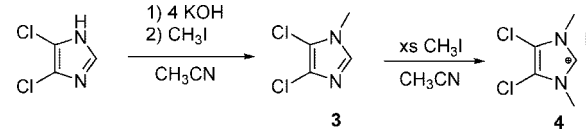
Based on the benefits of silver and the pressing interest in finding silver based antimicrobials active against resistant organisms, we have synthesized and investigated a variety of NHC silver complexes. Typically these complexes rapidly decompose in aqueous solutions.[5,11,12] Recently, a newly synthesized silver complex of caffeine showed promising antimicrobial properties as well as stability in water for several days (Scheme 1).[13]

These results led us to hypothesize that the presence of electron withdrawing groups on the 4 and 5 positions of the imidazole ring may lead to more stable silver systems. Our goal is to synthesize a silver carbene system that is stable enough to withstand systemic delivery prior to its degradation by salts or biological molecules present in the body.

Scheme 1. Synthesis of **2**



Scheme 2. Synthesis of **4**



In this paper, we report the preparation and characterization of several imidazolium salts with different substituents at the 4 and 5 positions of the imidazole ring and their silver complexes. The stability of the complexes in water is compared. Furthermore, the antimicrobial activity of two of the silver complexes against *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex species as well as control *Escherichia coli* strains is reported.

Results and Discussion

1-Methyl-4,5-dichloroimidazole (**3**) is formed from the deprotonation of 4,5-dichloroimidazole with potassium hydroxide and subsequent methylation with iodomethane in acetonitrile. The imidazolium salt 1,3-dimethyl-4,5-dichloroimidazolium iodide (**4**) is formed by the reaction of 1-methyl-4,5-dichloroimidazole (**3**) with excess iodomethane (Scheme 2).

The ^{1}H and ^{13}C NMR spectra of **4** are consistent with its molecular structure. In the ^{1}H NMR spectrum of **4**, the imidazolium proton appears at 9.42 ppm. This shift is consistent with the general C2−H acidic proton shift of imidazolium salts (δ 8–10 ppm).[14] The ^{13}C NMR shift of the N−C−N sp^2 carbon, which later becomes the carbene center, appears at 136.6 ppm for **4**.

Single crystals of **4** suitable for X-ray diffraction analysis were obtained by slow evaporation from a concentrated solution of acetonitrile. The molecular structure of the cationic part of **4** is depicted in Figure 1.

The in situ deprotonation of **4** with silver acetate in a 1:2 molar ratio in dichloromethane afforded the corresponding NHC

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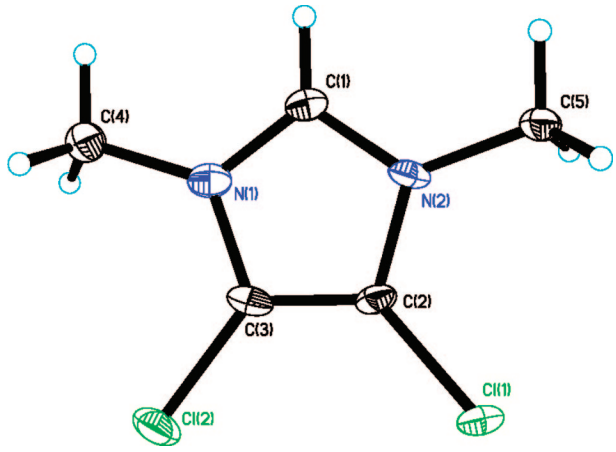


Figure 1. Crystal structure of the cationic portion of **4** with thermal ellipsoids shown at 50% probability.

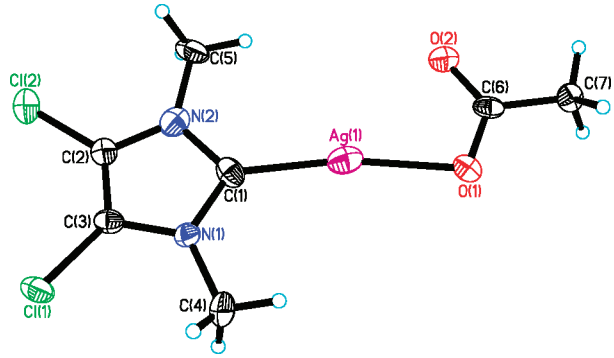
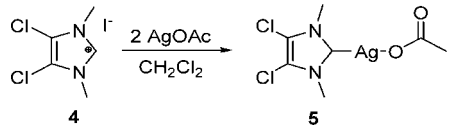


Figure 2. Crystal structure of [(**4**)AgOAc] (**5**). Thermal ellipsoids are shown at 50% probability.

silver acetate complex **5** in good yield (79%), eq 1. The most significant feature of the ^{13}C NMR spectrum of **5** is the signal at 179.7 ppm corresponding to the carbene carbon atom, which appears in the typical range of other NHC complexes of Ag(I), and the loss of the resonance at 136.6 ppm. The ^{1}H NMR spectrum shows a disappearance of the imidazolium proton signal at ca. 9 ppm, which further demonstrates the formation of the expected NHC silver acetate complex. The intensities of the signals in the ^{13}C NMR of the carbon atoms of the 4 and 5 positions of the imidazole ring are low due to the increase in the T_1 relaxation time upon replacement of hydrogen with chlorine.

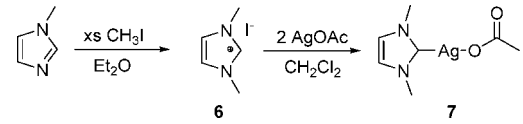


(1)

Single crystals of **5** were grown from a concentrated sample in THF (Figure 2). The asymmetric unit contains three molecules of water and one molecule of **5**. The geometry at the Ag atom deviates from linearity significantly with a C1−Ag−O1 bond angle of 168.9(2)°. The C1−Ag bond length is 2.030(8) Å.

To evaluate the stability introduced by the presence of electron withdrawing groups on the 4 and 5 positions of the imidazole ring, silver complex **5** was compared to two analogous silver complexes. Silver complexes **7** and **9** have hydrogen atoms and a methyl ester group on the alkene carbon atoms, respectively.

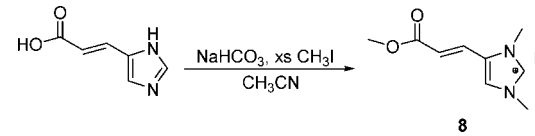
Scheme 3. Synthesis of **7**



1,3-Dimethylimidazolium iodide (**6**) was easily formed by the reaction of methylimidazole and excess iodomethane in diethylether by using a modified literature procedure.[15] The reaction of the imidazolium salt (**6**) with 2 molar equiv of silver acetate in refluxing methylene chloride for 40 h afforded silver complex **7** in 64% yield (Scheme 3).

The ^{1}H and ^{13}C NMR of **6** are consistent with the structure, with the imidazolium proton observed at 9.06 ppm in the ^{1}H NMR, and with the imidazolium carbon observed at 137.0 ppm in the ^{13}C NMR. The carbene carbon of **7** is observed at 178.8 ppm in the ^{13}C NMR spectrum, which is similar to the cases of **2** and **5**. The solid state structure of **7** was determined by single crystal X-ray diffraction (Figure 3). Crystals were obtained from slow evaporation of a saturated methylene chloride solution. The C6−Ag2 bond length is 2.064(6) Å, and the geometry at the Ag(I) atom is almost linear with a C6−Ag2−O3 bond angle of 175.97(18)°.

Treatment of urocanic acid with sodium bicarbonate and excess iodomethane in acetonitrile results in the formation of the iodide salt of dimethylated methyl urocanate (**8**), eq 2. In the ^{1}H NMR spectrum of **8**, the imidazolium proton (C2−H) appears at 9.16 ppm, which is consistent with the molecular structure of **8**. The imidazolium carbon (C2) appears at 138.7 ppm in the ^{13}C NMR spectrum. Single crystals of **8** suitable for X-ray diffraction studies were grown from a concentrated acetonitrile solution (Figure 4).



(2)

The silver(I) complex **9** was synthesized by the reaction of **8** with 2.4 molar equiv of silver acetate in dichloromethane. The reaction mixture was stirred for 1 h at room temperature to afford the NHC silver acetate complex **9** as a white solid in 88% yield, eq 3. In the ^{1}H and ^{13}C spectra of **9**, the loss of the imidazolium proton (C2−H) and the imidazolium carbon (C2) signals at 9.16 and 138.7 ppm, respectively, suggests the formation of the silver(I) NHC complex. In the ^{13}C NMR

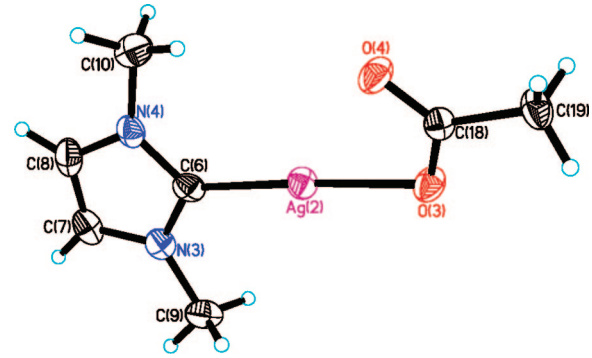


Figure 3. Crystal structure of [(**6**)AgOAc] (**7**). Thermal ellipsoids are shown at 50% probability.

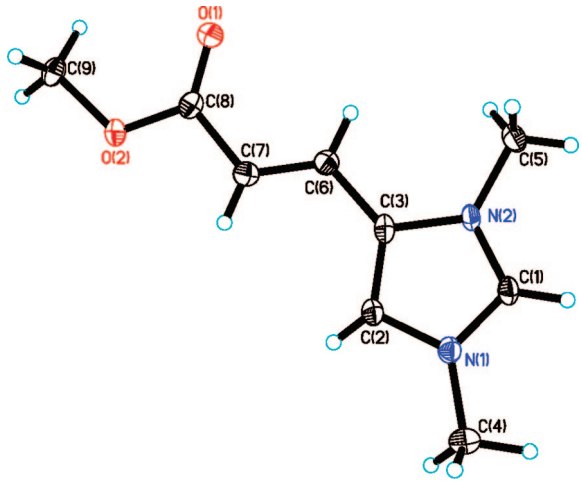


Figure 4. Crystal structure of the cationic portion of **8** with thermal ellipsoids shown at 50% probability.

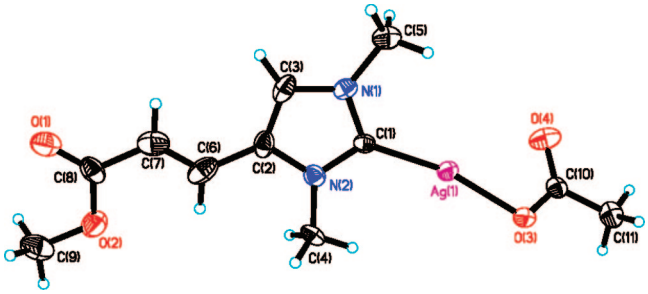


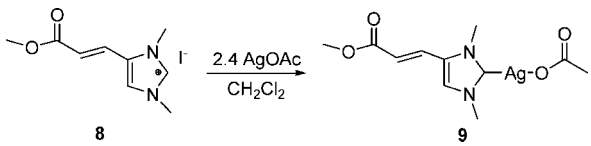
Figure 5. Crystal structure of [(**8**)AgOAc] (**9**). Thermal ellipsoids are shown at 50% probability.

Table 1. Stability of Silver Complexes in D_2O

silver complex	stability in D_2O
2	3 days
5	17 weeks +
7	2 h
9	1.5 h

spectrum of **9**, the carbene carbon (C2) signal was observed at 182.0 ppm, which is similar to other silver(I) NHC systems.

Single crystals of complex **9** suitable for X-ray diffraction were grown from a concentrated solution of acetonitrile. The solid state structure of **9** is shown in Figure 5. The geometry at the Ag(I) atom deviates from linearity, with a C1−Ag1−O3 bond angle of 167.28(18)° and Ag(1)−C(1) and Ag(1)−O(3) bond distances of 2.062(5) and 2.129(4) Å, respectively. The Ag−C distance of 2.062(5) Å is similar to that of the other reported silver NHC complexes. In the solid state structure, the conjugated methyl ester group appears to be in the plane of the silver acetate complex.



8 **9** (3)

Water Stability Studies. The stability of each silver complex in water is monitored by following the changes in the ^{13}C NMR and ^{1}H NMR spectra in D_2O (Table 1). A minor degradation is observed initially when complex **5** is added to D_2O. Approximately, 16% of silver complex **5** degrades into the imidazolium cation within the first hour. This degradation is accompanied by the appearance of the resonance for the methyl groups of **4** at 3.84 ppm in the ^{1}H NMR and 34.7 ppm in the ^{13}C NMR. After 2 weeks, approximately 22% of the imidazolium cation **4** is formed, and after 17 weeks, 34% of **5** has degraded to **4**. Complex **7** shows rapid decomposition within the first 2 h when added to D_2O, as approximately 95% degrades into the starting compound **6**. The appearance of the resonance at 7.47 ppm for the C−H protons on the 4 and 5 positions of the imidazolium cation and the simultaneous disappearance of the resonance at 7.29 ppm for the C−H protons on the 4 and 5 positions of the imidazole ring of complex **7** in the ^{1}H NMR are the basis for the above observation. Furthermore, complex **9** also proves to be quite unstable in D_2O, as approximately 94% of the imidazolium cation **8** is formed within 1.5 h.

Based on the above experiment, silver complex **5** bearing two Cl atoms shows improved stability when compared to those bearing hydrogens or a methyl ester group. We believe that the chlorine atoms in complex **5** stabilize the silver complex by acting as σ-withdrawing and π-donating substituents, thereby withdrawing electron density from the carbene carbon, making it less susceptible to attack by protons in an aqueous environment. The hydrogen atoms of complex **7** do not appear to contribute any stability. These results compliment the previous findings of Peris in that the chlorine substituents in the 4 and 5 positions of the imidazole ring lead to a reduction of σ-donor capability compared to the nonchlorinated equivalents.[16] The methyl ester substituent in complex **9** is both σ-withdrawing and π-withdrawing. This feature does not appear to add any water stability to the complex. This is most likely due to the free rotation of the methyl ester in solution, which serves to break the conjugation with the electronic framework of the imidazole ring.

MIC and MBC of 5. The minimum inhibitory concentration (MIC) of **5** against a panel of pathogens, primarily respiratory isolates of *P. aeruginosa* and the *Burkholderia* species, was determined in two different growth media, the Clinical and Laboratory Standards Institute (CLSI) standard Mueller-Hinton (M-H) as well as Luria broth (LB) (Table 2). A 10 mg/mL stock solution of **5** in water was prepared and diluted 1:1 into M-H or LB containing 10^5 colony forming units (CFU) of each bacterial strain to yield the appropriate test concentration (0, 1, 2, 4, 6, 8, or 10 μg of **5**/mL). The MIC_{50} and MIC_{90} for **5** against the panel of 36 respiratory pathogens grown in M-H were 1 and 4 μg/mL, respectively. The MIC_{50} for **5** against the same pathogens grown in LB was higher at 2 μg/mL, although the MIC_{90} was the same at 4 μg/mL. Although little difference was seen in the MIC_{50} and MIC_{90} of **5** against strains grown in M-H compared with LB, a few strains were very sensitive to growth inhibition by **5** in M-H but were not sensitive in LB at any of the concentrations tested. *E. coli* strains J53, a silver-sensitive strain, and J53+pMG101, a silver-resistant strain, were used as positive and negative controls. Determination of the minimum bactericidal concentration (MBC) of **5** against a subset of strains was performed and found to depend markedly on the growth media in which the tested strain was grown, as well as the strain itself (Table 2). Compound **5** appears to be bactericidal for numerous strains, particularly if grown in M-H, but only bacteriostatic for other strains at the concentrations tested. Thus, compound **5** appears capable of killing numerous bacterial strains at concentrations achievable clinically.

Comparison of the Antimicrobial Activities of 5 and 7. For a sample panel of four respiratory pathogens, two *P.*

Table 2. MIC and MBC of **5**

species (genomovar)	strain	MIC (μg/mL) M-H	MIC (μg/mL) LB	MBC (μg/mL) M-H	MBC (μg/mL) LB	note
Pseudomonas aeruginosa	PAO1-V	2	2	>10	>10	laboratory strain
	PAM57–15	4	1	6	2	mucoid CF clinical isolate
	PA 2192	1	6	2	8	mucoid CF clinical isolate
	PA 6294	2	2	>10	>10	invasive corneal clinical isolate
	PA N6	1	2	4	>10	nonmucoid CF clinical isolate
	PAJG3	2	2	4	8	nonmucoid CF clinical isolate
	PA N13	1	2	10	4	nonmucoid CF clinical isolate
	PA 1061	1	2	6	10	nonmucoid CF clinical isolate
	PA N8	1	2	>10	>10	nonmucoid CF clinical isolate
	PA 324	1	1	2	>10	mucoid CF clinical isolate
Burkholderia cepacia (I)	PC783	1	4	2	8	standard Bcc panel strain
Burkholderia multivorans (II)	HI2229	1	4	1	>10	standard Bcc panel strain
	AU8170	4	4	nd[a]	nd[a]	CF clinical isolate
	AU5735	2	2	nd[a]	nd[a]	CF clinical isolate
	AU7484	2	2	nd[a]	nd[a]	CF clinical isolate
	AU5248	4	2	nd[a]	nd[a]	CF clinical isolate
Burkholderia cenocepacia (III)	J2315	2	1	4	2	epidemic strain
	HI2718	2	>10	2	>10	standard Bcc panel strain
	ATTC BAA-245	1	1	1	4	LMG 16656, UK CF patient, 1989
Burkholderia stabilis (IV)	ATTC BAA-67	1	>10	6	>10	LMG 14294, Belgium CF patient, 1993
	HI2210	1	>10	2	>10	standard Bcc panel strain
Burkholderia vietnamiensis (V)	PC259	1	1	4	10	standard Bcc panel strain
Burkholderia dolosa (VI)	AU0645	1	2	1	6	standard Bcc panel strain
	ATTC BAA-246	1	2	4	6	LMG 18941, first CF *B. dolosa* isolate
	AU4459	1	1	4	6	CF clinical isolate
	AU5404	1	1	1	nd[a]	CF clinical isolate
	AU4298	2	2	nd[a]	nd[a]	CF clinical isolate
	AU3556	1	2	nd[a]	nd[a]	CF clinical isolate
	AU4881	4	4	nd[a]	nd[a]	CF clinical isolate
	AU9248	4	2	nd[a]	nd[a]	CF clinical isolate
	AU4894	4	2	nd[a]	nd[a]	CF clinical isolate
	AU3123	4	2	nd[a]	nd[a]	CF clinical isolate
	AU4750	4	2	nd[a]	nd[a]	CF clinical isolate
Burkholderia ambifaria (VII)	HI2468	2	1	4	>10	standard Bcc panel strain
Burkholderia anthina (VIII)	AU1293	1	2	1	4	standard Bcc panel strain
Burkholderia pyrocinia (IX)	BC11	1	1	1	1	standard Bcc panel strain
Escherichia coli	J53	2	4	>10	>10	susceptible strain
	J53+pMG101	>10	>10	>10	>10	J53 + plasmid with silver resistance genes

[a] nd = not done.

Table 3. MIC Comparison of **5** and **7**

species (genomovar)	strain	MIC (μg/mL) **5**	MIC (μg/mL) **7**	note
Pseudomonas aeruginosa	PAO1-V	2	1	laboratory strain
	PAM57-15	4	1	mucoid CF clinical isolate
Burkholderia cenocepacia (III)	J2315	2	1	epidemic strain
Burkholderia dolosa (VI)	AU4459	4	1	CF clinical isolate
Escherichia coli	J53	2	1	susceptible strain
	J53+pMG101	>10	>10	J53 + plasmid with silver resistance genes

aeruginosa strains and two strains of the *Burkholderia* species, the MIC_{50} of **5** was 2 μg/mL, and the MIC_{90} was 4 μg/mL. In contrast, the MIC of **7** was uniformly 1 μg/mL (Table 3) for all of the strains tested. *E. coli* strains J53, a silver-sensitive strain, and J53+pMG101, a silver-resistant strain, were again used as positive and negative controls. If compared on a molar basis, the MIC_{50} values of **5** and **7** against this panel of pathogens are essentially the same, namely 6.0×10^{-6} mmol/mL (MIC_{90} for **5** is 12×10^{-6} mmol/mL) and 3.8×10^{-6} mmol/mL, respectively. The result is somewhat surprising, given the significant difference in degradation rates between **5** and **7**, suggesting either that the degradation rate of **5** in bacteria-laden media is dramatically accelerated or that the intact complex has antimicrobial activity. In order to assess the stability of silver complexes **5** and **7**, 10 mg/mL stock solutions of **5** and **7** in D_2O were made and added in a 1:1 ratio to M-H and LB made in D_2O. This was done to mimic the conditions of the MIC determination experiments. Changes in the ^{1}H NMR spectrum were monitored at 0 °C, room temperature, and 37 °C. Complex **5** demonstrated stability in the M-H/D_2O broth mixture at 0 °C, room temperature, and 37 °C when monitored over a maximum of 72 h. It also appeared stable in the LB/D_2O broth mixture at room temperature when monitored over the same length of time. However, complex **7** demonstrated very fast degradation when mixed with the M-H/D_2O broth mixture at 0 °C. Based on the ^{1}H NMR spectrum, this compound degraded within the first minute after the initial mixing of the components. However, in support of the first hypothesis is the observation that a precipitate formed upon the addition of **5** to the M-H broth. Thus, a portion of **5** may degrade immediately, releasing an active silver cation, and the stable ^{1}H NMR spectrum may represent that of the remainder of intact **5**. These results strongly support the possibility that the intact compound has antimicrobial activity

given the equivalent MIC_{50} concentrations of the stable **5** and the unstable **7**.

Conclusion

In conclusion, we have shown that imidazolium salts **4**, **6**, and **8** did not act as antibacterial agents in low concentrations, but when combined with silver acetate the systems exhibited excellent bacteriostatic activity. Most of the silver complexes dissociated completely in the presence of water within a few hours or days with rapid removal of all silver. However, complex **5** showed a moderate initial dissociation, with continual release of silver over the time observed. Thus, we have presented evidence of the electronic tuning of an NHC ligand by introducing chlorine substituents on the 4 and 5 positions of the imidazole ring. The chlorine substituents act in a σ-withdrawing and π-donating fashion, thereby significantly stabilizing the NHC silver complex **5** compared to its nonchlorinated analogue **7** and its σ-withdrawing and π-withdrawing methyl ester analogue **9**.

We are currently exploring other systems bearing a variety of electron withdrawing groups as well as other halogens. Our goal is to explore if other halogens will act similarly to the chlorines in further stabilizing the NHC silver complexes and if other electron withdrawing groups that act strictly as σ-withdrawing and π-withdrawing groups will have a similar affect. This study will ultimately help us find the ideal silver NHC that may survive systemic delivery.

Experimental Section

General Procedures. All manipulations were carried out under aerobic conditions. 4,5-Dichloroimidazole and iodomethane were purchased from Alfa Aesar. Silver(I) oxide, silver acetate, and methylimidazole were purchased from Acros Organics. Silver nitrate was purchased from Fisher Scientific, and urocanic acid was purchased from TCI. LB Broth Miller (DIFCO) and Bacto-agar (DIFCO) were prepared according to manufacturer's instruction and sterilized before use. All solvents were obtained from a PureSolv solvent purification system. ^{1}H and ^{13}C NMR data were collected on a Varian Gemini 300 MHz instrument. ^{1}H NMR stability studies for complexes **5** and **7** in M-H/D_2O and LB/D_2O broth mixtures were done on a Varian INOVA 400 MHz instrument. The ^{1}H and ^{13}C NMR spectra obtained were referenced to the residual protons of the deuterated solvents and the solvent resonances, respectively. ^{109}Ag NMR data were collected on a Varian INOVA 750 MHz instrument. Mass spectrometry data were collected on a Bruker Daltons (Billerica, MA) Esquire-LC mass spectrometer equipped with ESI. Elemental analyses were performed at the Microanalyses Laboratory at The University of Illinois and Galbraith Laboratories.

X-ray Crystallographic Structure Determination Details. Crystals of **3**, **4**, **5**, **7**, **8**, and **9** were coated in paratone oil, mounted on a CryoLoop, and placed on a goniometer under a stream of nitrogen. X-ray data were collected using a Bruker Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data was integrated using SAINT. An empirical absorption correction and other corrections were applied by using multiscan SADABS. A Bruker SHELXTL package was used for the structure solution, refinement, and modeling of the crystals. The structures were determined by full-matrix least-squares refinement of F^2 and the selection of appropriate atoms from the generated difference map.

Synthesis of 1-Methyl-4,5-dichloroimidazole (3). 4,5-Dichloroimidazole (1.23 g, 9.00 mmol) and potassium hydroxide (2.24 g, 40.0 mmol) were stirred in acetonitrile (50 mL) for 2 h at room temperature. The excess KOH was filtered from the solution, and iodomethane (0.562 mL, 9.00 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The volatile components were removed, and the crude product was redissolved in dichloromethane. The solid, presumably KI, was filtered and discarded, and the volatile components were removed in vacuo to yield a yellow crystalline solid. Crystals suitable for single crystal X-ray diffraction studies were grown from acetonitrile. Yield: (1.32 g, 8.72 mmol, 97%); mp: 56–58 °C. Anal. Calcd for $C_4H_4Cl_2N_2$: C, 31.82; H, 2.67; N, 18.55. Found: C, 32.31; H, 2.74; N, 17.51. ESI-MS (*m*/*z*): calcd, 157.0, $[M + Li]^+$; found, 156.9. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.60 (s, 3H, CH_3), 7.76 (s, 1H, NCHN). ^{13}C {^{1}H} NMR (75 MHz, DMSO-d_6): δ 32.3 (NCH_3), 136.2 (NCHN), 124.0 (C−Cl), 112.6 (C−Cl).

X-ray crystal structure analysis of **3**: formula $C_4H_4Cl_2N_2$, MW = 150.99, colorless crystal 0.27 × 0.16 × 0.07 mm^3, a = 7.128(3) Å, b = 7.195(3) Å, c = 11.938(4) Å, α = 84.108(6)°, β = 78.185(5)°, γ = 88.354(6)°. T = 100(2) K, Z = 4, triclinic, space group $P\bar{1}$, V = 596.1(4) Å^3, D_{calc} = 1.682 Mg·m^{-3}, λ = 0.71073 Å, μ = 0.969 mm^{-1}, 4634 reflections collected, 2383 independent (R_{int} = 0.0334), 147 refined parameters, R1/*w*R2 ($I > 2\sigma(I)$) = 0.0415/0.1120 and R1/*w*R2 (all data) = 0.0445/0.1139, maximum (minimum) residual electron density 0.739(−0.358) e·Å^{-3}.

Synthesis of 1,3-Dimethyl-4,5-dichloroimidazolium Iodide (4). Compound **3** (1.32 g, 8.72 mmol) was dissolved in acetonitrile (30 mL), and excess iodomethane (2 mL, 32.1 mmol) was added. The mixture was refluxed at 85 °C for 2 days. The volatile components were removed in vacuo to yield a green crystalline solid. Crystals suitable for single crystal X-ray diffraction were grown from acetonitrile. Yield: (2.45 g, 8.37 mmol, 96%); mp: 179–182 °C. Anal. Calcd for $C_5H_7Cl_2N_2I$: C, 20.50; H, 2.41; N, 9.56. Found: C, 20.31; H, 2.27; N, 9.22. ESI-MS (*m*/*z*): calcd, 165.0, $[C_5H_7Cl_2N_2^+]$; found, 164.9. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 6H, CH_3), 9.42 (s, 1H, NCHN). ^{13}C {^{1}H} NMR (75 MHz, DMSO-d_6): δ 35.0 (NCH_3), 136.6 (NCHN), 118.9 (C−Cl).

X-ray crystal structure analysis of **4**: formula $C_5H_7Cl_2N_2I$, MW = 292.93, colorless crystal 0.50 × 0.10 × 0.05 mm^3, a = 7.1338(12) Å, b = 7.4652(13) Å, c = 8.7890(15) Å, α = 90°, β = 92.125(3)°, γ = 90°. T = 100(2) K, Z = 2, monoclinic, space group $P2(1)$, V = 467.74(14) Å^3, D_{calc} = 2.080 Mg·m^{-3}, λ = 0.71073 Å, μ = 3.928 mm^{-1}, 3971 reflections collected, 2104 independent (R_{int} = 0.0295), 93 refined parameters, R1/*w*R2 ($I > 2\sigma(I)$) = 0.0368/0.0909 and R1/*w*R2 (all data) = 0.0374/0.0913, maximum (minimum) residual electron density 2.252(−2.467) e·Å^{-3}.

Synthesis of (1,3-Dimethyl-4,5-dichloroimidazole-2-ylidene)-silver(I)acetate (5). Compound **4** (1.51 g, 5.15 mmol) was dissolved in dichloromethane (50 mL), and silver acetate (1.78 g, 10.7 mmol) was added. The mixture was stirred at room temperature for 2.5 h. The yellow precipitate, presumably AgI, was filtered and discarded. The volume of the reaction mixture was reduced under pressure to 5 mL. Hexane (1 L) was added, and the fine white precipitate was filtered and washed with 20 mL of hexane. Crystals suitable for single crystal X-ray diffraction studies were grown from THF. Yield: (1.35 g, 4.07 mmol, 79%); mp: 187–188 °C. Anal. Calcd for $C_7H_9AgCl_2N_2O_2$: C, 25.33; H, 2.73; N, 8.44. Found: C, 25.37; H, 2.69; N, 8.01. ESI-MS (*m*/*z*): calcd, 165.0, $[C_5H_7Cl_2N_2^+]$, 272.8 $[C_5H_6AgCl_2N_2^+]$, 436.8 $[C_{10}H_{12}AgCl_4N_4]$; found, 164.9, 272.7, and 436.7. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.79 (s, 3H, $COCH_3$), 3.77 (s, 6H, N−CH_3). ^{13}C {^{1}H} NMR (75 MHz, DMSO-d_6): δ 179.7 (C−Ag), 175.7 (C−O), 116.9 (C−Cl), 37.5 (N−CH_3), 23.4 ($COCH_3$). ^{109}Ag (35 MHz, DMSO-d_6): δ 352.55 (s, C−Ag−O).

X-ray crystal structure analysis of **5**: formula $C_7H_{15}AgCl_2N_2O_5$, MW = 385.98, colorless crystals 0.48 × 0.16 × 0.10 mm^3, a = 6.4992(10) Å, b = 13.491(2) Å, c = 15.744(3) Å, α = 90°, β = 90°, γ = 90°. T = 100(2) K, Z = 4, orthorhombic, space group $P2(1)2(1)2(1)$, V = 1380.4(4) Å^3, D_{calc} = 1.828 Mg·m^{-3}, λ = 0.71073 Å, μ = 1.856 mm^{-1}, 12260 reflections collected, 3298 independent (R_{int} = 0.0594), 157 refined parameters, R1/*w*R2 ($I > 2\sigma(I)$) = 0.0673/0.1767 and R1/*w*R2 (all data) = 0.0825/0.1876, maximum (minimum) residual electron density 4.484(−1.219) e·Å^{-3}.

Synthesis of (1,3-Dimethylimidazole-2-ylidene)silver(I)acetate (7). Compound **6** (0.91 g, 4.0 mmol) was dissolved in dichloromethane (50 mL), AgOAc (1.37 g, 8.20 mmol) was added, and the mixture was refluxed for 40 h. The precipitate, presumably AgI, was filtered, and a clear solution was obtained. The volatile

components were removed in vacuo to produce a white sticky solid. The solid was washed with diethyl ether (3 × 10 mL) and dried under reduced pressure for 7 h to yield **7**. Crystals suitable for single crystal X-ray diffraction studies were grown from methylene chloride. Yield: (0.67 g, 2.6 mmol, 64%); mp: 109–111 °C. Anal. Calcd for $C_7H_{11}N_2O_2Ag$: C, 31.96; H, 4.22; N, 10.65. Found: C, 32.39; H, 4.57; N, 10.05. ESI-MS (*m*/*z*): Calcd for $C_5H_8N_2Ag$ $[M-OAc]^+$: 202.97 and 204.97, found 203.00 and 205.00. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.41 (s, 2H, CH), 3.75 (s, 6H, CH_3), 1.77 (s, 3H, CH_3). ^{13}C {^{1}H} NMR (75 MHz, DMSO-d_6): δ 178.6 (s, C—Ag), 174.8 (s, *C*═O), 122.9 (s, *C*═C), 37.9 (s, CH_3), 23.2 (s, CH_3). ^{109}Ag (35 MHz, DMSO-d_6): δ 343.62 (s, C—Ag—O).

X-ray crystal structure analysis of **7**: formula $C_7H_{11}AgN_2O_2$, MW = 263.05, colorless crystal 0.11 × 0.05 × 0.04 mm^3, a = 10.508(2) Å, b = 10.521(2) Å, c = 12.881(3) Å, α = 95.984(4)°, β = 96.090(4)°, γ = 93.974(4)°, V = 1403.7(5) Å^3, D_{calc} = 1.867 Mg·m^{-3}, μ = 2.117 mm^{-1}, Z = 6, triclinic, space group $P\bar{1}$, λ = 0.71073 Å, T = 100 K, ω and φ scans, 12369 reflections collected, 6448 independent (R_{int} = 0.0337), 344 refined parameters, R1/wR2 ($I \geq 2\sigma(I)$) = 0.0534/0.1101 and R1/wR2 (all data) = 0.0801/0.1166, maximum (minimum) residual electron density 1.058 (−0.915) e·Å^{-3}.

Synthesis of 1,3-Dimethyl-4-acrylic Acid Methyl Ester Imidazolium Iodide (8). A solution of urocanic acid (0.497 g, 3.60 mmol) and sodium bicarbonate (1.2 g, 14 mmol) was refluxed in acetonitrile (150 mL) for 1 h. After the addition of excess iodomethane (12.77 g, 90.00 mmol), stirring was continued for 3 days under reflux. The excess sodium bicarbonate was filtered off, and the volatile components were removed in vacuo. The crude product was redissolved in 250 mL of dichloromethane and stirred for 2 h. The solid, presumably NaI, was filtered, and the volatile components were removed in vacuo to yield a white solid. Crystals suitable for single crystal X-ray diffraction were grown from acetonitrile. Yield: (0.9 g, 3 mmol, 81%); mp: 195–197 °C; Anal. Calcd for $C_9H_{13}N_2O_2I$: C, 35.08; H, 4.25; N, 9.09. Found: C, 35.08; H, 3.90; N, 8.91. ESI-MS (*m*/*z*): calcd for $C_9N_2O_2H_{13}I$ $[M-I]^+$ 181.1. Found 180.9. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.16 (s, 1H, NCHN), 8.32 (s, 1H, NCHC), 7.50 (d, 1H, CCH), 6.70 (d, 1H, CHC═O), 3.92 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 3.75 (s, 3H, CH_3). ^{13}C {^{1}H} NMR (75 MHz, DMSO-d_6): δ 165.6 (*C*═O), 138.7 (NCHC), 129.8 (*C*═C), 127.5 (*C*═C), 123.6 (*C*═C), 122.1 (*C*═C), 52.0 (OCH_3), 36.2 (N—CH_3), 34.0 (N—CH_3).

X-ray crystal structure analysis of **8**: formula $C_9H_{13}N_2O_2I$, MW = 308.11, colorless crystals 0.25 × 0.21 × 0.09 mm^3, a = 9.6726(16) Å, b = 9.4366(15) Å, c = 12.868(2) Å, α = 90°, β = 99.595(3)°, γ = 90°. T = 100(2) K, Z = 4, monoclinic, space group $P2(1)/n$, V = 1158.1(3) Å^3, D_{calc} = 1.767 Mg·m^{-3}, λ = 0.71073 Å, μ = 2.744 mm^{-1}, 9576 reflections collected, 2759 independent (R_{int} = 0.0638), 179 refined parameters, R1/*w*R2 ($I > 2\sigma(I)$) = 0.0375/0.0840 and R1/*w*R2 (all data) = 0.0460/0.0875, maximum (minimum) residual electron density 1.688(−0.812) e·Å^{-3}.

Synthesis of (1,3-Dimethyl-4-acrylic Acid Methyl Ester Imidazole-2-ylidene)silver(I)acetate (9). A mixture of **8** (0.20 g, 0.65 mmol) and silver acetate (0.26 g, 1.6 mmol) in dichloromethane (50 mL) was stirred at room temperature for 1 h. The reaction mixture was filtered through Celite to remove a yellow precipitate, presumably AgI, and the volatile components were removed in vacuo to yield a white solid. Crystals suitable for single crystal X-ray diffraction were grown from acetonitrile. Yield: (0.20 g, 0.57 mmol, 88%); mp: 122–123 °C. Anal. Calcd for $C_{11}H_{15}N_2O_4Ag$: C, 38.06; H, 4.36; N, 8.07. Found: C, 38.85; H, 4.68; N, 7.87. ESI-MS (*m*/*z*): calcd for $C_{11}N_2O_4H_{15}Ag$ $[M-OAc]^+$ 287.0, 289.0. Found 286.8, 288.8. Calcd for $C_{18}N_4O_4H_{24}Ag$ $[M]^+$ 467.1, 469.1. Found 467.0, 469.0. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.11 (s, 1H, NCHC), 6.50 (d, 1H, CCH), 7.51 (d, 1H, CHC═O), 3.88 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 1.83 (s, 3H, CH_3C═O). ^{13}C {^{1}H} NMR (75 MHz, DMSO-d_6): δ 182.0 (C—Ag), 174.0 (*C*═O), 166.2 (*C*═O), 130.0 (*C*═C), 129.4 (*C*═C), 124.4 (*C*═C), 118.7 (*C*═C), 51.7 (OCH_3), 38.5 (N—CH_3), 36.6 (N—CH_3), 22.4 ($COCH_3$).

X-ray crystal structure analysis of **9**: formula $C_{11}H_{15}AgN_2O_5$, MW = 363.11, colorless crystals 0.26 × 0.05 × 0.02 mm^3, a = 3.9393(11) Å, b = 13.676(4) Å, c = 14.281(4) Å, α = 63.854(4)°, β = 84.961(4)°, γ = 81.923(4)°. T = 100(2) K, Z = 2, triclinic, space group $P\bar{1}$, V = 683.5(3) Å^3, D_{calc} = 1.764 Mg·m^{-3}, λ = 0.71073 Å, μ = 1.492 mm^{-1}, 5856 reflections collected, 3152 independent (R_{int} = 0.0669), 176 refined parameters, R1/*w*R2 ($I > 2\sigma(I)$) = 0.0563/0.1250 and R1/*w*R2 (all data) = 0.0704/0.1277, maximum (minimum) residual electron density 1.428(−0.671) e·Å^{-3}.

Crystallographic data for compounds **3**, **4**, **5**, **7**, **8**, and **9** have been deposited with the Cambridge Crystallographic Data Center (666021, 655235, 655236, 655237, 655238, 655239).

Bacteria. All bacterial strains were maintained as glycerol stocks at −80 °C. For experiments, the bacteria were streaked onto either tryptic soy agar (TSA) or blood agar plates and incubated overnight at 37 °C. Dr. Simon Silver of Chicago, IL, kindly provided the silver sensitive and silver resistant *E. coli* strains. Dr. Thomas Ferkol of St. Louis, MO, provided PAM57-15. Dr. Gerald Pier of Boston, MA, provided all other strains of *P. aeruginosa*. *B. cepacia* complex species either were obtained from the Clinical Microbiology Laboratory at St. Louis Children's Hospital (CF clinical isolates of *B. multivorans*) or were provided by Dr. John LiPuma of Ann Arbor, MI, Dr. Johannes Huebner of Boston, MA (CF clinical isolates of *B. dolosa*), or the American Type Culture Collection.

Stock Solutions of 5 and 7. Compound **5** was diluted in sterile, <20 MΩ water at a concentration of 10 mg/mL and stored in small aliquots at −80 °C. Individual aliquots of stock solution **5** were thawed and stored at 4 °C for periods no longer than 2 weeks. Compound **7** was dissolved in dimethylsulphoxide (DMSO) to give a final concentration of 30 mg/mL. Stock solutions of **7** were not stored for longer than the day of the experiment.

In Vitro Antimicrobial Activity. Minimal inhibitory concentrations (MICs) were determined by broth microdilution as previously described by using standard CLSI (formerly the National Committee on Clinical Laboratory Standards, NCCLS) protocols.[13,17] Briefly, bacteria from fresh overnight plates were suspended in standard Mueller-Hinton broth (M-H), or for some experiments in Luria broth (LB), to an optical density at 650 nm (OD_{650}) of 0.2 and were grown in a shaking incubator to an OD_{650} of 0.4 (~2 × 10^8 CFU/mL). The bacteria were diluted in the broth to a concentration of 10^5 in 100 μL, which was added to triplicate wells, containing 100 μL of either **5** or **7** diluted in water to various concentrations from 10 mg/mL stocks. The final concentrations tested were 1, 2, 4, 6, 8, and 10 μg/mL. The MIC was the lowest of these concentrations, at which each of the triplicate wells was clear after incubation of the plate for 18–20 h at 37 °C. The MIC_{50} is, by definition, the concentration at which growth of 50% of the tested strains is inhibited, and similarly the MIC_{90} is the concentration at which 90% of the tested strains fail to grow. Minimal bactericidal concentrations (MBCs) of **5** were determined by plating all of the clear wells of the MIC experiments on TSA plates, incubating the plates overnight at 37 °C, and noting the concentration at which there were no colonies.

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Supporting Information Available: Melting point, elemental analysis, ^{1}H NMR, and ^{13}C NMR data for compounds **3**, **4**, **5**, **7**, **8**, and **9**. ^{109}Ag NMR data for compounds **5** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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