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Syntheses, Structures, and Antimicrobial Activity of New Remarkably Light-Stable and Water-Soluble Tris(pyrazolyl)methanesulfonate Silver(I) Derivatives of *N*-Methyl-1,3,5-triaza-7-phosphaadamantane Salt - [mPTA]BF_4

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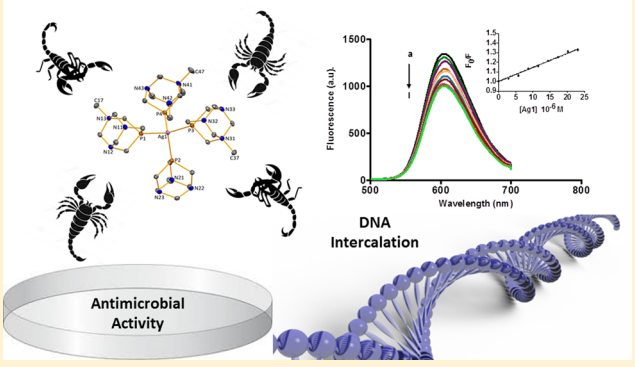
Supporting Information

ABSTRACT: Two new silver(I) complexes of formula [Ag(mPTA)$_4$](Tpms)$_4$(BF_4) (**1**) and [Ag(Tpms)(mPTA)](BF_4) (**2**) (mPTA = *N*-methyl-1,3,5-triaza-7-phosphaadamantane cation, Tpms = tris(pyrazol-1-yl)methanesulfonate anion) have been synthesized and fully characterized by elemental analyses, ^{1}H and ^{31}P{^{1}H} NMR, ESI-MS, and IR spectroscopic techniques. The single-crystal X-ray diffraction study of **1** discloses a noncoordinated nature of the Tpms species, existing as counterions around the highly charged metal center [Ag(mPTA)]$^{5+}$, **1** being the first reported coordination compound bearing a κ^0-Tpms. **1** features high solubility and stability in water ($S_{25\ °C} \approx 30$ mg·mL^{-1}). The two complexes interact with calf thymus DNA via intercalation mode, binding to the BSA with decrease of its tryptophan fluorescence with a static quenching mechanism. The two new silver complexes exhibit significant antibacterial and antifungal activities screened *in vitro* against the standard strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*.



INTRODUCTION

Silver complexes containing N- and P-donor ligands constitute a class of interesting compounds not only because of their wide application in fields of analytical chemistry,[1a] catalysis[1b] or in the materials industry[1c] but also because of their rich therapeutic applications.[2] Antimicrobial activities of Ag^+−X (X = P, S, O, and N) bonding complexes and other desirable properties (e.g., water solubility and light stability) can be tuned by varying the number and type of ligands coordinated to the metal center.[2−5] However, many silver materials applied as therapeutic drugs are sparingly soluble in water, limiting their uses in oil treatments and creams, as for the most used topical antibacterial agent silver sulfadiazine (SSD).[6]

To extend this research toward the synthesis and application of aqua-soluble and light-stable, bioactive silver complexes, the use of the aqua-soluble 1,3,5-triaza-7-phosphaadamantane (PTA) or highly hydrophilic derivative mPTA salts (*N*-methyl-1,3,5-triaza-7-phosphaadamantane) instead of the hydrophobic phosphines has been taken into account as a promising alternative. In recent years, the coordination chemistry of PTA has experienced a pronounced development justified by the search for water-soluble transition metal complexes.[7] Although Ru, Os, Au, Pt, and Cu compounds bearing PTA and derived ligands are known as rather potent antimicrobial and/or antitumor agents,[7,8] the biological properties of Ag-PTA derivatives remain poorly explored.[8,9] Within our recent research dealing with the use of PTA as a versatile *N,P* building block in crystal engineering, we have obtained a number of silver−PTA coordination networks supported by various arylcarboxylate ligands.[10]

The tris(1-pyrazolyl)methanesulfonate ligand $O_3SC(pz)_3$ (Tpms) was designed by analogy with tris(1-pyrazolyl)borate (Tp), both being able to act as monoanionic C_{3v}-symmetrical,

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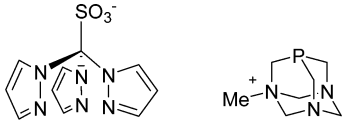
tripodal nitrogen-donor ligands.[11−13] However, unlike the tris(1-pyrazolyl)borate derivatives, insoluble in water and unstable toward hydrolysis, Tpms has a methanesulfonate unit, which imparts an increased solubility in polar solvents and a very good stability in aqueous media over a wide range of pH.[12] Moreover, as shown recently,[14] Tpms ligand can act not only as a tripodal but also as a bipodal ligand. In the former case, it can exhibit either a *N,N,N* or a *N,N,O* coordination, whereas as a bipodal ligand it can coordinate through either *N,N* or *N,O*,[14,15] hence being suitable ligands for the synthesis of structural models for *N,N,O*-binding in metalloenzymes.[16]

Now we report the synthesis and full characterization of the new silver(I) complexes $[Ag(mPTA)_4](Tpms)_4(BF_4)$ (**1**) and $[Ag(Tpms)(mPTA)](BF_4)$ (**2**), containing mPTA and Tpms (in **2**) ligands (Chart 1). The single-crystal X-ray diffraction study of (**1**), according to our knowledge, discloses the first example of a coordination compound with noncoordinated ions of Tpms species that occur as counterions, thus compensating the highly charged metal center $[Ag(mPTA)]^{5+}$. Moreover, compound **1** is able to fully preserve the antibacterial and antifungal activity of silver.

Chart 1. Structural Formulas of [Tpms] and [mPTA]



Tpms mPTA

EXPERIMENTAL SECTION

Materials and Methods. All synthetic work was performed under an inert atmosphere of dry oxygen-free dinitrogen, using standard Schlenk techniques. Solvents were dried and distilled prior to use. $AgBF_4$ was obtained from Aldrich and used as received, while *N*-methyl-1,3,5-triaza-7-phosphaadamantane tetrafluoroborate {$[mPTA](BF_4)$}[17] and $[O_3SC(pz)_3]Li$[12] were synthesized in accordance with literature methods. Elemental analyses were performed on a Vario EL III apparatus. The positive and negative electrospray ionization mass spectra (ESI-MS) were obtained with a Bruker MicroOTOF-Q instrument, using a methanol mobile phase. Infrared spectra (4000−400 cm^{-1}) were recorded on a Bruker IFS 1113v instrument in KBr pellets, whereas ^1H and $^{31}P\{^1H\}$ NMR spectra were measured on a Bruker 300 AMX spectrometer at ambient temperature (~25 °C). ^1H chemical shifts (δ) are expressed in ppm relative to $Si(Me)_4$, while $\delta(^{31}P)$ are relative to an external 85% H_3PO_4 aqueous solution. Coupling constants are in Hz; abbreviations: s, singlet; d, doublet; dd, doublet of doublets; br, broad. Bovine serum albumin (BSA), calf thymus DNA (ct-DNA), tris(hydroxymethyl)aminomethane (Tris), and ethidium bromide (EB) were purchased from Sigma−Aldrich. NaCl, NaH_2PO_4, and Na_2HPO_4 were purchased from Merck. All materials and solvents were of analytical reagent grade quality and were used without further purification. Tris−HCl and KH_2PO_4−Na_2HPO_4 buffers were prepared with twice-distilled water.

Synthesis and Characterization of Compounds 1 and 2. *$[Ag(mPTA)_4](Tpms)_4(BF_4)$·MeOH (**1**·MeOH).* To a solution of $AgBF_4$ (50 mg, 0.26 mmol) in methanol (30 mL) was added *N*-methyl-1,3,5-triaza-7-phosphaadamantane tetrafluoroborate $(mPTA)(BF_4)$ (269 mg, 1.04 mmol), and after 30 min, lithium salt of tris(1-pyrazolyl)-methanesulfonate $[O_3SC(pz)_3]Li$ (312 mg, 1.04 mmol). The resulting white suspension was stirred at room temperature for 3 h. Then was filtered off, washed with several small portions of methanol, and dried in vacuo to afford complex **1** as a white microcrystalline solid. Yield: 60% (326 mg), based on silver tetrafluoroborate. Anal. Calcd for $C_{69}H_{100}AgBF_4N_{36}O_{13}P_4S_4$ (2088.6): C, 39.68; H, 4.83; N, 24.14; S, 6.14%. Found: C, 39.02; H, 4.49; N, 23.97; S, 6.50%. IR (KBr, cm^{-1}): 3155w, 2978m, 1631w, 1519m, 1462m, 1420m, 1394m, 1323m, 1262s, 1097s, 1098s, 1062s, 1043s, 985s, 859m, 762m, 631m, 534m. ^1H NMR (deuterated dimethyl sulfoxide (DMSO-d_6), 298 K): δ, 8.06 (d, 12H, $H^{3,5}$ (pz)), 7.49 (d, 12H, $H^{3,5}$ (pz)), 6.37 (dd, 12H, H^4 (pz)), 4.97 and 4.84 ($J(H^AH^B)$ = 11 Hz, 16H, $NCH^AH^BN^+$), 4.50 and 4.30 ($J(H^AH^B)$ = 15 Hz, 8H, NCH^AH^BN), 4.45 (s, 8H, PCH_2N^+), 4.09 (q, 1H, MeOH), 4.06 and 3.87 ($J(H^AH^B)$ = 15.0 Hz, $^2J(H^A\text{-}P)$ = 7.5 Hz, $^2J(H^B\text{-}P)$ = 0, 16H, PCH^AH^BN), 3.16 (d, 3H, MeOH), 2.63 (s, 12H, N^+CH_3). $^{31}P\{^1H\}$ NMR (DMSO-d_6, 298 K): δ, −74.9 br. s (mPTA). ESI-MS (MeOH) (+): m/z 172.1 $[mPTA]^+$, 431.5 $[(mPTA)_2(BF_4)]^+$, 366.0 $[Ag(mPTA)(BF_4)]^+$. ESI-MS (MeOH) (−): m/z = 87.0 $[BF_4]^-$, 293.0 $[Tpms]^-$, 403.0 $[Na(Tpms)(BF_4)]$. Single crystals suitable for X-ray-analysis were grown from the reaction filtrate at 4 °C. ^1H NMR of free $[O_3SC(pz)_3]Li$ (DMSO-d_6, 298 K): δ, 8.09 (d, 3H, $H^{3,5}$ (pz)), 7.45 (d, 3H, $H^{3,5}$ (pz)), 6.32 (dd, 3H, H^4 (pz)).

*$[Ag(mPTA)(Tpms)](BF_4)$·MeOH (**2**·MeOH).* To a solution of $AgBF_4$ (101 mg, 0.52 mmol) in methanol (40 mL) was added lithium salt of tris(1-pyrazolyl)methanesulfonate $[O_3SC(pz)_3]Li$ (156 mg, 0.52 mmol), and after 1 h, *N*-methyl-1,3,5-triaza-7-phosphaadamantane tetrafluoroborate $(mPTA)(BF_4)$ (122 mg, 0.47 mmol). The resulting white suspension was stirred at room temperature for 1 h. Then was filtered off, washed with several small portions of cold methanol, and dried in vacuo to afford complex **2** as a white microcrystalline solid. Yield 80% (288 mg). Anal. Calcd for $C_{18}H_{28}Ag\ BF_4N_9O_4PS$ (692.2): C 31.23; H 4.08; N 18.21; S 4.63%. Found: C, 31.20; H, 4.11; N, 19.00; S, 6.70%. IR (KBr, cm^{-1}): 3131w, 29 858m, 2940m, 1631w, 1519m, 1456m, 1420m, 1389m, 1320m, 1234s, 1198w, 1122m, 1097s, 1053m, 1024m, 984m, 924w 896m, 861m, 813m 757m, 747m, 630s, 534m. ^1H NMR (DMSO-d_6, 298 K): δ, 8.06 s (3H, $H^{3,5}$ (pz)), 7.55 s (3H, $H^{3,5}$ (pz)), 6.40 s (3H, H^4 (pz)), 5.03 and 4.88 ($J(H^AH^B)$ = 12 Hz, 4H, $NCH^AH^BN^+$), 4.56 (s, 2H, PCH_2N^+), 4.61 and 4.30 ($J(H^AH^B)$ = 13 Hz, 2H, NCH^AH^BN), 4.09 (q, 1H, MeOH), 4.21 and 3.98 ($J(H^AH^B)$ = 15.0 Hz, $^2J(H^A\text{-}P)$ = 6.5 Hz, $^2J(H^B\text{-}P)$ = 0, 4H, PCH^AH^BN), 3.16 (d, 3H, MeOH), 2.67 (s, 3H, N^+CH_3). $^{31}P\{^1H\}$ NMR (DMSO-d_6, 298 K): δ, −66.9 br. s (mPTA). ESI-MS (MeOH) (+): m/z 172.1 $[mPTA]^+$, 431.5 $[(mPTA)_2(BF_4)]^+$, 366.0 $[Ag(mPTA)(BF_4)]^+$, 572.0 $[(Ag)(mPTA)(Tpms)]^+$. ESI-MS (MeOH) (−): m/z = 87.0 $[BF_4]^-$, 293.0 $[Tpms]^-$, 403.0 $[Na(Tpms)(BF_4)]$.

*X-ray Crystal Structure Determination of **1**.* The crystal data of **1** were collected at 150 K using a Bruker AXS-KAPPA APEX II diffractometer and graphite-monochromated Mo Kα radiation generated from X-ray diffraction tube operated at 50 kV and 20 mA. The images were indexed, integrated, and scaled using the Bruker data reduction package.[18] The structure was solved by direct methods using SHELXS97 and refined by the full-matrix least-squares method on all F^2 data.[19] Non-H atoms were included in the refinement with anisotropic displacement parameters, whereas H atoms were positioned geometrically and constrained to ride on their parent atoms. The data were corrected for absorption,[18] leading to min/max absorption coefficients of 0.8759 and 0.9160, respectively. Crystal data for **1**: $C_{68}H_{96}AgBF_4N_{36}O_{12}P_4S_4$, M = 2056.61, λ = 0.710 73 Å (Mo Kα), T = 150(2) K, monoclinic, space group $P2_1/c$, a = 15.3697(18), b = 22.755(3), c = 29.247(3) Å, β = 100.888(6), V = 10 045(2) $Å^3$, Z = 4, D_c = 1.360 g/cm^3, F_{000} = 4256, μ = 0.425 mm^{-1}, 146 439 reflections collected, 22 208 unique, $I > 2\sigma(I)$ (R_{int} = 0.0561), R_1 = 0.0489, wR_2 = 0.1110, GOF 1.039.

DNA Binding Study by Fluorescence Spectroscopy. DNA binding experiments, which include emission spectroscopy, were performed as previously reported.[9c] Fluorescence quenching spectra were recorded using an ISS-Greg 200 spectrofluorophotometer with an excitation wavelength of 490 nm and emission spectrum of 480−700 nm. The fluorescence spectra of a series of solutions with various concentrations of Ag-complex and a constant EB-ct-DNA complex were measured, and the fluorescence data were corrected according to the relationship (eq 1)

$$F_c = F_m \times e^{(A1+A2)/2} \quad (1)$$

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where F_c and F_m are the corrected and measured fluorescence, respectively. $A1$ and $A2$ are the absorbance of Ag complexes at the exciting and emission wavelengths. For fluorescence quenching experiments, the Stern−Volmer equation was used (eq 2):

$$F_0/F = 1 + k_Q\tau_0[D] = 1 + K_{sv}[D] \quad (2)$$

where F_0 and F represent the fluorescence intensity in the absence and in the presence of drug. $[D]$ is the concentration of the Ag complex, and K_{sv} is the linear Stern−Volmer constant. k_Q is the EB−DNA complex quenching rate constant; τ_0 is the average lifetime of fluorophore in the absence of quencher, and its value is $\sim 10^{-8}$ s. All experiments involving ct-DNA were performed in buffer solution (50 mM NaCl−5 mM Tris−HCl pH 7.1) at room temperature.

4,6-Diamidino-2-phenylindole (DAPI) displacement assays were carried out by monitoring the emission spectra of a solution of DNA (20 μM) and DAPI (15 μM), in 10 mM phosphate buffer pH 7.2, after addition of different concentrations of $AgNO_3$ (from 0 to 70 μM), complex **1** (from 0 to 6.6 μM), complex **2** (from 0 to 70 μM), Tmps (from 0 to 100 μM), and mPTA (from 0 to 100 μM), at room temperature after excitation at 338 nm and recording the emission spectra between 400 and 700 nm.[20]

Interaction with Bovine Serum Albumin. Silver complexes and bovine serum albumin (BSA) solutions were prepared in the buffer solution adjusted to pH 7.2 with 0.01 M Na_2HPO_4 and NaH_2PO_4 in pure aqueous medium. BSA stock solution (3×10^{-5} M, based on its molecular weight of 66 000) was prepared in 0.01 M phosphate buffer of pH 7.2 and was kept in the dark at 277 K. Triply distilled water was used throughout the experiment. Fluorescence measurements were carried out with an ISS-Greg 200 spectrofluorophotometer by keeping the concentration of BSA constant (3×10^{-5} M) while varying the silver complex concentration ([silver complex]/[BSA]) = 0.0, 0.1, 0.2, 0.3, 0.5, 0.7, 1, 1.2) at room temperatures. Fluorescence intensities were recorded after each successive addition of silver complex solution and equilibration (ca. 20 min).[21] Fluorescence data of the binding of Ag complexes with BSA were evaluated as previously described for the ct-DNA binding experiments.

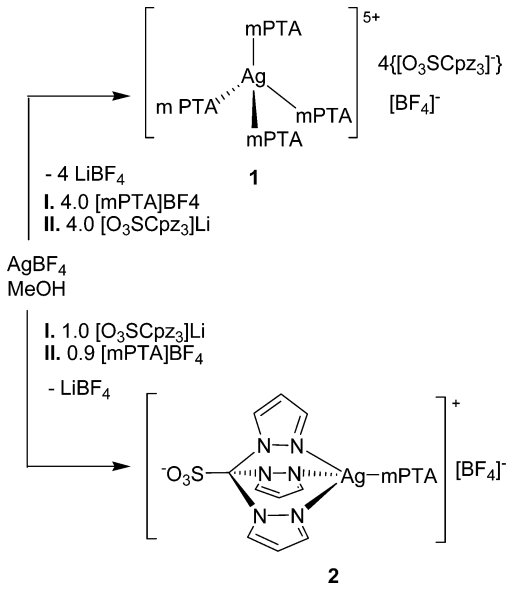
Enzymatic Activity. Enzymatic activity of adenosine deaminase (ADA) was determined spectrophotometrically by following the decrease in absorbance at 265 nm according to Kalcar.[22] One enzyme unit is the amount of enzyme that will convert 1 mmol of adenosine to inosine per minute at pH 7.2 and at 37 °C.

Antimicrobial Activity. The two silver compounds were tested against a panel of microorganisms including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 24433. Bacterial strains were cultured overnight at 37 °C in blood agar plates with the exception of *C. albicans*, which was grown in RPMI1640. Antibiotic susceptibility testing was performed by the microdilution methods, following the international guidelines of the CLSI. Briefly 2-fold serial dilutions of each compound were prepared in 96-well plates, starting from 256 μM in the cation adjusted Mueller Hinton broth or RPMI 1640 medium, depending on the microorganism tested. An equal volume of bacterial inoculum (10^6 cfu mL^{-1}), obtained by a direct colony suspension of an overnight culture, was added to each well of the microtiter plate containing 0.1 mL of the serially diluted test molecule. After incubation for 18−24 h at 35 °C (24−48 h in the case of *C. albicans*) in a normal atmosphere, the minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the compound able to inhibit the growth of the microorganisms. All tests were done in triplicate. Minimum bactericidal concentration (MBC) was subsequently determined as recommended by the CLSI guidelines.[23]

RESULTS AND DISCUSSION

Treatment of $AgBF_4$ with a 4-fold molar excess of *N*-methyl-1,3,5-triaza-7-phosphaadamantane tetrafluoroborate (mPTA)-(BF_4), in MeOH solution at ~25 °C, followed by the addition of an equimolar amount of tris(1-pyrazolyl)methanesulfonate $[O_3SC(pz)_3]Li$ (i.e., Ag/(mPTA)/(Tpms) molar ratio of 1:4:4), leads to $[Ag(mPTA)_4](Tpms)_4(BF_4)$ (**1**). However, reaction of $AgBF_4$ but using in reverse order Tpms and mPTA in around equimolar amounts (Ag/Tpms/mPTA molar ratio of 1:1:0.9) affords $[Ag(Tpms)(mPTA)](BF_4)$ (**2**) (Scheme 1).

Scheme 1. Synthetic Procedure for Compounds 1 and 2



The new products **1**−**2** were isolated as air- and light-stable, white, microcrystalline solids in ca. 60−80% yields based on $AgBF_4$ and were characterized by IR, ^1H and $^{31}P\{^1H\}$ NMR spectroscopies, ESI−MS(±), elemental analyses, and single-crystal X-ray diffraction analysis (for **1**). Both complexes are soluble in water and stable (including long exposition to sun light) and in addition show poor to medium solubility in DMSO, MeOH, and MeCN.

The solution ^1H NMR spectra of **1**−**2** exhibit four types of CH_2 and one of CH_3 protons in the mPTA region. Three of them: NCH_2N, NCH_2N^+, and PCH_2N (centered at δ 4.40−4.45, 4.90−4.95, and 3.97−4.10 for **1**−**2**, respectively) are of AB type, which are assigned to the $N-CH_{ax}-N$ and the $N-CH_{eq}-N$ protons, as reported in other compounds.[7,9,10] In case of PCH_2N^+ and N^+CH_3 the singlets at δ 4.45−4.56 and 2.63−2.67 for **1**−**2**, respectively, were observed. The ^1H NMR (DMSO-d_6, 298 K) spectrum of **1** also shows in the aromatic region three resonances due to pyrazolyl rings of Tpms, centered at δ 8.06, 7.49−7.55, and 6.37−6.40, respectively. Analogously in the aromatic region the ^1H NMR spectrum of **2** exhibits three broad singlets, suggesting a N_3-coordination to silver. The $^{31}P\{^1H\}$ NMR spectra of **1**−**2** are typical for silver−PTA derivatives, showing the corresponding broad singlets at δ −74.9 and −66.9 for **1**−**2**, respectively, which are shifted downfield in comparison with the uncoordinated mPTA. The $^{107}Ag-^{31}P$ and $^{109}Ag-^{31}P$ spin−spin coupling was not observed in all cases due to the fast ligand exchange.[24] The IR spectra of **1**−**2** exhibit related features with typical vibrations assigned to the Tpms and mPTA species, as well as a broad band due to BF_4 anion.

The asymmetric unit of **1** contains the cationic $[Ag(mPTA)_4]^{5+}$ monomeric block surrounded by one $[BF_4]^-$ and four $[Tpms]^-$ counteranions (Figure 1). The four-coordinate metal atom exhibits an almost perfect tetrahedral geometry (τ_4 = 0.96)[25] filled by the phosphorus atoms with

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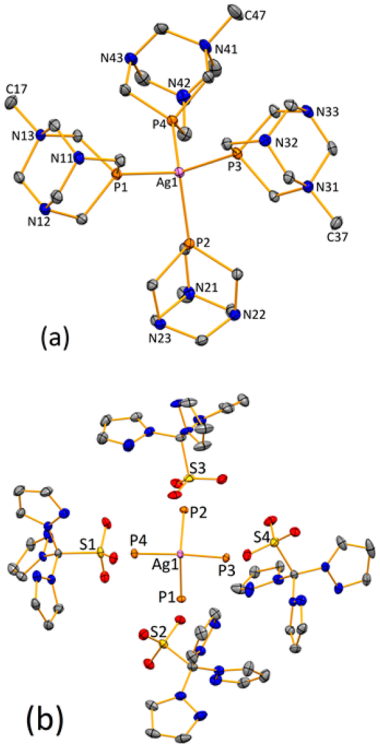

Figure 1. Ellipsoid plot (drawn at 30% probability level) of complex **1**, showing the $[Ag(mPTA)_4]^{5+}$ cation (a) and its $[Tpms]^-$ environment (b). H atoms, $[BF_4]^-$ (a, b), and cages of phosphaadamantane (b) are omitted for clarity. Color codes: Ag, magenta; N, blue; P, orange; O, red; S, yellow; C, gray.

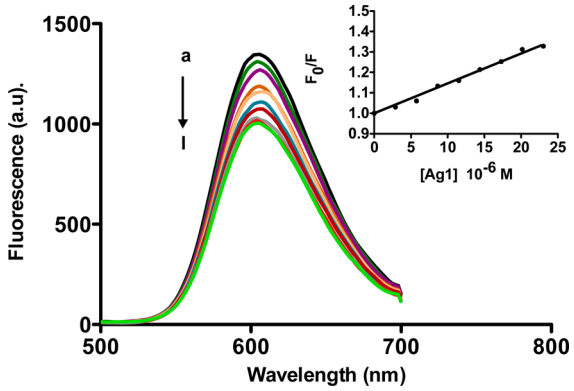

Figure 2. Fluorescence spectra of EB-ct-DNA in absence (a) and presence of different concentrations of complex **1**: from **a** to **j** (0; 2.8; 5.6; 8.4; 11.2; 14.0; 17.8; 20.6; 23.4; 32.6 μM). (inset) Stern–Volmer plot of the interaction of compound **1** with EB-ct-DNA.

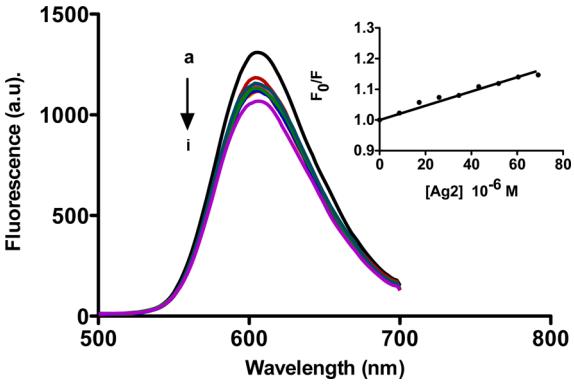

Figure 3. Fluorescence spectra of EB-ct-DNA in absence (a) and presence of different concentrations of complex **2**: from **a** to **i** (0; 8.6; 17.2; 25.8; 34.4; 43.0; 51.6; 60.6; 69.2 μM). (inset) Stern–Volmer plot of the interaction of compound **2** with EB-ct-DNA.

average Ag–P distances and P–Ag–P angles of 2.475(9) Å and 109.42(3)°, respectively.

DNA Binding Study by Fluorescence Spectroscopy. The biocidal effect of silver, with its broad spectrum of activity including bactericidal action, is particularly well-known, but silver is a metal of interest also in cancer therapy because its toxicity in humans is believed to be quite low. Silver ions induce errors in DNA transcription processes, which may cause disturbance of normal functionality of nucleic acids. To this purpose we evaluated the binding of the new synthesized silver complexes with DNA. No luminescence was observed for all the Ag complexes, neither in aqueous solution nor in the presence of calf thymus DNA. Hence the intercalation between the complexes and DNA cannot be directly observed in the emission spectra. The binding of Ag complexes to ct-DNA was therefore studied by competitive binding studies using EB. EB is weakly fluorescent in aqueous solution, but its emission intensity in the presence of DNA is greatly enhanced because of its strong intercalation between the adjacent DNA base pairs.[26]

A competitive ct-DNA binding of the Ag complexes displace the bound EB, and as a consequence the emission intensity of EB is likely to decrease. Addition of complexes **1** and **2** to ct-DNA pretreated with EB decreases in emission intensity of the EB-ct-DNA complex as shown in Figures 2 and 3.

These behaviors can be analyzed through Stern–Volmer equation (inset, Figures 2 and 3), and values of binding constants K_{sv} and k_Q for silver complexes **1** and **2** are reported in Table 1. The binding constants (K_A) and apparent binding sites (n) can be analyzed by the following eq 3:[21,27−29]

$$\log(F_0 - F)/F = \log K_A + n \log[D]_f \quad (3)$$

Table 1. Values of Binding Constants of Silver Complexes with DNA

compound	$K_{sv} \times 10^4$ M^{-1}	$K_Q \times 10^{12}$ M^{-1} s^{-1}	$K_A \times 10^4$ M^{-1}	n
1	1.37	1.37	3.46	0.97
2	0.31	0.31	0.12	0.76

where F_0 and F represent the fluorescence intensities in the absence and presence of quencher, respectively, K_A is the binding constant of the silver complex with ct-DNA, and n is the apparent number of binding sites, which is lower than the real number of binding sites. The plot of $\log(F_0 - F)/F$ versus $\log[D]_f$ is drawn and fitted linearly, and then the slope n and value of binding constant K_A can be obtained.[21,27−29] All values of association constants obtained for the different complexes are reported in Table 1. From the values of the K_{sv} and K_A constants, related to the binding of the different Ag^+ complexes to DNA, we can see that compound **1** shows significantly higher affinity for DNA when compared with that of **2**. The reported values of **n** (the Hill coefficient), are lower than 1, which suggests that the binding of one molecule of the silver complex to ct-DNA decreases the affinity for another molecule.

To obtain more information on the association ability of complexes **1** and **2** and also of the Ag^+ ion to ct-DNA, we performed competition binding on DNA using a different probe with respect to EB, namely, 4′,6-diamidino-2-phenyl-

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indole (DAPI), which is a well-known fluorogenic probe able to bind on the minor groove of ct-DNA. In the presence of another minor groove ligand, there would be competition between the new molecule and DAPI for binding to the DNA minor groove, with a corresponding decrease of fluorescence intensity of the DAPI-ct-DNA complex. Figure S1 (Supporting Information) shows fluorescence quenching data performed with complexes **1** and **2** and $AgNO_3$. For all silver species the addition of small aliquots do not induce a decrease on fluorescence of DAPI-ct-DNA complex but led to a fluorescence enhancement with a blue shift (~30 nm) of the emission maximum. All the silver species interact with DAPI-ct-DNA complex with different affinity, and the presence of isosbestic points indicates an equilibrium state between Ag^+ or complexes **1** and **2** and ct-DNA (Figure S1). The significant hyperchromic effects, as well as the moderate blue shift, support the existence of a strong interaction between the silver species and ct-DNA via intercalation within the ct-DNA double helix through electrostatic interaction especially at low ionic strength or, most probably, through partial electronic overlap among the silver complexes and the DAPI-ct-DNA complex, all of them producing its partial stabilization. As reported, no binding activity was observed for the free ligands (mPTA and Tmps, Figure S1). From such data we can observe that the complexes, or part of them if dissociated in solution, can interact with ct-DNA through a different affinity, which decreases as **1** > **2** > Ag^+ > Tpms = mPTA(Table S1, Supporting Information). Studies are in progress to characterize the species that can be involved on the binding with ct-DNA.

Interaction with BSA. The interaction between biomacromolecules and drugs has attracted great interest; among biomacromolecules, serum albumin is the major soluble protein constituent of circulatory system and has many physiological functions, in particular, as transport protein for many exogenous compounds. The drug–protein interaction may result in the formation of a stable drug–protein complex, which has important effects on the distribution, free concentration, metabolism, the efficacy of drugs, etc. Therefore, it is important to study the interaction of drugs with serum albumin, which plays an important role in pharmacology and pharmacodynamics. In this regard, bovine serum albumin (BSA) has been studied extensively, partly because of its structural homology with human serum albumin (HSA). Fluorescence of BSA originates from tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) residues, whereas the Trp residue alone mainly contributes the intrinsic fluorescence of BSA. Figures 4 and 5 show the fluorescence emission spectra of BSA with various amount of the Ag complexes following an excitation at 285 nm.

BSA exhibited a strong fluorescence emission band at 345 nm at pH 7.40, and its intensity decreased gradually with the addition of the Ag complexes. To investigate the quenching mechanism induced by the silver complexes, fluorescence quenching data were analyzed with the Stern–Volmer eq 2 (inset, Figures 4 and 5), and the values of quenching constants K_{sv} are reported in Table 2. We evaluated also the k_q constants (the quenching rate constant of biomolecule in eq 2) assuming τ_0 is the average lifetime of biomolecule without the quencher equal to 5 ns. Values for k_q (Table 2) are higher than the value of maximum diffusion collision quenching rate constant (2.0×10^{10} L mol^{-1} s^{-1}) of a variety of quenchers with biopolymer; therefore, they suggest that the fluorescence quenching process of the Ag complexes at low concentrations may be mainly

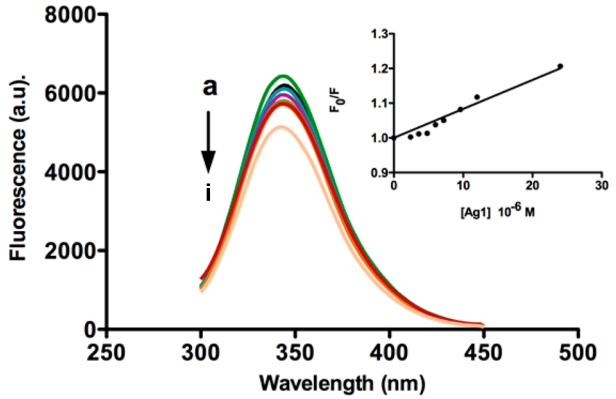

Figure 4. Fluorescence spectra of BSA at different concentrations of compound **1**; [BSA] = 3×10^{-5}: from **a** to **i** [compound **1**] = 0, 0.24, 0.36, 0.48, 0.6, 0.72, 1.0, 1.2, 2.4×10^{-5} M, respectively. (inset) Stern–Volmer plot of the interaction of compound **1** with BSA.

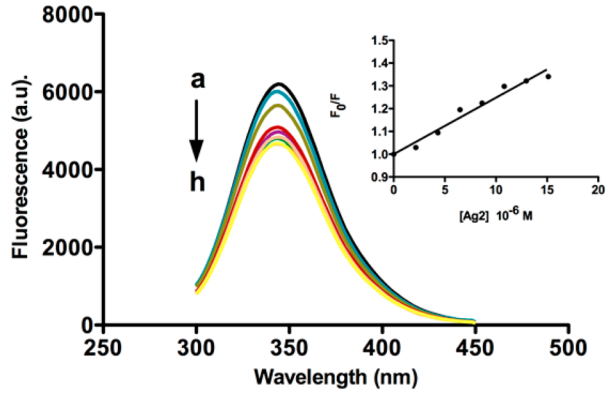

Figure 5. Fluorescence spectra of BSA at different concentrations of compound **2**; [BSA] = 3×10^{-5}: from **a** to **h** [compound **2**]; 0, 0.24, 0.48, 0.66, 0.86, 1.0, 1.3, 1.5×10^{-5} M, respectively. (inset) Stern–Volmer plot of the interaction of compound **2** with BSA.

Table 2. Values of Binding Constants of Silver Complexes with Bovine Serum Albumin

compound	$K_{SV} \times 10^4$ M^{-1}	$k_Q \times 10^{13}$ L mol^{-1} s^{-1}	$K_A \times 10^4$ M^{-1}	n
1	1.34	8.31	1.70	0.87
2	1.64	10.16	1.58	0.83

controlled by a static quenching mechanism owing to a complex formation rather than a dynamic quenching mechanism.

As previously reported,[21,27−32] eq 3 has been used to evaluate the binding constants and apparent binding sites of the two complexes on BSA (Table 2), which indicate that the two complexes show the same affinity for BSA.

Antimicrobial Activity. Table 3 shows the results for the antimicrobial activity of silver compounds against reference strains of important human pathogenic species as assessed by the microdilution method. Minimal inhibitory concentrations (MICs) of compound **1** (8–128 μM) are overall lower than those obtained for compound **2** (32–256 μM) against both bacteria and the yeast species. There is a higher activity against the Gram-negative species (*E. coli* and *P. aeruginosa*, MIC range = 16–128 μM) in respect to the Gram-positive ones (*S. aureus* and *E. faecalis*, MIC range = 64–256 μM). Also the growth of the yeast *C. albicans* is inhibited at low MIC values (8–32 μM). Comparison of silver compounds and the comparator $AgNO_3$

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Table 3. MIC (μM) and MBC (μM) Values of Silver Complexes against a Panel of Reference Bacterial Strains and Fungi

compound	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*
			MIC (μM)		
1	64	128	32	16–32	8
2	128–256	256	128	128	32
$AgNO_3$	32–64	64	16	8–16	8
			MBC (μM)		
1	128–256	>256	32	32	8
2	>256	>256	128	128–256	32
$AgNO_3$	64–128	>256	16	16	8

shows that compound **1** maintains the antibacterial activity at similar levels (8–64 μM for $AgNO_3$ and 8–128 μM for compound **1**). On the contrary, compound **2** loses some of the activity (32–256 μM). Also bactericidal activity confirms the general trend described for the inhibitory activity. Both silver compounds are bactericidal against the Gram-negative species and the yeast, but they seem only bacteriostatic against the Gram-positives, namely, *S. aureus* and *E. faecalis*. Hence, compound **2** structure seems to diminish the availability of silver that cannot fully exert its natural antibacterial activity. Compound **1** is able to preserve the potency of the silver ion. Noteworthy, it shows the best activity against Gram-negative species, which are notoriously more refractory to the action of biocides due to the presence of the cell wall outer membrane. The hydrophobic nature of the ligands used to generate the tested complexes, albeit with a different efficiency, is most probably favoring the interaction and the antimicrobial activity of the silver ion.

Enzymatic Activity. To evaluate the interaction of the silver complexes with thiol-rich proteins we measured their inhibition activity toward adenosine deaminase (ADA), an enzyme involved in purine metabolism, whose inhibitors are used as anticancer and antiviral drugs. As shown, adenosine deaminase is competitively inhibited by $AgNO_3$, such susceptibility to silver toxicity being due to the presence of a conserved cysteine residue (Cys262) on the enzyme, located in the entrance of the enzyme active site.[33] This residue can play an important role to maintain the active conformation of the enzyme,[34] and the binding of the SH reactive groups to metal ions of complexes can decrease the enzyme catalytic activity.[35−37]

We also evaluated the ability of complexes **1** and **2** and $AgNO_3$ in inhibiting calf intestine ADA, and results are reported in Table 4. It can be observed that, in the same experimental conditions (concentration of complexes and $AgNO_3$ in the assay are 6.5×10^{-8} M), a strong inhibiting effect is exerted by $AgNO_3$, while a minor effect was found for complex **1**; a very low inhibition on ADA activity was shown by complex **2**. Data from Table 4 seem to suggest also that dissolution of complexes **1** and **2** in water or buffer at neutral pH does not produce complete dissociation with silver ion loss; rather, a large amount of silver complexes remain unaltered in such conditions. These results are in accordance with the antibacterial activity of complexes **1** and **2**, and can be important in view of a potential biological use of our silver complexes. In fact, they do not inhibit the activity of enzymes important for the life of cells, an aspect which should be taken into account when biological properties of new substances are evaluated.

Table 4. Enzymatic Activity of Silver Complexes toward ADA Enzymatic Activity

compound[a]	inhibition (%)
blank	0
$AgNO_3$	86.7(±2.8)
1	64.1(±1.8)
2	17.1(±1.2)
Tpms	0
mPTA	0

[a]The concentration of $AgNO_3$ and Ag complexes (**1** and **2**) in the assay was 6.5×10^{-8} M, while concentration of ligands was of 0.1 mM.

CONCLUSIONS

Two new water-soluble silver complexes containing a scorpionate (Tpms = tris(pyrazol-1-yl)methanesulfonate anion) and a phosphine (mPTA = *N*-methyl-1,3,5-triaza-7-phospha-adamantane cation) have been synthesized and characterized. Of them, $[Ag(mPTA)_4](Tpms)_4(BF_4)$ is the first example of a coordination compound bearing a κ^0-Tpms species and a cationic $[Ag(mPTA)_4]^{5+}$ monomeric block. Both silver complexes exhibit significant antibacterial and antifungal activities. The activity of the complex $[Ag(mPTA)_4]^{5+}$ (**1**) is comparable to that of $AgNO_3$, while a lower activity has been observed for complex $[Ag(Tpms)(mPTA)]^+$ (**2**). Both complexes are able to interact with calf thymus DNA, and results obtained through displacement assays seem to indicate that strong electrostatic effects, more than the intercalation mode, could be responsible for the interactions observed. Moreover, they also display binding ability to the BSA, with decrease of its tryptophan fluorescence.

ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic file in CIF format, fluorescence spectra of DAPI–DNA complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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