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# A simple 2,6-diphenylpyridine-based fluorescence "turn-on" chemosensor for Ag<sup>+</sup> with a high luminescence quantum yield

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Intraligand Charge Transfer (ILCT)

A simple 2,6-diphenylpyridine (C^N^C)-based cyclometalated receptor as

"turn-on" fluorescent probe was designed and synthesized for selectively sensing

 $Ag^+$  with an unprecedented quantum yield in living cells.

# A simple 2,6-diphenylpyridine-based fluorescence "turn-on" chemosensor for Ag<sup>+</sup>

### with a high luminescence quantum yield

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### Abstract

A sensitive and simple 2,6-diphenylpyridine-based receptor servered as a "turn-on" fluorescent chemosensor was designed and synthesised for selectively sensing  $Ag^+$  ions in aqueous media with an anomalously quantum yield. The probe exhibits high selectivity toward  $Ag^+$  over other common metal ions, displaying a significant fluorescent turn on nature in the presence of  $Ag^+$ . The fluorescence response is explained by X-ray diffraction analysis, mass spectrometry, and theoretical calculations. The unprecedentedly high luminescence quantum yield can be attributed to intraligand charge transfer (ILCT) transitions with mixed some Ag(I) 5p orbitals perturbation characters. Finally, the results of cell experiments show that the probe can be used to selectively detect  $Ag^+$  in mammalian cells.

**Keywords:** Turn on; Fluorescent chemosensor; bioimaging; Silver ion; High luminescence quantum yield

#### **1. Introduction**

Fluorescence detection techniques have become powerful tools for sensing and imaging of trace amounts of heavy and transition-metal (HTM) ions due to their simplicity, high selectivity and sensitivity, and instantaneous response [1-14]. More importantly, most fluorescent sensors are ready for *in vivo* and *in vitro* cellular imaging to make the fluorescence approach superior to other analytical methods, such as atomic absorption and ICP atomic emission spectroscopy [15-19]. Design of chemosensors for  $Ag^+$  is a field of intense research activity, because they are potentially attractive for use in such areas as bioaccumulation, toxicity, enzymes, drug delivery, and antimicrobial activities, and so on [20-25]. Recently, synthetic strategies for constructing functional  $Ag^+$  receptors with various structures and novel binding properties have been well established [26-34, 20]. However, only a handful of fluorescence-on sensors for  $Ag^+$  with high

selectivity and sensitivity have been reported because of the characters of  $Ag^+$  with  $d^{10}$  electronic configuration which is known as fluorescence quencher *via* the electron transfer and facilitated intersystem crossing processes [35-41]. The poor binding selectivity for  $Ag^+$  over other HTM ions, such as  $Hg^{2+}$ ,  $Cu^{2+}$ , and  $Fe^{3+}$ , decrease the accuracy of  $Ag^+$  detection in the analysis media [42-45]. Additionally, all these reported sensors often display poor luminescence quantum yields (LQY) due to most Ag(I) complexes exhibit clearly the nature of non-emissive in the solution state or weak emission only at low temperature and/or in the solid state [46-52]. Ahn and co-workers have reported the highest LQY ( $\Phi_f = 0.76$ ) of reaction-based  $Ag^+$  sensor relied on the binding-promoted ring-opening process by the coordination of  $Ag^+$  to argentophillic element [13].

Therefore, development of chemosensors for  $Ag^+$  with high selectivity and sensitivity, clear confirmed luminescence mechanism, and excellent LQY have remained rare up to now. Recently, our group reported an unprecedented high LQY nature of two-coordinated silver(I) complex [Ag(4-(4-N,N-dimethylaminophenyl)-C^N^C)\_2]X (X = ClO<sub>4</sub><sup>-</sup>, C^N^C = 2,6-diphenylpyridine) due to the introducing of strong electron-donating substituent of N, N-dimethylamine (DMA) group ( $\Phi_f$  = 0.83) [53]. The successful applications of the DMA group encouraged us finding a new strongly electron-donating substituent to extend our research in the field of design novel fluorescence probe for Ag<sup>+</sup>. As a continuous investigations on the chemosensors [54-59], herein, we report the syntheses and metal binding properties of new simple C^N^C-type receptor (CNC-OMe) for fluorescent discriminating of Ag<sup>+</sup> over a wide range of tested metal ions with high LQY (Scheme 1). Indeed, the chromophore of *p*-methoxy (*p*-OMe) group was selected as the strongly electron-donating property of the substituent, while pyridine was selected as the unique two-coordinated Ag<sup>+</sup> receptors. Single-crystal X-ray diffraction analyses proved that the two necessary units were introduced as trigger sites to achieve efficient metal interactions and a consequently good fluorescence signal response. This "turn-on" nature of the **CNC-OMe** + Ag<sup>+</sup> can be assigned to intraligand charge transfer (ILCT)

transitions with mixed some Ag(I) 5p orbitals perturbation characters. Interestingly, the bright blue ( $\lambda \sim 300-500 \text{ nm}$ ) with high LQY ( $\Phi_f = 0.79$ ) and visible-light excitation of  $[Ag(CNC-OMe)_2]^+$  provide an opportunity to luminescent image Ag<sup>+</sup> in living cells. To the best of our knowledge, CNC-OMe was one of the brightest Ag<sup>+</sup>-binding chemosensors with high selectivity and sensitivity to date.

#### Scheme 1

#### 2. Experimental

#### 2.1 Materials and apparatuses

All materials were used as received. The solvents used for synthesis were of analytical grade. *Caution! Perchlorate salts are potentially explosive and should be handled with care and in small amounts.* 

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded using a Varian inova-400 spectrometer with chemical shifts reported as ppm (in DMSO- $d^6$ , TMS as internal standard). ESI-TOF mass spectra were obtained using a LC/Q-TOF MS spectrometer. Steady-state luminescence emission spectra were recorded using a SPEX Fluorolog-2 Model F111 fluorescence spectrophotometer. For all fluorescent measurements, both excitation and emission slit widths were 1 nm. Optical absorption spectra were measured using a Hitachi U-0080D spectrometer at room temperature. Luminescent QYs were referenced to quinine sulfate in 0.1 N H<sub>2</sub>SO<sub>4</sub> ( $\Phi_f = 0.546$ ) and extrapolated to infinite dilution (estimated error  $\leq 1\%$ ). All the spectroscopic measurements were performed at least in triplicate and averaged.

#### 2.2 General procedures for spectroscopy

ACCEPTED MANUSCRIPT Stock solutions  $(2 \times 10^{-2} \text{ M})$  of the perchlorate salts of Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup>, Mn<sup>2+</sup>, Al<sup>3+</sup>, Pb<sup>2+</sup>, and Ag<sup>+</sup> in acetonitrile were prepared. Stock solution of CNC-OMe (1 mM) was also prepared in acetonitrile solution. Test solutions were prepared by placing 40 µL of the probe stock solution into a quartz cell of 1 cm optical path length including 2 mL CH<sub>3</sub>CN:H<sub>2</sub>O (1:1, v/v, containing 0.01 M HEPES, pH = 7.21) solution, and then adding an appropriate aliquot of each metal stock solution with a micro-syringe. All the spectroscopic measurements were performed at least in triplicate and averaged.

<sup>1</sup>H NMR titration was measured using a Varian inova-400 spectrometer at 298 K. A solution (1 mM) of receptor CNC-OMe in DMSO- $d_6$  was titrated with a sufficient quantity of AgClO<sub>4</sub> using a micro-syringe. After shaking it for ten minutes, <sup>1</sup>H NMR spectrum was taken at room temperature. The chemical shift changes of the proton of CNC-OMe were monitored.

For Ag<sup>+</sup>-bound CNC-OMe, ESI-TOF spectrum was measured using a LC/Q-TOF MS spectrometry. The reaction mixture of AgClO<sub>4</sub> and CNC-OMe in a 1 : 2.05 molar ratio in 30 mL mixed solvents of acetonitrile and methanol (9:1, v/v) was stirred at room temperature for 2 h, then filtered and concentrated to 5 mL. Addition of diethyl ether gave the product as a colorless solid. The solid was filtered and washed with diethyl ether. Then, the product was redissolved in dichloromethane for the ESI-TOF spectrum.

#### 2.3 Computational details

All theoretical calculations were performed in Virtual Laboratory for Computational Chemistry, CNIC, CAS. All calculations were carried out with the Gaussian09 programs. The B3LYP calculations were carried out by the 6-31G\*\* basis set for the main group elements and the effective core potentials (ECP) such as Lanl2DZ for the Ag atom. We employed the density functional theory (DFT) with no symmetry constraints to investigate the optimized ground state (S<sub>0</sub>) geometries of the ligand **CNC-OMe** and [2**CNC-OMe** + Ag]<sup>+</sup> (GENECP). The first

excited-state ( $S_1$ ) geometrical optimization was performed using the Time dependent DFT (TDDFT, nstates = 30) method. Fluorescence emission energies were computed from TDDFT calculations (nstates = 30) based on the optimized geometry of the lowest excited state in acetonitrile. The contour plots of MOs were obtained with the Gaussian 09 view program.

#### 2.4 Cell incubation and imaging

HeLa cells were cultured in 1640 supplemented with 10% FCS (Invitrogen). Cells were seeded in 24-well flat-bottomed plates for Nikon eclipase TE2000-5 inverted fluorescence microscopy. After 12 h, HeLa cells were incubated with 5  $\mu$ M compound **CNC-OMe** (in the culture medium containing 0.5% DMSO) for 15 min at 37 °C under 5% CO<sub>2</sub> and then washed with phosphate-buffered saline (PBS) three times before incubating with 10 eq Ag<sup>+</sup> for another 30 min, and cells were rinsed with PBS three times again. The fluorescence imaging of intracellular Ag<sup>+</sup> in HeLa cells was observed under Nikon eclipase TE2000-5 inverted fluorescence microscopy with a 20 × objective lens (excited with blue light). For all images, the microscope settings, such as brightness, contrast, and exposure time were held constant to compare the relative intensity of intracellular Ag<sup>+</sup> fluorescence.

#### 2.5 X-ray structure determinations

Crystals mounted in a glass capillary were used for data collection at 28 °C on a MAR diffractometer with a 300 mm image plate detector using graphite monochromatized Mo-K<sub> $\alpha$ </sub> radiation ( $\lambda = 0.71073$ Å). Data collection was made with 1.5° oscillation step of  $\varphi$ , 5 or 10 minutes exposure time and scanner distance at 120 mm. 130 images were collected. The images were interpreted and intensity was integrated with the program DENZO. All structures were solved by direct methods employing SHELXS-97 program on a PC. Ag and many non-H atoms were located according to the direct methods. The positions of the other non-hydrogen atoms were found after successful refinement by

full-matrix least-squares using the SHELXL-97 program suite. In the final stage of least-squares refinement, disordered C atoms were refined isotropically, other non-H atoms were refined anisotropically. The positions of H atoms were calculated based on riding mode with thermal parameters equal to 1.2 times that of the associated C atoms, and participated in the calculation of final R-indices. CCDC 1444590 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

#### 2.5 Synthesis of CNC-OMe

A mixture of acetamide (45.5 g, 0.77 mol), ammonium acetate (29.3 g, 0.38 mol), acetophenone (6.0 mL, 51.5 mmol), and 4-4-methoxybenzaldehyde (25.7 mmol) was heated to reflux for 2 h and then cooled to 120 °C, and NaOH (22.5 g in 50 cm<sup>3</sup> of water) was added. After a further 2 h, the solution was cooled to room temperature. The suspension was diluted with water and extracted with dichloromethane. After evaporation of the solvent, the residue was washed by petroleum ether to give products with yield of 36%. Anal calc. for  $C_{24}H_{19}NO$ : C 85.43, H 5.68, N 4.15%. Found: C 85.37, H 5.72, N 4.14%; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.65 (s, 2H), 7.95 (d, *J* = 6.4, 4H), 7.68 (m, 6H), 7.16 (d, *J* = 6.4, 2H), 7.00 (d, *J* = 6.4, 2H), 3.87 (s, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  161.8, 159.5, 152.5, 141.2, 130.7, 129.9, 128.8, 128.1, 126.7, 119.9, 115.0, 55.6 ppm; MS: m/z 338.4 (M+H)<sup>+</sup>.

# 3. Results and discussion

#### 3.1 Design and synthesis

**CNC-OMe** was first reported by Krygowski [60]. In order to further examine and tune the fluorescence properties of 4'-phenyl terpyridines, Araki and co-workers had introduced several

substituents having different electron donating or withdrawing properties into the *p*-position of the 4'-phenyl unit [61]. Interestingly, *p*-OMe group has the similar  $\Delta\mu$  (the difference between ground and excited state dipole moment) as that of *p*-amino groups. Here, we adopt the one-step method reported by Collin et al [62]. and Cao et al [63]. **CNC-OMe** was synthesized by 1,4-Michael addition reaction of the unsaturated ketone intermediate produced by condensation of the 4-methoxybenzaldehyde and acetophenone in good yield (Scheme 1). **CNC-OMe** was characterized by EA, NMR and MS.

#### 3.2 Fluorescent detections of $Ag^+$

**CNC-OMe** exhibited two characteristic absorption band cenerted at 258 nm and 315 nm in CH<sub>3</sub>CN:H<sub>2</sub>O (1:1, v/v, containing 0.01 M HEPES, pH=7.21) solution (Fig. S1). **CNC-OMe** displayed a weak fluorescence band with a corresponding emission maximum at 349 nm ( $\Phi_f = 0.11$ ) in CH<sub>3</sub>CN:H<sub>2</sub>O (1:1, v/v, containing 0.01 M HEPES, pH=7.21) solution when excited at 315 nm. The addition of AgClO<sub>4</sub> resulted in a significant fluorescence enhancement until a plateau was reached ( $\Phi_f = 0.79$ ). Upon adding Ag<sup>+</sup>, the fluorescence intensity of **CNC-OMe** increased by ca. 12-fold (Fig. 1a). The association constant for Ag<sup>+</sup> binding to **CNC-OMe** was calculated as 3.17 ± 0.20 × 10<sup>6</sup> M<sup>-1</sup> [64]. Moreover, **CNC-OMe** featured a detection limit for Ag<sup>+</sup> of at least down to  $6.20 \times 10^{-6}$  M.

Under the same conditions, no drastic fluorescence enhancement of **CNC-OMe** (20  $\mu$ M) was observed in the presence of other tested metal salts of ClO<sub>4</sub><sup>-</sup> (Hg<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>3+</sup>, Fe<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Cr<sup>2+</sup>, Al<sup>3+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup>) (Fig. 1b). Furthermore, the competition experiments revealed that **CNC-OMe** retained the excellent Ag<sup>+</sup> specificity in the presence of a variety of other metal found in environmental and biological settings (Fig. S12), this means that the luminescence

enhancement induced by  $Ag^+$  was little affected by these metal ions. These results suggest that **CNC-OMe** could respond to  $Ag^+$  with high selectivity by a fluorescence output manner.

#### Fig. 1

#### 3.3 Nature of interaction

<sup>1</sup>H NMR titration displayed the chemical shift changes of **CNC-OMe** upon the addition of  $Ag^+$ , as shown in Fig. 2. Compared to the <sup>1</sup>H NMR spectrum of the free ligand **CNC-OMe** (Fig. 2a), the  $Ag^+$  binding caused significant shifts of almost all of the proton signals, especially for the protons on the pendant phenyl groups (Fig. 2b). And so forth, strongly suggested the coordination of the ligand to the  $Ag^+$  center [65]. The binding model was further supported by the ESI-MS spectra. In the case of a CH<sub>3</sub>CN solution of **CNC-OMe** in the presence of a sufficient amount of AgClO<sub>4</sub> (Fig. S2), an exact comparison of the most interesting experimental peak (which is observed at m/z 783.1) with the simulation results obtained on the basis of natural isotopic abundances reveals that the univalently charged species can be reasonably assigned to [(**CNC-OMe**)<sub>2</sub>+Ag]<sup>+</sup>, thus providing a direct evidence of a 2 : 1 stoichiometric host-guest complexation.

#### Fig. 2

Fortunately, the binding model was further supported by the single-crystal X-ray diffraction analysis. Crystals were grown by layering an CH<sub>3</sub>CN solution of 1 mM AgClO<sub>4</sub> on top of an CH<sub>3</sub>CN solution of 2 mM CNC-OMe (crystallographic data are summarized in Table S1). As shown in Fig. 3, the crystal structure reveals the Ag(I) center is coordinated to two pyridine nitrogens from two distinct CNC-OMe ligands in a slightly distorted linear geometry with the bond angles of N-Ag-N being 172.11(12)°. The steric hindrance of the pendant phenyl groups prevent the two pyridyl rings from being coplanar, and the dihedral angle between the two

pyridylplanes is approximately 76°. Meanwhile, The steric hindrance of the side phenyl groups effectively encapsulate the Ag(I) cation.

#### Fig. 3

The spatial arrangement of the side aromatic groups around the silver is clearly demonstrated by viewing the molecule along the N-Ag-N axis as shown in Fig. S3, which clearly shows how the Ag(I) cation is protected in the middle of the complexes. So, the steric hindrance of the pendant phenyl groups prevent the formation of the planar three-coordinate complexes or tetrahedral four-coordinate complexes. This phenomenon is the key role of the high selectivity due to Ag<sup>+</sup> is the only two-coordinate metal in environmental and biological settings. No intermolecular  $\pi$ - $\pi$  stacking are observed in the stacking diagrams of [Ag(CNC-OMe)<sub>2</sub>]<sup>+</sup> and the mono-nuclear units are only stacked by C-H<sup>...</sup>O interactions (Fig. S4). The feature of encapsulated and non-conjugated structure is helpful for the emitters to maintain high fluorescent efficiency due to the reducing nonradiative transition probability [66, 67]. To the best of our knowledge, [Ag(CNC-OMe)<sub>2</sub>]<sup>+</sup> was the third crystal structure for clearly confirming the luminescence mechanism of the Ag<sup>+</sup> recepter [24, 37].

## 3.4 Theoretical calculations for sensing mechanisms of $Ag^+$

In order to further rationalizing the anomalously strong fluorescence turn-on switch effect, as well as the ILCT effect of the probe, we optimized the  $S_1$  state structures of **CNC-OMe** and  $[Ag(CNC-OMe)_2]^+$  in acetonitrile solvent (Fig. S5). The result showed that in the  $S_1$  state  $[Ag(CNC-OMe)_2]^+$  exhibited a similar structure to that in the crystal state. The time-dependent density functional theory (TDDFT) calculations on the UV-vis absorption (vertical excitation) and the emission are summarized in Table S3 and S4. The calculated absorbance and fluorescence emission of **CNC-OMe** and  $[Ag(CNC-OMe)_2]^+$  are in good agreement with the experimental

results (Fig. 1 and S1).  $[Ag(CNC-OMe)_2]^+$  complex showed that the first  $(S_1 \rightarrow S_0, \text{HOMO} \rightarrow \text{LUMO}, \lambda_{em} = 378.68 \text{ nm}, \text{Osc.} = 0.8094)$  and second  $(S_2 \rightarrow S_0, \text{HOMO} - 2 \rightarrow \text{LUMO}, \text{HOMO} - 1 \rightarrow \text{LUMO} + 1, \lambda_{em} = 345.60 \text{ nm}, \text{Osc.} = 0.3616)$  emissive states were relevant to the fluorescence emission maxima with predominant ILCT character (Table. S4). As shown in Fig. 4, the distributions of the FMOs for  $[Ag(CNC-OMe)_2]^+$ , the HOMO are mainly located on the  $\pi$  orbitals of one independent CNC-OMe ligand, whereas the LUMO are mainly localized on the  $\pi^*$  orbitals of the two pyridine rings from distinct ligands and the unnoticeable  $p_{\pi}$  orbitals of metal centers. The computed electronic transitions in  $S_1$  state reveal that the emission process is mainly due to  $\pi^* \rightarrow \pi$  transition from LUMO to HOMO. By comparison, the shapes of the HOMO and LUMO involved in  $S_1$  state are similar with that of that of  $S_0$  state (Fig. S9). The distribution of the FMOs suggests the mainly ILCT nature of the emission process in Ag(I) binding with CNC-OME. The result of theoretical calculations is high similar to that of our previous work [53]. So, this "turn-on" nature of the CNC-OME+Ag<sup>+</sup> can be exactly assigned to ILCT transitions with mixed some Ag(I) 5p orbitals perturbation characters.

#### Fig. 4

Based on these observations, it should be noted that the strongly electron-donating substituent of p-OMe portions play an important role in the high LQY of **CNC-OMe**+Ag<sup>+</sup>. The strong fluorescence was due to a reduced electron-charge density at the pyridine site after the binding with Ag(I), which reduced the "push-pull" nature of the ILCT excited state of **CNC-OMe** (caused by the electron-donating p-OMe and the electron-withdrawing pyridine) [68]. Ultimately, the steric hindrance of the pendant phenyl groups help to improve the selectivity of the recognition process.

#### 3.5 Fluorescent detection of $Ag^+$ in living organisms

As we know, only a handful of cellular sensors for  $Ag^+$  have been reported because of the mechanism of fluorescence quenching by water [69, 15-19]. However, preliminary inverted fluorescence microscopy fluorescent images of **CNC-OMe**-loaded HeLa cells in the absence and presence of  $Ag^+$  was examined (Fig. 5). HeLa cells incubated with **CNC-OMe** for 30 min at room temperature showed a weak blue intracellular fluorescence, which suggested that **CNC-OMe** was cell permeable. The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments. A weak but remarkable fluorescence enhancement was observed when cells stained were further incubated with  $AgClO_4$  for 30 min by contrast of the fluorescence titration, indicating the possible usage in fluorescence images involving  $Ag^+$  within living cells.

#### Fig. 5

#### 4. Conclusions

In conclusion, a simple 2,6-diphenylpyridine-based cyclometalated ligand showing "turn on" fluorescent sensing response for  $Ag^+$  ion found in aqueous media. Further binding model studies by <sup>1</sup>H NMR spectroscopy, mass spectroscopy, crystal structure, and TDDFT calculations demonstrated that the receptor **CNC-OMe** formed a 2 : 1 complexation with  $Ag^+$ . The "turn-on" nature of the **CNC-OMe**+ $Ag^+$  with high LQY can be assigned to ILCT transitions with mixed some Ag(I) 5p orbitals perturbation characters. Due to its excellent sensitivity, high selectivity, and favorable spectroscopic properties, **CNC-OMe** could act as efficient sensing probe for the detection of  $Ag^+$  in living cells, over biologically-relevant metal ions.

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#### **Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version at http://\*\*

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#### Scheme Title and Captions of Figures

Scheme 1. Synthesis of receptor CNC-OMe.

**Fig. 1.** (a) Fluorescence titrations of **CNC-OMe** (20  $\mu$ M) with AgClO<sub>4</sub> in CH<sub>3</sub>CN:H<sub>2</sub>O(1:1, v/v, containing 0.01 M HEPES, pH=7.21). [Ag<sup>+</sup>]: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 equiv.  $\lambda_{ex}$ = 315 nm. Inset: liner of log ((F-F<sub>0</sub>)/(F<sub>lim</sub>-F) vs. log [Ag<sup>+</sup>]. (F present fluorescence of **CNC-OMe** at 362 nm). (b) Fluorescence changes of **CNC-OMe** (20  $\mu$ M) upon titration with 10.0 equiv. of each of the guest metal ions in CH<sub>3</sub>CN:H<sub>2</sub>O (1:1, v/v, containing 0.01 M HEPES, pH=7.21). Condition: excitation: 315 nm, emission: 362 nm.

Fig. 2. Partial <sup>1</sup>H NMR spectra for pure CNC-OMe (a) and CNC-OMe +AgClO<sub>4</sub> (sufficit quantum)
(b) in DMSO-*d*<sub>6</sub>, respectively.

**Fig. 3.** X-ray crystal structure of  $[Ag(CNC-OMe)_2]^+$  complex. Thermal displacement ellipsoids are drawn at the 30% probability level. The anions, solvent molecules and H atoms are omitted for clarity.

**Fig. 4.** Plots of HOMOs and LUMOs for the  $S_1$  state of  $[Ag(CNC-OMe)_2]^+$  by TDDFT methods.in acetonitrile solvent.

**Fig. 5.** Fluorescence images of HeLa cells ( $\lambda_{ex} = 320 \text{ nm}$ ). (a) Cells supplemented with **CNC-OMe** for 15 min. (b) Cells supplemented with **CNC-OMe** for 15 min and then incubated with Ag<sup>+</sup> for 30 min.



Scheme 1. Synthesis of receptor CNC-OMe.

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**Fig. 1.** (a) Fluorescence titrations of **CNC-OMe** (20  $\mu$ M) with AgClO<sub>4</sub> in CH<sub>3</sub>CN:H<sub>2</sub>O(1:1, v/v, containing 0.01 M HEPES, pH=7.21). [Ag<sup>+</sup>]: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 equiv.  $\lambda_{ex}$ = 315 nm. Inset: liner of log ((F-F<sub>0</sub>)/(F<sub>lim</sub>-F) vs. log [Ag<sup>+</sup>]. (F present fluorescence of **CNC-OMe** at 362 nm). (b) Fluorescence changes of **CNC-OMe** (20  $\mu$ M) upon titration with 10.0 equiv. of each of the guest metal ions in CH<sub>3</sub>CN:H<sub>2</sub>O (1:1, v/v, containing 0.01 M HEPES, pH=7.21). Condition: excitation: 315 nm, emission: 362 nm.



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# Highlights

**1. CNC-OMe** was one of the brightest Ag<sup>+</sup>-binding chemosensors with high selectivity and sensitivity to date.

- 2. The sensing mechanism is proposed on the basis of the experimental and theoretical studies.
- **3.** CNC-OMe could act as efficient sensing probe for the detection of  $Ag^+$  in living cells.

# ACCEPTED MANUSCRIPT Supporting Information

# A simple 2,6-diphenylpyridine-based fluorescence "turn-on" chemosensor for Ag<sup>+</sup> with a high luminescence quantum yield

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- **3. Table S1** Crystal data and structure refinements for [Ag(**CNC-OMe**)<sub>2</sub>]<sup>+</sup>ClO<sub>4</sub><sup>-</sup>.
- **4. Table S2** Selected bond lengths and angles for  $[Ag(CNC-OMe)_2]^+$ .
- **5. Figure S3** The encapsulate structure of  $[Ag(CNC-OMe)_2]^+$ .
- **6. Figure S4** Crystal-packing diagram of [Ag(**CNC-OMe**)<sub>2</sub>]<sup>+</sup>ClO<sub>4</sub><sup>-</sup>.
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- **11. Figure S7** The theoretical UV-vis spectrum of  $[Ag(CNC-OMe)_2]^+$  and the theoretical fluorescence spectrum of  $[Ag(CNC-OMe)_2]^+$ .
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- **14. Figure S10** Electron density plots of the frontier molecular orbitals for  $S_0$  state of **CNC-OMe** and plots of HOMO and LUMO for the  $S_1$  state of **CNC-OMe**.
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- 17. Table S6 Atomic coordinates calculated for CNC-OMe from TDDFT calculations.
- 18. Figure S12 Competition experiments of of CNC-OMe + Ag(I) toward other metal ions.





# ACCEPTED MANUSCRIPT **2. Figure S2** ESI-TOF spectrum of **CNC-OMe** + $Ag^+$ .



# ACCEPTED MANUSCRIPT **3. Table S1** Crystal data and structure refinements for $[Ag(CNC-OMe)_2]^+ClO_4^-$ .

	$[Ag(CNC-OMe)_2]^+ClO_4^-$
Formula	$C_{49}H_{40}AgCl_3N_2O_6$
Formula weight	967.05
Space group	P 2 <sub>1</sub> /n
Crystal system	Monoclinic
$a(\text{\AA})$	10.411(2)
$b(\text{\AA})$	20.962(4)
$c(\text{\AA})$	20.356(4)
a(deg)	90
$\beta(\text{deg})$	90.54(3)
y(deg)	90
$V(Å^3)$	4442.2(15)
Z	4
<i>T</i> (K)	301(2)
$\rho_{\rm calc}(\rm g\ cm^{-3})$	1.446
$\theta_{\rm rang}(\rm deg)$	2.18-25.66
$\mu(\text{mm}^{-1})$	0.686
GOF	0.929
no. unique	8024
R <sub>int</sub>	0.0531
no. parameters	522
$R1^{a}$	0.0458
$WR2^a$	0.1204
Max, min	0.473, -0.416
Peaks (e Å <sup>-3</sup> )	

<sup>*a</sup>I>2σ(I)*. R1 =  $\Sigma ||F_o| - |F_c||\Sigma|F_o|$ . wR2 = { $\Sigma [w(F_o^2 - F_c^2)^2]/\Sigma [w(F_o^2)^2]$ }</sup> 

# ACCEPTED MANUSCRIPT **4. Table S2** Selected bond lengths and angles for $[Ag(CNC-OMe)_2]^+$ .

Bond	lengths	Bond	angles
Ag1-N1	2.173(3)	C1-N1-C5	119.0(3)
Ag1-N2	2.160(3)	N1-Ag1-N3	172.11(12)
N1-C5	1.348(4)	Ag1-N1-C1	113.7(2)
N1-C1	1.356(5)	Ag1-N1-C5	125.2(2)
N2-C25	1.360(5)	Ag1-N2-C25	120.4(2)
N2-C29	1.343(5)	Ag1-N2-C29	19.5(2)
		N1-C1-C2	121.0(4)
		C2-C1-C6	121.7(3)
		N1-C1-C6	117.4(3)
		C1-C2-C3	121.3(3)
		C2-C3-C18	120.5(3)
		C2-C3-C4	116.4(3)
		C4-C3-C18	123.1(3)
		C3-C4-C5	120.8(3)
		N1-C5-C4	121.3(3)
		N1-C5-C12	117.1(3)
		C4-C5-C12	121.7(3)
		C1-C6-C11	121.9(4)
	Ć		

**5. Figure S3** The encapsulate structure of  $[Ag(CNC-OMe)_2]^+$  showing the spatial orientation of the side aromatic groups around the central Ag(I) cation and view along the N-Ag-N axis.





**ACCEPTED MANUSCRIPT 7. Figure S5**  $S_1$  state structures of **CNC-OMe** (top) and  $[Ag(CNC-OMe)_2]^+$  (bottom) in acetonitrile solvent.

a)

b)



**8. Table S3** Selected parameters for the vertical excitation (UV-vis absorption) of **CNC-OMe** and  $[Ag(CNC-OMe)_2]^+$  obtained by the TDDFT//B3LYP/LANL2DZ, based on the DFT//B3LYP/LANL2DZ optimized ground state geometries (acetonitrile was employed as a solvent in all the calculations)

Compound	Electronic transition <sup>a</sup>	Energy (eV)	$f^{b}$	Composition	$CI^{c}$	Character
<b>CNC-OMe</b> <sup>d</sup>	$S_0 \rightarrow S_1$	4.0458 (306.45 nm)	0.1238	HOMO-1→LUMO	0.55525	ILCT
				HOMO→LUMO+1	0.41096	ILCT
	$S_0 \rightarrow S_2$	4.1705 (297.29 nm)	0.5171	HOMO→LUMO	0.69181	ILCT
	$S_0 \rightarrow S_3$	4.3244 (286.71 nm)	0.2082	HOMO-1→LUMO	0.41085	ILCT
				HOMO→LUMO+1	0.56737	ILCT
	$S_0 \rightarrow S_4$	4.5290 (273.76 nm)	0.1193	HOMO-6→LUMO	0.36777	ILCT
				HOMO-5→LUMO	0.24994	ILCT
				HOMO-3→LUMO	0.27571	ILCT
				HOMO-2→LUMO	0.27427	ILCT
				HOMO-1→LUMO+1	0.36830	ILCT
	$S_0 \rightarrow S_5$	4.5463 (272.72 nm)	0.1746	HOMO-6→LUMO	0.25038	ILCT
				HOMO-5→LUMO	0.17776	ILCT
				HOMO-2→LUMO	0.21309	ILCT
				HOMO-1→LUMO+1	0.57798	ILCT
	$S_0 \rightarrow S_6$	4.6762 (265.14 nm)	0.0352	HOMO-6→LUMO+1	0.39793	ILCT
				HOMO-5→LUMO+1	0.24752	ILCT
				HOMO-2→LUMO+1	0.27907	ILCT
				HOMO-1→LUMO+1	0.41501	ILCT
			Y			
$[Ag(CNC-OMe)_2]^+$	$S_0 \rightarrow S_1$	3.3456 (370.58 nm)	0.7201	HOMO→LUMO	0.70011	ILCT
	$S_0 \rightarrow S_2$	3.6124 (343.22 nm)	0.1766	HOMO-2→LUMO	0.15124	ILCT
				HOMO-1→LUMO	0.66086	ILCT
				HOMO-1→LUMO+1	0.17837	ILCT
	$S_0 \rightarrow S_3$	3.6630 (338.48 nm)	0.1607	HOMO-2→LUMO	0.63286	ILCT
				HOMO-1→LUMO	0.15043	ILCT
				HOMO→LUMO+2	0.24139	ILCT
	$S_0 \rightarrow S_4$	3.7421 (331.32 nm)	0.0187	HOMO→LUMO+1	0.70106	ILCT
	$S_0 \rightarrow S_5$	3.7881 (327.30 nm)	0.4548	HOMO-1→LUMO	0.19601	ILCT
				HOMO-1→LUMO+1	0.66958	ILCT
	$S_0 \rightarrow S_6$	3.8483 (322.18 nm)	0.0192	HOMO-4→LUMO	0.59272	MLCT
				HOMO-3→LUMO	0.30809	ILCT
				HOMO→LUMO+2	0.13262	ILCT
	$S_0 \rightarrow S_7$	3.9400 (314.68 nm)	0.0530	HOMO-4→LUMO	0.24147	MLCT
				HOMO-3→LUMO	0.18534	ILCT
				HOMO-3→LUMO+1	0.12738	ILCT
				HOMO-2→LUMO	0.23109	ILCT
				HOMO→LUMO+2	0.56517	ILCT
	$S_0 \rightarrow S_8$	3.9418 (314.54 nm)	0.0717	HOMO-4→LUMO	0.22600	MLCT
				HOMO-3→LUMO	0.50283	ILCT
				HOMO-3→LUMO+1	0.24914	ILCT

	ACCEPTED M	ANUSC	CRIPT		
			HOMO-2→LUMO	0.10381	ILCT
			HOMO-1→LUMO+3	0.15284	ILCT
			HOMO→LUMO+2	0.29740	ILCT
$S_0 \rightarrow S_9$	4.0824 (303.70 nm)	0.0648	HOMO-3→LUMO	0.32784	ILCT
			HOMO-3→LUMO+1	0.46710	ILCT
			HOMO-2→LUMO+1	0.11461	ILCT
			HOMO-1→LUMO+3	0.37536	ILCT
$S_0 \rightarrow S_{10}$	4.1517 (298.64 nm)	0.0294	HOMO-4→LUMO+1	0.29941	MLCT
			HOMO-2→LUMO+1	0.61299	ILCT
			HOMO-1→LUMO+3	0.11782	ILCT
$S_0 \rightarrow S_{12}$	4.2561 (291.31 nm)	0.0995	HOMO-4→LUMO+1	0.15693	MLCT
			HOMO-3→LUMO+1	0.40145	ILCT
			HOMO-1→LUMO+2	0.10526	ILCT
			HOMO-1→LUMO+3	0.51394	ILCT
			HOMO→LUMO+3	0.14637	ILCT
$S_0 \rightarrow S_{16}$	4.3728 (283.53 nm)	0.0169	HOMO-10→LUMO	0.14314	ILCT
			HOMO-8→LUMO	0.23276	ILCT
			HOMO-6→LUMO	0.55101	ILCT
			HOMO-5→LUMO	0.27487	ILCT

<sup>*a*</sup> Only the main configuration are presented (absorbance bands > 250 nm and f > 0.01). <sup>*b*</sup> Oscillator strengths. <sup>*c*</sup> The CI coefficients are in absolute values. <sup>*d*</sup> L1 obtained by the TDDFT//B3LYP/6-31G\*\*(d, p), based on the DFT//B3LYP/6-31G\*\*(d, p) optimized ground state geometries.

**9. Table S4** Selected parameters for the fluorescence emission of **CNC-OMe** and  $[Ag(CNC-OMe)_2]^+$  obtained by the TDDFT//B3LYP/LANL2DZ, based on the DFT//B3LYP/LANL2DZ optimized ground state geometries (acetonitrile was employed as a solvent in all the calculations)

Compound	Electronic transition <sup>a</sup>	Energy (eV)	$f^{b}$	Composition	$\mathrm{CI}^c$
<b>CNC-OMe</b> <sup>d</sup>	$S_1 \rightarrow S_0$	3.5399 (350.25 nm)	0.3195	HOMO-1→LUMO+1	0.21788
				HOMO→LUMO	0.66080
	$S_2 \rightarrow S_0$	3.7645 (329.35 nm)	1.0069	HOMO-1→LUMO	0.67807
				HOMO→LUMO+1	0.13821
	$S_3 \rightarrow S_0$	3.9951 (310.34 nm)	0.2149	HOMO-1→LUMO+1	0.64909
				HOMO→LUMO	0.22163
				HOMO→LUMO+1	0.13478
	$S_4 \rightarrow S_0$	4.0730 (304.41 nm)	0.2585	HOMO-1→LUMO	0.13810
				HOMO-1→LUMO+1	0.13724
				HOMO→LUMO+1	0.67048
$[Ag(CNC-OMe)_2]^+$	$S_1 \rightarrow S_0$	3.2741 (378.68 nm)	0.8094	HOMO→LUMO	0.69877
	$S_2 \rightarrow S_0$	3.5875 (345.60 nm)	0.3616	HOMO-2→LUMO	0.15759
			$ \rightarrow $	HOMO-1→LUMO	0.61234
				HOMO-1→LUMO+1	0.30219

<sup>*a*</sup> Only the main configuration are presented (emission bands > 300 nm and f > 0.001). <sup>*b*</sup> Oscillator strengths. <sup>*c*</sup> The CI coefficients are in absolute values. <sup>*d*</sup> L1 obtained by the TDDFT//B3LYP/6-31G\*\*(d, p), based on the DFT//B3LYP/6-31G\*\*(d, p) optimized ground state geometries.

ACCEPTED MANUSCRIPT **10. Figure S6** (a) The theoretical UV-vis spectrum of **CNC-OMe**. (b) The theoretical fluorescence spectrum of **CNC-OMe**.



**ACCEPTED MANUSCRIPT 11. Figure S7** (a) The theoretical UV-vis spectrum of  $[Ag(CNC-OMe)_2]^+$ . (b) The theoretical fluorescence spectrum of  $[Ag(CNC-OMe)_2]^+$ . **(a)** 



ACCEPTED MANUSCRIPT **12. Figure S8** Plots of HOMO and LUMO for the  $S_1$  state of  $[Ag(CNC-OMe)_2]^+$ . Only the main configurations are presented.



**13. Figure S9** Electron density plots of the frontier molecular orbitals for  $S_0$  state of  $[Ag(CNC-OMe)_2]^+$  (isocontour value 0.02 au). Only the main configurations are presented.





HOMO-10 (-0.27189)

**14. Figure S10** Electron density plots of the frontier molecular orbitals for  $S_0$  state of **CNC-OMe** (isocontour value 0.02 au) and plots of HOMO and LUMO for the  $S_1$  state of **CNC-OMe**. Only the main configurations are presented.  $S_0$  state



HOMO-5 (-0.25832)

HOMO-6 (-0.26340)

# $S_1$ state



LUMO+1 (-0.05262)



LUMO (-0.06177)



HOMO-1 (-0.21800)



180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 f1 (ppm)

Atom Type	Х	Y	Z	
С	0. 13849	-1.13502	-3.26274	-
С	1.23549	-1.24202	-4.08274	
С	2.33349	-2.02502	-3.72274	
С	2.24349	-2.71202	-2.51974	
С	1.14549	-2.54502	-1.69474	
С	-1.06851	-0.38602	-3.67674	
С	-2.33751	-0.92002	-3.44674	
С	-3. 47351	-0.23802	-3.81574	
С	-3.38051	0.96098	-4.46674	
С	-2.15151	1.47898	-4.72974	
С	-0.99151	0.81098	-4.34274	
С	1.05049	-3.23702	-0.37874	
С	-0.14651	-3.65202	0.13926	
С	-0.21651	-4.31102	1.35126	
С	0.93649	-4.57902	2.05926	
С	2.14549	-4.15102	1.57426	
С	2.21549	-3.49102	0.37126	
С	3. 49049	-2.13602	-4.61774	
С	4.30649	-3.27302	-4.61474	
С	5.37549	-3.36702	-5.46774	
С	5.66149	-2.33602	-6.34274	
С	4.87549	-1.21202	-6.36074	
С	3. 79649	-1.11402	-5. 49774	
С	7.18249	-1.44702	-7.96174	
С	-3. 44949	-0.51098	1.31974	
С	-4.09149	0.10702	2.36774	
С	-3.75449	1.40102	2.77174	
С	-2.77949	2.04502	2.02174	
С	-2.16549	1.40302	0.95674	
С	-3. 80649	-1.87098	0.88074	
С	-4. 09749	-2.11898	-0.46026	
С	-4. 45149	-3.39998	-0.86226	
С	-4. 52549	-4.41898	0.04174	
С	-4. 21749	-4.17798	1.36674	
С	-3. 86549	-2.91498	1.77374	
С	-0.05807	2.84878	0.95277	
С	0.86493	3.57378	0.21877	
С	0.72993	3.66178	-1.16423	
С	-0. 32907	3.02478	-1.81123	
С	-1.25107	2.29978	-1.07623	
С	-4.39249	2.06002	3.91974	

16. Table S5 Atomic coordinates calculated for  $[Ag(CNC-OMe)_2]^+$  from TDDFT// B3LYP/LANL2DZ excited state geometry optimization.

	ACCEPTE	ED MANUSCRIPT
C -5.	68849 1.72402	2 4. 30674
C –6.2	25849 2.28402	2 5. 41574
C –5.	53449 3.17502	2 6. 18174
C -4.2	26649 3, 54102	2 5.81774
C –3 (	69649 2 99102	2 4 68374
C –7 ·	3 <i>4</i> 2 <i>4</i> 9 3 <i>4</i> 9202	7 71574
Δα -1	28316 = 0.0037	-0 71529
	76040 = 252802	-7 11874
0 5.	10949 2. $32002$	7.11014
-5. N	12049 $3.75002$	
N U.	12049 -1.74102	2 -2.04974
N -2.4	4/849 0.13/02	
	24149 -0.78502	
Н 2.5	92649 -3.29202	2 -2.26274
Н —2.4		
H -4.3	30951 -0.59202	2 -3. 62174
Н –4.	14951 1.41798	3 -4. 72974
Н —2.0	08551 2.29198	3 -5. 17374
Н —0.	16051 1.17698	3 -4. 53574
Н —0.9	93151 -3. 48502	2 -0. 33874
Н —1.0	03951 - 4.57102	2 1.68626
Н 0.8	89849 -5.04902	2 2. 86126
Н 2.9	92349 -4.31102	2 2. 06526
Н 3.0	04049 -3.21202	2 0.05426
Н 4.	12549 -3.97302	2 -4. 03174
Н 5.9	90949 -4.12802	2 -5.45674
Н 5.0	06449 -0.51702	2 -6.94674
Н 3.1	26949 -0.34702	2 -5. 51374
Н 6.4	46349 -1.20802	2 -8. 55674
Н 7.9	94349 -1.71802	2 -8. 47274
Н 7.4	41949 -0.68802	2 -7.41874
Н —4. 1	76249 -0. 34998	3 2.81674
Н —2.	53649 2.91802	2 2 23574
Н —4 (	05349 -1 43198	3 -1 08426
Н —4	64149 -3 56598	3 -1 75726
Н –4	78149 -5 26998	3 -0. 23326
н –4	24649 - 4 87198	8 1 98374
н _2 и	66449 -2 76008	8 2 66974
н 0.	03103 2.70030 03103 2.70030	8 1 87777
	57203 <i>1</i> 00178	Q 0 64077
	24602 4 14070	-1.65692
	JHUYJ 4.148/8	5 - 1.00025 5 - 9.79599
	41907 3.08378	5 - 2.13023
н –1.9	96007 1.87178	
н –6.	17349 1.10902	2 3.80174
Н —7.1	12949 2.06202	2 5.64974
Н -3. ′	78949 4.15702	2 6. 33174
Н —2.8	84149 3. 24902	2 4. 43274
Н —7.9	94349 3.84002	2 7.04674

	A	CCEPTED	MANUSCRIPT
Н	-7.51749	3.91602	8. 55674
Н	-7.47149	2.54502	7.79774
С	-1.03615	2.15127	0. 22441

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17.	Table	<b>S6</b>	Atomic	coordinates	calculated	for	<b>CNC-OMe</b>	from	TDDFT/B3LYP
exci	ited stat	te ge	eometry o	ptimization.					

Atom Type	Х	Y	Ζ	
С	1.26922	1. 18627	-0.07636	-
С	-0.13288	1.1484	-0.09572	
С	-0.79764	-0.08479	-0.07284	
С	-0.00624	-1.2407	-0.03833	
С	1.3924	-1.12929	-0.02446	
С	2.01303	2.47827	-0.09747	
С	3. 31651	2.54539	0. 42349	
С	4.02411	3.74708	0.42547	
С	3. 44787	4.90622	-0.10317	
С	2.15778	4.84987	-0.63677	
С	1.44754	3.64799	-0.63364	
С	1.82961	-3.58575	-0.44109	
С	2.66305	-4.70412	-0.38301	
С	3.953	-4.59308	0.14219	
С	4.40425	-3.3514	0.60011	
С	3. 57325	-2.23327	0.53679	
С	-2.2793	-0.1645	-0.06352	
С	-3.05356	0.75611	0.65593	
С	-4. 44945	0.68683	0.67581	7
С	-5.10279	-0.32311	-0.04189	
С	-4.34507	-1.25311	-0. 77089	
С	-2.95893	-1.17226	-0.77635	
С	-7.28279	0.43141	0.61334	
0	-6.45697	-0.48564	-0.09545	
Ν	2.00882	0.06499	-0.03163	
Н	-0.70452	2.06847	-0.12401	
Н	-0.47734	-2.21379	0.03485	
Н	3. 76251	1.64242	0.82433	
Н	5.02779	3.779	0.84008	
Н	4. 00026	5.84147	-0.10482	
Н	1.70539	5.73982	-1.06509	
Н	0.45725	3.62059	-1.07742	
Н	0. 83996	-3.68891	-0.87498	
Н	2. 30626	-5.66003	-0.75584	
Н	4. 60147	-5.46321	0.18895	
Н	5. 40657	-3.25364	1.00771	
Н	3. 92162	-1.26715	0.88342	
Н	-2.56309	1.53075	1.23801	
Н	-5.00627	1.41188	1.25718	
Н	-4.86463	-2.02316	-1.33178	
Н	-2.39248	-1.88783	-1.36477	

ACCE	PTED MANU	SCRIPT		
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	9633       1. 6918         1419       0. 42362         5683       0. 24841         3409       0. 02325			
			R	
R				

**18. Figure S12** Competitive experiments of  $Ag^+$  (10 equiv) with **CNC-OMe** (20  $\mu$ M) in CH<sub>3</sub>CN : H<sub>2</sub>O (1 : 1, v/v, containing 0.01 M HEPES, pH = 7.21) solution in the presence of various metals (10 equiv).

