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Highly selective and sensitive fluorescence detection of Zn²⁺ and Cd²⁺ ions by using an acridine sensor†

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Fluorescence spectroscopy investigations of the new acridine derivative bis(*N,N*-dimethylaminemethylene)acridine (**3**) show remarkable selectivity and sensitivity towards Zn²⁺ and Cd²⁺ ions in methanol and for the latter even in water. Through the chelation of the metal ions the present PET effect is quenched, significantly enhancing the emission intensity of the fluorophore. In solution, the bonding situation is studied by fluorescence and NMR spectroscopy, as well as ESI-TOF mass-spectrometry measurements. The solid state environment is investigated by X-ray diffraction and computational calculations. Here, we can show the complexation of the zinc and cadmium ions by the methylene bridged amine receptors as well as by the nitrogen atom of the acridine system.

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Introduction

The detection of metal ions in solution has always been an important topic in analytical chemistry, environmental protection and medicinal applications. Metal ions like magnesium, calcium, or zinc are essential components of many enzymes in the human body.¹ Especially Zn²⁺ plays a fundamental role in many different areas; *e.g.* in the emergence of Alzheimer's disease.² Moreover, its level of concentration could help in diagnosing the growth of tumour cells in the prostate.³ Zinc deficiency increases the susceptibility to a variety of pathogens and is of central importance for the immune system.⁴

The heavier homologue Cd²⁺ is known to be a very toxic metal ion. Over several decades in the first half of the 20th century, hundreds of people in Japan were affected by the deadly *itai-itai* disease due to a cadmium polluted river.⁵ Therefore, it is vital to have a fast working and very sensitive method for the detection of metal ions in *e.g.* blood or water supplies. Fluorescence spectroscopy combined with suitable

fluorescent sensors is a well-recognized method for such analysis. Sensor molecules should be able to switch instantaneously between fluorescent on/off states or change their emission wavelength significantly upon addition of an analyte. To achieve this response several different mechanisms can be employed. Besides intramolecular charge transfer (ICT),⁶ metal–ligand charge transfer (MLCT)⁷ or excimer formation,⁸ an extensively investigated concept is the photoinduced electron transfer (PET)⁹ effect. Here a poor or non-fluorescent ligand starts to emit light upon coordination of an analyte under UV light irradiation.

The great interest in molecular sensors is reflected by the multitude of publications.¹⁰ Acridine derivatives in particular are commonly applied in the detection of bioorganic compounds. For example, it is frequently used as a fluorescent dye for DNA intercalation.¹¹ Acridine based sensors for cationic analytes are less commonly used¹² since they often do not show a strong enhancement of the fluorescence emission.¹³ In the following, we present the characterization in the solid state and in solution of an interesting new acridine based sensor.

Results and discussion

Synthesis

Many of the molecular sensors reported in the literature contain elaborated side arms working as the receptor unit.^{10a,14} However, another requirement to be met by a sensor is the economic aspect of feasibility and cheap availability. Therefore, we chose a short and effective synthetic route to

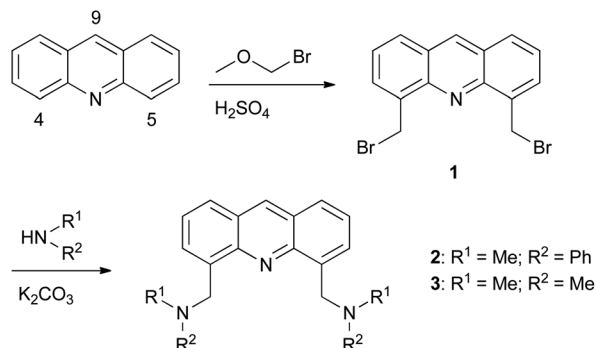
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Scheme 1 Reaction pathway to 4,5-substituted acridine derivatives.

produce derivatives of acridine systems with small amine side arms (Scheme 1). The desired sensor system with the concept “fluorophore–spacer–receptor” can be obtained in a quick two-step synthesis. The two bromomethylene units can be easily introduced at the 4- and 5-positions employing bromomethyl methyl ether (BMME) and a strong acid like conc. H_2SO_4 .¹⁵ The bromine atoms can straightforwardly be substituted by a variety of amines. In this publication we present two new acridine derivatives with distinct amine moieties: 4,5-bis(*N*-methyl-*N*-phenylaminemethylene)acridine (**2**) and 4,5-bis(*N,N*-dimethylaminemethylene)acridine (**3**). Although the two molecules are very similar, only the latter shows extraordinary fluorescence properties.

The introduction of two receptor units directly neighbored to the aromatic nitrogen atom holds the option to coordinate a target cation with the side arms as well as with the fluorophore. This is a great advantage compared to its lighter congener, the widely used fluorophore anthracene.^{13,14b}

Fluorescence studies

The fluorescence measurements were carried out in methanol in which both the ligands and the metal salts are readily soluble. Moreover, this protic and very polar solvent exhibits similar properties to water, which is important for possible future applications. The employed structural design (fluoro-

phore–spacer–receptor) results in a very efficient electron transfer which is favourable for a molecular off/on switch: the methylene units (spacer) allow the amines (receptor) to rotate easily about their N–C and C–C bonds. This provides the conformational freedom to interact electronically with the π -system of the acridine (fluorophore) and results in an effective quenching of the fluorescence.¹⁶ At the same time, the amines are able to employ their lone pair in complexation of analytes. If this is the case, the PET is hindered and the emission of light is facilitated. Fig. 1 shows the excitation and emission spectra of the ligands **2** and **3** in methanol.

The emission intensities of both ligands are very weak, whereas they show a strong absorption behaviour (ESI Fig. S4.1†) which hints at a working PET. Interestingly, the intensity of the methyl derivative **3** is considerably higher than the phenyl derivative **2**. This observation could indicate impurities and will be discussed later in full detail. In Fig. 1, left, the highest peak at 398 nm in both spectra is attributed to Raman scattering of the used solvent methanol.¹⁷

In the next step, a metal ion screening was conducted to check the sensing ability of the ligands. Here, sixteen different metal salts were dissolved in methanol and each was titrated to the 1×10^{-5} M concentrated solutions of the ligands, respectively. To afford a good comparability, the metal ion concentrations were increased stepwise in the same manner (1, 5, 15, 100 eq.). Additionally, only bromides were used to standardize the possible interference of the counter ion. With **2** as the ligand system, no significant change in the emission intensity is observed for the tested metal salts (ESI Fig. S5.1†). To check whether the structural motif nevertheless is suitable as a sensor in general, we used the less polar and donating solvent dichloromethane. In this solution, we observed a massive fluorescence enhancement by adding ZnBr_2 to the ligand (ESI Fig. S5.2†). Since this solvent is not in the focus of our interest, we concentrated on the dimethylamine system **3**. In Fig. 2, we compare the intensity maxima of its emission spectra during the metal ion screening. Among the tested sixteen metal salts, this ligand shows a very high selectivity towards Zn^{2+} and Cd^{2+} ions. The emission intensity increases enormously. The only minute emission enhancement with AlBr_3 and SnBr_2 is solely

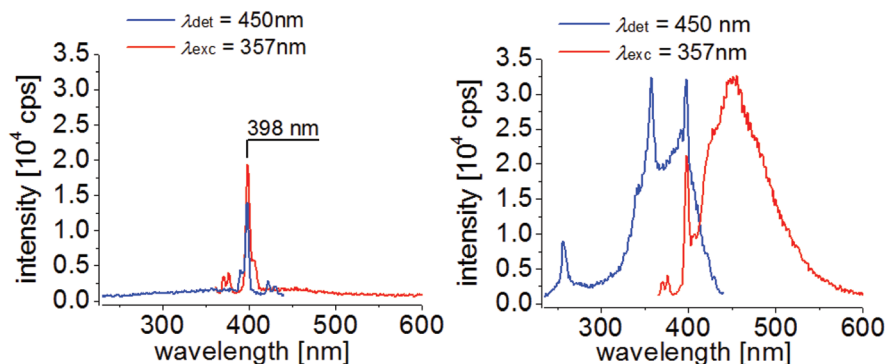


Fig. 1 Excitation (blue, $\lambda_{\text{det}} = 450$ nm) and emission spectra (red, $\lambda_{\text{exc}} = 357$ nm) of **2** (left) and **3** (right) in a 10^{-5} M methanol solution.



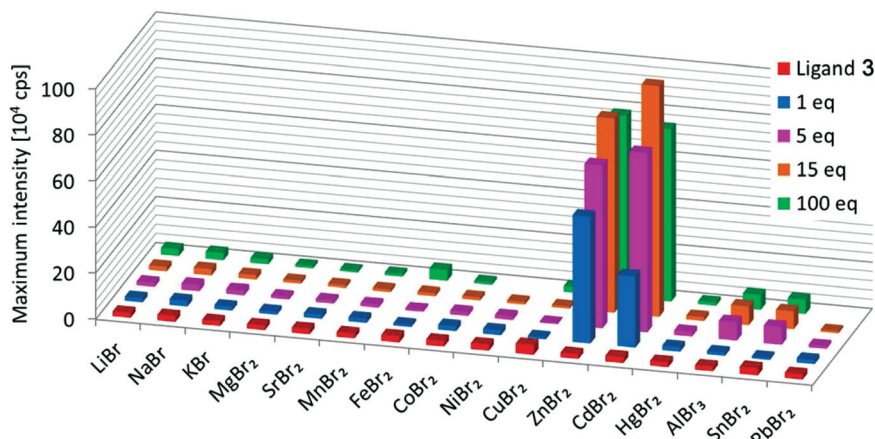


Fig. 2 Metal ion screening with ligand **3** in methanol ($c = 1 \times 10^{-5}$ M). The maximum emission intensity is depicted for each metal ion addition ($\lambda_{\text{exc}} = 357$ nm).

due to the strong Lewis acidity of these metal ions which leads to slight protonation of the ligand. This can be evidenced by the different resulting emission wavelengths. If the ligand is protonated, the wavelength of the emission maximum is blue-shifted to 425 nm (ESI Fig. S5.4†), whereas in the case of Zn^{2+} or Cd^{2+} coordination, the maximum is observed at 457 nm and 445 nm, respectively. In this context it should be mentioned that although the emission shift between the two metal ions is only 12 nm, this is sufficient to differentiate the metals by fluorescence spectroscopy. A closer look at the detailed emission spectra depicted in Fig. 3 additionally demonstrates the high sensitivity of this sensor molecule.

After adding only 0.1 eq. of ZnBr_2 to the ligand solution, a significant increase of emission intensity is observed. Besides the standard titration of the analytes, we checked the reversibility of the metal complexation. Therefore, we alternately added a specified amount of metal salt and ethylenediamine (en) to the ligand (CdBr_2 is depicted in Fig. 5, for ZnBr_2 see the ESI Fig. S5.6†). With 1 eq. of CdBr_2 in the cuvette, the emission intensity rises from 0.2 to 3.5×10^5 cps and is afterwards quenched to 0.1×10^5 cps when 10 eq. of the chelating amine are added.

This experiment can be repeated several times, confirming the great reversibility of the complexation of the two metal ions. Moreover, the starting intensity of the pure ligand (red line) is about twice as high as the intensity after adding ethylenediamine. This indicates possible traces of impurities by preparing the ligand solutions. The methanol was purchased from VWR Chemicals® (AnalaR NORMAPUR) which provides analytical data for their solvents. Each kilogram contains impurities of <0.2 mg Zn^{2+} and <0.01 mg Cd^{2+} ions.

Converted to the employed volume and concentration for the measurements, every sample could contain an amount of <0.3 eq. Zn^{2+} ions and <0.009 eq. Cd^{2+} ions with respect to the ligand. This is a significant amount one has to consider when comparing the results. But again, this underlines the sensitivity of the synthesized sensor system which enables even to detect the traces of impurities. In addition, this could explain the higher excitation and emission spectrum of **3** in comparison with **2** (Fig. 1), which is not sensitive to the mentioned metal ions.

In addition to the bromide salt, we investigated the influence of $\text{Zn}(\text{OAc})_2$ and $\text{Zn}(\text{NO}_3)_2$ on the fluorescence properties of **3**. With small additions of the nitrate salt, the emission

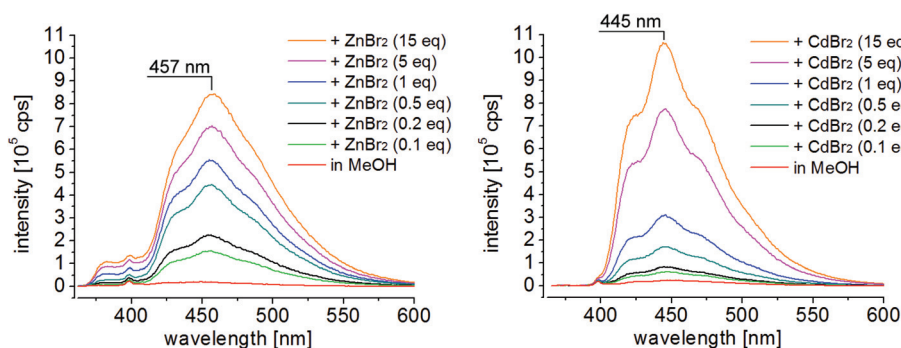


Fig. 3 Emission spectra of **3** titrated with ZnBr_2 (left) and CdBr_2 (right) in methanol; both were irradiated at 357 nm.



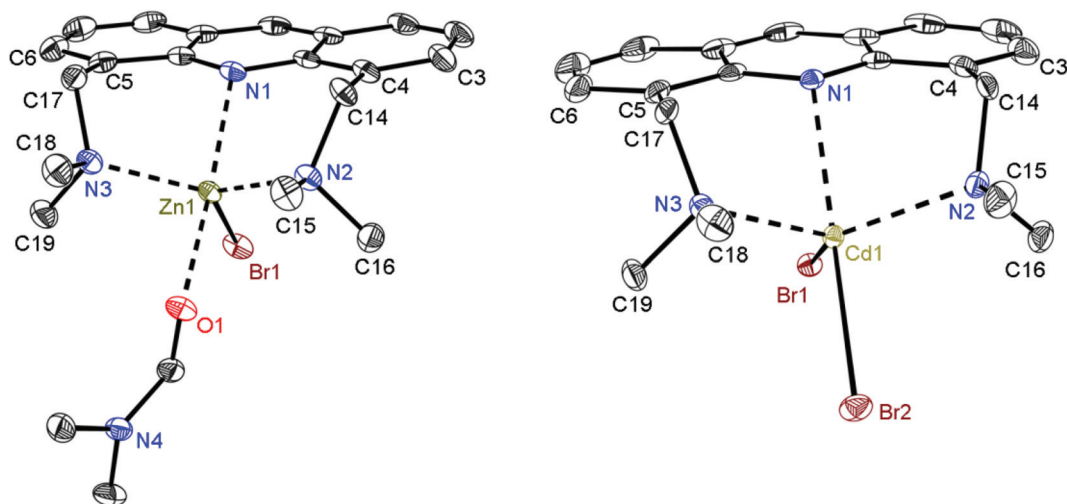


Fig. 4 Solid state structures of $[(\text{dmf})\text{ZnBr}\{(\text{Me}_2\text{NCH}_2)_2\text{Acr}\}]^+$ (**4a**, left) and $[\text{CdBr}_2\{(\text{Me}_2\text{NCH}_2)_2\text{Acr}\}]$ (**5**, right); crystallized from a THF/DMF mixture. The omitted counter anion in the asymmetric unit of the zinc structure is a solvent-separated $[(\text{dmf})\text{ZnBr}_3]^-$ unit. All hydrogen atoms are omitted for clarity. Anisotropic displacement parameters are depicted at the 50% probability level.

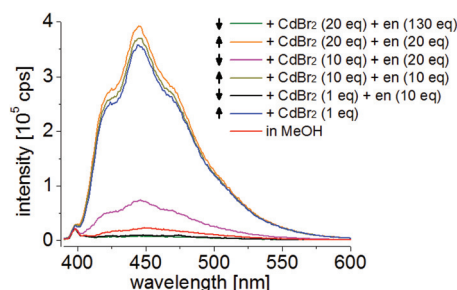


Fig. 5 Reversibility of metal complexation of **3** with alternating addition of CdBr_2 and ethylenediamine (en); all substances are dissolved in methanol ($\lambda_{\text{exc}} = 357 \text{ nm}$).

intensity rises as usual whereas an excess quickly leads to its reduction. However, using acetate as the counter anion, the fluorescence is increased strongly even resulting in an eight times higher emission maximum than with ZnBr_2 (ESI Fig. S5.5†).

For a better assessment of the measured fluorescence values, the relative emission intensities are compared with the typical fluorescent compound anthracene. In Table 2, the maximum intensities of the used compounds and their relative intensities divided by the fluorescence of anthracene are shown.

Investigations of the metal complexes

To gain a better understanding of the observed fluorescence properties, it is necessary to know how the metal ions are linked to the ligand. Therefore, we synthesized the two metal complexes and investigated them with different analytical methods. For the synthesis of **4**, the ligand and ZnBr_2 were dissolved in THF whereas MeOH was used for the preparation of **5** (Scheme 2). In both cases, one equivalent of the metal salts

was added dropwise to **3**, resulting in an immediate precipitation of the metal complexes. The crystallization of **3** with zinc acetate to give **7** was endeavoured to check whether complexes with different zinc salts behave the same which is indeed the case here. The comparison of the crystal structures containing zinc ions is provided in the ESI.† The single crystals of **4a** and **5** were obtained from crystallization in a THF/DMF mixture. By X-ray diffraction experiments, we could determine their solid state structures (Fig. 4). The zinc complex **4a** crystallizes in the monoclinic space group $P2_1/c$ and the cadmium derivative in the chiral orthorhombic space group $P2_12_12_1$. Both complexes are almost isostructural. In the zinc structure one bromide ion is substituted by a coordinating solvent molecule DMF. The positively charged complex is counterbalanced by a solvent-separated $[\text{ZnBr}_3]^-$ anion in the asymmetric unit (ESI Fig. S7.1†).

The solid state structures indicate the coordination of the Zn^{2+} and Cd^{2+} ions by all three present nitrogen atoms. This binding motif where the methylene bridged amines at the 4- and 5-positions and the aromatic nitrogen atom of the acridine are all involved in metal ion coordination is currently not present in the CSD. Only two examples with methylene bridged phosphorus atoms coordinating a ruthenium ion can be found in the database.¹⁸ Overall, a structural search concerning coordination of an acridine unit to any metal ion merely results in 56 hits.¹⁹

From the nitrogen–metal bond lengths in Table 1, it is obvious that the average distances from the amine nitrogen atoms to the metal ion (208.49(17) for Zn^{2+} and 232.01(19) pm for Cd^{2+}) are shorter than that from the acridine nitrogen atom (230.39(16) and 254.07(18) pm, respectively). The shorter distances are in perfect agreement with the literature values found in the CSD, compared to general $\text{N-Zn}^{2+}/\text{Cd}^{2+}$ bonds. The longer distances can still be found in the database quoted as bonds but less frequently. One reason for this different

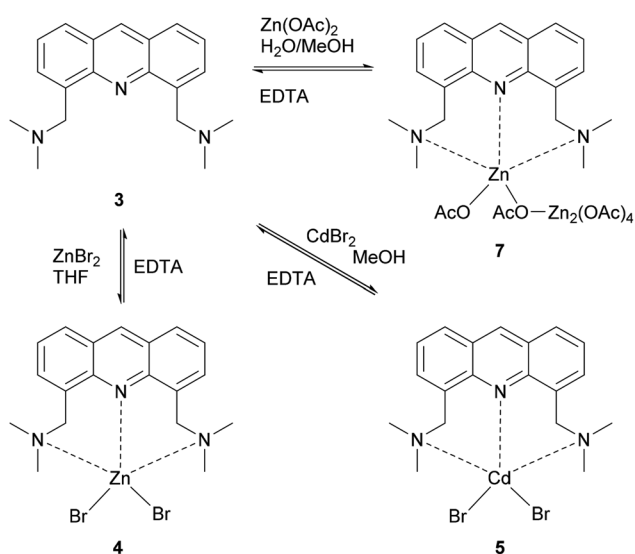


Table 1 Selected bond lengths [pm] and angles [°]. The angular sums arise from the C–N–C angles

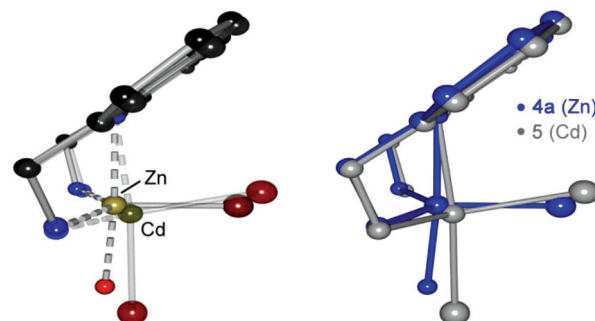
Bond [pm]	4a (Zn)	5 (Cd)	Angle [°]	4a (Zn)	5 (Cd)
N1–M	230.39(16)	254.07(18)	N1–M–N2	90.05(6)	82.91(6)
N2–M	207.89(16)	232.31(19)	N1–M–N3	89.81(6)	82.81(6)
N3–M	209.09(17)	231.70(19)	N2–M–N3	128.68(6)	120.87(7)
Br1–M	239.83(4)	259.13(4)	N1–M–O1/Br2	175.31(6)	174.08(4)
O1/Br2–M	218.82(14)	266.16(4)	Angular sum of N2	325.0(5)	327.1(6)
			Angular sum of N3	324.0(5)	327.0(6)
			C3–C4–C14–N2	–114.8(2)	–105.3(3)
			C6–C5–C17–N3	112.7(2)	105.9(3)

Table 2 The maximum emission intensities of the metal complexes of **3** and their relative emission intensities compared with anthracene. All measurements were done with 10^{-5} M solutions in methanol

Compound	λ_{exc} [nm]	Intensity [10^4 cps]	Relative intensity
3 + ZnBr ₂	357	84.15	0.12
3 + CdBr ₂	357	105.83	0.15
3 + Zn(OAc) ₂	357	678.63	0.95
Anthracene	373	711.91	1.00

**Scheme 2** Reaction pathways to the metal ion complexes **4**, **5** and **7**.

bonding situation results from the rotational ability of the amines to improve the orbital overlap with the target ions. The C–N–C angles of the sp^3 -hybridized amines are very close to the ideal tetrahedral angle. The resulting best geometry forces the metal ion underneath the acridine plane which reduces the dative bonding to the ring nitrogen atom. Moreover, the lone pair of this sp^2 -hybridized nitrogen atom is partly delocalized in the π -system of the aromatic system and has therefore a reduced donor capacity. The N–M bond encloses a relatively acute angle of 44.5° (**4a**) and 38.5° (**5**), to the plane of the central acridine ring. A closer look at the bonding situation and the angle values in Table 1 reveals the coordination poly-

**Fig. 6** Superpositions of both metal ion structures (**4a**, **5**), showing the trigonal bipyramidal geometry of the coordination motif. The methyl groups at the amines and the main part of the DMF molecule are omitted for clarity.

hedron of a trigonal bipyramid at each metal atom. This geometry is depicted in Fig. 6.

Although the longer N–M²⁺ distances can still be found in the CSD, the solid state investigations are not sufficient evidence for the existence of a bonding situation.²⁰ In order to judge on the main binding forces of the metal ion coordination, and to relate the findings to the chemistry in solution, we conducted a series of electronic structure calculations on the Zn²⁺ and Cd²⁺ compounds. All structures were optimized at the B3LYP-D3/def2-TZVPP²¹ level of theory. Included were the complexes **4a** and **5**, identified in the solid state structures, as well as the hypothetical complex **4**. The structures obtained are in good agreement with the crystal data (ESI†). A natural bond orbital (NBO) analysis²² was carried out to gain insight into the coordination of the metals to the acridine derivative **3**. The data in Table 3 give the second order perturbation theory energies²³ for the interaction between the lone pairs of the

Table 3 NBO second-order perturbation theory analysis of the metal ion (Zn/Cd) coordination to **3** at the B3LYP-D3/def2-TZVPP level of theory

$E^{(PT2)}$ [kcal mol ^{−1}]	N1	N2	N3
[(dmf)ZnBr{(Me ₂ NCH ₂) ₂ Acr}] ⁺ (4a)	17.0	25.4	25.1
[ZnBr ₂ {(Me ₂ NCH ₂) ₂ Acr}] (4)	5.6	24.7	25.4
[CdBr ₂ {(Me ₂ NCH ₂) ₂ Acr}] (5)	4.7	22.8	22.7
[(MeOH) ₂ Zn{(Me ₂ NCH ₂) ₂ Acr}] ²⁺ (in MeOH)	21.9	25.4	26.5
[(MeOH) ₂ Cd{(Me ₂ NCH ₂) ₂ Acr}] ²⁺ (in MeOH)	15.0	32.1	31.8



different nitrogen atoms and the zinc or cadmium ion. As expected, in these structures the metal has a weaker coordination to N1, compared to the other nitrogen atoms.²⁴ Substitution of one bromide anion by a DMF solvent molecule increases this value from 5.6 to 17.0 kcal mol⁻¹ (**4** in comparison with **4a**). Another possibility would be that the bulky bromide anion pulls the metal away from the acridine ring nitrogen atom.

The NPA charges for the two compounds are 1.49 (**4**) and 1.60 (**4a**), so that the charge difference is not very significant. The second possibility, hence the interaction with a second bromide to weaken the coordination to the acridine seems to be the most likely explanation for this effect. Furthermore, we conducted calculations on the hypothetical complexes formed in solution, [(MeOH)₂Zn{(Me₂NCH₂)₂Acr}]²⁺ and [(MeOH)₂Cd{(Me₂NCH₂)₂Acr}]²⁺. Since we are interested in replicating the conditions in solution as close as possible the COSMO continuum solvation model²⁵ was applied (for standard methanol solution conditions). The NBO results show that the coordination to the acridine nitrogen is strengthened (21.9 and 15.0 kcal mol⁻¹ for Zn²⁺ and Cd²⁺, respectively). This is in line with the observations made when comparing **4** and **4a**. The complexes formed in the solid state have a weaker binding to the acridine moiety due to the crystallization with bromide anions. Our computed structures show smaller N1–Zn/Cd distances in agreement with this observation. The values are 216 and 237 pm, for Zn²⁺ and Cd²⁺ respectively. In methanol solution, sizeable interactions between the metal ion and the N1 nitrogen atom can be confirmed. The interactions with the side arm nitrogens are relatively constant, just slightly enhanced in solution. In Fig. 7, the computed difference electron density map of **4a** is illustrated. The electron densities of the individual parts (**3**, Zn²⁺, Br⁻, and DMF) were computed and subtracted from the electron density of **4a**; all with the same geometry. This facilitates us to illustrate the regions where the electron density is enhanced due to the interaction of the relevant atoms.

The green cloud represents the accumulated electron density. Here, the interaction of the lone pairs of all three nitrogen atoms with the metal ion is accentuated. When com-

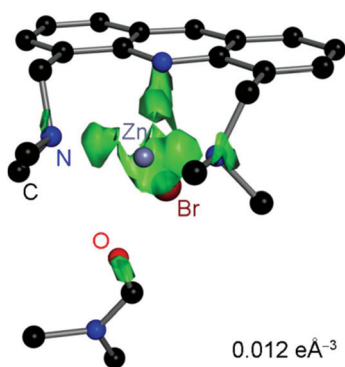


Fig. 7 Computed difference electron density map of **4a** at an isosurface level of 0.012 e Å⁻³.

paring the results of the computational studies with the fluorescence properties, it is apparent from the difference density plot that the metal coordination does not affect the π -density significantly in agreement with the almost unchanged emission wavelength. A possible explanation would be that the coordination does not influence the π -system significantly. Considering the geometry optimisations for the complex in methanol solution, both metal ion interactions with the aromatic nitrogen atom become stronger.

This should also be the case for the titration experiment of the fluorescence measurements. For a deeper insight into the liquid phase, we recorded several ¹H NMR spectra depicted in Fig. 8. In comparison with the spectrum of the pure ligand, the complexes show significantly broadened signals. This is often caused by dynamic processes. Consequently, we measured the spectrum of **4** at different temperatures (Fig. 9). The increase of the temperature sharpens the broad signals until one definite set of signals is obtained (353 K). At lower temperatures, the spectrum splits into two different sets of signals with a maximum intensity at around 253 K. At this temperature they exhibit an intensity ratio of around 2 : 1 which can be monitored by the singlet of the H-9 proton at 9.57 ppm and 9.17 ppm, respectively. This splitting is most likely to be attributed to the exchange of the bromine atoms with the solvent DMF. To prove this statement, we carried out a pseudo 2D ¹H DOSY experiment at low temperatures and added an excess of NaBr to the NMR sample. After the addition, a third set of signals appears whereby the intensity ratio of the other two sets of signals is reversed (ESI Fig. S3.13†). The diffusion coefficient of the rising signals decreases slightly which can be explained by the smaller radius/mass ratio of a bromine atom compared to a DMF molecule.²⁶ Consequently, the latter should represent the dibrominated species (**4**). The new signals have a diffusion coefficient which lies between the two others and are therefore assigned to the monobrominated compound (**4a**). Furthermore, the signals at room temperature are no longer broadened after the addition of NaBr. That is why the dynamic processes can be attributed to this solvent/bromide exchange.

At the coalescence temperature of nearly 303 K, the diastereotopicity of the CH₂ protons becomes visible. The signal of the four methyl groups at the amines splits into two signals. The velocity of their hindered rotation caused by the coordination of the zinc ion lies underneath the NMR timescale at lower temperatures and can be monitored. Zinc ions are spectroscopically silent which makes it difficult to detect a ligand–metal interaction by NMR spectroscopy. However, we measured the ¹⁵N NMR chemical shifts of the free ligand and of the zinc complex. The comparison of the resulting shifts shows a small but significant deshielding of $\Delta\delta = 8.6$ ppm.²⁷

In principle, the ¹H NMR spectra of **5** show similar dependencies upon temperature variation (ESI Fig. S3.17†). The coalescence temperature in DMF-d₇ is around 313 K where the diastereotopicity of the CH₂ protons becomes visible as well as the mentioned splitting of the signal of the methyl groups. But in this case, no second set of signals appears and the



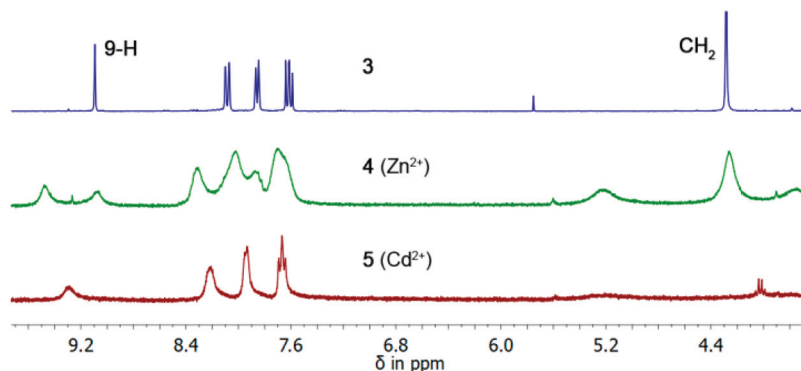


Fig. 8 ^1H NMR spectra of compounds 3–5 in $\text{DMSO}-d_6$ at ambient temperature.

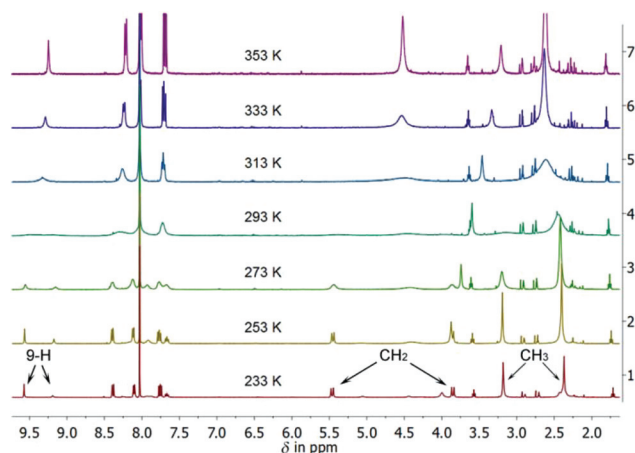


Fig. 9 Temperature-dependent ^1H NMR spectra of 4 in $\text{DMF}-d_7$. The discussed signals are marked at 233 K.

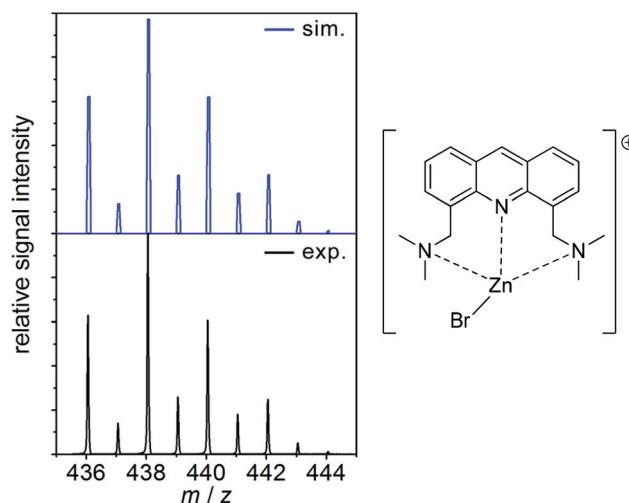


Fig. 10 Extract from the mass spectrum of the cation 4, showing the simulated (blue) and the experimental (black) isotope pattern of the positively charged complex.

spectrum is less broadened. The advantage of this complex is the NMR active ^{113}Cd nucleus. Employing a 2D ^{113}Cd , ^1H -HMBC experiment at low temperatures, we could identify vicinal couplings of the methyl and methylene protons to the metal ion (ESI Fig. S3.18†). The chemical shift of the cadmium isotope is -345 ppm, referenced to Me_2Cd . Interestingly, a coupling with the ^{113}Cd nucleus was only observed for one proton of each methylene group. The coupling with the other proton is likely not to be observed due to an unfavourable angle between the related atoms.²⁸ From the NMR spectroscopy experiments it is clear that the complexes adopt the same contact ion pairs in solution as observed in the solid state. Another analytical method which allows for investigating the transferability of the solid state structure to solution is mass spectrometry. We chose time-of-flight (TOF) spectrometry in combination with the mild electrospray ionisation method because of the poor solubility in most of the common solvents (e.g. THF, MeOH, MeCN). The measurements were performed in pure THF for the zinc complex and in THF/ H_2O for the cadmium complex due to the even lower solubility. However, both complexes were sufficiently present in solution for TOF spectrometry. Fig. 10 depicts a comparison of the two

mass patterns. The black lines represent the experimental data from the zinc complex, whereas the blue lines illustrate the simulated spectrum for the proposed structural motif (in the positive ion mode one bromide anion was omitted for the calculated spectrum). The comparison demonstrates a perfect match of the predicted and observed species. Furthermore, the full mass spectrum is free of any ionic fragments because only signals generated by the acridine derivative, with and without metal ions, and related fragments can be detected. The same is valid for the Cd^{2+} complex (ESI Fig. S6.5†). Both methods, NMR spectroscopy and mass spectrometry, emphasize that the solid state structure is maintained in solution. As mentioned in the introduction, molecular sensors play a key role in a wide range of areas, especially in medicinal or analytical applications. In this publication, we have mainly shown the possibility of using our sensor molecules in very polar solvents (fluorescence measurements in MeOH; NMR in DMF; ESI-MS in THF). However, using water as a solvent frequently evokes serious challenges. Despite the poor solubility of organic molecules, the self-ionization of water mainly results in the proto-



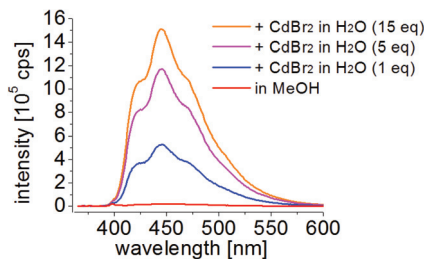


Fig. 11 Emission spectra of **3** in methanol for various concentrations of CdBr_2 dissolved in purified water ($\lambda_{\text{exc}} = 357 \text{ nm}$).

nation of the amine side-arm receptor units. This dramatically hampers the coordination of analytes. Additionally, the pK_a value of acridine rises from 5.45 to 10.7 when becoming excited in the fluorescence spectrometer,²⁹ an even higher value than the aliphatic amine (~ 9.7 , value of the related *N,N*-dimethylbenzylamine).³⁰ The formation of the protonated species can be easily monitored by fluorescence and NMR spectroscopy. In the laboratory, the addition of hydrochloric acid to a clear solution of **3** in toluene resulted in a yellow precipitate. The solubility in this solvent is reduced due to the induced charge in the product. After purification, a ^1H NMR spectrum in DMSO-d_6 shows the attached proton and the related coupling constants. Furthermore, the solid state crystal structure of this salt could be determined (ESI Fig. S3.19 and S7.2†). With **2**, the protonation experiment was not feasible. On account of the aniline like structure, the lone pair of the nitrogen atom is partly delocalized in the phenyl ring which results in a lower basicity (+M effect).³¹

To achieve an enhancement of the emission intensity, a large excess of hydrochloric acid was needed, whereas for **3**, already two equivalents of the acid resulted in a notable change of the spectrum (ESI Fig. S5.3–4†). The latter was expected for this compound since with regard to the lone pair the bonding to a proton is equivalent to the coordination of a metal ion. In both cases, the PET is quenched and the emission of light is facilitated. According to these results, measurements in water were only reasonable with a buffer system in a high pH region (>11). However, we could prevent these difficulties by dissolving the ligand in methanol and the analyte in water. In Fig. 11, the good sensitivity of the sensor system towards Cd^{2+} ions is still present even in the presence of water.

Conclusions

The fluorescence spectra of ligand **3** show a remarkable metal ion selectivity and an increase in fluorescence emission upon titration with dissolved ZnBr_2 and CdBr_2 in methanol/water. We could demonstrate the high sensitivity of the acridine based sensor molecule and the good stability of the systems. The solid state contact ion pairs **4** and **5** determined by X-ray structure analyses were confirmed to be present both in the gas phase by computational chemistry and in solution by NMR

spectroscopy and mass spectrometry. This features the 4,5-bis(*N,N*-dimethylaminemethylene)acridine as a valuable ligand in the medically and environmentally important photochemical detection of zinc and cadmium ions. Compared to the numerous publications concerning zinc sensors, only a very few provide structural evidence of the metal ion coordination, although this is inevitable for the accurate understanding of fluorescence processes.

Experimental section

Materials and measurements

All materials were purchased from Sigma-Aldrich, VWR International, and ABCR Chemicals and used without further purification – except water which was purified first through a Millipore water purification system Milli-RO 3 plus and finally with a Millipore ultrapure water system Milli-Q plus 185. The NMR measurements were performed on a Bruker Avance III 300 and Bruker Avance III HD 400 spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to the residual proton signals of the incompletely deuterated solvents. The fluorescence measurements were carried out on a Horiba Jobin-Yvon Fluoromax-4 spectrometer. All emission spectra were recorded with an excitation wavelength of 357 nm. For electrospray ionization mass spectrometry studies, sample solutions of $c \approx 5 \text{ mm}$ were continuously administered into the ESI source of a microTOF-Q II mass spectrometer (Bruker Daltonik) by using a step motor-driven gas-tight syringe (flow rate: 0.5 ml h^{-1}). This instrument combines a quadrupole mass filter with a time-of-flight analyzer. The simulated isotope patterns were calculated using the COMPASS® software package from Bruker Daltonik.

Single-crystal structural analysis

Suitable single crystals for X-ray structural analysis were selected from a Schlenk flask under an argon atmosphere and covered with perfluorinated polyether oil on a microscope slide, which was cooled with a nitrogen gas flow using the X-TEMP2 device.³² An appropriate crystal was selected using a polarizing microscope, mounted on the tip of a MiTeGen MicroMount, fixed to a goniometer head and shock-cooled by using the crystal cooling device. The data for **4a** were collected on an INCOATEC Mo microsource³³ with mirror optics and $\text{Mo-K}\alpha$ radiation with $\lambda = 71.073 \text{ pm}$. The data for **5** were collected at an INCOATEC Ag microfocuss source with INCOATEC Quazar mirror optics and Ag radiation at $\lambda = 56.086 \text{ pm}$. Both diffractometers were equipped with an APEX II detector with a D8 goniometer and a low-temperature device. The data for **4a** and **5** were integrated with SAINT³⁴ and an empirical absorption correction (SADABS)³⁵ was applied. The structures were solved by direct methods using SHELXT³⁶ and refined by full-matrix least-squares methods against F^2 (SHELXL)³⁷ within the SHELXLE GUI.³⁸ The hydrogen atoms were refined isotropically on calculated positions using a riding model with their U_{iso} values constrained to equal 1.5 times the U_{eq} of their



pivot atoms for terminal sp^3 carbon atoms and 1.2 times for all the other carbon atoms. The disordered moieties were refined using bond length restraints and anisotropic displacement parameter restraints.³⁹ Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. The CCDC numbers, crystal data, and experimental details for the X-ray measurements are listed in Table S7.2 of the ESI.†

Computational studies

All geometry optimizations were carried out at the B3LYP-D3/def2-TZVPP^{21,40} level of theory (including Becke–Jones type damping⁴¹ of the dispersion correction^{21f}). In the case of Cd, the Stuttgart/Dresden ECP28MDF⁴² pseudopotential was used. The electronic densities and the corresponding NBO analysis^{22,23} were computed at the same level. All calculations were carried out with the ORCA⁴³ program package. B3LYP calculations were performed under the RIJCOSX approximation.⁴⁴

Synthesis of 4,5-bis(bromomethylene)acridine (1). A solution of acridine (4.06 g, 22.6 mmol, 1.0 eq.) in conc. H_2SO_4 (50 ml) was heated up to 50 °C when BMME (7.4 ml, 90.6 mmol, 4.0 eq.) was added. After stirring for 17 h at this temperature, the reaction mixture was cooled to rt and the formed excess of bromine gas was discharged through an aqueous solution of thiosulfate. The crude product was precipitated by pouring into ice (400 g) and stirring for 1 h. The yellow precipitate was filtered, washed several times with dem. water and dissolved in chloroform (400 ml). The solution was washed with brine (3 × 200 ml) and the gathered aqueous phases were extracted with chloroform (100 ml). The organic phases were dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure. For purification, the crude dark yellow product was recrystallized from chloroform two times to obtain a light yellow powder (2.54 g, 6.96 mmol, 30.7%). 1H NMR (300 MHz, $CDCl_3$): δ 8.78 (s, 1 H, H_9), 7.99 (d, $^3J = 8.5$ Hz, 2 H, $H_{1,8}$), 7.94 (d, $^3J = 6.8$ Hz, 2 H, $H_{3,6}$), 7.51 (dd, $^3J = 8.5$ Hz, 6.9 Hz, 2 H, $H_{2,7}$), 5.43 (s, 4 H, CH_2). $^{13}C\{^1H\}$ NMR (75.5 MHz, $CDCl_3$): δ 145.8 (2 C, $C_{4a,10a}$), 136.8 (1 C, C_9), 136.5 (2 C, $C_{4,5}$), 131.3 (2 C, $C_{3,6}$), 129.2 (2 C, $C_{1,8}$), 126.9 (2 C, $C_{8a,9a}$), 126.0 (2 C, $C_{2,7}$), 30.3 (2 C, CH_2). EI-MS: m/z (%) 365 (34) $[M]^+$, 286/284 (76/76) $[M - Br]^+$, 205 (100) $[M - Br_2]^+$. Anal. Calcd for $C_{15}H_{11}Br_2N$: C, 49.35; H, 3.04; N, 3.84; Br, 43.78. Found: C, 48.86; H, 3.06; N, 3.78; Br, 43.30.

Synthesis of 4,5-bis(*N*-methyl-*N*-phenylaminemethylene)acridine (2). To a suspension of 1 (0.50 g, 1.37 mmol, 1.0 eq.) and K_2CO_3 (0.66 g, 4.78 mmol, 3.5 eq.) in MeCN (30 ml), methylphenylamine (0.30 ml, 2.76 mmol, 2.0 eq.) was added. After stirring the reaction mixture for 4 h at rt, the volatile components were removed under reduced pressure. The crude product was dissolved in ethyl acetate and aqueous NaCl solution and washed three times with brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. For further purification, a recrystallization from methanol was implemented. Thereby, the impurities were dissolved and filtered off. 2 (0.49 mg, 1.17 mmol, 85.4%) could be obtained as a yellow solid. 1H NMR (300 MHz, $DMSO-d_6$):

δ 9.15 (s, 1 H, H_9), 8.07 (d, $^3J = 8.0$ Hz, 2 H, $H_{1,8}$), 7.55 (dd, $^3J = 8.0$ Hz, $^3J = 6.9$ Hz, 2 H, $H_{2,7}$), 7.47 (d, $^3J = 6.9$ Hz, 2 H, $H_{3,6}$), 7.12 (dd, $^3J = 8.6$ Hz, $^3J = 7.2$ Hz, 4 H, *meta* H_{Ph}), 6.73 (d, $^3J = 8.6$ Hz, 4 H, *ortho* H_{Ph}), 6.58 (dd, $^3J = 7.2$ Hz, $^3J = 7.2$ Hz, 2 H, *para* H_{Ph}), 5.38 (s, 4 H, CH_2), 3.19 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (75.5 MHz, $DMSO-d_6$): δ 149.2 (2 C, *ipso* C_{Ph}), 145.8 (2 C, $C_{4a,10a}$), 136.7 (1 C, C_9), 135.7 (2 C, $C_{4,5}$), 129.0 (4 C, *meta* C_{Ph}), 127.0 (2 C, $C_{1,8}$), 126.8 (2 C, $C_{3,6}$), 126.1 (2 C, $C_{8a,9a}$), 125.6 (2 C, $C_{2,7}$), 115.6 (2 C, *para* C_{Ph}), 111.7 (4 C, *ortho* C_{Ph}), 52.6 (2 C, CH_2), 38.9 (2 C, CH_3). EI-MS: m/z (%) 417.2 (14) $[M]^+$, 310.1 (100) $[M - NMePh]^+$, 205.1 (84) $[M - (NMePh)_2]^+$. Anal. Calcd for $C_{29}H_{27}N_3$: C, 83.42; H, 6.52; N, 10.06. Found: C, 82.31; H, 6.33; N, 9.92.

Synthesis of 4,5-bis(*N,N*-dimethylaminemethylene)acridine (3). The synthesis procedure for 3 is the same as that for 2 with 1 (1.55 g, 4.25 mmol, 1.0 eq.), K_2CO_3 (1.76 g, 12.75 mmol, 3.0 eq.), a solution of dimethylamine in THF (2.0 M, 4.5 ml, 9.0 mmol, 2.1 eq.), and MeCN (40 ml). The desired product (0.78 g, 2.66 mmol, 62.6%) could be obtained as a yellow-brown solid. 1H NMR (300 MHz, $DMSO-d_6$): δ 9.09 (s, 1 H, H_9), 8.08 (dd, $^3J = 8.5$ Hz, $^4J = 1.6$ Hz, 2 H, $H_{1,8}$), 7.86 (dd, $^3J = 6.8$ Hz, $^4J = 1.3$ Hz, 2 H, $H_{3,6}$), 7.61 (dd, $^3J = 8.5$, 6.8 Hz, 2 H, $H_{2,7}$), 4.28 (s, 4 H, CH_2), 2.35 (s, 12 H, CH_3). $^{13}C\{^1H\}$ NMR (75.5 MHz, $DMSO-d_6$): δ 146.2 (2 C, $C_{4a,10a}$), 136.6 (2 C, $C_{4,5}$), 135.9 (1 C, C_9), 130.0 (2 C, $C_{3,6}$), 127.4 (2 C, $C_{1,8}$), 125.9 (2 C, $C_{8a,9a}$), 125.6 (2 C, $C_{2,7}$), 58.2 (2 C, CH_2), 45.4 (4 C, CH_3). ^{15}N -HMBC (40.6 MHz, $DMF-d_7$): δ -81.2 (N_{arom}), -355.3 (NMe_2). EI-MS: m/z (%) 293 (4) $[M]^+$, 250 (64) $[M - NMe_2]^+$, 205 (100) $[M - (NMe_2)_2]^+$. Anal. Calcd for $C_{19}H_{23}N_3$: C, 77.78; H, 7.90; N, 14.32. Found: C, 76.45; H, 7.77; N, 13.94.

Synthesis of 4,5-bis(*N,N*-dimethylaminemethylene)acridine dibromido zinc(II) (4). 2 (175 mg, 596 μ mol, 1.0 eq.) was dissolved in THF (1 ml) and a solution of $ZnBr_2$ (134 mg, 596 μ mol, 1 eq.) in THF (0.2 ml) was added dropwise. Thereby, the zinc complex precipitated immediately. The suspension was filtered and washed several times with THF. The zinc complex (145 mg, 280 μ mol, 47.0%) was isolated as a yellow powder. Suitable crystals for X-ray analysis could be grown after recrystallization from a THF/DMF (9 : 1) mixture. The crystals were formed at rt after two weeks. 1H NMR (400 MHz, $DMF-d_7$, 353 K): δ 9.25 (s, 1 H, H_9), 8.22 (d, $^3J = 8.3$ Hz, 2 H, $H_{1,8}$), 8.01 (d, $^3J = 7.1$ Hz, 2 H, $H_{3,6}$), 7.69 (dd, $^3J = 8.3$, 7.1 Hz, 2 H, $H_{2,7}$), 4.52 (4 H, CH_2), 2.62 (s, 12 H, CH_3). $^{13}C\{^1H\}$ NMR (100 MHz, $DMF-d_7$, 243 K): δ 148.7 (2 C, $C_{4a,10a}$), 142.0 (1 C, C_9), 135.5 (2 C, $C_{3,6}$), 130.5 (2 C, $C_{1,8}$), 127.7 (2 C, $C_{8a,9a}$), 126.4 (2 C, $C_{2,7}$), 63.1 (2 C, CH_2), 48.9 (2 C, CH_3), 46.4 (2 C, CH_3). ^{15}N NMR (40.6 MHz, $DMF-d_7$, 243 K): δ -346.7 (NMe_2). ESI-TOF: m/z : 813.15 $[(C_{19}H_{23}N_3)_2ZnBr_2H]^+$, 438.05 $[(C_{19}H_{23}N_3)ZnBr]^+$, 294.20 $[(C_{19}H_{23}N_3)H]^+$. Anal. Calcd for $C_{19}H_{23}N_3ZnBr_2$: C, 44.00; H, 4.47; N, 8.10; Br, 30.81. Found: C, 44.00; H, 4.50; N, 8.10; Br, 30.00.

Synthesis of 4,5-bis(*N,N*-dimethylaminemethylene)acridine dibromido cadmium(II) (5). 2 (141 mg, 480 μ mol, 1.0 eq.) was dissolved in methanol (0.5 ml) and a solution of $CdBr_2$ (131 mg, 480 μ mol, 1.0 eq.) in methanol (1.5 ml) was poured into. Upon addition, the cadmium complex precipitated



immediately. Another 5 ml of methanol was added and the suspension was filtered. The residue was washed several times with methanol (3 × 5 ml) and dried under reduced pressure. The pure product (245 mg, 433 μmol, 90.2%) was obtained as a yellow powder. The product was dissolved in a THF/DMF (3 : 2) mixture, heated up to the boiling point of THF and then cooled to rt overnight. The crystals thus formed were suitable for X-ray diffraction. ¹H NMR (400 MHz, DMF-d₇, 353 K): δ 9.32 (s, 1 H, H₉), 8.28 (d, ³J = 8.5 Hz, 2 H, H_{1,8}), 8.08–7.99 (m, 2 H, H_{3,6}), 7.71 (dd, ³J = 8.5, 6.8 Hz, 2 H, H_{2,7}), 4.56 (s, 4 H, CH₂), 2.74 (s, 12 H, CH₃). ¹¹³Cd, ¹H-HMBC (66.6 MHz, DMF-d₇, 243 K): δ –345.5. ESI-TOF: *m/z*: 861.15 [(C₁₉H₂₃N₃)₂CdBr₂H]⁺, 486.02 [(C₁₉H₂₃N₃)CdBr]⁺, 294.21 [(C₁₉H₂₃N₃)H]⁺. Anal. Calcd for C₁₉H₂₃N₃CdBr₂: C, 40.35; H, 4.10; N, 7.43; Cd, 19.87. Found: C, 40.28; H, 4.31; N, 7.20; Cd, 19.00.

Note added in proof

During the production of this paper related acridine complexes were published: E. V. Solovyeva, G. L. Starova, L. A. Myund, A. S. Denisova, *Polyhedron*, 2016, **106**, 1–9.

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