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Two Acridone Units in One Crown Ether

Incorporating a Second Acridone

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Abstract

We present here a critical overview on the effects of the second heterocyclic subunit in a bisacridino-crown ether by the discussion of its role in modulating optochemical behavior and preference in molecular recognition. The preparation of a new bisacridono-crown ether is presented including synthetic difficulties, and its fluorescence properties and selectivity in formation of inclusion complexes with various cations are evaluated in the light of reported analogues.

Keywords

acridone, macrocycle, fluorescence, molecular recognition

1 Introduction

Many derivatives of bisacridines can be obtained from natural sources and a significant proportion of them shows cytotoxic properties [1–3]. Recently, these bisheterocycles also play an important role in the synthetic chemical research as their extended aromatic systems can result in highly curved, boat-shaped molecules, which demonstrate interesting properties both in increasing the molecular dimensions and triggering unique physicochemical features [4, 5].

In the field of supramolecular chemistry, the strong coordination tendency of acridines to DNA is often exploited through the development of applications based on complex formation. Over the years, several macrocyclic hosts, especially cyclo-bis-intercorporated type ligands containing two acridine units in the macroring have been synthesized for similar purposes [6–11]. Using these cage compounds allows fine-tuning of molecular recognition by their diverse and multiple-coordinated inclusion complexes. Some examples are shown in Fig. 1.

Macrocycles containing a rigid linker proved to be more advantageous as they showed an enhanced selectivity, a reduced potential for self-stacking between their aromatic units and a higher antitumor activity [10, 11]. In addition, the structural rigidity of the host molecules is generally considered to result in higher selectivity toward a guest molecule as these kinds of structures are less able to distort upon the coordination of a competitor [12, 13].

Another class of acridino- and acridono-macrocycles is oligoaza-crown ethers. The acridino- and acridono-crown ethers contain *O*- or *N*-atoms or both of them as additional

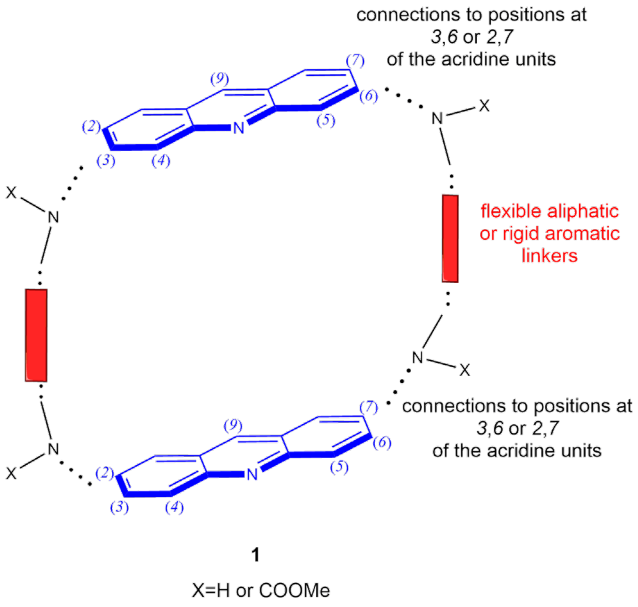

Fig. 1 Cyclo-bis-intercorporations containing acridine subunits linked by different spacers as most commonly used bisacridino-macrocycles with a nucleic acid-specific applicability

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centers for intermolecular coordination. Besides the size of the macrocyclic cavity and many other factors, the nature of these heteroatoms strongly influences recognition abilities of the host molecules. Some previously reported bisacridino- and bisacridono-crown ethers are shown in Fig. 2 [14–16].

A comparative study, which discussed the complexation and optical signaling ability of the macrocycles as a function of the number of acridine units in the macroring (see **2** in Fig. 2) has recently showed, that in addition to the quite different selectivities, the fluorescence could either not be enhanced by the increasing number of incorporated heterocycles, because the greater number of their possible vibration modes inhibited it [16]. The macrocycles containing *NH*-groups with aliphatic chains (**2** in Fig. 2) tend to coordinate anions when two or more of their basic nitrogens are in their cationic form [14, 17, 18]. In these cases, the acridine-*N* is not involved in the complexation and the coordination of anions takes place only by the ammonium units with the aliphatic chains. The acridinium cation can only be present in excited state attributed to an intramolecular proton transfer, unless an anion is complexed by the macrocycle [17, 18]. Moreover, in the fully protonated state a strong electrostatic repulsion takes place, which also prevents the acridines to accept a proton [15, 17, 18]. In contrast, if *O*-atoms are also parts of the macroring as nucleophile centers, coordination of cations is preferred [15, 19, 20] and anions are not complexed even in partially protonated forms of the macrocycles [15, 19].

Acridono-crown ethers are preferred over their acridino-analogues in cation-selective chemosensing applications as the former ones typically show less pH-dependence and more advantageous photophysical properties compared to the latter ones [20]. To the best of our knowledge, acridono-macrocycles **3** in Fig. 2 are the first and only bisacridono-hosts. They were synthetized with moderate yields (12–18%) starting from *N*-methyl-2,7-dihydroxy-acridone in a 2:2 type macrocyclization, but as intermediates for photoswitchable receptors, their applicability was not discussed [14, 21].

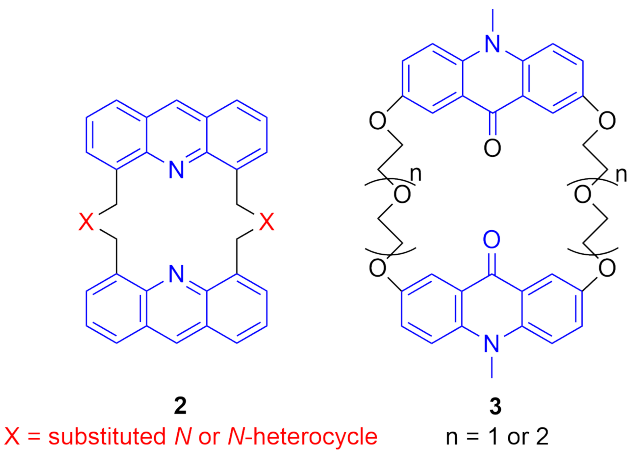


Fig. 2 Macrocycle-analogues containing two acridine or two acridone units (made by Panna Vezse based on the synthesis in [14–16])

In order to study the contribution of a second heterocyclic subunit to improving selectivity and photophysical properties, we have synthesized a bisacridono-macrocycle as an analogue of the previously reported monoacridono-crown ethers, which showed an excellent fluorescence and high selectivity toward Pb^{2+} [20, 22, 23] and also as an analogue of **3** with a smaller cavity size and without *N*-methyl groups. The applicability of the new bisacridino-macrocycle as an optochemical sensor molecule was critically discussed.

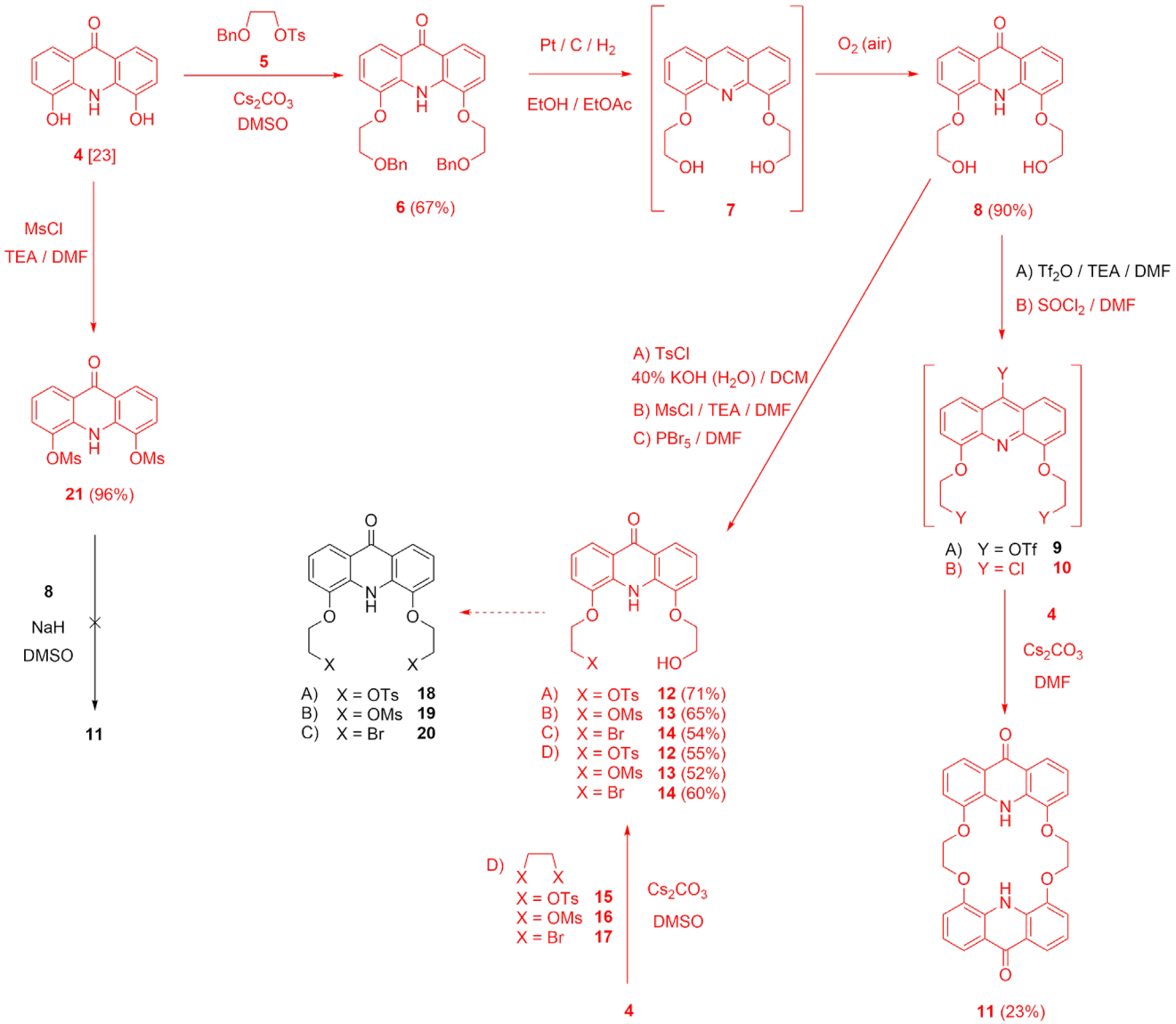
2 Results and discussion

2.1 Synthesis

The synthesis of the desired bisacridono-crown ether was started from acridone-4,5-diol (**4**), which was prepared based on a reported procedure [24]. Numerous synthetic pathways had to be attempted due to unexpected difficulties. The main procedures are outlined in Scheme 1.

Since the 2:2 type macrocyclization of acridone-4,5-diol (**4**) with ethylene glycol ditosylate (**15**) (Cs_2CO_3/DMSO), which would have been the shortest procedure of all could not be performed even in extremely high dilution conditions (not reported in Scheme 1), the synthesis of macrocycle **11** was attempted *via* 1:1 type macrocyclizations of the corresponding intermediates.

Initially, acridonediol **4** had to be connected with glycols, which would serve as the ether linkers between the two heterocycles of the host. First this reaction was also attempted by using bifunctional ethanes (**15-17**) as reagents (see procedure D) in Scheme 1), but the reactions resulted in the corresponding monohydroxy derivatives (**12-14**) as main products. Thus, the same reaction was carried out using monoprotected glycol **5** [25], when product **6** was obtained with a medium yield. The benzyl protecting groups of bis(benzyloxy)-derivative **6** were removed by catalytic hydrogenation to get acridone **8**. A small amount of the reduced acridine form (**7**) was also obtained, but it underwent spontaneously into the corresponding acridone **8** in the air. Then, the functionalization of the hydroxyl groups was carried out to obtain ditosylates **18**, dimesylate **19** and dibromide **20**. It is worth mentioning, that acridone **8** proved to be



Scheme 1 Synthetic pathways to obtain the desired symmetric bisacridono-18-crown-6 ether (**11**) (Compounds in square brackets were not characterized due to their instability, while derivatives indicated in black color were not isolated.)

extremely insoluble, even in dipolar aprotic solvents, thus its reactions were carried out as suspensions. Surprisingly we found, that the main products were the monofunctionalized derivatives (**12**, **13**, **14**, respectively) in each case. Although LC-MS studies showed that the expected bifunctionalized products (**15-17**) were formed, but their amounts proved to be negligible. Many efforts were made using large excess of reagents (TsCl, MsCl, PBr_5) in harder conditions to enhance the conversion of the second hydroxyl group, but only the monohydroxy derivatives (**12-14**) could be gained as main products. The replacement of the reported two-phase-tosylation (procedure A) in Scheme 1) by conventional methods using pyridine or triethylamine as solvents or using *Appel*-reaction (CBr_4/PPh_3, not reported in Scheme 1) instead of bromination with PBr_5 were also unsuccessful and led to the same results. May be these derivatives containing two leaving groups (**18-20**) are extremely prone to hydrolysis or the reaction is sterically inhibited.

In addition, a *Mitsunobu*-reaction of intermediates **4** and **8** (diisopropyl azodicarboxylate/PPh_3/Cs_2CO_3/DMSO/THF, not reported in Scheme 1) and the macrocyclization of diol **8** with heteroaromatic dimesylate **21** were also attempted to prepare the crown ether **11**, but the desired product could not be isolated.

Finally, crown ether **11** could be successfully prepared *via* trichloro-intermediate **10**. Thionyl chloride was used as a reagent instead of the generally applied $POCl_3$ or PCl_5 [26] to avoid extraction and simplify the work-up.

Trichloro-compound **10** was obtained with an almost quantitative yield in contrast to triflate **9**, which attempted synthesis resulted in several by-products, thus this pathway was also neglected. In addition to the halogenation of the aliphatic hydroxyl groups, the substitution of the heteroaromatic ring at position *9* with a simultaneous conversion of the acridone to acridine also takes place in the cases of compounds **9**, **10**, **14** and **20**. However, these 9-substituted acridines (**9**, **10**, **14** and **20**) decompose quickly, thus only the corresponding acridin-9(10*H*)-one derivative can be isolated. As the macrocyclization of trichloro-compound **10** with diol **4** took 2 weeks to complete, only the acridin-9(10*H*)-one form of **10** takes part in the reaction. Host molecule **11** was obtained in a moderate yield, which can be considered typical for 1:1 macrocyclizations. The acridone units of the host molecule provides diverse opportunities for the subsequent modification of the macrocycle backbone [26], thus electronic, physicochemical and photochemical features are widely tailorable.

The lowest energy conformation state was calculated by theoretical methods. These results including additional discussion on structural flexibility can be found in the Supplement.

2.2 Fluorescence properties

The spectral properties of the new macrocycle (**11**) were investigated by UV/Vis and fluorescence spectroscopies. The absorption and fluorescence emission spectra of the sensor molecule are shown in the Supplement. The absorption and emission peak-wavelengths were 253 nm and 440 nm, respectively. An outstandingly high *Stokes*-shift of 187 nm was observed [26], which reduces the possibility for self-absorption. For the purpose of studying the applicability of the reported bisacridono-host (**11**) as an optochemical sensor molecule, its fluorescence quantum yield was also determined and found as 0.39 in acetonitrile, indicating a fluorescence of the free ligand similar to acridone (ϕ = 0.41, [27]). It is seen, that the second acridone unit could not strengthen the fluorescence. What is more, the quantum yield of the bisacridono-macrocycle is inferior to that of many analogues containing only one acridone unit [26]. Probably it is due to the increased number of possible vibration modes as observed similarly by dos Santos Carlos et al. [16].

2.3 Spectrophotometric studies on cation-selectivity

Bisacridono-crown ether **11** showed a rather poor solubility even in diprotic apolar solvents similarly to its diol precursor **8**. The maximum solubility was found as 1 mM in DMSO. Studies on metal ion selectivity were performed by adding 10 equivalents of 24 different metal ions as 50 mM aqueous solutions separately to the solution of macrocycle **11** in DMSO (detailed information can be found in Section 3.3). The results are shown in Fig. 3.

As the fluorophore subunits are parts of the macrocyclic cavity, coordination of a guest cation would inevitably cause a significant change in the photophysical propertiesof the host molecule. Changes in absorption spectra indicated complex formation with Hg^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Ag^{+}, Cr^{3+}, which ions caused an enhancement in optical signal in the corresponding order. In the cases of the other 18 cations, no spectral change was observed, indicating that complexation did not take place.

Optochemical response upon complex formation was also investigated by fluorescence spectroscopy. The same procedure was used as in the case of UV-studies on selectivity, 10 equivalents of 24 different metal ions as 50 mM aqueous solutions were added separately to macrocycle **11** in DMSO. Spectral changes are shown in Fig. 4.

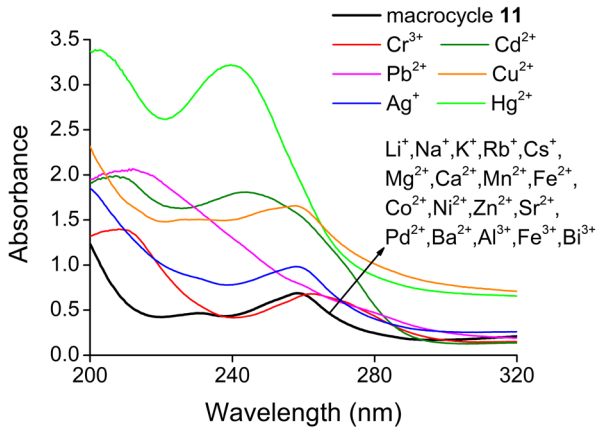


Fig. 3 Studies on metal ion-selectivity of new fluoroionophore **11** (c_{host} = 1 mM in DMSO, $c_{metal\ ions}$ = 10 mM in water or ethanol in the case of Pd^{2+})

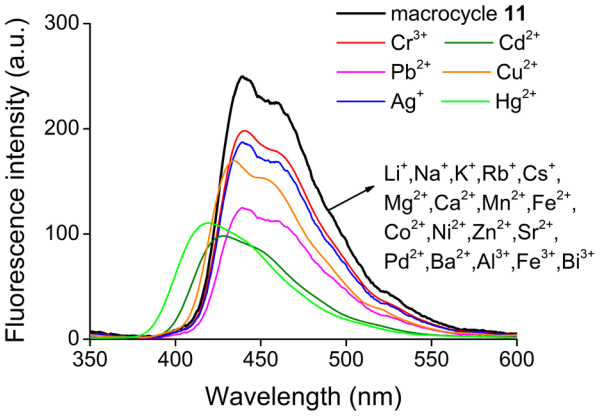


Fig. 4 Fluorescence studies on metal ion-selectivity of new macrocycle **11** (c_{host} = 10^{-7} M in DMSO, $\lambda_{ex.}$ = 265 nm)

The results are fully consistent with those of obtained by UV/Vis spectroscopy. Upon coordinating the preferred metal cations partial fluorescence quenching was observed with a minimal hypsochromic shift (0–25 nm) in some cases. In the presence of the other metal ions the lack of photophysical changes indicates the absence of significant guest-coordination.

All of the preferred cations are considered as soft ones. Although they have quite different ionic radii, they are similarly preferred for complexation due to their similar character and the soft nucleophile heteroaromatic-*N* atoms (in the 9-hydroxyacridine form) of the host molecule. Moreover, the two electron-rich heteroaromatic moieties can also contribute to the coordination of the soft cations *via* the formation of cation-π interactions.

3 Experimental

3.1 General

Starting materials and reagents were purchased from Sigma-Aldrich Corporation (USA, owned by Merck, Darmstadt, Germany) and used without further purification unless otherwise noted. Solvents were dried and purified according to well established methods [28]. Silica Gel 60 F254 (Merck, Germany) and aluminum oxide 60 F254 neutral type E (Merck, Germany) plates were used for thin-layer chromatography (TLC). All reactions were monitored by TLC and visualized by UV-lamp. Silica Gel 60 (70–230 mesh, Merck) was used for column chromatography. Purifications by preparative thin-layer chromatography (PTLC) were carried out using Silica gel 60 F254 (Merck, Germany) plates of 2 mm layer thickness (art No.: 1.05744) or aluminum oxide 60 F254 neutral type E (Merck, Germany) plates of 0.25 mm layer thickness (art No.: 1.05727). Ratios of solvents for the eluents are given in volumes (mL/mL). Evaporations were carried out under reduced pressure unless otherwise stated.

The new compounds were characterized by their physical constants such as melting point, thin-layer chromatography retention factor (R_f), infrared, ^{1}H-NMR and ^{13}C-NMR spectroscopies and HRMS spectrometry. In the cases of isolated by-products and intermediates, which were unsuccessfully applied for the preparation of the macrocycle only ^{1}H-NMR, IR and LC-MS methods were used for characterization. Melting points were taken on a Boetius micro-melting point apparatus and are uncorrected. Infrared spectra were recorded on a Bruker Alpha-T FT-IR spectrometer (Bruker Corporation, Billerica, MA, USA) using KBr disks. ^{1}H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Bruker 300 Avance spectrometer (Bruker Corporation, USA). ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were taken on a Bruker DRX-500 Avance spectrometer (Bruker Corporation, USA). LC-MS studies were performed on an Agilent-1200 Quadrupole LC-MS Instrument (Agilent, USA) equipped with Phenomenex Kinetex C18 2.6 µm 100 A column (50×3 mm ID; water - acetonitrile gradient, ammonium-formate additive; flow rate: 0.5 mL/min, column temperature: 40 °C; UV-detector α: 254 nm). Measurements were performed both in positive and negative electrospray ionization modes. In the cases of characterizing the targeted host molecule and their important precursors, LC-MS measurement were supplemented with HRMS method. The HRMS analysis was carried out on a Thermo Velos Pro Orbitrap Elite (Thermo Fisher Scientific, Dreieich, Germany) system. The ionization method was ESI and was operated in positive ion mode. The protonated molecular ion peak was fragmented by CID at a normalized collision energy of 35–45%. The sample was dissolved in methanol. Data acquisition and analysis were accomplished with Xcalibur software version 2.2 [29].

3.2 Synthesis

3.2.1 Preparation of 4,5-bis(2-(benzyloxy)ethoxy) acridin-9(10*H*)-one (6, Scheme 1)

A mixture of acridonediol **4** [24] (500 mg, 2.20 mmol), finely powdered anhydrous caesium carbonate (4.30 g, 13.20 mmol) and dry and pure DMSO (50 mL) was stirred vigorously under argon at room temperature for 30 min, then glycol **5** (2.02 g, 6.60 mmol) in dry and pure DMSO (50 mL) was added dropwise. The temperature of the reaction mixture was raised to 70 °C and the reaction mixture was kept at this temperature for 4 days. Water (500 mL) was added to the reaction mixture and it was extracted with ethyl acetate (5 × 200 mL). The combined organic phase was shaken with water (9 × 500 mL) and then with saturated aqueous sodium chloride solution (1 × 500 mL) to remove DMSO. The organic phase was dried over magnesium sulphate, filtered and the solvent was evaporated. The crude product was purified by column chromatography on silica gel using ethyl acetate:hexane (1:1) as an eluent to give **6** (731 mg, 67%) as a bright yellow solid.

M.p. = 109–110 °C. R_f = 0.45 (SiO_2 TLC, ethyl acetate:hexane 1:1). R_f = 0.77 (SiO_2 TLC, dichloromethane:-methanol 20:1). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm]: 9.32 (s, 1H), 8.09 (d, J = 7.3 Hz, 2H), 7.49–7.26 (m, 10H), 7.25–6.92 (m, 4H), 4.69–4.50 (m, 4H), 4.36 (t, J = 4.6 Hz, 3H),

3.88 (t, J = 4.7 Hz, 3H), 3.83–3.73 (m, 1H), 3.69–3.58 (m, 1H). ^{13}C-NMR (75.5 MHz, DEPT 135, $CDCl_3$): δ [ppm]: 128.46, 127.80, 127.63, 120.73, 118.88, 113.27, 73.47, 68.76, 68.41. IR: ν_{max} [cm^{-1}]: 3421, 3063, 3030, 2934, 2869, 2816, 1627, 1597, 1536, 1492, 1453, 1273, 1226, 1110, 1086, 967, 821, 745, 697, 587, 456. HRMS: m/z = [MH$^+$]: 496.2053, (Calcd. for $C_{31}H_{29}NO_5$, 495.2046).

3.2.2 Preparation of 4,5-bis(2-hydroxyethoxy)acridin-9(10*H*)-one (8, Scheme 1)

A solution of dibenzyloxy-derivative **6** (500 mg, 1.01 mmol) in a mixture of ethyl acetate and ethanol (50 mL, 1:1) was hydrogenated in the presence of Pt/C catalyst (50 mg, Pt/charcoal; activated, 10% Pt) at 60 °C. Perchloric acid was added to the reaction mixture in a catalytic amount. After 5 h the reaction was completed. The catalyst was filtered off and washed with ethanol (2 × 10 mL). The filtrate and washings were evaporated to give diol **7** (could not be isolated due to its spontaneous, immediate conversion to **8**) and **8**. Diol **8** (287 mg, 90%) was a pale yellow solid and it did not need to be purified for using in the next reaction.

M.p. = 255–256 °C. R_f = 0.55 (SiO_2 TLC, dichloromethane:methanol 10:1). ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): δ [ppm]: 9.65 (s, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.42 (d, J = 7.7 Hz, 2H), 7.22 (t, J = 8.0 Hz, 2H), 4.27 (t, J = 4.7 Hz, 4H), 3.86 (q, J = 5.1 Hz, 4H). ^{13}C-NMR (125 MHz, DEPT 135, $(CD_3)_2SO$): δ [ppm]: 181.69, 152.14, 136.60, 126.46, 126.38, 122.90, 120.00, 76.87, 64.75. IR: ν_{max} [cm^{-1}]: 3420 (broad), 3283, 2928, 1625, 1573, 1535, 1489, 1422, 1275, 1225, 1068, 1012, 910, 827, 748, 611. HRMS: m/z = [MH$^+$]: 316.1112, (Calcd. for $C_{17}H_{17}NO_5$, 315.1107).

3.2.3 Preparation of 9-chloro-4,5-bis(2-chloroethoxy) acridine (10, Scheme 1)

A suspension of diol **8** (400 mg, 1.27 mmol), thionyl chloride (20 mL, 275.70 mmol) and DMF (5 mL) was stirred in an oil bath under argon. The external temperature was raised to 90 °C and the suspension was stirred at this temperature for 6 h. The excess reagent and solvent were removed to gain trichloro-compound **10** as an orange solid, which was used immediately in the next step without characterization and purification.

R_f = 0.90 (SiO_2 TLC, dichloromethane:methanol 10:1). The ^{1}H-NMR spectrum of crude **10** can be found in the Supplement. NMR analysis was consistent with MS measurements and indicated a mixture of products including the desired trichloro-derivative **10** and its transformed products (acridono-analogues) with different numbers of chlorine atoms.

3.2.4 Preparation of 2-((5-(2-hydroxyethoxy)-9-oxo-9,10-dihydroacridin-4-yl)oxy)ethyl-4-methylbenzenesulfonate (12, Scheme 1, procedure A)

To a stirred mixture of diol **8** (500 mg, 1.59 mmol) in dichloromethane (15 mL) and potassium hydroxide solution (20 mL, 40 m/m%), 4-toluenesulfonyl chloride (1.22 g, 6.36 mmol) dissolved in 15 mL of dichloromethane was added dropwise in 15 min at 0 °C. After addition, the mixture was stirred at room temperature for 2 days. The pH of the reaction mixture was adjusted to 7 with aqueous HCl solution (5 m/m%). The phases were separated, and the aqueous phase was extracted with dichloromethane (3 × 100 mL). The combined organic phase was dried over magnesium sulfate, filtered, and the solvent was removed. The crude product was purified by PTLC on silica gel using dichloromethane as an eluent to get monotosylate **12** (531 mg, 71%) as a dark yellow solid.

M.p. = 143–144 °C. R_f = 0.83 (SiO_2 TLC, dichloromethane:methanol 20:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm]: 8.22 (dd, J = 8.0, 1.6 Hz, 1H), 8.07 (dd, J = 8.0, 1.7 Hz, 1H), 7.79 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.25 (dd, J = 7.7, 1.7 Hz, 1H), 7.18 (td, J = 7.9, 6.3 Hz, 2H), 7.10 (dd, J = 7.8, 1.6 Hz, 1H), 4.83 (t, J = 4.9 Hz, 2H), 4.49–4.45 (m, 2H), 4.42 (t, J = 4.9 Hz, 2H), 4.33–4.29 (m, 2H), 2.42 (s, 3H). IR: ν_{max} [cm^{-1}]: 3397 (broad), 2959, 2919, 2872, 2851, 1735, 1629, 1596, 1586, 1501, 1472, 1449, 1429, 1353, 1299, 1260, 1213, 1188, 1173, 1094, 1067, 1018, 922, 877, 813, 743, 661, 574, 552, 425. LC-MS: m/z = [MH$^+$]: 470.1, (Calcd. for $C_{24}H_{23}NO_7S$, 469.1).

3.2.5 Preparation of 2-((5-(2-hydroxyethoxy)-9-oxo-9,10-dihydroacridin-4-yl)oxy)ethyl-methanesulfonate (13, Scheme 1, procedure B)

A suspension of diol **8** (500 mg, 1.59 mmol), methanesulfonyl chloride (1.0 mL, 12.72 mmol), triethylamine (3.5 mL, 25.44 mmol) and DMF (10 mL) was stirred in an oil bath under argon. The external temperature was raised to 70 °C and the reaction mixture was stirred at this temperature for 2 days. The volatile components were evaporated. The residue was purified by PTLC on silica gel using dichloromethane as an eluent to get monomesylate **13** (407 mg, 65%) as a yellowish brown solid.

M.p. = 133 °C. R_f = 0.80 (SiO_2 TLC, dichloromethane:-methanol 20:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm]: 8.10 (dd, J = 8.0, 1.6 Hz, 1H), 7.96 (dd, J = 8.0, 1.6 Hz, 1H), 7.14 (dd, J = 7.6, 1.7 Hz, 1H), 7.09–7.04 (m, 2H), 7.00 (dd, J = 7.8, 1.6 Hz, 1H), 4.76 (t, J = 4.8 Hz, 2H), 4.60–4.55 (m, 2H), 4.35 (t, J = 4.8 Hz, 2H), 4.26–4.22 (m, 2H), 3.04 (s, 3H). IR: ν_{max} [cm^{-1}]: 3397 (broad), 2959, 2920, 2872,

2850, 1735, 1629, 1597, 1586, 1500, 1472, 1450, 1429, 1353, 1298, 1260, 1213, 1189, 1173, 1095, 1067, 1018, 923, 877, 813, 744, 661, 575, 552, 424. LC-MS: m/z = [MH^+]: 394.2, (Calcd. for $C_{18}H_{19}NO_7S$, 393.1).

3.2.6 Preparation of 4-(2-bromoethoxy)-5-(2-hydroxyethoxy)acridin-9(10*H*)-one (14, Scheme 1, procedure C)

A suspension of diol **8** (500 mg, 1.59 mmol), phosphorus pentabromide (2.74 g, 6.36 mmol) and DMF (50 mL) was stirred under argon atmosphere at 80 °C for 1 day. Then the reaction mixture was cooled down to room temperature and it was slowly poured into a vigorously stirred cold mixture of 400 mL of 40 m/m% aqueous trimethylammonium hydroxide solution with an external ice cooling. This mixture was extracted with chloroform (5 × 500 mL). The combined organic phase was dried over magnesium sulphate, filtered and the solvent was removed. After the work-up only the acridone form of the product was isolated due to the fast hydrolysis of 4,5-substituted-9-bromoacridines [26]. The crude product was purified by PTLC on silica gel using dichloromethane as an eluent to get **14** (325 mg, 54%) as a brown solid.

M.p. = 96 °C. R_f = 0.84 (SiO_2 TLC, dichloromethane:-methanol 20:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm]: 8.21 (dd, J = 7.9, 2.2 Hz, 1H), 8.07 (dd, J = 8.2, 1.7 Hz, 1H), 7.24 (dd, J = 7.8, 1.6 Hz, 1H), 7.18 (td, J = 8.0, 4.7, 2.4 Hz, 2H), 7.12 (dd, J = 7.9, 2.6 Hz, 1H), 4.95 (t, J = 4.3 Hz, 2H), 4.48 (t, J = 3.8, 2.9 Hz, 2H), 4.44 (t, J = 4.5, 3.8 Hz, 2H), 3.79 (t, J = 5.3 Hz, 2H). IR: ν_{max} [cm^{-1}]: 3397 (broad), 3094, 3000, 2936, 2916, 2837, 2074, 1644, 1602, 1524, 1489, 1468, 1439, 1352, 1274, 1229, 1128, 1083, 1027, 955, 753, 706, 632. LC-MS: m/z = [MH^+]: 378.0, 380.0 (Calcd. for $C_{17}H_{16}BrNO_4$, 377.0).

3.2.7 General method for synthesizing compounds 12, 13 and 14 by procedure D) (Scheme 1)

A mixture of acridonediol **4** [24] (500 mg, 2.20 mmol), finely powdered anhydrous caesium carbonate (4.30 g, 13.20 mmol) and dry and pure DMSO (50 mL) was stirred vigorously under argon atmosphere at room temperature for 30 min, then the corresponding reagent (**15** or **16** or **17**, 8.80 mmol) in dry and pure DMSO (50 mL) was added. The temperature of the mixture was raised to 70 °C and it was kept at this temperature for 4 days. Water (500 mL) was added to the reaction mixture, which was extracted with ethyl acetate (5 × 200 mL). The combined organic phase was shaken with water (9 × 500 mL) and then with saturated aqueous sodium chloride solution (1 × 500 mL) to remove DMSO. The organic phase was dried over magnesium sulphate, filtered and the solvent was evaporated. The crude product was purified by PTLC on silica gel using dichloromethane as an eluent to get compounds **12**, **13** and **14** with a yield of 55%, 52% and 60%, respectively. All of the physical and spectroscopic data were identical to those reported in Sections 3.2.4–3.2.6.

3.2.8 Preparation of 9-oxo-9,10-dihydroacridine-4,5-diyl-dimethanesulfonate (21, Scheme 1)

Acridonediol **4** (500 mg, 2.20 mmol) was dissolved in DMF (10 mL). Methanesulfonyl chloride (1.0 mL, 12.72 mmol) and triethylamine (3.5 mL, 25.44 mmol) were added to this solution under argon. The temperature of the reaction mixture was raised to 50 °C and the mixture was stirred at this temperature for 1 day. The volatile components were evaporated and the crude product was purified by PTLC on silica gel using dichloromethane as an eluent to get **21** (810 mg, 96%) as a yellowish brown solid.

M.p. = 156 °C. R_f = 0.62 (SiO_2 TLC, dichloromethane:-methanol 20:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm]: 8.46 (dd, J = 8.7, 1.4 Hz, 2H), 7.92 (dd, J = 7.5, 1.1 Hz, 2H), 7.74 (t, J = 8.9, 7.3 Hz, 2H), 3.49 (s, 6H). IR: ν_{max} [cm^{-1}]: 3001, 2958, 2918, 2872, 2850, 1636, 1621, 1568, 1543, 1536, 1432, 1429, 1352, 1300, 1067, 1017, 923, 732, 542. LC-MS: m/z = [MH^+]: 384.1, (Calcd. for $C_{15}H_{13}NO_7S_2$, 383.0).

3.2.9 Preparation of 6,9,23,26-tetraoxa-16,33-diazaheptacyclo[29.3.1.1^{14,18}.0^{5,34}.0^{10,15}.0^{17,22}.0^{27,32}] hexatriaconta-1,3,5(34),10(15),11,13,17,19,21,27,29,31-dodecaene-35,36-dione (11, Scheme 1)

A mixture of acridonediol **4** [24] (100 mg, 0.44 mmol), finely powdered anhydrous caesium carbonate (1.43 g, 4.40 mmol) and dry and pure DMSO (50 mL) were stirred vigorously under argon atmosphere at room temperature for 30 min, then freshly prepared crude chloride **10** (163 mg, 0.44 mmol) in dry and pure DMSO (50 mL) was added dropwise. The temperature of the mixture was raised to 40 °C and the reaction mixture was stirred at this value for 14 days. Water (500 mL) was added to the reaction mixture, which was extracted with ethyl acetate (3 × 200 mL). The combined organic phase was shaken with water (9 × 500 mL) and then with saturated aqueous sodium chloride solution (1 × 500 mL) to remove DMSO. The organic phase was dried over magnesium sulphate, filtered and the solvent was evaporated. The crude product was purified by PTLC on aluminum oxide with

dichloromethane:methanol (5:1) as an eluent, then recrystallized from ethanol to give macrocycle **11** (51 mg, 23%) as a dark yellow solid.

M.p. = >320 °C. R_f = 0.63 (SiO_2 TLC, dichloromethane:-methanol 10:1). ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): δ [ppm]: 9.62 (s, 2H), 7.65 (d, J = 8.0 Hz, 4H), 7.38 (d, J = 8.4 Hz, 4H), 7.06 (t, J = 7.9 Hz, 4H), 4.24 (t, J = 4.7 Hz, 8H). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): δ [ppm]: 176.93, 147.39, 131.84, 121.71, 121.63, 118.13, 115.28, 72.11. IR: ν_{max} [cm^{-1}]: 3425, 2985, 1628, 1582, 1500, 1472, 1371, 1224, 1210, 1153, 1090, 950, 746, 609, 554, 501. HRMS: m/z = [MH$^+$]: 507.1480, (Calcd. for $C_{30}H_{22}N_2O_6$, 506.1478).

3.3 Spectrophotometric studies

UV/Vis spectra were recorded on a UNICAM UV4-100 spectrophotometer controlled by VIZION software version 3.4 [30]. Fluorescence emission spectra were recorded on a Perkin-Elmer LS50B luminescent spectrometer (PerkinElmer Inc., Waltham, USA) and were corrected by FL Winlab spectrometer software version 3.0 [31]. Quartz cuvettes with a path length of 1 cm were used in all cases. Spectroscopic measurements were carried out at room temperature (25±1 °C). Polarizers were not applied. A 290 nm cut off type bandpass filter and 2.5 nm excitation and emission slits were used in the cases of fluorescent studies while exciting at 265 nm. During studies on selectivity, the solutions were added with a *Hamilton*-syringe to the ligand in DMSO. The reported spectra were corrected in each case with the background signal of the added solutions and concentration values were also corrected corresponding to the caused dilution. OriginPro version 8.6 [32] software was used for evaluation and visualization of the spectroscopic results.

Relative quantum yield was determined in acetonitrile according to a literature method [33] based on a comparison with acridone as a standard (absolute quantum efficiency ϕ = 0.41 in acetonitrile) [27]. The excitation and emission spectra were recorded in the same conditions and instrument settings as in the case of the standard. (The excitation wavelength for the ionophore was chosen to be 265 nm, because of better comparability with the previously reported fluorophore subunits and macrocycle analogues [26].)

4 Conclusions

A new bisacridono-macrocycle was synthesized, investigated as a cation-selective optical sensor molecule and compared to its monoacridono-analogues with similar size of macrocyclic cavity. Despite the increased structural rigidity caused by the introduction of the second heteroaromatic unit, a reduced selectivity was observed compared to the previously reported highly Pb^{2+}-selective monoacridono-18-crown-6 ethers [20, 22, 23]. The bisacridono-host is tending to coordinate most of the soft electrophilic cations regardless of their ionic radius, especially those with multiple charges. The soft cation-π and nucleophilic-electrophilic interactions override coordination forces resulting from cavity-size-compatibility. Thus, it cannot be effectively applied in heavy-metal analysis. In addition to the loss of selectivity, the photophysical properties of the new fluorescence macrocycle could not be exploited either as a very similar quantum yield and complexation-induced optochemical signal was obtained.

Although the new crown ether is difficult to synthetize, its acridone units provide several opportunities for post-synthetic modifications, especially *via* the substitution at position *9* of the acridone [26], which may result in acridino-analogues of an improved selectivity. Apart from that the new fluoroionophore proved not to be favorable for optochemical metal-ion- sensing, the replacement of the ethereal-*O* atoms by *N* ones in the macroring can result in macrocycles showing selectivity toward anions or nucleic acids as reported many times in the cases of azacrown-analogues [6–11, 14, 17, 18].

Sections in the Supplement

Further information can be found in the Supplement regarding the following topics:

1. ^{1}H-NMR and ^{13}C-NMR spectra of the new compounds
2. Theoretically estimated conformation and structural flexibility
3. Spectrophotometric investigation of new macrocycle **11.**

Author contributions

Panna Vezse: Investigation, Formal analysis, Visualization, Writing - Original Draft; Dániel Ster: Investigation; Ádám Golcs: Conceptualization, Methodology, Investigation, Writing - Original Draft, Project administration; Péter Huszthy: Writing - Review & Editing, Supervision, Funding acquisition, Resources; Tünde Tóth: Writing - Review & Editing.

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Conflicts of interest

The authors declare no conflicts of interest. The funding institution had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript and in the decision to publish the results.

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