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The synthesis of azacrown ethers with quinoline-based sidearms as potential zinc(II) fluorophores

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Abstract—Two novel fluorophores, **12** and **13**, composed of diaza-18-crown-6 ligands containing two quinoline-based sidearms which are structurally similar to Zn(II) fluorophores TSQ (**1**) and Zinquin (**2**) were synthesized as potential Zn(II) fluorophores by reductive amination of 8-benzenesulfonamido-2-quinoline carboxaldehyde **6** and 8-benzenesulfonamido-6-quinolyloxyacetate-2-carboxaldehyde **11** with diaza-18-crown-6 using sodium triacetoxyborohydride ($NaBH(OAc)_3$) as the reducing agent. Preliminary photophysical properties of ligands **12** and **13** show that they possess the properties necessary to be effective chemosensors for Zn^{2+}. The solid state structure of **12** is also reported.
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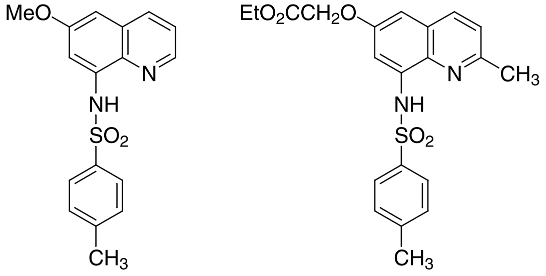
1. Introduction

Zinc is an essential element for humans and plays an important role in biochemical and nutritional processes.[1] Approximately 300 enzymes contain zinc(II) as an essential component, either for structural purposes or as part of a catalytic site.[2] Furthermore, zinc is a global excess burden on the environment due to human activities. Increased levels of zinc are the result of smelting, refining, and manufacturing processes, but domestic waste waters also can contain high local zinc concentrations especially in aquatic ecosystems.[3] The level of zinc in seawater[3,4] is of importance since this metal meets the needs of many species for survival, and for the productivity of plankton.[5] Therefore, there is a need for reliable, fast, and low-cost methods for the quantitative analysis and monitoring of zinc levels in environmental and biological systems.

While development of fluorescent chemosensors has been made for other biologically important divalent metal ions, in particular Ca^{2+} and Mg^{2+} with several selective fluorophores,[6] there are few Zn^{2+} selective fluorophores. Several quinoline-based compounds, such as 6-methoxy-8-*p*-toluenesulfonamido)-quinoline **1** (TSQ)[7,8] and its ester ether derivative **2** (Zinquin)[9] (Fig. 1), are currently the most widely used zinc-activated fluorophores. However, for quantitative analysis of Zn^{2+} either in living cells or in other environments, TSQ and Zinquin are not satisfactory because they are not selective for Zn^{2+} but form complexes with many metal ions with varying fluorescence intensities.[10]



1 (TSQ) **2** (Zinquin)

Figure 1. Structure of zinc-active fluorophores.

A new type of selective and efficient Zn^{2+} fluorophore, a dansylamide-substituted macrocyclic tetraamine (dansylamidoethylcyclen), has been developed by Kimura and co-workers.[11] Recently, we reported an efficient and convenient one-step method to introduce the dansyl chromophone onto macrocyclic polyamines.[12] The success of the dansylamidoethylcyclen fluorophore is due to a high affinity of the aza macrocycle for Zn^{2+}, a strong affinity for aromatic sulfonamides by the Zn^{2+}–cyclen complex, and the strong luminescence property of the dansyl chromophore. Herein, we report the synthesis of two diaza-18-crown-6 ligands containing two quinoline-based sidearms which are structurally similar to TSQ **1** and Zinquin **2**. We expected that attachment of TSQ or Zinquin analogues

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Keywords: chemosensors; fluorophores; crown ethers; quinoline; reductive amination.

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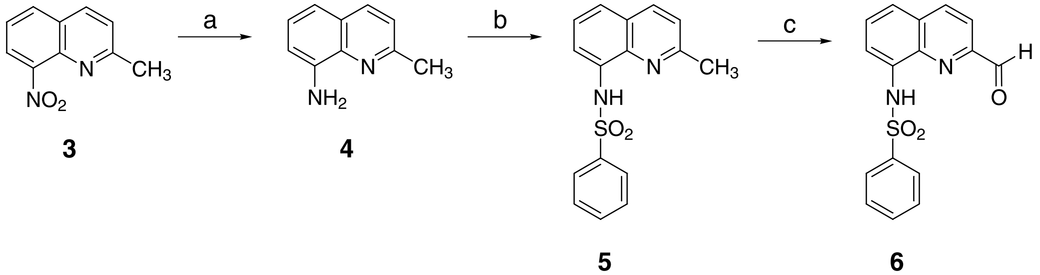
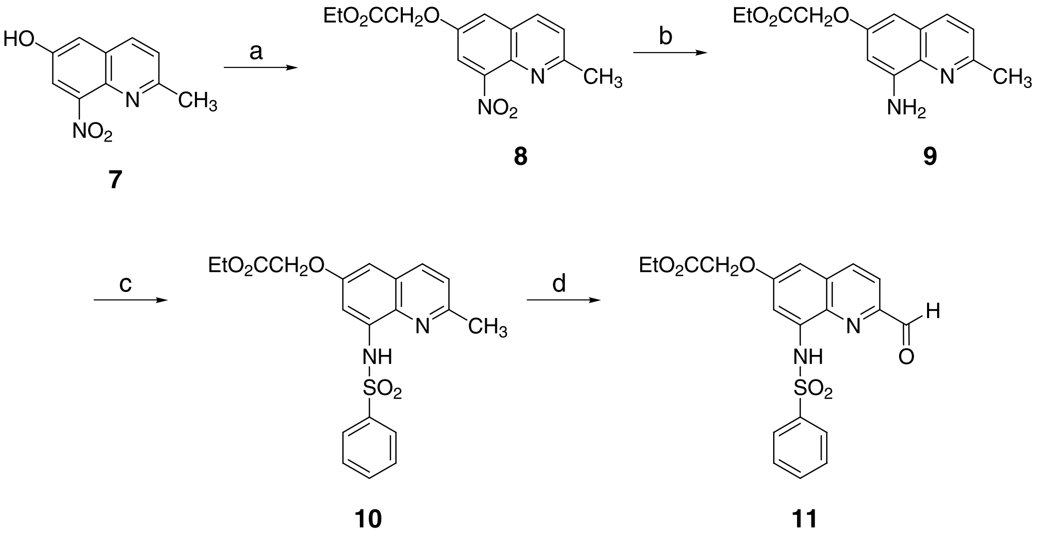
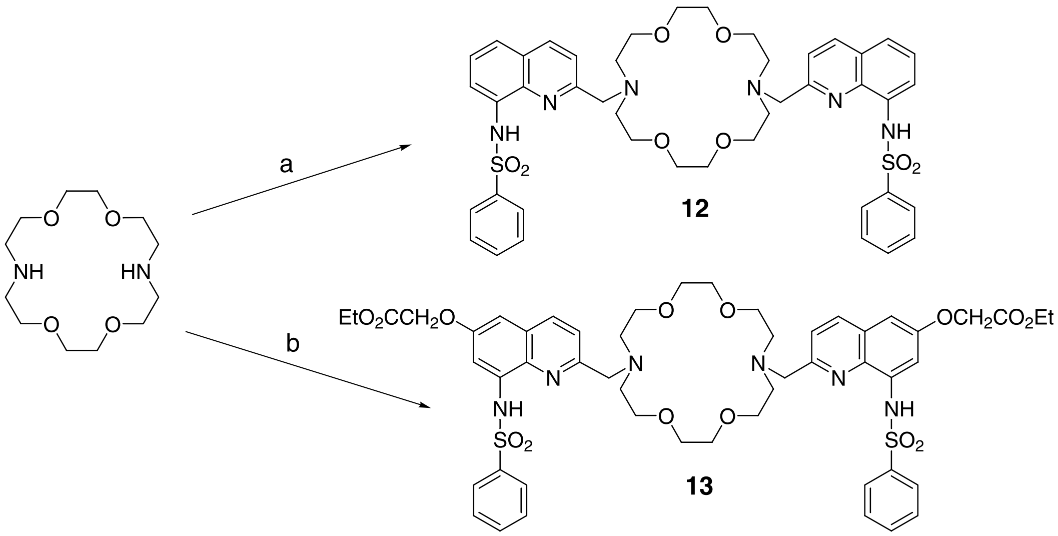


Scheme 1. (a) H_2/Pd-C/EtOH; (b) Benzenesulfonyl chloride/pyridine/0–5°C; (c) SeO_2/1,4-dioxane.



Scheme 2. (a) $BrCH_2CO_2Et/(i\text{-}Pr)_2NEt$; (b) H_2/Pd-C/EtOH; (c) Benzenesulfonyl chloride/pyridine/0–5°C; (d) SeO_2/1,4-dioxane.



Scheme 3. (a) **6**/$NaBH(OAc)_3$/DCE; (b) **11**/$NaBH(OAc)_3$/DCE.

to crown ethers to form **12** and **13** would improve affinities of the ligands for Zn^{2+} or other heavy metal ions. An X-ray crystal structure of ligand **12** is also described. Preliminary photophysical studies show that these two ligands can be effective chemosensors for Zn^{2+}.

2. Results and discussion

The synthesis of ligands **12** and **13**, employing reductive amination,[13] is outlined in Scheme 3. The preparation of quinolinecarboxaldehyde precursor **6** (Scheme 1) began

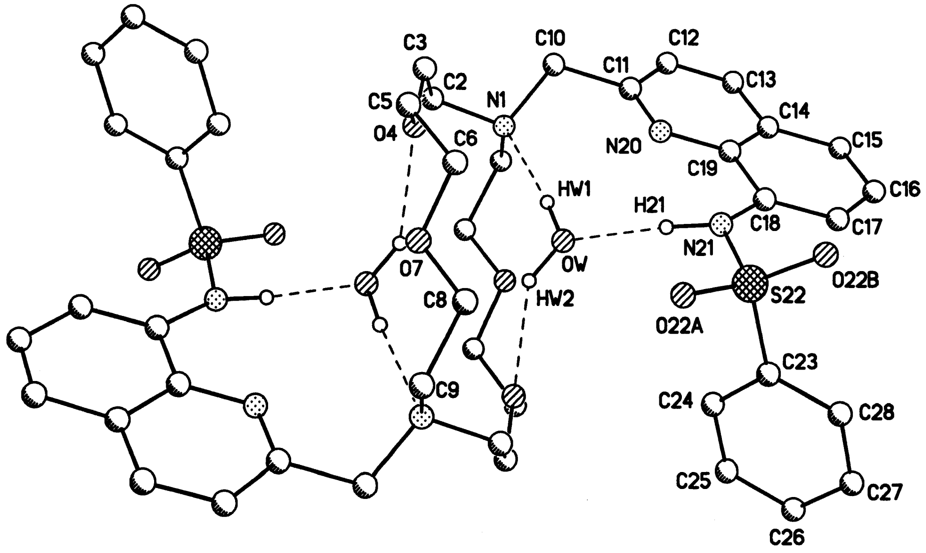


Figure 2. The solid state structure of **12** with hydrogen atoms omitted except for those that are involved in hydrogen bonding.

with commercially available 8-nitroquinaldine (**3**). Reduction of **3** to amino compound **4** was accomplished in 95% yield by hydrogenation using Pd–C as the catalyst. Treatment of **4** with benzenesulfonyl chloride in pyridine at 0–5°C gave **5** in 97% yield. Oxidation of **5** was accomplished with SeO_2 in 1,4-dioxane to provide aldehyde **6** in a 90% yield. Key quinolinecarboxaldehyde precursor **11** (Scheme 2) was prepared from commercially available 6-hydroxy-8-nitroquinaldine (**7**). Intermediates **8** and **9** were prepared by reported methods.[9a] Reaction of **9** with benzensulfonyl chloride in pyridine at 0–5°C gave Zinquin analogue **10** in 90% yield. Oxidation of **10** with SeO_2 in 1,4-dioxane gave aldehyde **11** in 88% yield. Finally, diaza-18-crown-6 was treated with aldehydes **6** and **11** in the presence of $NaBH(OAc)_3$ in DCE to give ligands **12** and **13** in 85 and 78% yields, respectively.

The solid state structure of **12** is shown in Fig. 2. The structure lies on a center of inversion and contains two water molecules of hydration, each interacting with **12** through three hydrogen bonds. It is interesting to note that each water is hydrogen bonded to an oxygen atom and a nitrogen atom of the macrocycle and the amide hydrogen atom of the sidearm. This suggests the possibility that this ligand could bind to two cations such as Zn^{2+} with each interacting with a combination of donor atoms of the macrocycle and the sidearms.

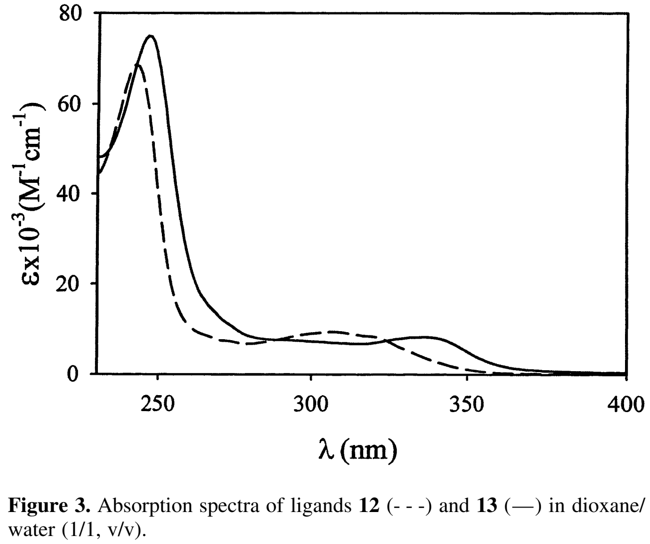


Figure 3. Absorption spectra of ligands **12** (- - -) and **13** (—) in dioxane/water (1/1, v/v).

Preliminary photophysical properties of ligands **12** and **13** and their complexes with Zn^{2+}, Cd^{2+}, and the alkaline earth metal ions have been investigated. All the complexation experiments were carried out in dioxane/water mixtures (1/1, v/v) because of the low solubilities of both compounds in water. Dioxane was preferred to acetonitrile since during some preliminary titrations in MeCN/water, precipitation of the metal complexes was observed. The solutions were buffered at pH 7.4 with 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO).

The two bis(8-benzenesulfonamido)quinoline-containing ligands (**12** and **13**) present quite different absorption spectra; the introduction of an ethereal oxygen on the quinoline moiety resulting in **13** causes a red shift of the two bands (242 and 307 nm) typical of this chromophore (Fig. 3 and Table 1). Both compounds show only a very weak fluorescence originating from a short-lived excited state (Table 1).

The responses of the two ligands to the addition of metal ions are very similar. As can be seen from Figs. 4 and 5, addition of Zn^{2+} to a solution of **12** or **13** leads to strong changes of the absorption spectra until 1 equiv. of metal ion has been added. No further changes were observed for Zn^{2+} concentrations greater than 1 equiv. A different pattern is observed from the fluorescence spectra as can be seen from Figs. 5 and 6. The fluorescence intensity increases in an almost linear manner up to the addition of 2 equiv. of Zn^{2+}. All these findings can be rationalized in terms of the formation in solution of two different complexes with 1:1 and 1:2 (ligand/metal) stoichiometries, respectively; a possibility that was also suggested, as discussed above, from the analysis of X-ray data. Qualitatively, the changes

Table 1. Absorption and luminescence data relative to compounds **12** and **13** and their metal ion complexes in dioxane/water (1/1, v/v) buffered at pH 7.4 with MOPSO

	Absorption			Luminescence		
	λ_{max} (nm)	ε_{max} ($M^{-1}cm^{-1}$)	λ_{max} (nm)	Φ	τ (ns)	log β[a]
12	242	69,000	420	0.003	<0.4	
	307	9400				
12 Zn	261	65,800	503	0.17	15	8.3±0.3
	357	7400				
12 Zn$_2$	260	58,600	514	0.33	21	12.9±0.3
	365	6900				
12 Cd	261	64,300	499	0.15	13	8.3±0.5
	358	8100				
12 Ba	244	51,700	490	0.08	17	4.7±0.1
	258	36,100				
	310	7500				
13	246	73,700	420	0.004	<0.4	
	336	7900				
13 Zn	264	70,300	495	0.19	16	8.8±0.1
	360	8080				
13 Zn$_2$	264	64,800	500	0.37	24	14.6±0.2
	362	7600				
13 Cd	264	68,000	489	0.19	16	8.6±0.6
	360	9000				
13 Ba	251	48,400	480	0.13	15	4.9±0.1
	259	46,000				
	346	7500				

[a] Apparent global association constant.

observed in the absorption and luminescence spectra are very similar to those reported for TSQ and Zinquin at the same pH. For these two ligands at neutral pH, the complexation process was associated with the deprotonation of the sulfonamido group,[7–9] leading to the observed large spectral changes. From the changes in the absorption spectra, it is evident that inclusion of the first metal ion leads to deprotonation of both of the chromophoric groups, while the formation of the bimetallic complex causes only minor changes in the spectra. The increased charge density close to the two chromophoric groups could be sufficient to further increase the luminescence band that, having charge transfer character, is very sensitive to the polarity of the environment. The increase in the observed excited state lifetime on going from the mononuclear to the binuclear complex is consistent with this hypothesis.

It is worth noting that the luminescence quantum yields (φ), as shown in Table 1, increase about a hundred-fold upon complexation, while the increase in the luminescence intensity upon excitation in the 350–400 nm region, where the absorbance of the free ligand is negligible, can be more than a thousand-fold. For this reason, the two ligands are both good OFF/ON luminescent chemosensors for zinc ions since they are almost non-luminescent when uncomplexed and give strongly luminescent complexes with this metal ion.

These ligands also form luminescent complexes with Cd^{2+}. With this ion, no evidence of a 1:2 (ligand/metal) was found. Both the absorption and luminescence data recorded during the titration could be fitted simply for formation of a complex with a 1:1 stoichiometry.

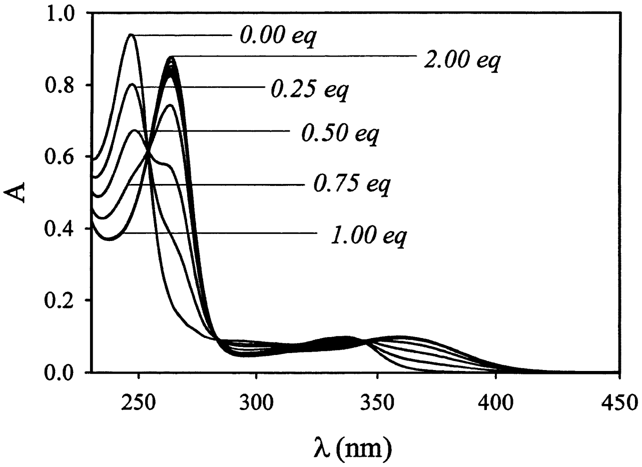


Figure 4. Absorption spectra of a 1.27×10^{-5} M solution of **13** and upon addition of increasing amounts of Zn(ClO$_4$)$_2$.

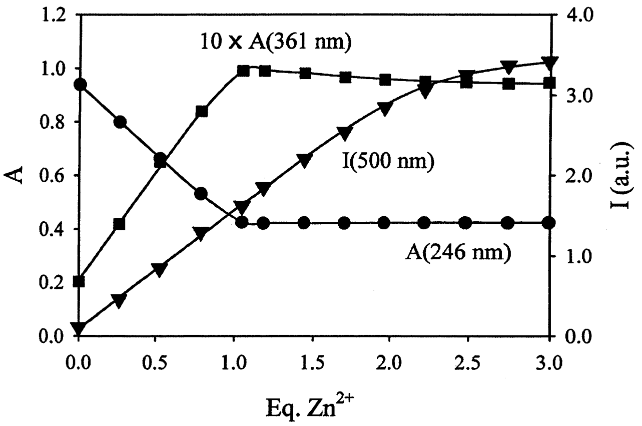


Figure 5. Changes in the absorbance (246 nm, ●, and 361 nm, ■) and in the fluorescence spectra (λ_{ex}=330 nm; λ_{ex}=500 nm, ▲) upon addition of increasing amounts of Zn(ClO$_4$)$_2$ to **13**.

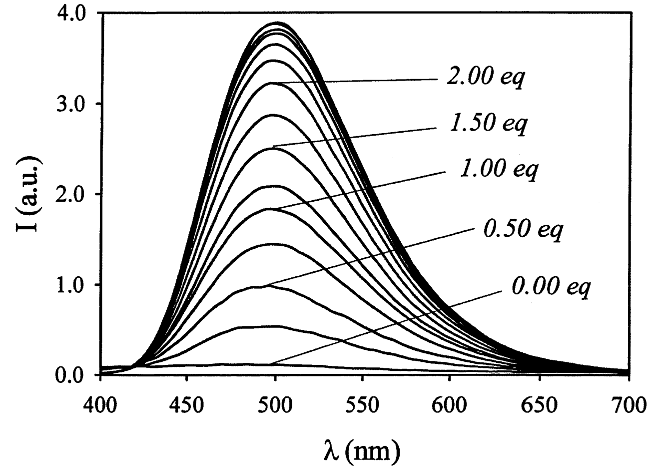


Figure 6. Luminescence spectra (λ_{ex}=330 nm) of a 1.27×10^{-5} M solution of **13** and upon addition of increasing amounts of $Zn(ClO_4)_2$.

Table 2. Crystal data and experimental details for **12**

Formula	$C_{44}H_{50}N_6O_8S_2\cdot 2H_2O$
Formula weight	891.05
Crystal system	Monoclinic
Space group	$P2_1/n$
a (Å)	11.267 (4)
b (Å)	11.757 (2)
c (Å)	16.743 (4)
β (Å)	93.02 (2)
V (Å^3)	2214.7 (9)
Z	2
F(000)	944
D_x (g cm^{-3})	1.336
Crystal size (mm^3)	0.4×0.4×0.2
μ (mm^{-1})	0.185
Temperature (K)	293
$2\theta_{max}$(°)	50.0
Total data	4503
Independent data	3866 (R_{int}=0.0352)
Total parameters	284
Goodness of fit on F^2	1.065
$R[I>2\sigma(I)]$	0.0609
R (all data)	0.1020
Largest diff. peak (e Å^{-3})	0.350
Largest diff. hole (e Å^{-3})	−0.265

Considering alkaline earth metal ions, only barium ions gave stable complexes (see Table 1). Addition of up to 5 equiv. of Mg^{2+}, Ca^{2+}, and Sr^{2+} caused only a very small increase in the luminescence band. From excited state lifetime measurements, it was possible to verify that in all three cases, the new emission was due to a very small amount of a quite strongly emitting species (t=16 ns). This could imply that complexes with Mg^{2+}, Ca^{2+}, and Sr^{2+} are luminescent but have a low stability (log β<3) or that at this pH only a small amount of the complex is present in a form having the two 8-benzenesulfonamidoquinoline groups deprotonated. Some preliminary competition experiments confirmed that the presence of these three metal ions does not cause interference with the determination of Zn^{2+}.

In conclusion, we have attached Zn(II) fluorophores TSQ and Zinquin to diaza-18-crown-6. The synthetic procedure presented here is efficient and convenient, which should make it useful for the synthesis of other new fluorophores. Preliminary photophysical properties of **12** and **13** show that they are effective chemosensors for Zn^{2+}.

3. Experimental

Melting points were taken on a Thomas–Hoover melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded at 200 or 300 MHz and 50 or 75 MHz in $CDCl_3$ unless otherwise indicated. IR spectra were recorded on a Perkin–Elmer paragon 500 Ft-IR spectrometer. HRMS spectra were obtained on a Finnegan 8430 high-resolution mass spectrometer using fast atom bombardent (FAB) and chemical ionization (CI) methods. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. Solvents and starting materials were purchased from commercial sources where available. Compounds **4**,[14] **8**,[9a] and **9**[9a] were prepared by the reported methods. The solvent for photophysical measurements was dioxane/water (1/1, v/v). Absorption spectra were recorded on a Perkin–Elmer lambda 40 spectrophotometer. Uncorrected emission and corrected excitation spectra were obtained on a Fluorolog spectrofluorimeter equipped with a R928 phototube. In order to allow comparison of emission intensities, corrections for instrumental response, inner filter effects,[15] and phototube sensitivity were performed. A correction for differences in the refraction index was introduced when necessary.

3.1. Synthesis of new ligands

3.1.1. 8-Benzenesulfonamidoquinaldine (5). Benzenesulfonyl chloride (2.48 g, 14 mmol) was added dropwise to a cooled, stirred solution of 8-aminoquinaldine (**4**) (1.85 g, 11.7 mmol) in 10 mL of pyridine and the mixture was stirred in an ice bath for 3 h. Ice water was then added and the resulting precipitate was filtered, washed well with water, dried and dissolved in CH_2Cl_2. The resulting solution was washed with 1 M HCl and the aqueous phase was extracted with CH_2Cl_2. The resulting solution was washed with 1 M HCl and the aqueous phase was extracted with CH_2Cl_2 (3×15 mL). The organic layers were combined, washed with a saturated solution of Na_2CO_3, dried ($MgSO_4$) and the solvent was removed under reduced pressure. Crystallization of the residue from EtOH gave 3.38 g (97%) of **5** as white crystals; mp 158–159°C; ^{1}H NMR: δ 9.23 (s, 1H), 7.98–7.74 (m, 4H), 7.44–7.25 (m, 6H), 2.69 (s, 3H); ^{13}C NMR δ 159.7, 145.1, 141.0, 139.7, 138.8, 133.8, 129.0, 128.6, 128.0, 126.9, 125.2, 123.2, 122.8, 25.4; IR (KBr) 3065, 1605, 1439, 1370, 1173, 1068, 795 cm^{-1}; HRMS m/z: calcd for $C_{16}H_{15}N_2O_2S$ $(M+1)^+$: 299.0856, found: 299.0836. Anal. calcd for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73. Found: C, 64.49; H, 4.59.

3.1.2. 8-Benzenesulfonamido-2-quinolinecarboxaldehyde (6). To a stirred suspension of freshly sublimed SeO_2 (3.33 g, 30 mmol) in 150 mL of dioxane at 50–55°C was added a solution of **5** (4.89 g, 16.4 mmol) in 50 mL of dioxane during the course of 1.5 h. The mixture was heated to 90–95°C overnight, filtered, and the dioxane was removed under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to give 4.61 g (90%) of **6** as white needles; mp

194–195°C; ^{1}H NMR: δ 10.13 (s, 1H), 9.11 (s, 1H), 8.22 (d, J=8.4 Hz, 1H), 8.05 (d, J=8.4 Hz, 1H), 7.95–7.87 (m, 3H), 7.61–7.33 (m, 5H); ^{13}C NMR δ 192.6, 150.9, 139.3, 138.2, 137.9, 134.7, 133.3, 130.3, 129.9, 129.2, 127.3, 122.3, 118.2, 116.2; IR (KBr) 2842, 1710, 1503, 1468, 1157, 841, 744 cm^{-1}; HRMS m/z: calcd for $C_{16}H_{12}N_2O_3S$ (M$^+$): 312.0569, found: 312.0562. Anal. calcd for $C_{16}H_{12}N_2O_3S$: C, 61.52; H, 3.87. Found: C, 61.34; H, 3.91.

3.1.3. 2-Methyl-8-(benzenesulfonamido)-6-quinolyloxyacetate (10). Compound **10** was prepared as above for **5** from **9**. Crude compound **10** was purified by column chromatography on silica gel with CH_2Cl_2/hexane (3:1) as eluent followed by recrystallization (CH_2Cl_2/hexane) to give a 90% yield of **10** as a white solid: mp 136–137°C; ^{1}H NMR: δ 9.25 (br, 1H), 7.95–7.83 (m, 3H), 7.53–7.22 (m, 5H), 6.62 (d, J=2.7 Hz, 1H), 4.65 (s, 2H), 4.27 (q, J=7.2 Hz, 2H), 2.63 (s, 3H), 1.29 (t, J=7.2 Hz, 3H); ^{13}C NMR δ 168.5, 155.5, 155.4, 143.7, 136.3, 135.2, 134.4, 134.3, 129.5, 127.1, 126.7, 123.3, 106.7, 101.0, 65.5, 61.5, 24.7, 14.1; IR (KBr) 3252, 2984, 1767, 1607, 1505, 1374, 1341, 1204, 1175, 1160, 1092, 840, 665, 544 cm^{-1}; HRMS m/z: calcd for $C_{20}H_{21}N_2O_5S$ (M+1)$^+$: 401.1172, found: 401.1161. Anal. calcd for $C_{20}H_{20}N_2O_5S$: C, 59.98; H, 5.03. Found: C, 59.79; H, 5.23.

3.1.4. 8-Benzenesulfonamido-6-quinolyloxyacetate-2-carboxaldehyde (11). Compound **11** was prepared as that for **6** from **10**. Crude compound **11** was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to give an 88% yield of **11**; mp 154–155°C, ^{1}H NMR: δ 10.13 (s, 1H), 9.15 (s, 1H), 8.12–7.95 (m, 4H), 7.65–7.40 (m, 4H), 6.73 (d, J=2.6 Hz, 1H), 4.68 (s, 2H), 4.30 (q, J=7.2 Hz, 2H), 1.30 (t, J=7.2 Hz, 3H); ^{13}C NMR δ 192.6, 168.0, 151.0, 139.5, 138.3, 138.0, 134.8, 133.5, 130.4, 130.0, 129.3, 127.6, 122.4, 118.5, 116.4, 65.8, 61.7, 14.1; IR (KBr) 3260, 3085, 1720, 1630, 1590, 1361, 1138, 872, 782 cm^{-1}; HRMS m/z calcd for $C_{20}H_{18}N_2O_6SNa$ (M+Na)$^+$: 437.0784, found: 437.0781. Anal. calcd for $C_{20}H_{18}N_2O_6S$: C, 57.96; H, 4.38. Found: C, 57.71; H, 4.38.

3.1.5. 7,16-Bis[(8-benzenesulfonamido)-2-quinolinylmethyl]diaza-18-crown-6 (12). A mixture of diaza-18-crown-6 (393 mg, 1.5 mmol) and **6** (1.03 g, 3.3 mmol) in 25 mL of dichloroethane was stirred with NaBH(OAc)$_3$ (848 mg, 4.0 mmol) under nitrogen at room temperature for 5 h. The reaction was then quenched with saturated Na_2CO_3 (15 mL) and the mixture was extracted with CH_2Cl_2 (3×10 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with acetone as eluent to give 1.09 g (85%) of **12** as white crystals; mp 184–185°C; ^{1}H NMR: δ 9.15 (br, 2H), 8.03–7.70 (m, 10H), 7.41–7.32 (m, 10H), 4.0 (s, 4H), 3.69 (t, J=5.5 Hz, 8H), 3.65 (s, 8H), 2.92 (t, J=5.5 Hz, 8H); ^{13}C NMR: δ 160.1, 139.7, 137.7, 136.4, 133.4, 132.8, 128.8, 127.2, 126.2, 122.1, 122.0, 115.5, 70.9, 70.0, 62.1, 54.5; IR (KBr) 3235, 2871, 1600, 1571, 1505, 1472, 1353, 1167, 1124, 1090, 930, 845, 754, 686, 583 cm^{-1}; HRMS m/z calcd for $C_{44}H_{48}N_6O_8S_2Na_3$ (M−2H+3Na)$^+$: 921.2671, found: 921.2667. Anal. calcd for $C_{44}H_{50}N_6O_8S_2$: C, 61.80; H, 5.89. Found: C, 61.96; H, 5.81.

3.1.6. 7,16-Bis[(8-benzenesulfonamido-6-oxyacetate)-2-quinolinylmethyl]diaza-18-crown-6 (13). Macrocycle **13** was prepared as above for **12** from diaza-18-crown-6 (393 mg, 1.5 mmol) and **11** (1.37 g, 3.3 mmol). Crude **13** was purified by column chromatography on silica gel with acetone/CH_2Cl_2 (3:1) as eluent to give a 78% yield of the pure product: mp <35°C; ^{1}H NMR: δ 7.97–7.84 (m, 8H), 7.62–7.39 (m, 10H), 6.64 (s, 1H), 6.63 (s, 1H), 4.68 (s, 4H), 4.29 (q, J=7.2 Hz, 4H), 3.95 (s, 4H), 3.67 (t, J=5.5 Hz, 8H), 3.63 (s, 8H), 2.89 (t, J=5.5 Hz, 8H), 1.31 (t, J=7.2 Hz, 6H); ^{13}C NMR: δ 168.4, 157.7, 155.9, 139.6, 135.4, 134.7, 134.2, 132.9, 128.9, 127.9, 127.2, 122.7, 107.4, 101.7, 70.8, 70.0, 65.7, 61.8, 61.5, 54.4, 14.1; IR (KBr) 3260, 3236, 2875, 1605, 1570, 1510, 1470, 1471, 1351, 1165, 1123, 1090, 848, 750, 685, 581 cm^{-1} HRMS: m/z calcd for $C_{52}H_{62}N_6O_{14}S_2Na$ (M+Na)$^+$: 1081.3667, found: 1081.3678. Anal. calcd for $C_{52}H_{62}N_6O_{14}S_2$: C, 58.96; H, 5.90. Found: C, 58.75; H, 5.77.

3.2. X-Ray crystallography data

Crystal data and experimental details are listed in Table 2. Single crystal data were collected using a Bruker P4 automated diffractometer which utilized graphite monochromated Mo Kα radiation. The structure was solved using direct methods and refined on F^2 using a full-matrix, least-squares procedure. All non-hydrogen atoms were refined anisotropically. Positions for hydrogen atoms with the exception of those bonded to C9, N21 (the amide nitrogen) and the water oxygen were calculated. The positions for the other hydrogens were obtained from a difference map. All hydrogen atoms were allowed to ride on their neighboring heavy atoms during the refinement. The structure was solved, refined and displayed using SHELXTL PC[16] computer program package.

3.3. Experimental data for UV–Vis and fluorescence titration experiments

In a typical experiment, a solution of ligand (concentration 2×10^{-5} M) in dioxane/water (1/1, v/v) was titrated by increasing amounts of a solution of the perchlorate salt of the cation of interest (concentration 5×10^{-3} M) at room temperature. After each addition of aliquots, the UV–Vis and fluorescence spectra of the solution were recorded. When the titration was completed, the spectra were implemented into the Specifit software.[17]

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