



Diaminoterephthalate-EDTA and -EGTA Conjugates – "Turn on"-Fluorescence Sensors for Zinc Ions

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Dedicated to Professor K. Peter C. Vollhardt on the occasion of his 75th birthday

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Graphic for the Table of Contents

Fluorescence sensors.



 $λ_{abs}$ = 427 nm, $λ_{em}$ = 511 nm, Φ = 0.07 $\square 2$ Φ = 0.41

Text for the table of contents: Zinc turns it on! Conjugation of the fluorescence dye diaminoterephthalate with EDTA results in a constitutionally very simple sensor $(DAT)_2EDTA$ with remarkable selectivity towards Zn^{2+} ions. In the presence of one equivalent of $Zn(OAc)_2$ in solution, the fluorescence quantum yield is increased by factor of about six!

Keywords: Diaminoterephthalates; Zinc ions; Fluorescence; Sensors; Chelate ligands

Abstract: A highly effective "turn-on" fluorescence sensor with high selectivity for Zn^{2+} ions is reported. Its constitution simply consists of two units of the fluorescence dye diaminoterephthalate (DAT) and the chelate ligand EDTA. In total, four products (DAT)₂EDTA and (DAT)₂EGTA were prepared by conjugation of DATs with chelate ligands EDTA and EGTA as sensors for metal ions in solution. The fluorescence of all four compounds is strongly quenched by coordination of Cu²⁺ and Ni²⁺ ions. For one EDTA-derivative, the fluorescence

quantum yield is increased by the factor of five with ten equivalents of ScCl₃. For both EDTA derivatives, the fluorescence quantum yield increased by the factor of five with one equivalent $Zn(OAc)_2$. Therefore, the $(DAT)_2EDTA$ structures define fluorescence sensors with a high selectivity for Zn^{2+} accompanied by a strong "turn-on" effect with Zn^{2+} ions. Furthermore, the coordination geometry of $(DAT)_2EDTA$ with Zn^{2+} was elucidated by X-ray single crystal structure analysis of the trinuclear complex $[ZnCl_2]{Zn(OH_2)[(DAT)_2EDTA - 2 H]}_2 \cdot 6.5 H_2O$.

Introduction

Zinc is one of the essential trace elements for metabolism.^[1] Zinc ions are found in all mammalian cells, either free or bound to a large number of proteins.^[2] Zinc is a cofactor for numerous enzymes and fulfills many different functions in the body.^[3] It plays a key role in the metabolism of carbohydrates, fat, and proteins and is involved in the development of genetic material and cell growth.^[4] Both the immune system^[5] and many hormones need zinc to function.^[6] Monitoring zinc ions in environmental or biological matrices by fluorescence assays is therefore an important issue.^[7, 8]

Fluorescence-based analytical techniques are valuable tools in different branches of chemistry and biology.^[9] Molecular fluorescence sensors are of particular interest, because of their high sensitivity down to the molecular level combined with "on-off" switchability.^[10] Such sensors for Zn²⁺ as a protoypical d¹⁰ ion are well known.^[8, 11] A so far underrated class of fluorescence dyes are 2,5-diaminoterephthalates (DAT **1**, Figure 1) which defines a chromophore with relatively simple constitution.^[12] With holding two carboxylate and two amino functions, the DAT structural motif allows for straightforward functionalization by amide coupling. EDTA (**2**, ethylenediamine tetraacetic acid) and EGTA [**3**, ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid] are chelate ligands exhibiting strong affinity to divalent ions like Ca²⁺ and Mg²⁺, but are also known to bind Zn²⁺. In continuation of our research projects on DAT fluorescence sensors for biochemical applications,^[13] we propose the conjugation of the chromophore **1** with chelate ligands **2** or **3** to obtain fluorescence or increase the quantum yield upon binding of a metal ion; actually to either quench the fluorescence or increase the quantum yield upon binding of a metal ion; actually, the latter concept of a "turn-on probe" was already achieved with a DAT chromophore before.^[14]



Figure 1. The prototypical example **1** of the fluorescence dye diaminoterephthalate (DAT) and chelate ligands EDTA (**2**) and EGTA (**3**).

Results and Discussion

Organic synthesis. The obvious strategy for ligation of DAT (1) with EDTA (2) and EGTA (3) is amide formation from the amino function of **1** with one carboxylate function of either **2** or **3**. The diesters 6a and 6b with one primary (NH₂) and one secondary (NHR') amino function have therefore been prepared starting from dimethyl and diethyl terephthalates 4a^[11] and 4b (Scheme 1), which were accessed from the respective dialkyl succinyl succinates according to Sinnreich.^[15] Conversion with Boc₂O gave the monocarbamates **5a** (70%, 94% based on recovered starting material; brsm) and **5b** (83%).^[16] In the series leading to *N*-methyl compound 6a, the alkylation was accomplished with one equivalent of Mel. Compound 7a was obtained in 46% yield (79% brsm); di- or oligomethylated species could not be detected. The N-benzylation of compound **5b** was accomplished by reductive amination with benzaldehyde according to a literature protocol^[17] (92% yield of compound **7b**). Finally, the Boc-protective groups were cleaved with TFA in CH₂Cl₂ to furnish primary amines **6a** (94%) and **6b** (100%). These compounds were then converted with commercially available EDTA-anhydride (8)[18] to furnish the amides (DAT)₂EDTA 9a (65%) and 9b (85%). Actually, the product 9b was prepared and investigated first. Since we were hoping for enhanced quantum yields when minimizing vibrational and rotational degrees of freedom, we have prepared the compound 9a with three methyl groups instead of ethyl and benzyl afterwards.



Scheme 1. Synthesis of DATs 6a and 6b and conjugation with EDTA-anhydride 8; reagents and conditions: (a) 1.1 equiv. Boc₂O, CH₂Cl₂, 23°C, 2 d; (b) for 5a: 1.0 equiv. MeI, 2.0 equiv. K₂CO₃, MeCN, 82°C, 16 h; for 5b: 1.5 equiv. PhCHO, 0.5 equiv. ZnCl₂, 1.5 equiv. NaBH₃CN, CH₂Cl₂, 23°C, 16 h; (c) TFA, CH₂Cl₂, 23°C, 16 h; (d) 2 equiv. 6a or 6b, 1 equiv. 8, DMF, 23°C, 17 h.

EGTA-anhydride **10** was prepared from EGTA (**3**) and Ac₂O according to a literature protocol.^[19] It was then converted with DATs **6a** and **6b** to furnish the conjugates **11a** (53%) and **11b** (76%, Scheme 2).



Scheme 2. Conjugation of DATs 6a and 6b with EGTA-anhydride 10; conditions (a) for 6a: DMF-MTBE, 50°C, 18 h; for 6b: DMF, 23°C, 20 h.

Spectroscopy. Being typical push-pull aromatic systems, all DAT derivatives are colored materials (yellow, orange, or red) showing pronounced fluorescence in solution (Table 1). The absorption and emission wavelengths are sensitively tuned by the electron-accepting or -donating nature of the nitrogen-substituents at the aromatic ring. Starting materials 4a and 4b show the absorption at 432–434 nm with a Stokes shift of approx. 100 nm (emission at 532–534). Introduction the Boc-group as an acceptor at one nitrogen atom in compounds 5a and 5b results in an hypsochromic shift of both, absorption and emission band (408-411 nm and 488-494 nm, resp.; Stokes shift 80-83 nm). A bathochromic shift of 11-25 nm of both, absorption and emission wavelengths, is achieved by introduction of the alkyl residue at one nitrogen atom (compounds 7a and 7b). After deprotection, i.e. without N-acceptor moiety, N-monoalkyl (compounds 6a and 6b), a further bathochromic shift is observed towards the absorption at 453-459 nm and emission at 550-558 nm; the Stokes shift rises to its initial value of approx. 100 nm. The quantum yields of all compounds range between 0.069 and 0.38. Except for compound 5a and 5b, the quantum yields of the compounds in the "trimethyl a-series" are lower than the values for the "benzyldiethyl b-series", although it was expected the opposite, since compounds of the "bseries" possess more vibrational and rotational degrees of freedom. Values for compound with a Boc-goup (5a, 5b, 7a, 7b) are higher than for compounds without Boc-group (4a, 4b, 6a, 6b, 9a, 9b). An exception are the EGTA-conjugates 11a and 11b.

Compound	$\lambda_{ ext{max}}$ / nm	lg (ϵ / dm ³ mol ⁻¹ cm ⁻¹)	λ_{em} / $nm^{[a]}$	$\Phi^{[b]}$
4a	434	3.72	534	0.10
4b ^[14]	432	3.75	532	0.18
5a	408	3.75	488	0.38
5b	411	3.78	494	0.34
6a	459	3.73	558	0.06
6b	453	3.62	550	0.23
7a	433	3.80	511	0.23
7b	428	3.88	505	0.41
9a ^[c]	427	4.03	511	0.069
9b ^[c]	424	4.05	505	0.099
11a ^[c]	422	3.95	512	0.23
11b ^[c]	416	3.94	505	0.36

Table 1. Spectroscopic properties of diaminoterephthalates in CH₂Cl₂ ($c = 0.1 \text{ g L}^{-1}$).

[a] Excitation at λ_{max} of the absorption band. [b] Quantum yields were determined according to the Parker Rees method^[20] using rhodamine B in EtOH as standard [λ_{max} = 544 nm, lg(ϵ / dm³ $mol^{-1} cm^{-1}$) = 3.23, λ_{em} = 569 nm, Φ = 0.46].^[21] [c] in DMSO as solvent (c = 0.1 g L⁻¹).

Complexation of metal ions. The influence of several metal ions on the absorption and emission spectra of the four dyes 9a, 9b, 11a, and 11b was investigated by adding 10 equiv. of the respective metal chloride to a stock solution of the dyes in DMSO, i.e. n(metal ion)/n(dye) 10:1 with c(dye) = 0.136 mmol L⁻¹. As metal salts LiCl, NaCl, KCl, MgCl₂ · 6 H₂O, CaCl₂ · 2 H₂O, SrCl₂ · 6 H₂O, BaCl₂ · 2 H₂O, ScCl₃ · 6 H₂O, VCl₃, CrCl₃ · 6 H₂O, MnCl₂ · 4 H₂O, FeCl₃ · 6 H₂O, CoCl₂ · 6 H₂O, NiCl₂ · 6 H₂O, CuCl₂ · 2 H₂O, ZnCl₂, CdCl₂ · H₂O, HgCl₂, PdCl₂, and AlCl₃ · 6 H₂O were applied. Table 2 lists the absorption (λ_{max}) and emission wavelengths λ_{em} together with the fluorescence quantum yield Φ if the latter differs significantly (±10%) from the value Φ_0 (listed in the first line of each compound section, i.e. entries 1, 13, 25, 33) of the dye without metal ion additive (for all data see Supporting Information). The column Φ/Φ_0 lists the relative change of the quantum yields if it is smaller than 0.9 or larger than 1.1. The values for molar absorption coefficient do not change significantly from the data listed in Table 1, thus, Ig ε is not given in Table 2. First of all, we would like to mention that the addition of Fe³⁺ or Pd²⁺ ions results

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in an unspecific absorption spectrum, thus, neither values for λ_{max} nor quantum yields Φ can be determined (entries 4, 11, 16, 23, 27, 32, 37, 41). Secondly, the addition of alkali or alkaline earth salts does not affect the emission spectra of (DAT)₂EDTA 9a and 9b as well as (DAT)₂EGTA **11a**, although EDTA and EGTA are known to be typical chelators for Mg²⁺ and Ca²⁺. Therefore, we presume that these ions actually bind to the ligands, but do not effect the quantum yields. However, for (DAT)₂EGTA **11b**, Li⁺ and Na⁺ result in slightly lower quantum yields ($\Phi/\Phi_0 = 0.85-0.88$; entries 34 and 35). Furthermore, Co²⁺ results in a small decrease of the quantum yields for dyes **9a**, **9b**, **11a**, and **11b** ($\Phi/\Phi_0 = 0.34-0.84$, entries 5, 17, 28 and 38); additionally, the EGTA-derivatives **11a** and **11b** show the same effect with Cr³⁺ (entry 36) and Ni²⁺ (entries 29 and 39). However, Cu²⁺ is a strong fluorescence guencher ($\Phi/\Phi_0 < 0.15$) for all four dyes 9a, 9b, 11a, and 11b (entries 7, 19, 30 and 40); additionally, the EDTA-derivatives 9a and **9b** are strongly guenched with Ni²⁺ (entries 6 and 18). Cu²⁺ (d⁹) and Ni²⁺ (d⁸) are known to quench fluorescence when bound in proximity of a respective dye. Most sensors for Cu²⁺ are based of this process.^[22] On the other hand, the fluorescence quantum yield increases a little bit for the three dyes **9a**, **9b**, and **11a** in the presence of V³⁺ ($\Phi/\Phi_0 = 1.2-1.7$, entries 3, 15, and 26). Such a small effect is also observed for the EDTA-derivatives **9a** and **9b** with Cd²⁺, Hg²⁺, and Al³⁺ (entries 9, 10 12, 21, 22, 24) as well as for EDTA-derivative **9b** with Sc³⁺ (entry14) and EGTA-derivative **11a** with Hg²⁺ (entry 31).

Up to that point, the results are more or less unspectacular. A significant increase of the fluorescence quantum (by the factor of $\Phi/\Phi_0 = 2.6$) yield was observed when treating EDTA-derivatives **9a** and **9b** with Zn²⁺ (entries 8 and 20). An even larger effect ($\Phi/\Phi_0 = 4.1$) is observed with Sc³⁺ at compound **9a** (entry 2). For dyes **11a** and **11b** no increase of fluorescence quantum yield was overserved with any metal ion investigated. As mentioned in the introduction, zinc ions are of great interest in biological environment, thus, the high selectivity of the "turn-on" effect on the fluorescence quantum yields can be considered as highly interesting. In contrast to zinc, scandium ions do not play a role in a biological context and are actually not present in such matrices, thus, the cross-selectivity of compound **9a** towards Sc³⁺ is not relevant for further investigations. Anyhow, Table 2 collects data for the ratio $n(Zn^{2+})/n(dye) = 10$. Therefore, we next investigated the dependence of this "turn-on" effect on stoichiometry and on the counter ion.

Table 2. Changing the photophysical data by addition various metal chlorides to solutions of the dyes **9a**, **9b**, **11a**, and **11b** in DMSO [c(dye) = 0.136 mmol L⁻¹ with n(metal ion)/n(dye) 10:1]. Data are only listed when the relative change of the quantum yield is lower than $\Phi/\Phi_0 = 0.9$ (color coded in red) or larger than $\Phi/\Phi_0 = 1.1$ (color coded in blue), which Φ_0 being the quantum yield of the dye without salt additive.

Entry	Dye	Metal salt	Φ ^[a]	Φ/Φ ₀	λ_{em} / $\text{nm}^{[b]}$	λ_{max} / nm
1	9a	_	0.069	1	511	427
2	9a	ScCl ₃ · 6 H ₂ O	0.283	4.1	503	394
3	9a	VCI ₃	0.114	1.7	512	409
4	9a	FeCl ₃ · 6 H ₂ O	_	_	_	_
5	9a	$CoCl_2 \cdot 6 H_2O$	0.024	0.35	512	398
6	9a	NiCl ₂ · 6 H ₂ O	0.009	0.13	506	395
7	9a	$CuCl_2 \cdot 2 H_2O$	0.007	0.10	507	392
8	9a	ZnCl ₂	0.176	2.6	511	406
9	9a	$CdCl_2 \cdot H_2O$	0.090	1.3	510	425
10	9a	HgCl ₂	0.115	1.7	512	423
11	9a	PdCl ₂	-	_	_	_
12	9a	AICI3 · 6 H ₂ O	0.109	1.6	512	421
13	9b	_	0.099	1	505	424
14	9b	ScCl ₃ · 6 H ₂ O	0.145	1.5	496	392
15	9b	VCI ₃	0.173	1.7	506	406
16	9b	FeCl ₃ · 6 H ₂ O	-	_	_	_
17	9b	$CoCl_2 \cdot 6 H_2O$	0.042	0.42	504	395
18	9b	NiCl ₂ · 6 H ₂ O	0.013	0.13	500	392
19	9b	CuCl ₂ · 2 H ₂ O	0.011	0.11	503	390
20	9b	ZnCl ₂	0.258	2.6	503	404
21	9b	CdCl ₂ · H ₂ O	0.132	1.3	505	420
22	9b	HgCl ₂	0.147	1.5	505	421
23	9b	PdCl ₂	-	-	_	_
24	9b	AICI ₃ · 6 H ₂ O	0.139	1.4	505	419
25	11a	_	0.233	1	512	422
26	11a	VCI ₃	0.268	1.2	510	415
27	11a	FeCl ₃ · 6 H ₂ O	_	-	_	-

28	11a	CoCl ₂ · 6 H ₂ O	0.196	0.84	512	419
29	11a	NiCl ₂ · 6 H ₂ O	0.188	0.81	511	412
30	11a	$CuCl_2 \cdot 2 H_2O$	0.027	0.12	511	391
31	11a	HgCl ₂	0.285	1.2	512	418
32	11a	PdCl ₂	_	_	_	_
33	11b	_	0.358	1	505	416
34	11b	LiCl	0.304	0.85	503	417
35	11b	NaCl	0.314	0.88	505	417
36	11b	CrCl ₃ · 6 H ₂ O	0.311	0.87	505	418
37	11b	FeCl ₃ · 6 H ₂ O	-	-	-	-
38	11b	CoCl ₂ · 6 H ₂ O	0.292	0.82	506	415
39	11b	NiCl ₂ · 6 H ₂ O	0.271	0.76	504	410
40	11b	CuCl ₂ · 2 H ₂ O	0.041	0.11	504	385
41	11b	PdCl ₂	-	-	-	_

[a] see footnote [b] of Table 1. [b] excitation at the wavelengths λ_{max} of the dye without added metal salt.

For dyes **9a** and **9b**, we have investigated the dependence of the fluorescence quantum yield on the ratio Zn^{2+}/dye as well as the counter ion, chloride vs. acetate. In Table 3, the relative values Φ/Φ_0 are listed. For ZnCl₂, the values are for one equiv. ZnCl₂ not significantly enhanced $(\Phi/\Phi_0 = 1.4-1.5, \text{ entry 3})$. For 10 equiv., the value is as already depicted in Table 2 (entry 4). It further rises for 100 (entry 5) and 1000 equiv. ZnCl₂ (entry 6) up to $\Phi/\Phi_0 = 5.9$. More interesting is the effect of the counter ion: While 0.1 equiv. Zn(OAc)₂ has no significant effect, it reaches $\Phi/\Phi_0 > 5$ for both dyes **9a** and **9b** already for one equiv. Zn(OAc)₂. For 10 equiv. Zn(OAc)₂ is then lower again, which is however due to incomplete dissolution of the Zn(OAc)₂ (even after ultrasonication of the sample) which leads to significant scattering of the excitation beam. For more than 1000 equiv. Zn(OAc)₂, emission spectra could not be recorded anymore due to the same reason. While ZnCl₂ is a Brønsted acid in the presence of moisture, the acetate is surely not. Thus, coordination to the carboxylate functions of the EDTA-part of the dye is enhanced by deprotonation.

The next step for the development of an operating sensor system will be to investigate whether other metal ions are affecting the "turn-on" effect caused by Zn²⁺. Preliminary, qualitative experiments indeed resulted in a small change on fluorescence quantum yields when apart from

Zn²⁺ one equivalent of Na⁺ and K⁺ is present. With Ca²⁺ and Mg²⁺ this change is almost insignificant.

Φ/Φ ₀		ZnCl ₂	ZnCl ₂	Zn(OAc) ₂	Zn(OAc) ₂
entry	n(Zn ²⁺)/n(dye)	dye 9a	dye 9b	dye 9a	dye 9b
1	0.01	1.0	1.1	1.1	1.1
2	0.1	1.2	1.2	1.3	1.4
3	1	1.5	1.4	5.9	5.3
4	10	2.6	2.6	5.0	4.3
5	100	5.0	4.8	4.0	3.9
6	1000	5.9	5.3	-	_

Table 3. Dependence of the relative quantum yield Φ/Φ_0 on the concentration and the counterion of the Zn²⁺ salt; c(dye) = 0.136 mmol L⁻¹ in DMSO.

In Figure 2, the absorption and emission spectra of compound **9a** without and with one equiv. $Zn(OAc)_2$ as additive are shown. While both absorption spectra are about equal in intensity (actually, in the presence of Zn^{2+} , there is a small hypsochromic shift of $\Delta\lambda = 25$ nm), the two graphs for the emission spectra clearly show the "turn-on" effect of the Zn^{2+} ions since an almost sixfold increase of fluorescence intensity is observed. This "turn-on" effect is visible with the bare eye when irradiating the sample with blue LED light (see Supporting Information for a video).



Figure 2. Absorbance and emission spectra of compound **9a** without and with one equiv. $Zn(OAc)_2$ added. All data were recorded with c(dye) = 0.136 mmol L⁻¹ solutions in DMSO; emission spectra were excited at 427 nm. Color code: without Zn^{2+} , absorbance violet, emission blue; with Zn^{2+} absorbance green, emission red.

Crystallographic investigation. The coordination geometry of the $(DAT)_2EDTA$ chromophore to the Zn²⁺ center was established by single crystal X-ray structure determination.^[23] A crystalline complex of the twice deprotonated $(DAT)_2EDTA$ -dianion $(\mathbf{9b} - 2 H)^{2-}$ and Zn²⁺ was grown from a solution of compound $\mathbf{9b}$ and 10 equiv. of ZnCl₂ in EtOH by slow evaporation. The material contained single crystals suitable for a structure determination. A trinuclear complex with the stoichiometry $[ZnCl_2][Zn(OH_2)(\mathbf{9b} - 2 H)]_2 \cdot 6.5 H_2O$ (C₉₆H₁₂₅Cl₂N₁₂O_{36.5}Zn₃) was obtained. However, the two $[Zn(OH_2)(\mathbf{9b} - 2 H)]_2 \cdot 6.5 H_2O$ (C₉₆H₁₂₅Cl₂N₁₂O_{36.5}Zn₃) was obtained. However, the two $[Zn(OH_2)(\mathbf{9b} - 2 H)]$ units are crystallographically not equal. Therefore, three different zinc-polyhedra (Zn1, Zn2, and Zn3) are observed (Figure 3). Zn3, located in the center of the structure, is tetrahedrally coordinated by two chloro atoms and two oxygen atoms O6 and O21 from carboxylate groups of different ligands. Therefore, the two different $[(DAT)_2EDTA - 2 H]^{2-}$ units are linked by this $[ZnCl_2O_2]$ tetrahedron. The second oxygen atom of the carboxylate groups, O5 or O20, coordinates either to Zn1 or Zn2 forming a five-membered chelate ring with a nitrogen atom N3 or N9. These carboxylates are therefore bridging Zn3 with Zn1 or Zn2, respectively.



Figure 3. The ORTEP-representation of the structure of compound $[ZnCl_2][Zn(OH_2)(9b - 2 H)]_2$ • 6.5 H₂O in the solid state; hydrogen atoms and crystal water are omitted for clarity; color code: C colorless, O red, N dark blue, Zn light blue, Cl green. ((please two column format))

The zinc center Zn1 is coordinated by two nitrogen and four oxygen atoms in a distorted octahedron (Figure 4). The EDTA-nitrogen centers N2 and N3 are in *cis*-arrangement and form a five membered chelate ring. A water molecule with O15 is coordinating *trans* to N2; *trans* to N3 is carboxamide oxygen O1. The axial positions are occupied by carboxylate oxygens O3 and O5, with O5 belonging to the bridging carboxylate group which coordinates with O6 to Zn3.



Figure 4. Coordination sphere of Zn1. ((please two column format))

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The coordination sphere of zinc Zn2 is very similar to Zn1, with N8 and N9 in *cis*-arrangement (Figure 5). A water molecule is *trans* to N8 (with oxygen O30), while oxygen O16 of a carboxamide function is in *trans*-arrangement to N9. The two carboxylates are again in *trans*-positions with O18 and O20; the latter belongs to the bridging carboxylate coordination with O21 to Zn3. The most significant difference of this structure to the octahedral coordination of EDTA to divalent cations is that one water occupies a position instead of a carbonyl oxygen atom. It can be assumed, that coordination of a second carboxamide oxygen atom to the Zn ion would place the two DAT moieties in close proximity to each other. This seems to be avoided in the present structure either due to steric hindrance or by disadvantageous packing factors within the unit cell.



Figure 5. Coordination sphere of Zn2. ((please two column format))

Conclusion

The fluorescence dye diaminoterephthalate (DAT) was conjugated with chelate ligands EDTA and EGTA. Four products (DAT)₂EDTA **9a** and **9b** and (DAT)₂EGTA **11a** and **11b** were prepared as potential sensor compounds for metal ions. The effect of various metal chlorides on the fluorescence quantum yield was investigated. While some metal salts lower quantum yields by a factor of 0.35–0.88, Cu²⁺ is a strong fluorescence quencher of all four dyes **9a**, **9b**, **11a** and **11b** and Ni²⁺ for EDTA-derivatives **9a** and **9b**. More interesting was the enhancement of quantum yields, which was observed for EDTA-derivatives **9a** and **9b**, but not for EGTA-derivatives **11a** and **11b**. This effect was moderate with V³⁺, Cd²⁺, Hg²⁺ and Al³⁺, but significant with Sc³⁺ for compound **9a** (fourfold increase) and Zn²⁺ for both, **9a** and **9b** (increase by factor

2.5–2.6). Importantly, this effect was observed for ZnCl₂ with a Zn²⁺/dye-ratio of 10:1; with 1 equiv. of Zn²⁺ this effect was lower (only factor 1.4–1.5). However, with only one equiv. of Zn(OAc)₂, the "turn-on" effect of the fluorescence quantum yield is significantly boosted to a factor of 5.9 (for **9a**) and 5.3 (for **9b**). While Sc³⁺ is surely without particular interest in a biological or environmental context, the combination of Zn(OAc)₂ and fluorescence dye **9a** seems to be a promising approach for the development of a Zn²⁺ sensor system. Furthermore, the coordination geometry of (DAT)₂EDTA **9b** with Zn²⁺ was elucidated by X-ray single crystal structure analysis of the complex [ZnCl₂][Zn(OH₂)(**9b** – 2 H)]₂ · 6.5 H₂O. While one Zn²⁺ is tetrahedrally coordinated by two chloro and two oxgen atoms (the latter from carboxylate groups), the two other metal centers are found in a distorted octahedron with two nitrogen atoms and four oxygens coordinating. However, only three of these oxygen atoms belong to carbonyl groups, the fourth one is a water molecule. This differs from the normal coordination geometry of a EDTA-complex of a divalent metal ion which is characterized by five five-membered chelate rings with all coordinating oxygen atoms belonging to carboxylate groups.

Experimental Section

General Methods. Preparative column chromatography was carried out using Merck SiO₂ (35– 70 µm, type 60A) with hexanes (mixture of isomers, bp. 64–71°C) and EtOAc as eluents. TLC was performed on aluminum plates coated with SiO₂ F₂₅₄. ¹H and ¹³C NMR spectra were recorded on Bruker Avance DRX500 and DRX300 instruments. Multiplicities of carbon signals were determined with DEPT experiments. MS and HRMS spectra were obtained with a Waters Q-TOF Premier (ESI) or Thermo Scientific DFS (EI) spectrometers. IR spectra were recorded on a Bruker Tensor 27 or a Shimadzu IR Spirit T spectrometer equipped with diamond ATR units. UV/Vis spectra were recorded with a Shimadzu UV-1800, fluorescence spectra with a Shimadzu RF-5301PC spectrometer. Compounds **4a**,^[13] **4b**^[14] and **10**^[19] were literature known and prepared accordingly. All other starting materials were commercially available.

Dimethyl 2-amino-5-(*N-tert*-butyloxycarbonylamino)terephthalate (5a). A solution of Boc₂O (3.99 g, 18.3 mmol, 1.1 eq) in CH₂Cl₂ (20 ml) was dropwise added at 0°C and over a period of 1 h to a solution of DAT **4a** (3.73 g, 16.6 mmol, 1.0 eq) in CH₂Cl₂ (100 ml). The resulting mixture was allowed to warm to ambient temperature and then stirred at this temperature for 2 d. Subsequently, H₂O (100 ml) was added to the reaction mixture and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 50 ml). The combined organic layers were dried over MgSO₄, filtered, and the solvent was evaporated in vacuo. The residue was purified

by column chromatography (SiO₂, hexanes/EtOAc 2:1 with 1 vol% NEt₃, R_f = 0.33). The product **5a** (3.77 g, 11.6 mmol, 70%, 94% brsm) was obtained as a green-yellow solid, mp. 116–117°C. ¹H NMR (300 MHz, CDCl₃): δ = 1.51 (s, 9H), 3.88 (s, 3H), 3.89 (s, 3H), 5.49 (br. s, 2H), 7.32 (s, 1H), 8.81 (s, 1H), 9.53 (br. s, 1H) ppm. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ = 28.50 (3 x CH₃), 52.20 (CH₃), 52.62 (CH₃), 80.28 (C), 115.75 (C), 118.77 (CH), 120.64 (C), 121.82 (CH), 130.86 (C), 144.29 (C), 153.21 (C), 167.68 (C), 168.00 (C) ppm. IR (ATR): \tilde{v} = 3480 (w), 3372 (w), 2979 (w), 2953 (w), 1693 (s), 1626 (w), 1567 (m), 1526 (s), 1439 (m), 1420 (m), 1393 (w), 1367 (m), 1316 (m), 1296 (w), 1269 (m), 1226 (s), 1213 (s), 1192 (w), 1157 (s), 1106 (s), 1074 (w), 1043 (w), 1020 (m), 969 (w), 913 (w), 851 (w), 793 (m), 733 (m) cm⁻¹. MS (EI, 70 eV): *m/z* (%) 324 (10) [M⁺], 268 (20), 250 (30), 224 (100), 219 (20), 192 (20), 164 (25), 132 (30), 84 (35). HRMS (EI, 70 eV): calcd. 324.1316 (for C₁₅H₂₀N₂O₆⁺), found 324.1328 [M⁺]. UV/Vis (CH₂Cl₂): λ_{max} (log ϵ) 408 nm (3.75). Fluorescence (CH₂Cl₂): λ_{em} 488 nm (λ_{ex} 408 nm), Φ = 0.38. C₁₅H₂₀N₂O₆ (324.33 g mol⁻¹).

Diethyl 2-amino-5-(*N-tert*-butyloxycarbonylamino)terephthalate (5b). According to the procedure reported above for compound **5a**, DAT **4b** (2.48 g, 9.83 mmol, 1.0 eq) was converted with Boc₂O (2.36 g, 10.8 mmol, 1.1 eq.) to furnish the title compound **5b** (2.89 g, 8.20 mmol, 83%) as a green-yellow solid (mp. 122–123°C) after chromatography (SiO₂, hexanes/EtOAc 5:1 with 1 vol% NEt₃, R_f = 0.18). ¹H-NMR (300 MHz, CDCl₃): δ = 1.40 (t, *J* = 7.1 Hz, 3H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.52 (s, 9H), 4.36 (q, *J* = 7.1 Hz, 2H), 4.37 (q, *J* = 7.1 Hz, 2H), 5.48 (br s, 2H), 7.33 (s, 1H), 8.78 (s, 1H), 9.53 (br. s, 1H) ppm. All other data are in accordance with the literature.^[16] UV/Vis (CH₂Cl₂): λ_{max} (log ϵ) 411 nm (3.78). Fluorescence (CH₂Cl₂): λ_{em} 494 nm (λ_{ex} 411 nm), Φ = 0.34. C₁₇H₂₄N₂O₆ (352.39 g mol⁻¹).

Dimethyl 2-(*N***-tert-butyloxycarbonylamino)-5-(***N***-methylamino)terephthalate (7a).** Methyl iodide (353 mg, 2.49 mmol, 1.0 eq) was added to a suspension of DAT **5a** (807 mg, 2.49 mmol, 1.0 eq) and K₂CO₃ (688 mg, 29.8 mmol, 2.0 eq) in MeCN (3 ml). The reaction mixture was stirred in a tightly closed reaction vial at 90°C for 16 h. After cooling to ambient temperature, brine (50 ml) was added to the suspension and the layers were separated. Subsequently, the aqueous layer was extracted with CH₂Cl₂ (3 x 20 ml). The combined organic layers were dried over MgSO₄, filtered, and the solvent was evaporated in vacuo. The residue was purified by column chromatography (SiO₂, hexanes/EtOAc 3:1 with 1 vol% NEt₃, R_f = 0.38). The title compound **7a** (386 mg, 1.14 mmol, 46%, 79% brsm) was obtained as an orange solid, mp. 200–201°C. ¹H NMR (300 MHz, CDCl₃): δ = 1.52 (s, 9H), 2.91 (s, 3H), 3.88 (s, 3H), 3.94 (s, 3H), 7.25 (s, 1H), 7.30 (br. s, 1H), 8.86 (s, 1H), 9.52 (br. s, 1H) ppm. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ =

28.54 (3 x CH₃), 29.95 (CH₃), 52.15 (CH₃), 52.64 (CH₃), 80.18 (C), 112.50 (CH), 114.83 (C), 121.01 (C), 122.57 (CH), 129.44 (C), 146.34 (C), 153.31 (C), 168.08 (C), 168.51 (C) ppm. IR (ATR): $\tilde{v} = 3403$ (w), 3306 (w), 2973 (w), 2953 (w), 1714 (s), 1696 (s), 1584 (w), 1541 (s), 1473 (w), 1450 (m), 1437 (m), 1429 (m), 1404 (m), 1394 (m), 1372 (w), 1364 (w), 1339 (w), 1240 (s), 1220 (s), 1209 (s), 1186 (m), 1162 (m), 1147 (m), 1102 (m), 1064 (m), 1016 (m), 967 (w), 913 (w), 860 (w), 786 (s), 770 (m), 749 (m), 710 (m), 597(m) cm⁻¹. MS (EI, 70 eV): *m/z* (%) 338 (5) [M⁺], 282 (20), 264 (5), 238 (100), 233 (5), 206 (10), 178 (25), 146 (20), 118 (10). HRMS (EI, 70 eV): calcd. 338.1472 (for C₁₆H₂₂N₂O₆⁺), found 338.1470 [M⁺]. UV/Vis (CH₂Cl₂): λ_{max} (log ε) 433 nm (3.80). Fluorescence (CH₂Cl₂): λ_{em} 511 nm (λ_{ex} 433 nm), Φ = 0.23. C₁₆H₂₂N₂O₆ (338.36 g mol⁻¹).

Diethyl 2-(N-benzylamino)-5-(N-tert-butyloxycarbonylamino)terephthalate (7b). A solution of PhCHO (271 mg, 2.55 mmol, 1.5 eq) in CH₂Cl₂ (8 ml) was added to a solution of DAT 5b (600 mg, 1.70 mmol, 1.0 eq) in CH₂Cl₂ (8 ml). The reaction mixture was stirred for 1 h at ambient temperature. Subsequently, NaBH₃CN (160 mg, 2.55 mg, 1.5 eq) and ZnCl₂ (116 mg, 0.850 mmol, 0.5 eq) were successively added. The resulting mixture was stirred at ambient temperature for 16 h. Subsequently, sat. aqueous NaHCO₃ solution (50 ml) was added. The layers were separated and the aqueous layer was extracted with MTBE (3 x 20 ml). The combined organic layers were dried over MgSO₄, filtered and the solvent evaporated in vacuo. After column chromatography (SiO₂, hexanes/EtOAc 5:1 with 1 vol% NEt₃, R_f = 0.33), compound **7b** (693 mg, 1.57 mmol, 92%) was obtained as an orange solid, mp. 177–178°C. ¹H NMR (300 MHz, CDCl₃): δ = 1.32 (t, J = 7.1 Hz, 3H), 1.38 (t, J = 7.2 Hz, 3H), 1.49 (s, 9H), 4.29 (g, J = 7.1 Hz, 2H), 4.35 (q, J = 7.1 Hz, 2H), 4.41 (d, J = 4.8 Hz, 2H) 7.19–7.38 (m, 6H), 7.80 (t, J = 5.2 Hz, 1H), 8.81 (s, 1H), 9.46 (br. s, 1H) ppm. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ = 14.22 (CH₃), 14.50 (CH₃), 28.55 (3 x CH₃), 47.61 (CH₂), 61.14 (CH₂), 61.59 (CH₂), 80.12 (C), 113.69 (CH), 115.53 (C), 121.17 (C), 122.72 (CH), 127.37 (CH), 127.46 (2 x CH), 128.80 (2 x CH), 129.82 (C), 138.99 (C), 145.17 (C), 153.28 (C), 167.48 (C), 168.15 (C) ppm. IR (ATR): \tilde{v} = 3363 (w), 3302 (w), 3124 (w), 3066 (w), 3032 (w), 2982 (m), 2972 (m), 2933 (w), 2906 (w), 2869 (w), 1717 (s), 1674 (s), 1542 (s), 1469 (m), 1423 (s), 1390 (m), 1366 (s), 1327 (m), 1243 (m), 1216 (s), 1204 (m), 1159 (m), 1122 (w), 1106 (m), 1066 (m), 1043 (w), 1023 (s), 907 (m), 870 (m), 821 (m), 786 (s), 757 (m), 747 (m), 700 (s), 594 (s), 516 (m) cm⁻¹. HRMS (ESI, pos. mode): calcd. 443.2177 (for $C_{24}H_{31}N_2O_6^+$, found 443.2183 [M + H⁺]. UV/Vis (CH₂Cl₂): λ_{max} (log ϵ) 428 nm (3.88). Fluorescence (CH₂Cl₂): λ_{em} 505 nm (λ_{ex} 428 nm), Φ = 0.41. C₂₄H₃₀N₂O₆ (442.51 g mol⁻¹).

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Dimethyl 2-amino-5-(N-methylamino)terephthalate (6a). TFA (4 ml) was added to a solution of carbamate 7a (594 mg, 1.76 mmol, 1.0 eq) in CH₂Cl₂ (15 ml) at 0°C. The reaction mixture was warmed to ambient temperature and stirred at this temperature for 16 h. Subsequently, EtOAc (50 ml) was added to the solution. The resulting mixture was carefully added to a sat. aqueous NaHCO₃ solution (40 ml). The layers were separated and the organic layer was washed with sat. aqueous NaHCO₃ solution (3 x 40 ml). The combined aqueous layers were then re-extracted with EtOAc (1 x 20 ml). The combined organic layers were dried over MgSO₄, filtered and the solvent evaporated in vacuo. After column chromatography (SiO₂, hexanes/EtOAc 2:1 with 1 vol% NEt₃, $R_f = 0.30$), title compound **6a** (394 mg, 1.65 mmol, 94%) was obtained as a red solid, mp. 119–120°C. ¹H NMR (300 MHz, CDCl₃): δ = 2.89 (s, 3H), 3.86 (s, 3H), 3.90 (s, 3H), 5.56 (br. s, 2H), 7.19 (s, 1H), 7.34 (s, 1H) ppm; the signal of the NH proton was not observed. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ = 30.31 (CH₃), 51.91 (CH₃), 51.98 (CH₃), 112.51 (CH), 116.43 (C), 117.75 (C), 119.84 (CH), 139.21 (C), 143.37 (C), 168.07 (2 x C) ppm. IR (ATR): $\tilde{v} = 3480$ (w), 3408 (w), 3377 (m), 2953 (w), 2803 (w), 1683 (s), 1567 (m), 1477 (w), 1436 (m), 1407 (m), 1303 (m), 1260 (m), 1247 (w), 1206 (m), 1206 (w), 1103 (m), 963 (w), 897 (w), 854 (w), 784 (m), 597 (w) cm⁻¹. MS (EI, 70 eV): *m/z* (%) 238 (100) [M⁺], 207 (12), 178 (35), 146 (30), 118 (25). HRMS (EI, 70 eV): calcd. 238.0948 (for C₁₁H₁₄N₂O₄⁺), found 238.0955 [M⁺]. UV/Vis (CH₂Cl₂): λ_{max} (log ϵ) 459 nm (3.73). Fluorescence (CH₂Cl₂): λ_{em} 558 nm (λ_{ex} 459 nm), $\Phi = 0.06$. C₁₁H₁₄N₂O₄ (238.24 g mol⁻¹).

Diethyl 2-amino-5-(*N***-benzylamino)terephthalate (6b).** According to the procedure reported above for compound **6a**, carbamate **7b** (673 mg, 1.52 mmol, 1.0 eq) was converted with TFA (7 ml) to furnish the title compound **6b** (520 mg, 1.52 mmol, 100%) as a red solid (mp. 65–66°C) after chromatography (SiO₂, hexanes/EtOAc 4:1 with 1 vol% NEt₃, R_f = 0.33). ¹H NMR (300 MHz, CDCl₃): δ = 1.38 (t, *J* = 7.1 Hz, 3H), 1.41 (t, *J* = 7.1 Hz, 3H), 4.33 (q, *J* = 7.2 Hz, 2H), 4.36 (q, *J* = 7.2 Hz, 2H), 4.42 (s, 2H), 5.05 (br. s, 2H), 7.27–7.45 (m, 7H) ppm; the signal of the NH proton was not observed. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ = 14.35 (CH₃), 14.41 (CH₃), 48.12 (CH₂), 60.86 (CH₂), 60.93 (CH₂), 113.84 (CH), 117.04 (C), 117.94 (C), 119.81 (CH), 127.22 (CH), 127.57 (2 x CH), 128.71 (2 x CH), 139.49 (C), 139.52 (C), 142.16 (C), 167.59 (C), 167.77 (C) ppm. IR (ATR): \bar{v} = 3477 (m), 3375 (m), 3067 (w), 3033 (w), 2989 (w), 2906 (w), 2844 (w), 1677 (s), 1583 (m), 1559 (m), 1512 (m), 1472 (m), 1422 (m), 1389 (w), 1364 (m), 1297 (m), 1297 (m), 1256 (w), 1197 (s), 1117 (m), 1097 (s), 1023 (m), 897 (w), 859 (w), 787 (s), 741 (m), 696 (m) cm⁻¹. MS (EI, 70 eV): *m/z* (%) 342 (100) [M⁺], 51 (15), 314 (54), 297 (55), 267 (55), 205 (53), 195 (46), 179 (52), 133 (95), 125 (25), 105 (51), 91 (55), 77 (27), 65 (43). HRMS (EI, 70 eV): calcd. 342.1574 (for C₁₉H₂₂N₂O₄⁺), found 342.1570 [M⁺]. UV/Vis (CH₂Cl₂): λ_{max} (log ε) 453

nm (3.62). Fluorescence (CH₂Cl₂): λ_{em} 550 nm (λ_{ex} 453 nm), Φ = 0.23. C₁₉H₂₂N₂O₄ (342.40 g mol⁻¹).

3,6-Bis(carboxymethyl)-3,6-diazaoctandioic acid N,N'-bis[2,5-di(methoxycarbonyl)-4-(Nmethylamino)phenyl]diamide (9a). DAT 6a (667 mg, 2.80 mmol, 2.0 eq) was added to a solution of EDTA bisanhydride 8 (359 mg, 1.40 mmol, 1.0 eq) in DMF (5 ml). The resulting mixture was stirred for 17 h at ambient temperature. The precipitated solid was collected on a glass frit. The residue was first washed with DMF (1 x 2 ml) and then with hexanes (4 x 10 ml). The title compound **9a** (667 mg, 0.910 mmol, 65%) was obtained as a vellow solid (mp. 243–244°C) after drying under high vacuum. Remaining traces of DMF were removed by (repeated, if necessary) evaporation with CHCl₃ (5 mL). ¹H NMR (500 MHz, DMSO-d₆): δ = 2.75 (d, *J* = 4.8 Hz, 6H), 2.93 (s, 4H), 3.38 (s, 4H), 3.45 (s, 4H), 3.76 (s, 6H), 3.84 (s, 6H), 6.79 (s, 2H), 7.25 (br. q, J = 4.8 Hz, 2H), 8.96 (s, 2H), 11.0 (s, 2H), 12.4 (br. s, 2H) ppm. ¹³C{¹H} NMR (125 MHz, DMSO-d₆, T = 353 K): $\delta = 28.86 (2 \times CH_3), 51.35(2 \times CH_3), 51.90 (2 \times CH_3), 52.31 (2 \times CH_2), 54.77 (2 \times CH_2), 59.06$ (2 x CH₂), 111.70 (2 x CH), 112.77 (2 x C), 123.58 (2 x C), 123.75 (2 x CH), 125.92 (2 x C), 146.20 (2 x C), 165.93 (2 x C), 166.87 (2 x C), 168.66 (2 x C), 171.42 (2 x C) ppm. IR (ATR): v = 3493 (w), 3386 (w), 3196 (w), 2960 (w), 2880 (w), 2533 (w), 1743 (m), 1690 (s), 1633 (m), 1537 (s), 1477 (w), 1447 (w), 1433 (m), 1406 (m), 1340 (w), 1317 (w), 1243 (s), 1217 (s), 1190 (s), 1154 (w), 1130 (m), 1097 (s), 1003 (w), 960 (m), 934 (w), 917 (w), 887 (w), 861 (w), 823 (w), 790 (s), 783 (s), 684 (m), 599 (w), 574 w), 513 (w) cm⁻¹. HRMS (ESI, pos. mode): calcd. 733.2675 (for C₃₂H₄₁N₆O₁₄⁺), found 733.2684 [M + H⁺]. UV/Vis (DMSO): λ_{max} (log ε) 427 nm (4.03). Fluorescence (DMSO): λ_{em} 511 nm (λ_{ex} 427 nm), Φ = 0.07. C₃₂H₄₀N₆O₁₄ (732.70 g mol⁻ ¹).

3,6-Bis(carboxymethyl)-3,6-diazaoctandioic acid *N,N'-bis*[**4-(***N*-benzylamino)-**2,5-di(eth-oxycarbonyl)phenyl]diamide (9b).** According to the procedure reported above for compound **9a**, DAT **6b** (250 mg, 0.730 mmol, 2.0 eq) was converted with EDTA bisanhydride **8** (94 mg, 0.365 mmol, 1.0 eq) to furnish the title compound **9b** (292 mg, 0.310 mmol, 85%) as a yellow solid (mp. 222–223°C) ¹H NMR (500 MHz, DMSO-d₆): δ = 1.25 (t, *J* = 7.1 Hz, 6H), 1.37 (t, *J* = 7.1 Hz, 6H), 2.93 (s, 4 H), 3.37 (s, 4H), 3.43 (s, 4H), 4.15 (q, *J* = 7.1 Hz, 4 H), 4.33–4.40 (m, 8H), 6.92 (s, 2H), 7.25–7.31 (m, 2H), 7.32–7.38 (m, 8H), 7.84 (t, *J* = 5.4 Hz, 2H), 9.06 (s, 2H), 11.1 (s, 2H), 12.4 (br. s, 2H) ppm. ¹³C{¹H} NMR (125 MHz, DMSO-d₆, T = 353 K): δ = 13.29 (2 x CH₃), 13.60 (2 x CH₃), 46.21 (2 x CH₂), 52.36 (2 x CH₂), 54.76 (2 x CH₂), 59.04 (2 x CH₂), 60.25 (2 x CH₂), 60.73 (2 x CH₂), 112.91 (2 x CH), 113.49 (2 x C), 123.43 (2 x C), 123.67 (2 x CH), 126.59 (2 x CH), 126.60 (2 x C), 126.75 (4 x CH), 128.05 (4 x CH), 138.50 (2 x C), 145.12

(2 x C), 165.38 (2 x C), 166.60 (2 x C), 168.72 (2 x C), 171.34 (2 x C) ppm. IR (ATR): \tilde{v} = 3365 (w), 3246 (w), 2976 (w), 2880 (w), 2707 (w), 2662 (w), 2506 (w), 1717 (m), 1706 (w), 1682 (s), 1530 (m), 1489 (w), 1470 (w), 1419 (m), 1406 (w), 1369 (m), 1329 (m), 1254 (m), 1245 (m), 1200 (s), 1129 (w), 1104 (s), 1016 (w), 954 (m), 954 (m), 923 (m), 873 (w), 866 (w), 791 (s), 744 (w), 717 (m), 694 (m), 610 (w), 541 (w), 512 (w) cm⁻¹. HRMS (ESI, neg. mode): calcd. 939.3782 (for C₄₈H₅₅N₆O₁₄⁻), found 939.3767 [M - H⁺]. UV/Vis (DMSO): λ_{max} (log ϵ) 423 nm (4.11). Fluorescence (DMSO): λ_{em} 503 nm (λ_{ex} 423 nm), Φ = 0.13. C₄₈H₅₆N₆O₁₄ (941.00 g mol⁻¹).

EGTA-bisanhydride (10). Under nitrogen atmosphere, EGTA (**3**, 3.80 g, 10 mmol, 1.0 eq) was added to a mixture of Ac₂O (9.19 g, 90 mmol, 9.0 eq) and freshly distilled pyridine (10 ml). The reaction mixture was stirred for 24 h at 65°C. After evaporation of all volatiles in vacuo at a temperature of 60°C, EGTA-bisanhydride **10** was obtained as a brown oil (3.47 g, 10 mmol, 100%). ¹H NMR (300 MHz, CDCl₃): δ = 2.82 (t, *J* = 4.7 Hz, 4H), 3.56 (s, 4H), 3.61 (t, *J* = 4.7 Hz, 4H), 3.68 (s, 8H) ppm. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ = 53.66 (4 x CH₂), 55.01 (2 x CH₂), 69.28 (2 x CH₂), 70.41 (2 x CH₂), 164.71 (4 x C) ppm. IR (ATR): \tilde{v} = 2870 (w), 1813 (m), 1759 (s), 1674 (m), 1633 (w), 1436 (w), 1353 (w), 1332 (w), 1306 (w), 1254 (m), 1222 (m), 1090 (s), 947 (s), 924 (s), 846 (w), 811 (w), 784 (m), 636 (w), 613 (m), 547 (m) cm⁻¹. MS (EI, 70 eV): *m/z* (%) 344 (10) [M⁺], 316 (90), 213 (55), 187 (30), 156 (50), 128 (100), 114 (55), 100 (65), 86 (30), 71 (35), 56 (70), 42 (80). HRMS (EI, 70 eV): calcd. 344.1214 (for C₁₄H₂₀N₂O₈⁺), found 344.1200 [M⁺]. C₁₄H₂₀N₂O₈ (344.32 g mol⁻¹).

3,12-Bis(carboxymethyl)-6,9-dioxa-3,12-diazatetradecandioic acid *N,N***-bis[2,5-di(meth-oxycarbonyl)-4-(***N***-methylamino)phenyl]diamide (11a). Under nitrogen atmosphere, DAT 6a** (557 mg, 2.34 mmol, 2.0 eq) was added to a solution of EGTA bisanhydride **10** (403 mg, 1.17 mmol, 1.0 eq) in abs. DMF/MTBE (1:1, 6 ml). The resulting mixture was stirred at 50°C for 18 h. Subsequently, the reaction mixture was cooled to ambient temperature until a precipitate has formed. The resulting solid was filtered off and washed with MTBE (1 x 2 ml) and hexanes (3 x 5 ml). The solid was dissolved in CH₂Cl₂ (2 ml) and the solution was dropwise added into a mixture of MTBE/hexanes (1:1, 40 ml). The resulting solid was filtered off. After drying in vacuo, title compound **11a** (509 mg, 0.620 mmol, 53%) was obtained as a yellow-green solid, mp. 98–99°C. ¹H NMR (500 MHz, DMSO-d₆): δ = 2.83 (t, *J* = 5.5 Hz, 4H), 2.86 (d, *J* = 4.6 Hz, 6H), 3.35 (s, 4H), 3.36 (s, 4H), 3.48 (t, *J* = 5.8 Hz, 4H), 3.49 (s, 4H), 3.82 (s, 6H), 3.86 (s, 6H), 7.14 (s, 2H), 7.32 (br. q, *J* = 4.3 Hz, 2H), 8.83 (s, 2H), 10.8 (br. s, 2H), 12.3 (br. s, 2H) ppm. ¹³C{¹H} NMR (125 MHz, DMSO-d₆): δ = 29.34 (2 x CH₃), 51.89 (2 x CH₃), 52.51 (2 x CH₃), 53.88 (2 x CH₂), 55.56 (2 x CH₂), 59.29 (2 x CH₂), 69.30 (2 x CH₂), 69.40 (2 x CH₂), 112.10 (2 x CH),

112.68 (2 x C), 124.04 (2 x CH), 124.42 (2 x C), 125.96 (2 x C), 146.58 (2 x C), 166.36 (2 x C), 167.15 (2 x C), 169.35 (2 x C), 172.28 (2 x C) ppm. IR (ATR): \tilde{v} = 3386 (w), 3269 (w), 3000 (w), 2952 (w), 2869 (w), 1686 (s), 1619 (m), 1533 (s), 1474 (w), 1434 (m), 1404 (m), 1333 (w), 1227 (s), 1146 (w), 1102 (m), 964 (w), 914 (w), 866 (w), 789 (m), 746 (s), 694 (m), 666 (m) cm⁻¹. HRMS (ESI, pos. mode): calcd. 843.3019 (for C₃₆H₄₈N₆NaO₁₆⁺), found 843.3017 [M + Na⁺]. UV/Vis (DMSO): λ_{max} (log ϵ) 422 nm (3.95). Fluorescence (DMSO): λ_{em} 512 nm (λ_{ex} 422 nm), Φ = 0.23. C₃₆H₄₈N₆O₁₆ (820.81 g mol⁻¹).

3,12-Bis(carboxymethyl)-6,9-dioxa-3,12-diazatetradecandioic acid N,N'-bis[4-(N-benzylamino)-2,5-di(ethoxycarbonyl)phenyl]diamide (11b). Under nitrogen atmosphere, DAT 6b (616 mg, 1.80 mmol, 2.0 eq) was added to a solution of EGTA bisanhydride **10** (310 mg, 0.901 mmol, 1.0 eq) in abs. DMF (5 ml). The resulting mixture was stirred for 20 h at ambient temperature. Subsequently, the reaction mixture was diluted with EtOAc (60 ml) and extracted with sat. aqueous NaHCO₃ solution (3 x 60 ml). The combined aqueous layers were acidified to pH < 2 with conc. hydrochloric acid (ca. 10 ml) and extracted with CH₂Cl₂ (4 x 100 ml). Then the solvent was removed in vacuo and the residue was diluted in CH₂Cl₂ (about 5 ml). The extract was added dropwise into a mixture of hexanes/MTBE (1:1, 70 ml). The resulting solid was filtered off and washed with hexanes (2 x 5 ml). After drying in vacuo, title compound **11b** (702 mg, 0.682 mmol, 76%) was obtained as a yellow-green solid, mp. 145–146°C. ¹H NMR (500 MHz, DMSO-d₆): δ = 1.23 (t, J = 7.1 Hz, 6H), 1.29 (t, J = 7.1 Hz, 6H), 2.87 (s, 4H), 3.34 (s, 4H), 3.39-3.51 (m, 8H), 3.55 (s, 4H), 4.23 (q, J = 7.0 Hz, 4H), 4.30 (q, J = 7.1 Hz, 4H), 4.44 (s, 4H), 7.16 (s, 2H), 7.21–7.27 (m, 2H), 7.30–7.38 (m, 8H), 7.90 (br. s, 2H), 8.75 (s, 2H), 10.8 (br. s, 2H) ppm, signals for the NH protons of the amines were not observed. ¹³C{¹H} NMR (125 MHz, DMSO-d₆, T = 343 K): δ = 13.55 (2 x CH₃), 13.76 (2 x CH₃), 46.23 (2 x CH₂), 53.88 (2 x CH₂), 55.47 (2 x CH₂), 58.82 (2 x CH₂), 60.41 (2 x CH₂), 60.95 (2 x CH₂), 68.68 (2 x CH₂), 69.24 (2 x CH₂), 113.07 (2 x CH), 113.34 (2 x C), 124.50 (2 x CH), 125.11 (2 x C), 125.93 (2 x C), 126.71 (2 x CH), 126.86 (4 x CH), 128.18 (4 x CH), 138.65 (2 x C), 145.61 (2 x C), 165.54 (2 x C), 166.63 (2 x C), 168.64 (2 x C), 171.25 (2 x C) ppm. IR (ATR): \tilde{v} = 3373 (w), 3262 (w), 2980 (w), 1683 (s), 1539 (m), 1420 (m), 1367 (w), 1329 (w), 1213 (m), 1102 (m), 1017 (w), 910 (w), 866 (w), 790 (w), 736 (w), 697 (w) cm⁻¹. HRMS (ESI, pos. mode): calcd. 1051.4271 (for $C_{52}H_{64}N_6NaO_{16}^+$), found 1051.4270 [M + Na⁺]. UV/Vis (DMSO): λ_{max} (log ϵ) 416 nm (3.94). Fluorescence (DMSO): λ_{em} 505 nm (λ_{ex} 416 nm), Φ = 0.36. C₅₂H₆₄N₆O₁₆ (1029.11 g mol⁻¹).

Investigation of metal salt additives. A stock-solution of the respective dye (c = 0.136 mmol L⁻¹) was prepared by dissolving compounds **9a** (25.0 mg), **9b** (32.1 mg), **11a** (28.0 mg) or **11b** (35.1 mg) in DMSO (250 mL). A solution of the respective metal salt (0.1 mL, c = 136 mmol L⁻¹, 13.6 µmol) was added to the stock-solution of the dye (10 mL, 1.36 µmol) and the resulting mixture was submitted to fluorescence spectroscopy. The absorption wavelengths λ_{max} of the respective dye without metal additive was used as excitation wavelength λ_{ex} , i.e. 427 nm (**9a**), 424 nm (**9b**), 422 nm (**11a**) or 416 nm (**11b**). Additionally, absorption spectra were recorded from these solutions.

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Conflict of interests

The authors declare no conflict of interests.

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