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Rigid Luminescent Bis-Zinc(II)–Bis-Cyclen Complexes for the Detection of Phosphate Anions and Non-Covalent Protein Labeling in Aqueous Solution

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A series of water-soluble bis- and tetrakis-Zn^{II}–cyclen complexes with rigid structures was prepared to enhance the carboxylate and phosphate ion binding response in contrast to analogues with less-confined molecular structures. Boc-protected 6-chloro-1,3,5-triazine–bis-cyclen was coupled to different aryl and alkyl moieties in moderate to high yields and subsequently converted into the corresponding bis- or tetrakis-Zn^{II}–cyclen complexes. The bis-Zn^{II}–cyclen moiety is known for its affinity to anions. Depending on the arene substituent some of the synthesized synthetic receptors are luminescent. They were studied by absorption and emission spectroscopy for their response to the presence of phosphate anions of biological relevance in buffered aqueous solution at neutral pH and for their affinity to the genetically encodable oligoaspartate and glutamate sequences (D_4 and E_4 tag) recently introduced. The rigid structures of the compounds enhance the electronic coupling between the metal complex binding site and the reporter dye. This leads to an increased anion binding response in homogeneous aqueous solution.

Introduction

The ubiquitous presence of phosphates in nature makes their molecular recognition under physiological conditions of immense interest.[1] Phosphates are vividly present in RNA and DNA, in phosphorylated saccharides, and in phosphorylated proteins.[2] The nucleotide adenosine triphosphate (ATP), is the universal energy currency for metabolism and is involved in intracellular energy transport for various metabolic processes including biosynthetic reactions, mobility, and cell division. The hydrolysis of ATP in cells produces pyrophosphate (PPi) along with adenosine monophosphate (AMP) and it plays an important role in intracellular signaling.[3] Hence, the development of artificial phosphate anion receptors is of great interest.

Transition metal complexes with vacant coordination sites are well suited to serve as phosphate ion binding sites.[4] Some widely used binding receptor moieties in phosphate recognition are zinc(II)–dipicolylamine (Dpa) complexes as demonstrated by Hamachi,[5] Hong,[6] Koike,[7] and Smith[8] and macrocyclic 1,4,7,10-tetraazacyclododecane (cyclen) transition metal complexes reported by Kikuchi[9] and Kimura.[10]

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We have recently reported the application of 1,3,5-triazine-based bis-Zn^{II}–cyclen complexes in phosphate ion recognition.[11] However, the so-far prepared receptors have a rather flexible molecular structure, and even though the complexes bear luminescent labels, they did not respond to the presence of phosphate anions in homogeneous solution. As the rigidity of a molecule and the distance and connection between a binding site and a luminescent probe may be crucial to achieve high analyte binding response, we have designed and synthesized modified bis-Zn^{II}–cyclen receptors, which are more rigid in their molecular structure.

Furthermore, the selective luminescent labeling of proteins by markers is an important quest and an ongoing challenge in molecular biology.[12] Covalent labeling uses reactive dyes or genetic fusion of fluorescent proteins, for example, green fluorescent proteins (GFPs).[13] Non-covalent labeling strategies employ antibodies or snap tags.[14] Recently, pairs of protein-fused peptide tags and complementary fluorescent chemical probes were reported for applications in protein labeling.[15]

The Hamachi group developed a new high affinity peptide tag – artificial probe pair orthogonal to the His tag – Ni^{II}–NTA pair, for protein labeling, employing coordination chemistry and multivalent interaction between a genetically encodable oligoaspartate sequence (D4-tag) and a corresponding oligonuclear Zn^{II}–Dpa complex.[16] Because artificial zinc dipicolylamine (Zn^{II}–Dpa) receptors[17] and zinc 1,4,7,10-tetraazacyclododecane (Zn^{II}–cyclen) complex derivatives[18] are both known for their application as phosphate binders under physiological conditions, we considered using Zn^{II}–cyclen derivatives as alternative artificial probes

for the developed artificial probes for oligoaspartate (D4-tag) and oligoglutamate (E4-tag) tag sequences. Although the coordination geometry of Zn^{II}–Dpa and Zn^{II}–cyclen is rather different, Zn^{II}–cyclen derivatives show a comparable high binding affinity and fluorescence response and may therefore be used as an alternative artificial non-covalent protein marker.

Results and Discussions

Syntheses of Zn^{II}–Cyclen Complexes

The rigid bis-Zn^{II}–cyclen based receptors are synthesized mainly by using Suzuki–Miyaura cross-coupling reactions as shown in Scheme 1. Threefold Boc-protected bis-cyclen and compound **1** were synthesized following an earlier reported procedure.[19] The protection of the cyclen azamacrocycle reduces polarity and prevents multi-*N*-substitution. Trichlorotriazine reacts with two equivalents of threefold Boc-protected cyclen to yield **1** in a clean twofold nucleophilic aromatic substitution. The remaining chloro substituent of triazine bis-cyclen **1** was then used for coupling with different boronic acids **2** by palladium-catalyzed Suzuki–Miyaura reaction. Synthesized ligands **3** are listed in Table 1 with their isolated yields. Obtained Boc-protected cyclen ligands **3** were deprotected with trifluoroacetic acid to give the corresponding ammonium salts in quantitative yields. Finally, complexation of the azamacrocyclic amines with $ZnCl_2$ gave Zn^{II}–cyclen complexes **4**.

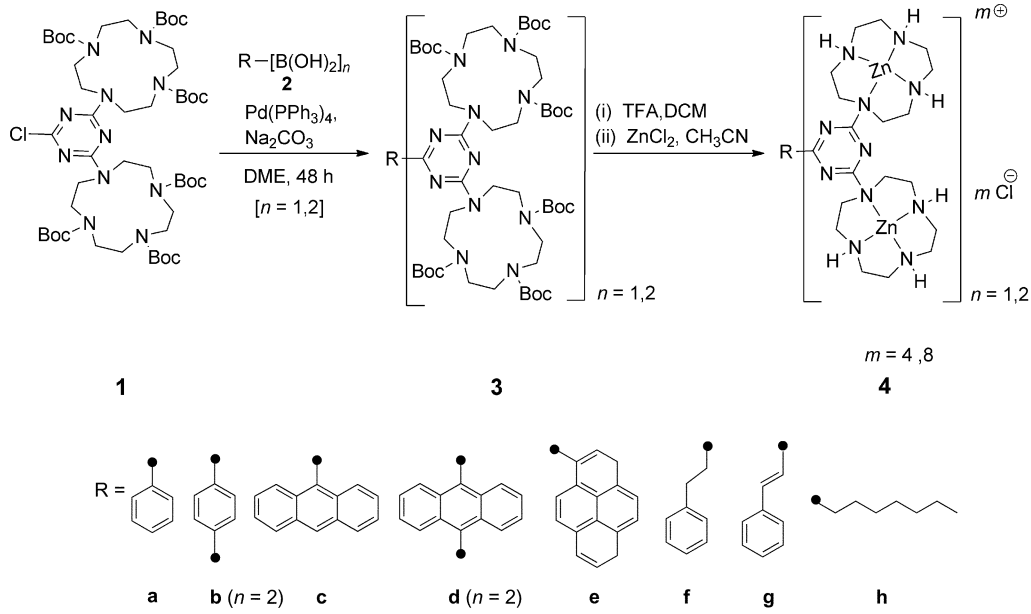
Complex **4c** was characterized with the help of single-crystal diffraction analysis. In order to obtain suitable crystals we used a small amount of zinc(II) chloride present in the water solution for crystallization. These conditions led to the formation of the complex $[(\mathbf{4c}\text{-}2Cl^-)^{2+}][ZnCl_4^{2-}]$ (Figure 1). The cyclene moieties coordinate to zinc cations with typical Zn–N distances of 2.1 Å to the three aliphatic nitrogen atoms and one longer Zn–N distance of 2.6 Å to the triazine nitrogen. The anthracene ring is twisted from the triazine plane by a torsion angle of 68°.

In attempts to include rigidity in the bis-Zn^{II}–cyclen complexes, apart from a Suzuki–Miyaura cross-coupling strategy, two other different strategies, Sonogashira cross-coupling and the click reaction, were also tried. These approaches are outlined in Schemes 2 and 3, but have not been further developed due to the unsatisfactory chemical yields of some of the reactions. Detailed experimental procedures and analytical data of all prepared compounds are provided in the Experimental Section and in the Supporting Information.

UV/Vis and Luminescent Properties of Zn^{II}–Cyclen Complexes

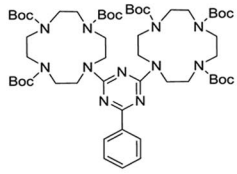
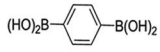
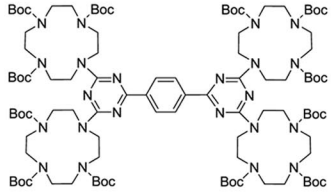
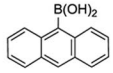
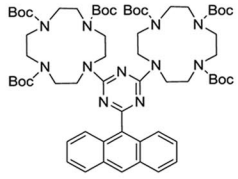
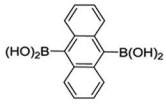
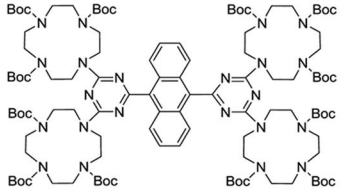
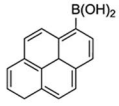
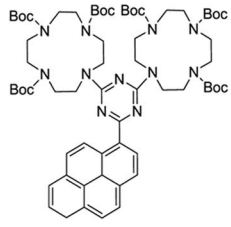
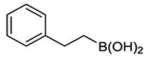
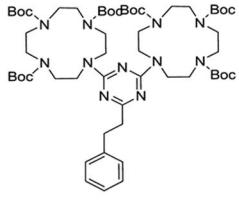
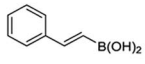
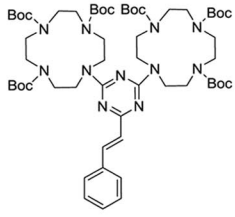
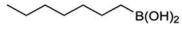
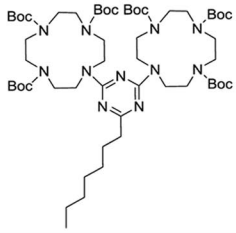
Some of the synthesized complexes **4** bear fluorophores, and their UV/Vis and luminescent properties were investigated. The data are summarized in Table 2.

The highest quantum yields were observed for compounds **4b**–**e**. These compounds were studied in detail by using fluorescence titrations in 25 mM HEPES buffer, pH 7.4 at 25 °C and binding affinities for carboxylates and phosphates were determined. The concentration of HEPES



Scheme 1. Synthesis of triazine bis-Zn^{II}–cyclen complexes **4a**–**h**.

Table 1. Structures and isolated yields of the synthesized bis- and tetrakis-Boc-protected cyclen ligands **3a**–**h** obtained by Suzuki–Miyaura cross coupling strategy.

Entry	Boronic acid **2**	Protected ligand **3**		Isolated Yield [%]
1			**3a**	50
2			**3b**	31
3			**3c**	50
4			**3d**	30
5			**3e**	54
6			**3f**	52
7			**3g**	78
8			**3h**	47

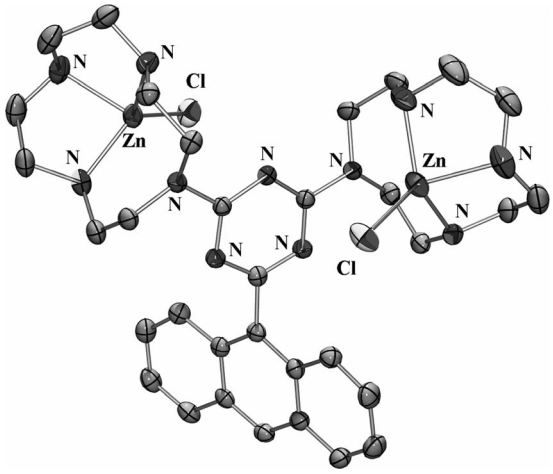
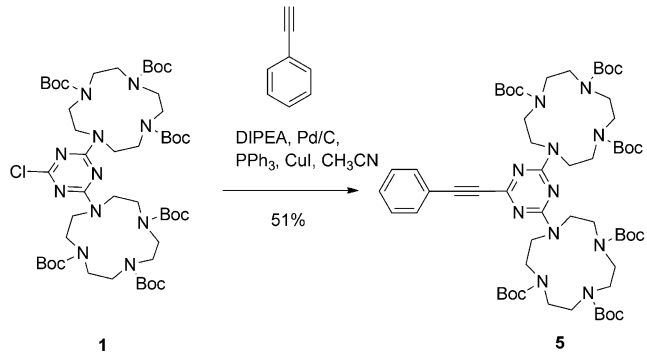


Figure 1. ORTEP rendered view of the molecular structure of complex **4c**. Hydrogen atoms, methanol, and the $ZnCl_4^{2-}$ anion are omitted for clarity. Ellipsoids are shown at the 50% probability level.

buffer was chosen to cover the range of binding affinities which are characteristic for complexes **4b**–**e**. We were interested to compare the selectivities of binding and emission responses of rigid bis- and tetra-Zn^{II} complexes arising from the interaction with different anions. The studies performed by the Hamachi group using Zn^{II}–Dpa–Tyr and a series of oligoaspartate peptides (Boc-D_n-NH_2, n = 2–5) and oligoglutamate peptides (Boc-E_n-NH_2, n = 3, 4) revealed that D_4 tag (n = 4) has the highest affinity.[21] Based on the reported results, we selected D_4 and E_4 tag sequences for our investigations. The derived binding affinities of the complexes with the carboxylates, pyrophosphate (PPi), and *O*-phospho-L-serine are shown in Table 3.

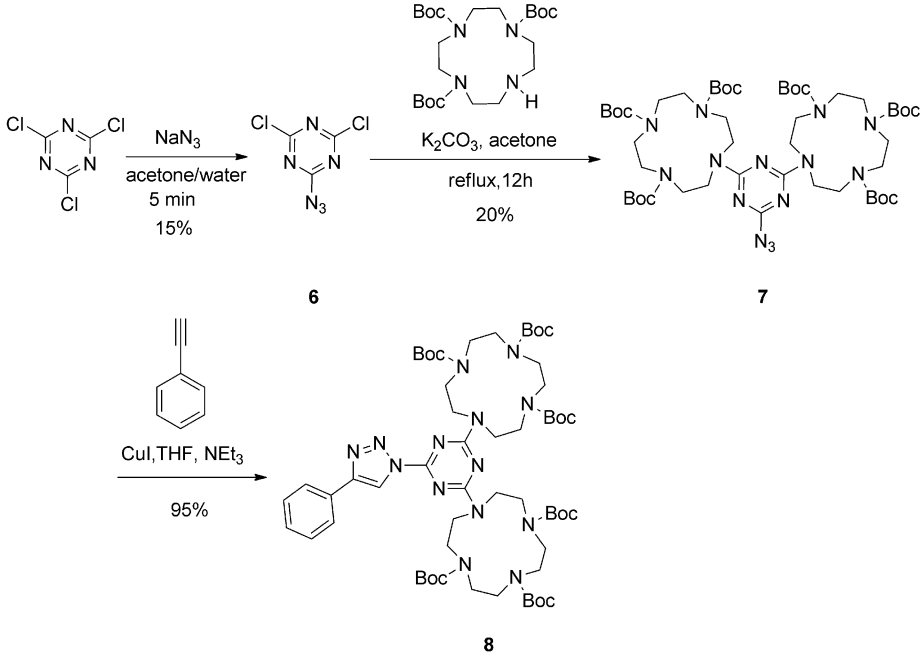


Scheme 2. Synthesis of modified triazine bis-cyclen ligand **5**, for potential metal complexation by using a Sonogashira cross-coupling strategy.

Table 2. Absorption and emission data of synthesized complexes **4a**–**e** and **4g**. All compounds have similar emission maxima in HEPES buffer. The emission quantum yields of the synthesized complexes were measured in methanol as solvent with quinine sulfate as reference to explore variations in the photophysical properties.

Complex	Absorption[a]		Emission[b]		
	λ_{max}	log ε	$\lambda_{excitation}$ [nm]	λ_{max} [nm]	Φ_{rel} [%][c]
4a	224	4.44	230	457	0.02
4b	285	4.53	290	385	0.2
4c	365	3.90	364	455	2.0
4d	370	3.36	372	456	20
4e	350	4.16	354	453	5.9
4g	220	4.10	220	424	0.02

[a] Measured in HEPES buffer at a concentration of c = 10^{-4} mol L^{-1}. [b] Measured in HEPES buffer at a concentration of c = 10^{-5} mol L^{-1}. [c] Relative quantum yields were determined by using quinine sulfate ($\Phi_{quinine\ sulfate}$ = 58%).[20]



Scheme 3. Synthesis of modified triazine bis-cyclen ligand **8**, for potential metal complexation by using click chemistry.

Table 3. Binding characteristics of complexes **4b**–**e** measured as 0.01–0.05 mM solutions in 25 mM HEPES buffer, pH 7.4, 25 °C.

	Boc–E_4–NH_2	Boc–D_4–NH_2	PPi	*O*-Phospho-L-serine
4d	$\log\beta_{21}$ = 13.46(12) $\log\beta_{11}$ = 7.21(6)	$\log\beta_{21}$ = 16.22(8) $\log\beta_{11} > 7$	[b]	$\log\beta_{11} < 3$
H/G ratio, F/F_0[a]	1:1, 6.8	1:1, 4.8	1:2, 5.5	1:1, 1.21
4b	$\log\beta_{21}$ = 12.24(8) $\log\beta_{11}$ = 6.74(3)	$\log\beta_{11} > 7$ $\log\beta_{12}$ = 13.38(8)	[b]	[c]
H/G ratio, F/F_0[a]	1:1, 2.2	1:2, 1.3	1:2, 0.1	
4c	$\log\beta_{11}$ = 3.41(1)	$\log\beta_{11}$ = 3.31(1)	[b]	[c]
H/G ratio, F/F_0[a]	1:1, 1.3	1:1, 1.8	1:1, 3.4	
4e	$\log\beta_{11} < 3$	$\log\beta_{11} < 3$	$\log\beta_{21}$ = 11.05(7) $\log\beta_{22}$ = 16.55(12)	[c]
H/G ratio, F/F_0[a]	1:1, 0.9	1:1, 0.9	1:1, 1.2	

[a] Stoichiometry of host (H)/guest (G) ratio was determined according to Job's method; F/F_0 = changes in fluorescence of zinc complex upon addition of one equivalent of a guest. [b] Good fitting of the experimental curve was not possible; each stepwise binding constant was in the range $>10^6$ M^{-1}. [c] Changes in fluorescence response upon titration with guest were negligible.

Analysis of fluorescence responses (F/F_0) in Table 3 reveals that in most cases the coordination of a guest to a zinc complex leads to an increase in emission. This is in good agreement with reports on Zn^{2+}–DPA complexes.[17d] According to our ESI mass spectrometry investigations, at the concentration required for fluorescence measurement some di- and trinuclear zinc complexes are formed from the parent tetranuclear zinc complexes. They are weakly fluorescent due to quenching by photoinduced electron transfer (PET) from the uncomplexed aliphatic nitrogen atoms. The coordination of an anion induces complete Zn^{2+} cation coordination to the nitrogen ligand and thus decreases PET quenching,[22] which results in a turn-on response. The presence of this mechanism was proved by addition of an excess amount of zinc(II) chloride to the complexes, in this case a slight increase (1.1–1.4 fold) in fluorescence was observed. However, the coordination of the E_4 and D_4 tags to complex **4d** led to much larger increases in fluorescence – 6.8 and 4.8, respectively. This data indicate that another turn-on mechanism is also present: The coordination of a guest rigidifies the structure, leading to a significant emission increase.[a] The stoichiometry of the host (H)/guest (G) ratio was determined according to Job's method; F/F_0 = changes in fluorescence of zinc complex upon addition of one equivalent of a guest.[b] Good fitting of the experimental curve was not possible; each stepwise binding constant was in the range $>10^6$ M^{-1}.[c] Changes in fluorescence response upon titration with guest were negligible.

Coordination of Tetraaspartate (Boc–D_4–NH_2) and Tetraglutamate (Boc–E_4–NH_2) Peptide Sequences

Coordination of D_4 or E_4 peptide sequences to bis-Zn^{II} complexes **4c** and **4e** is rather weak and only small changes in fluorescence are observed upon addition of ca. 10 equiv. of a guest. The tetra-Zn^{II} complexes have much higher binding affinities for both E_4 and D_4 tags with stepwise binding constants of ca. 10^7 M^{-1}. Such a dramatic increase is explained by the increased coulombic interaction in a host–guest complex. The characteristic changes in emission and the binding isotherm for tetra-Zn^{II} complex **4d** are shown in Figure 2.

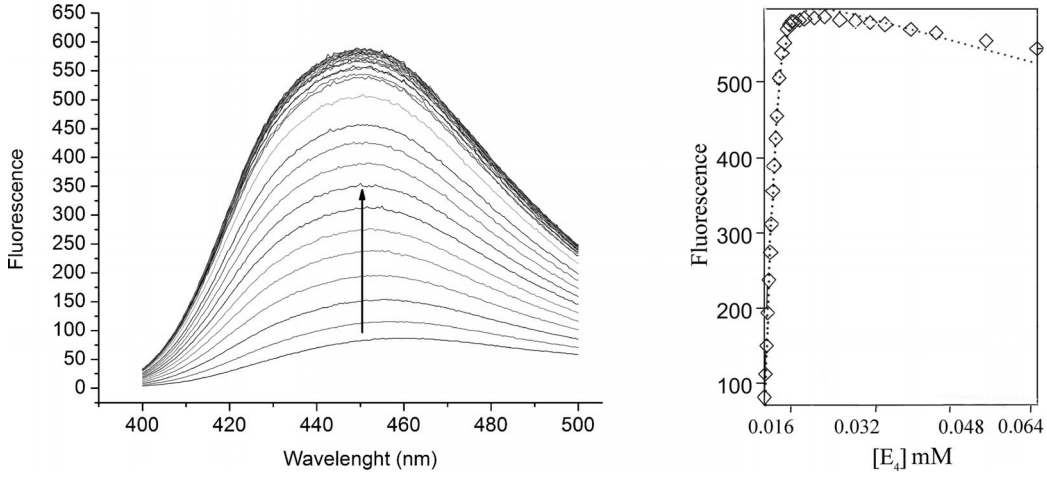


Figure 2. Changes in fluorescence of tetra-Zn^{II} complex **4d** upon addition of the E_4 tag. The titrations were carried out in 25 mM HEPES buffer, pH 7.4, 25 °C, λ_{ex} = 320 nm, $[\mathbf{4d}]_0$ = 0.015 mM. The titration isotherm is shown in the right graph: rhombuses – experimental data; dashed line – fitting curve.

Binding isotherms were fitted to a model of stepwise 2:1, 1:1, and 1:2 binding. Aggregate formation with a 2:1 stoichiometry appears only due to the addition of a mM solution of the guest to the μM solution of a host. During first additions the complex is in large excess, thus several molecules of the latter can coordinate to the tetracarboxylate. The other binding stoichiometries were observed in solution and confirmed by ESI spectrometry. Comparison of the calculated and experimentally found isotope distribution of the molecular ions is shown in Figure 3.

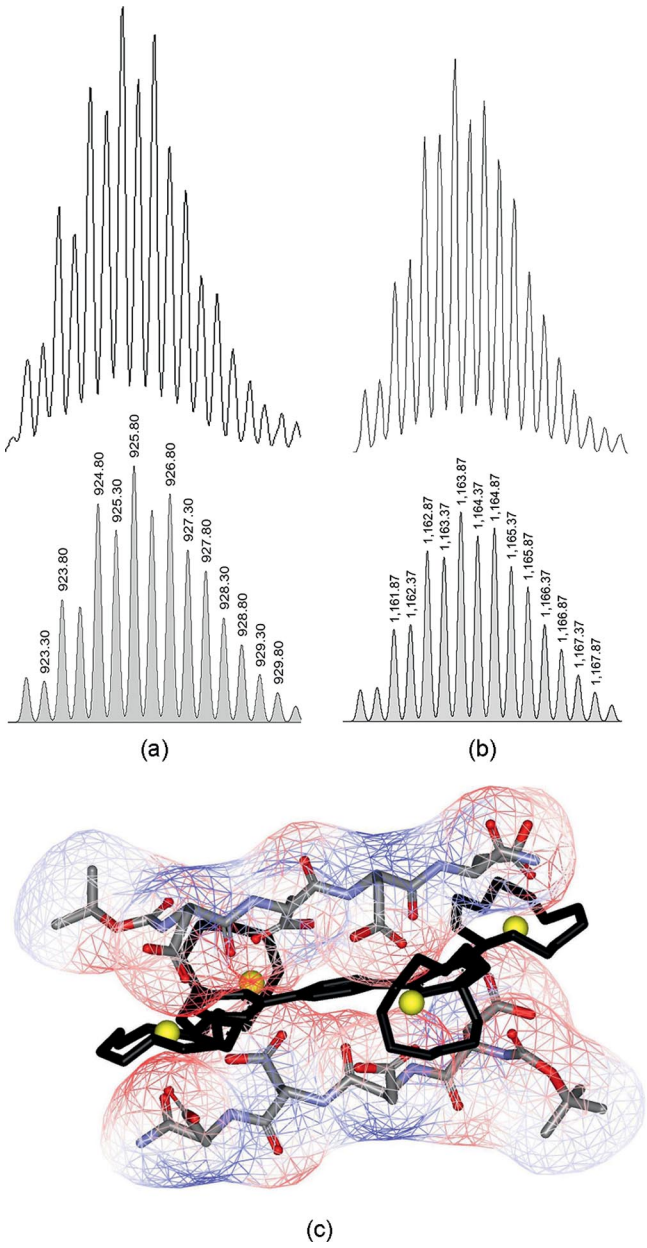


Figure 3. Comparison of the calculated (lower) and observed (upper) isotope distribution for ions: (a) m/z = 923 [**4d** – 8Cl$^-$ + D_4 – 6H]$^{2+}$ and (b) m/z = 1160 [**4b** – 8Cl$^-$ + 2D_4 – 6H]$^{2+}$; (c) schematic geometry of the receptor–peptide tag aggregate of **4b** with two molecules of D_4 tag.

In order to schematically depict the mode of interactions between the oligopeptide and the tetrazinc complexes we have conducted DFT (B3LYP, 6-31G*) calculations using Spartan06 (Wavefunction Inc.). According to the calculations, the scaffold of the two benzene-linked binuclear Zn^{II}–cyclen complexes (i.e., **4b**) perfectly matches the oligopeptide tags (D_4, E_4) structure and charge distribution. In a sandwich-like aggregate of **4b** and the tag protein, strong electrostatic interactions are likely, resulting in the observed high apparent binding affinity. The electrostatic potential surface is displayed as a wired mesh of the D_4 tag in Figure 3. The red color indicates a high density of negative charges at the carboxylate residues of the tag. The cationic Zn^{II}–cyclen favorably interacts with the anionic carboxylate residues. The figure shows the formation of the sandwich-like 1:2 receptor/peptide-tag aggregate of **4b** and the D4 tag. The energy-minimized structure of **4d** shows that unlike **4b**, where the benzene and triazine moieties are in one plane, the plane of anthracene is perpendicular to the plane of the triazine. This creates steric hindrance, which may be responsible for the observed 1:1 receptor to peptide binding stoichiometry in **4d**. The predicted twisted conformation of the triazine anthracene moiety of complex **4d** is in a good agreement with the crystal structure of bis-Zn^{II}–anthracene complex **4c**.

Coordination of Phosphate Anions

Bis-Zn^{II}–cyclen complexes show high affinity for phosphate anions in aqueous solution, which is of interest for potential applications in biological phosphate recognition. However, the so-far developed bis-Zn^{II}–cyclen based synthetic receptors have flexible spacers between the receptor moiety and the signaling unit. Due to this flexibility, the luminescent group cannot respond effectively to the binding event by a change in its emission properties. Figure 4 shows one of our previously reported complexes with a flexible linker between fluorophore and the receptor and its negligible change emission properties in the presence of analytes in homogeneous solution.[23]

In the here-reported synthesized bis-Zn^{II}–cyclen complexes, the rigid molecular structure and the direct conjugation of the central triazine unit to the arenes allows transmission of the anion binding event at the cyclen complex to the signaling unit, which responds by changes in the emission properties (Table 3). The affinities of the complexes for monophosphate anions such as phenylphosphate or *O*-phospho-L-serine are rather small, but they are excellent for polyphosphates. The most efficient response was observed for bis-Zn^{II}–anthracene containing complex **4c**, whose fluorescence is 3.4-fold increased upon addition of 1 equiv. of pyrophosphate. The sensitivity for pyrophosphate is even higher for tetra-Zn^{II} complexes: 5.5- and 0.1-fold change in fluorescence for anthracene- (**4d**) and benzene-containing (**4b**) complexes, respectively (Table 3). Complexes **4b** and **4d** have opposite responses: the presence of pyrophosphate quenches the fluorescence of **4b**, but strongly increases the fluorescence of **4d** (Figure 5).

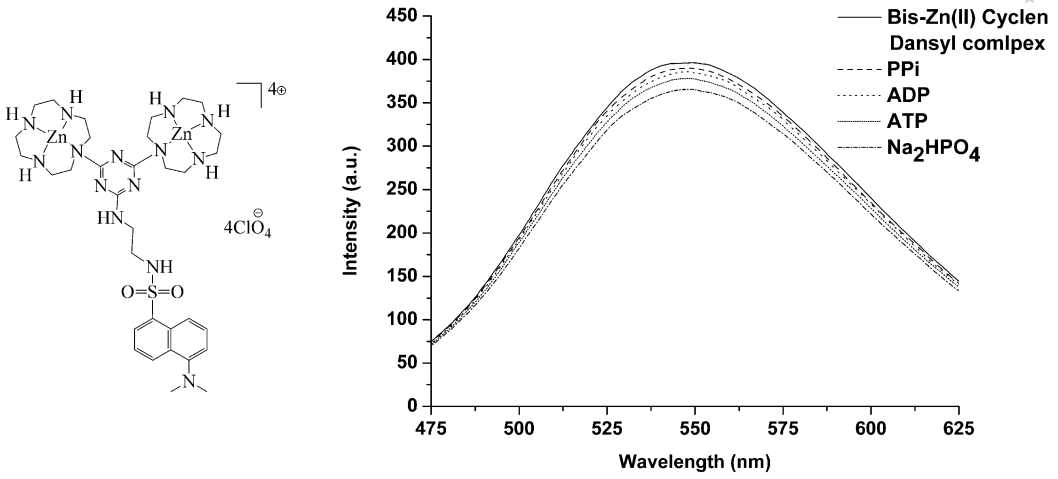


Figure 4. Dinuclear Zn^{II}–cyclen dansyl complex with flexible linker between receptor and fluorophore (left); emission intensity changes of binuclear Zn^{II}–cyclen dansyl complex (80 μM in HEPES buffer, pH 7.4, λ_{ex} = 330 nm, 25 °C) upon addition of various nucleotides and phosphates (right).

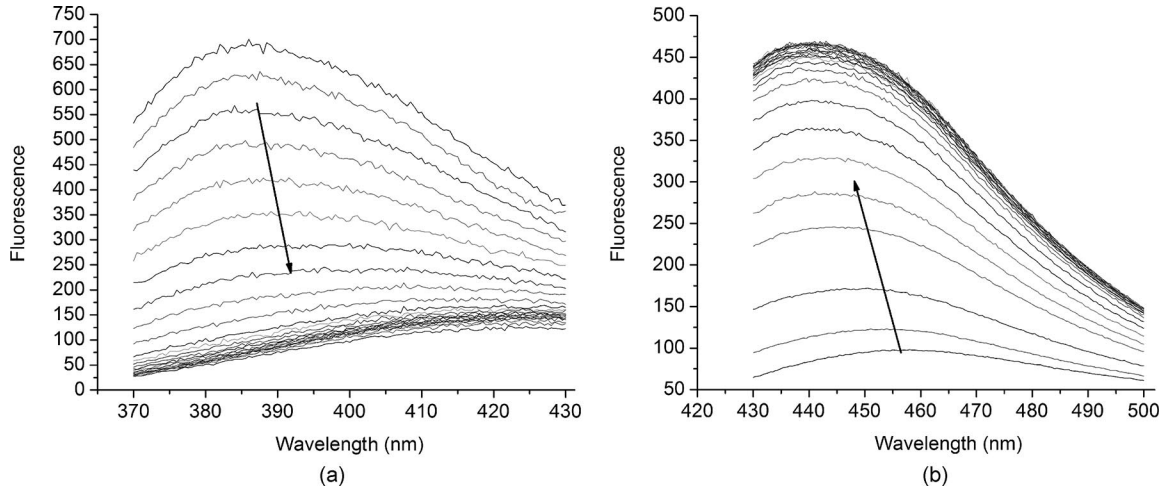


Figure 5. Fluorescence response upon addition of sodium pyrophosphate to the solution of (a) complex **4b** and solution of (b) complex **4d**. Titrations were carried out in 25 mM HEPES buffer, pH 7.4, 25 °C.

Though the affinities of tetra-Zn^{II} complexes for pyrophosphate were good, it was difficult to fit binding isotherms with typical models. Job's plot analysis showed that tetra-Zn^{II} complexes bind pyrophosphate in a 1:2 stoichiometry. Using this model we were able to obtain a stepwise binding constant in the order 10^7 M^{-1} (Table 3). The binding isotherm of bis-Zn^{II} complex **4c** was successfully fitted using a 2:1 and 2:2 stepwise binding model. Additional proof for the formation of the complex with a 2:2 stoichiometry was obtained by ESI mass spectrometry, where the complex with the composition $[(\mathbf{4c} - 4Cl^- + PPi^{4-} + Na^+)_2]^{2+}$ was one of the major peaks (Supporting Information, Figure S26). Thus, coordination of pyrophosphate to **4c** induces dimerization of the complex. This observation led us to suggest that strong quenching of the fluorescence of complex **4b** upon interaction with pyrophosphate can arise from a similar dimerization resulting in π–π stacking interactions of planar benzene–triazine moieties (Figure 6c). This could not be the case for the anthracene-containing tetra-Zn^{II} complex, because it does not have a planar structure according to DFT calculations of **4d** and the X-ray structure of **4c**, thus close interaction of the anthracene rings is not sterically favorable. To prove the dimerization of **4b**, we conducted ESI measurements of both complexes **4b** and **4d** in the presence of an excess amount of pyrophosphate in aqueous solution. Though the major peaks for both complexes corresponded to $[\mathbf{4b} - 8Cl^- + 2PPi^{4-} + 2Na^+]^{2+}$ and $[\mathbf{4d} - 8Cl^- + 2PPi^{4-} + 2Na^+]^{2+}$, the presence of the dimer $[(\mathbf{4b} - 8Cl^- + 2PPi^{4-} + Na^+)_2]^{2+}$ was clearly observed, allowing the isotope distribution to be compared with the predicted one (Figure 6). For anthracene-containing tetra-Zn^{II} complex **4d**, the corresponding dimer was also observed, but with much lower intensity, which did not allow the isotopic distribution to be resolved experimentally (see the Supporting Information).

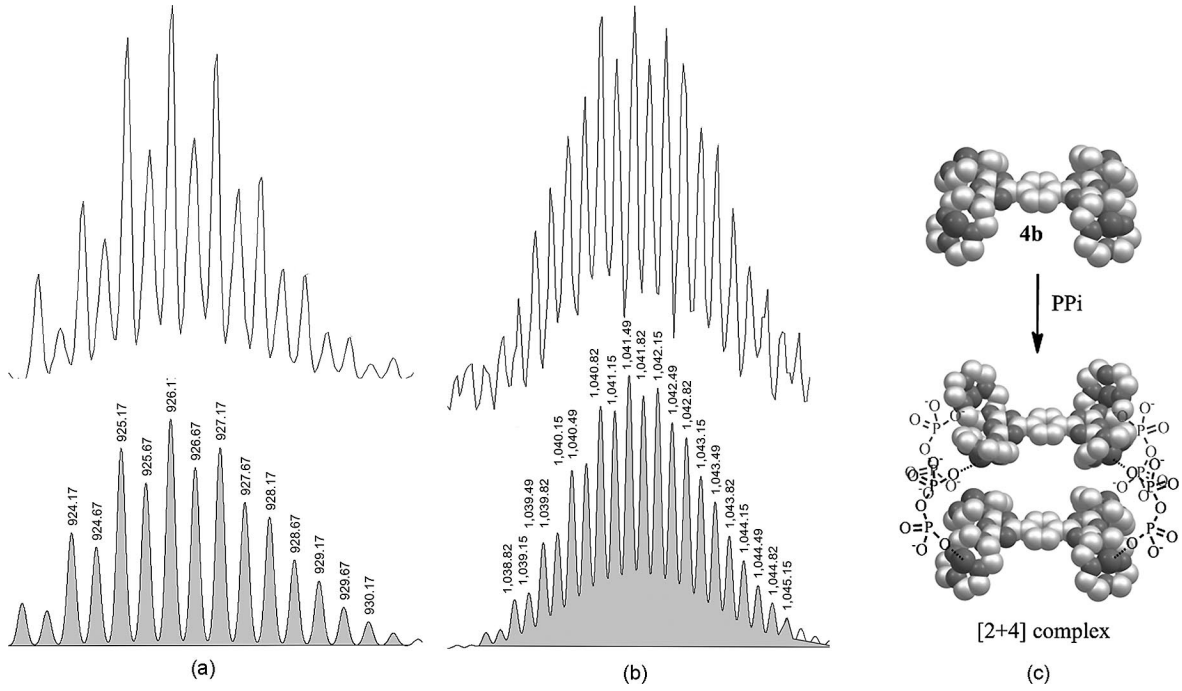


Figure 6. Isotope distribution for ions: (a) m/z = 923 $[(\mathbf{4c} - 4Cl^- + PPi^{4-} + Na^+)_2]^{2+}$, (b) m/z = 1036 $[(\mathbf{4b} - 8Cl^- + 2PPi^{4-} + Na^+)_2 + Na^+]^{3+}$, and (c) proposed structure of dimer $[\mathbf{4b} - 8Cl^- + 2PPi^{4-}]_2$.

Conclusions

We have obtained luminescent synthetic receptors based on 1,3,5-triazine bis-Zn^{II}–cyclen binding sites and arenes by transition-metal-mediated cross-coupling reactions. The synthesized complexes are rigid in structure and show excellent analyte response in buffered aqueous solution. Complexes **4b**, **4c**, and **4d** have high affinities for pyrophosphate and oligocarboxylate (Boc–D_4–NH_2, Boc–E_4–NH_2) anions with changes in emission response reaching one order of magnitude. Though the coordination geometry of Zn^{II}–cyclen is different in comparison to that of Zn^{II}–Dpa, the affinities for the target anions are comparable. Thus, our complexes can be considered as alternative probes for polyphosphates and polycarboxylates.

Experimental Section

General: All reagent-grade chemicals were used without purification unless otherwise specified. Phenylboronic acid, benzene-1,4-diboronic acid, phenyl acetylene, cyanuric chloride, ATP, ADP, *O*-phospho-L-serine, and phenyl phosphate were obtained from Aldrich and used as received. UV/Vis absorption spectra were recorded by using a Cary 50 Bio spectrophotometer, and emission spectroscopy was performed by using a Varian Cary Eclipse fluorescence spectrophotometer. Except for phenylboronic acid (**2a**) and benzene-1,4-diboronic acid (**2b**), all other boronic acids (i.e., **2c**–**h**) were synthesized following reported procedures.[24,25]

General Procedure for the Synthesis of Complexes 4: To a mixture of Boc-protected bis-cyclen chlorotriazene **1** (100 mg, 0.1 mmol) and $Pd(PPh_3)_4$ (10 mg, 0.012 mmol, 12 mol-%) in DME was added required aryl boronic acid **2** (1.5 equiv. of monoboronic acid and 0.5 equiv. of diboronic acid), which was immediately followed by the addition of aqueous Na_2CO_3 (2 M, 2 mL). The mixture was heated at reflux for 48 h under a N_2 atmosphere. After cooling, the solvent was evaporated under reduced pressure to dryness. THF was added, and the suspension was shortly placed in an ultrasonication bath. The mixture was then filtered, washed thoroughly with THF, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel [ethyl acetate (EA)/petroleum ether (PE)] to afford pure products **3** as solids. Obtained ligand **3** was Boc-deprotected with trifluoroacetic acid (14 equiv. per Boc group) and passed through a basic ion exchange column. The Boc-deprotected azamacrocycles, except in the case of **4d**, were dissolved in acetonitrile. To this solution was slowly added anhydrous $ZnCl_2$ (2.5 equiv. for **4a**, **4c**, **4e**–**h** and 4.5 equiv. for **4b**, **4d**) dissolved in methanol, leading to the formation of a white precipitate. For the synthesis of **4d**, Boc-deprotected **3d** was dissolved in methanol/water (4:1), and to this solution was slowly added anhydrous $ZnCl_2$ (4.5 equiv.) dissolved in methanol. The reaction mixture was heated at reflux overnight, which dissolved the precipitate. The hot solution is then decanted in a conical flask. Upon cooling a white precipitate was obtained, which was filtered and analyzed by NMR spectroscopy.

Compound 4a: The Boc-protected ligand was obtained as a colorless solid (53 mg, 50%). R_f = 0.68 (EA/PE, 50:50). It was then Boc deprotected by using TFA to yield the free base (23 mg, 97%) followed by zinc complexation (30 mg, 100%). Data for **3a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 54 H, CH_3-Boc), 3.41–3.91 (br. m, 32 H, CH_2-cyclen), 8.441 (d, J = 6.9 Hz, 2 H), 7.469 (t, 1 H), 7.438 (t, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.5 (+, CH_3-Boc), 49.1, 49.7, 50.2 (–, CH_2-cyclen), 76.5, 78.9 (C_q, C-Boc), 156.4, 157.0 (C_q, C=O Boc), 168.6 (C_q, triazine-C_{aryl}-N), 126.9, 127.430, 130.2, 135.5 (benzene) ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): m/z (%) = 1094 (100) $[M + H]^+$. Data for **4a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.492 (t, 2 H), 7.512–7.623 (m, 3 H), 4.433–4.508 (m, 2 H), 4.149 (br. s, 2 H), 2.721–3.554 (m, 28 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 42.4, 43.3, 44.5, 45.5,

45.6, 46.4, 47.6, 47.9 (–, CH_2-cyclen), 128.1, 128.5, 132.2, 132.4, 135.1, 135.3, 170.3, 170.8 ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 372.6 (100) $[M^{4+} + 2CH_3COO^-]^{2+}$. M.p. 235–238 °C. IR (ATR): $\tilde{\nu}$ = 3100, 2940, 1350, 1154, 968, 820 cm^{-1}. UV (HEPES pH 7.4 25 mM): λ_{max} (log ε) = 224 nm (4.4). MF: $[C_{25}H_{43}N_{11}Zn_2]^{4+}4ClO_4^-$. FW: 1026.25 g mol^{-1}.

Compound 4b: The Boc-protected compound was obtained as a colorless solid (64 mg, 31%). R_f = 0.68 (EA/PE, 50:50). It was then Boc-deprotected by using TFA (27 mg, 96%) followed by Zn complexation (41 mg, 100%). Data for **3b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.391 (s, 108 H, CH_3-Boc), 3.370–3.594 (br. m, 64 H, CH_2-cyclen), 8.368 (s, 4 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 28.52 (+, CH_3-Boc), 50.24 (–, CH_2-cyclen), 79.91 (C_q, C-Boc), 128.11, 156.62, 169.46 ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc) *m/z* (%) = 1060 (100) $[M + 2H^+]^{2+}$, 1010 (50) $[M + 2H^+ – Boc]^{2+}$, 2119.2 (10) $[M + H^+]$. Data for **4b**: ^{1}H NMR (300 MHz, $[D_6]$DMSO): δ = 1.042–1.992 (br. m, 58 H), 2.925 (br., 6 H), 6.878 (s, 4 H) ppm. ^{13}C NMR (75 MHz, $[D_6]$DMSO): δ = 44.2, 46.3, 129.5, 140.5, 168.9, 171.4 ppm. MS (ESI, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 490.7 (100) $[M^{8+} + 5CH_3COO^-]^{3+}$. M.p. 255–257 °C. IR (ATR): $\tilde{\nu}$ = 3398, 2933, 1680, 1524, 1347, 1193, 1132, 1087, 971, 813, 723 cm^{-1}. UV (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 285 nm (4.530). MF: $[C_{44}H_{92}N_{22}Zn_4]^{8+}Cl_8$. FW: 1474.50 g mol^{-1}.

Compound 4c: The Boc-protected compound was obtained as a colorless solid (56 mg, 50%). R_f = 0.68 (EA/PE, 50:50). It was then Boc deprotected by using TFA (25 mg, 95%) followed by Zn complexation (36 mg, 100%). Data for **3c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.451 (s, 54 H, CH_3-Boc), 3.440–3.661 (br. m, 32 H, CH_2-cyclen), 7.316–7.439 (m, 4 H), 7.903 (br. d, 2 H), 8.003 (d, 2 H), 8.445 (s, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.88, 26.30, 26.87, 27.48, 28.43, 48.95, 49.99, 79.05, 123.93, 124.28, 125.17, 126.12, 127.31, 127.82, 130.27, 133.22 ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1023 (100) $[M + 2H^+]^{2+}$, 1199 (65) $[M + H^+]$, 1053 (35) $[M + 2H^+ – Boc]^{2+}$. Data for **4c**: ^{1}H NMR (300 MHz, $[D_6]$DMSO): δ = 2.647–3.231 (br. m, 32 H), 4.249 (br. s, 2 H), 4.815 (br. s, 4 H), 7.445–7.573 (m, 4 H), 8.025 (d, 2 H), 8.168 (d, 2 H), 8.725 (s, 1 H) ppm. ^{13}C NMR (150 MHz, $[D_6]$DMSO): δ = 30.6, 44.8, 46.8 (CH_2-cyclen), 125.4, 126.2, 128.1, 130.7, 132.8, 133.1, (CH, aromatic), 158.0, 169.2, 172.5, 173.0 ppm. MS (ESI, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 423.6 (100) $[M^{4+} + 2CH_3COO^-]^{2+}$. M.p. 274–282 °C. IR (ATR): $\tilde{\nu}$ = 3090, 2943, 1345, 1087, 1130, 963, 825 cm^{-1}. UV (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 365 nm (3.9). MF: $[C_{33}H_{47}N_{11}Zn_2]^{4+}Cl_4^-$. FW: 870.37 g mol^{-1}.

Compound 4d: The Boc-protected compound was obtained as a colorless solid (65 mg, 30%). R_f = 0.68 (EA/PE, 50:50). It was then Boc deprotected by using TFA (12 mg, 95%) followed by Zn complexation (18 mg, 100%). Data for **3d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.457 (s, 108 H, CH_3-Boc), 3441–3.653 (br. m, 64 H, CH_2-cyclen), 7.283–7.317 (dd, 4 H), 7.834–7.868 (br., 4 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 27.5 (+, CH_3-Boc), 48.9 (–, CH_2-cyclen), 79.0 (C_q, C-Boc) ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1110.6 (100) $[M + 2H^+]^{2+}$, 2220 (6) $[M + H^+]$. Data for **4d**: ^{1}H NMR (300 MHz, D_2O): δ = 2.667–2.965 (m, 46 H), 2.987–3.011 (br., 3 H), 3.192–3.337 (br., 18 H), 7.4921–7.953 (dd, 4 H) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 43.6, 44.8, 46.1, 47.6, 113.4, 115.3, 117.2, 119.2, 169.8, 173.9 ppm. MS (ESI, H_2O/MeCN): *m/z* (%) = 355.0 $[M^{8+}+4Cl^-]^{4+}$(100), 485.2 (50) $[M^{8+}+5Cl^-]^{3+}$. M.p. 292–296 °C. IR (ATR): $\tilde{\nu}$ = 3025, 2893, 1640, 1585, 1125, 980, 720 cm^{-1}. UV (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 370 nm (3.36). MF: $[C_{52}H_{84}N_{22}Zn_4]^{8+}Cl_8^-$. FW: 1562.52 g mol^{-1}.

Compound 4e: The Boc-protected compound was obtained as a colorless solid (62 mg, 54%). R_f = 0.68 (EA/PE, 50:50). It was then Boc deprotected by using TFA (30 mg, 96%) followed by Zn complexation (43 mg, 100%). Data for **3e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.466 (s, 54 H, CH_3-Boc), 3.150–3.896 (br. m, 32 H, CH_2-cyclen), 8.015–8.216 (m, 7 H), 8.571 (br. d, 1 H), 9.109 (br. s, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 50.3, 51.0, 80.1, 124.3, 124.7, 125.2, 125.4, 125.9, 127.3, 127.5, 128.3, 130.71, 131.2 ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1223 (100) $[M + H^+]$, 1022.9 (20) $[M + H^+ – 2Boc]$. Data for **4e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.730–2.941 (br. m, 28 H), 3.472 (br. s, 2 H), 4.391 (br. s, 4 H), 4.935 (br. d, 4 H), 8.150 (t, 1 H), 8.290–8.421 (m, 6 H), 8.593 (d, 1 H), 9.042 (d, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 43.9, 45.0, 46.3, 47.3, 123.6, 123.9, 124.5, 125.4, 125.6, 126.0, 126.6, 127.3, 128.2, 128.7, 128.8, 130.1, 130.7, 132.2, 132.2, 169.3, 172.7 ppm. MS (ESI, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 434.8 (100) $[M^{4+} + 2CH_3COO^-]^{2+}$. M.p. 285–287 °C. IR (ATR): $\tilde{\nu}$ = 3125, 2890, 1650, 1545, 1175, 980, 880, 715 cm^{-1}. UV (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 350 nm (4.16). MF: $[C_{35}H_{47}N_{11}Zn_2]^{4+}Cl_4^-$. FW: 894.39 g mol^{-1}.

Compound 4f: The Boc-protected compound was obtained as a colorless solid (83 mg, 78%). R_f = 0.58 (EA/PE, 50:50). It was then Boc deprotected by using TFA (37 mg, 97%) followed by Zn complexation (72 mg, 100%). Data for **3f**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.478 (s, 54 H, CH_3-Boc), 2.84 (t, 2 H, CH_2), 3.12 (t, 2 H, CH_2), 3.31–3.87 (br. m, 32 H, CH_2-cyclen), 7.22–7.26 (m, 5 H, aromatic protons) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.1 (CH_2), 28.4, 28.5 2 (CH_3-Boc), 41.1 (CH_2), 50.1 (CH_2-cyclen), 79.86 (C_q, C-Boc), 125.5, 128.2 (aromatic C), 144.2 (C_q, aromatic C), 176.7 (C_q, triazine) ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1126.7 (100) $[M + H^+]$, 1164.6 (40) $[M + K^+]$. Data for **4f**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.84 (t, 2 H, CH_2), 3.12 (t, 2 H, CH_2), 3.29–3.55 (br. m, 32 H, CH_2-cyclen), 7.22–7.26 (m, 5 H, aromatic protons) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.3 (CH_2), 41.1 (CH_2), 50.1 (CH_2-cyclen), 79.8 (C_q, C-Boc), 125.5, 128.2 (aromatic C), 144.2 (C_q, aromatic C), 176.7 (C_q, triazine) ppm. MS (ESI, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 386.5 (100) $[M^{4+} + 2CH_3COO^-]^{2+}$. M.p. 290–293 °C. IR (ATR): $\tilde{\nu}$ = 3290, 2942, 2971, 2863, 1645, 1470, 1385, 980, 824, 728 cm^{-1}. MF: $[C_{27}H_{47}N_{11}Zn_2]^{4+}Cl_4^-$. FW: 798.3 g mol^{-1}.

Compound 4g: The Boc-protected compound was obtained as a colorless solid (50 mg, 47%). R_f = 0.71 (EA/PE, 50:50). It was then Boc deprotected by using TFA (23 mg, 96%) followed by Zn complexation (40 mg, 100%). Data for **3g**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.445 (s, 54 H, CH_3-Boc), 3.428–4.085 (br. m, 32 H, CH_2-cyclen), 6.839 (d, CH-ethylene, J = 15.9 Hz), 7.338–7.358 (m, 3 H, aromatic protons), 7.568 (d, J = 7.2 Hz, 2 H, aromatic protons), 7.959 (d, J = 15.9 Hz, 1 H, CH-ethylene) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.3, 28.6 (CH_3-Boc), 49.1, (CH_2-cyclen), 78.8 (C_q, C-Boc), 126.6, (C=C), 127.6, 127.9 (aromatic C), 135.0 (C=C), 168.9 (C_q, triazine) ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1124.5 (100) $[M + H^+]$. Data for **4g**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.67–3.76 (br. m, 38 H), 6.35 (d, CH-ethylene), 7.18–7.55 (m, aromatic protons), 7.79 (d, CH-ethylene) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 43.9, 45.0, 46.3, 47.3, 123.6, 123.9, 124.4, 125.4, 125.6, 125.9, 126.6, 127.3, 128.2, 128.7, 128.8, 130.1, 130.7, 132.2, 132.2, 169.2, 172.7 ppm. MS (ESI, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 385.5 (100) $[M^{4+} + 2CH_3COO^-]^{2+}$. IR (ATR): $\tilde{\nu}$ = 3385, 3083, 2966, 2896, 1680, 1644, 1525, 1465, 1182, 965, 812, 720 cm^{-1}. UV (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 220 nm (4.10). MF: $[C_{27}H_{45}N_{11}Zn_2]^{4+}Cl_4^-$. FW: 796.29 g mol^{-1}.

Compound 4h: The Boc-protected compound was obtained as a colorless solid (56 mg, 52%). R_f = 0.68 (EA/PE, 50:50). It was then

Boc deprotected by using TFA (24 mg, 92%) followed by Zn complexation (48 mg, 100%). Data for **3h**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, 3 H, CH_3), 1.455 (s, 54 H, CH_3-Boc), 1.576–1.711 (m, 10 H, CH_2), 2.458 (t, 2 H, CH_2), 3.22–3.76 (br. m, 32 H, CH_2-cyclen) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.5, 28.7 (CH_3-Boc), 49.5 (CH_2-cyclen), 78.8 (C_q, C-Boc) ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1120.9 (100) [M + H^+]. Data for **4h**: ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 0.86 (t, 3 H, CH_3-alkyl chain) 1.26 (br. s, 8 H), 1.75 (s, 7 H), 2.1 (s, 6 H), 2.89 (br. m, 16 H), 3.4 (s, 13 H) ppm. ^{13}C NMR (75 MHz, [D_6]-DMSO): δ = 13.7 (CH_3-alkyl chain), 17.8, 21.8, 22.1, 22.2, 26.2, 28.3 (CH_2-alkyl chain), 30.9, 39.1 ppm. MS (ESI, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 383.6 (100) [M^{4+} + 2CH_3COO^-]$^{2+}$. IR (ATR): $\tilde{\nu}$ = 2921, 2852, 1693, 1561, 1525, 1465, 1420, 1346, 1282, 1085, 812 cm^{-1}. MF: [$C_{26}H_{53}N_{11}Zn_2$]$^{4+}Cl_4^-$. FW: 792.35 g mol^{-1}.

Synthesis of Compound 5: In a two-necked flask, Boc-protected biscyclen chlorotriazene **1** (0.2 g, 0.19 mmol), Pd/C (10%, Fluka), PPh_3, and CuI in a 1:0.04:0.16:0.04 molar ratio were placed under a nitrogen atmosphere along with DIPEA (1.5 mL) as the suitable base. After accurately purging with nitrogen, a solution of phenyl acetylene (0.05 mL, 0.48 mmol) in CH_3CN was added, the temperature was raised, and the mixture was vigorously stirred overnight. After cooling and filtering the mixture on a short plug of Celite, the solution was evaporated under reduced pressure. The crude reaction mixture was purified by column chromatography (40% EA in PE) to get pure product **5** (51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 54 H, CH_3-Boc), 3.24–4.00 (br. m, 32 H, CH_2-cyclen), 7.5–7.57 (dd, 2 H, benzene ring), 7.29–7.43 (br. m, 3 H, benzene ring) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.5, 48.9, 87.6, 120.8, 127.2, 128.3, 131.4, 158.0 ppm. MS (ESI, DCM/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1122.8 (100) [M + H^+]. IR (ATR): $\tilde{\nu}$ = 3385, 3083, 2966, 2896, 2250, 1680, 1512, 1465, 1182, 965, 812 cm^{-1}. MF: $C_{57}H_{91}N_{11}O_{12}$. FW: 1122.4 g mol^{-1}.

Synthesis of Compound 7: The monoazide derivative of cyanuric chloride, **6** was synthesized following a reported procedure.[26] In a round-bottomed flask, the monoazide derivative of trichlorotriazine (0.2 g, 1.08 mmol) and K_2CO_3 (0.6 g, 4.32 mmol) were suspended in acetone (70 mL). To this suspension was slowly added a solution of threefold Boc-protected cyclen (1.02 g, 2.16 mmol) in acetone (30 mL). The reaction mixture was then heated under reflux for 12 h. The solvent was removed under reduced pressure, and the crude mixture was purified by column chromatography (30% EA in PE) to get pure product **7** (0.15 g, 20%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 (s, 54 H, CH_3-Boc), 3.04–3.75 (br. m, 32 H, CH_2-cycle) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.4, 31.7, 50.0, 53.8, 69.4, 79.9, 156.1, 168.2, 210.7 ppm. MS (ESI-Q1MS): *m/z* (%) = 1063.8 (100) [M + H^+]. M.p. 87–89 °C. IR (ATR): $\tilde{\nu}$ = 2974, 2928, 2865, 1694, 1540, 1361, 1169 cm^{-1}. MF: $C_{48}H_{84}N_{14}O_{12}$. FW: 1048.64 g mol^{-1}.

Synthesis of Compound 8: To a stirred solution of **7** (0.28 g, 0.29 mmol) in degassed dry THF (3 mL) was added phenylacetylene (0.05 mL, 0.48 mmol), CuI (6 mol-%), and TEA (0.1 mL, 0.72 mmol), and the reaction mixture was stirred at room temperature for 12 h. Then the product was extracted with ethyl ether (3 × 10 mL). After removal of the solvent, the crude product was purified by column chromatography (EA/PE, 1:1) to get **8** (0.29 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.49 (s, 54 H, CH_3-Boc), 3.27–3.85 (br. m, 32 H, CH_2-cyclen), 7.29–7.52 (br. m, 5 H, benzene ring), 7.97 (s, 1 H, triazole ring) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.5, 50.1, 80.1, 80.1, 80.2, 104.4, 126.0, 128.2, 128.7, 130.2, 147.3 ppm. MS (ESI-Q1MS): *m/z* (%) = 283.1 (100) [M + 2H]$^{2+}$, 565.3 (40) [M + H^+]. M.p. 95–98 °C. IR (ATR): $\tilde{\nu}$ = 2973, 2923, 1667, 1541, 1249, 1201, 1136, 901, 844 cm^{-1}. MF: $C_{27}H_{44}N_{14}$. FW: 564.39 g mol^{-1}.

X-ray Structure Determination: Crystal data for $C_{33}H_{47}C_{l2}N_{11}Zn_2{\cdot}CH_3OH{\cdot}ZnCl_4$: M = 1038.73 g mol^{-1}, monoclinic, $P2_1/c$, a = 13.33784(16) Å, b = 29.7188(4) Å, c = 10.88517(13) Å, α = 90°, β = 94.0245(11)°, γ = 90°, V = 4304.07(9) Å^3, Z = 4, 18752 reflections measured, 8828 independent (R_{int} = 0.0208), which were used in all calculations. The final wR_2 was 0.0972 (all data). Intensity data were collected with graphite-monochromated Mo-K_α radiation (λ = 1.54184 Å) at 123 K with a Goniometer Xcalibur, detector: Ruby (Gemini ultra Mo). For data collection and structure solution and refinement the following programs were used: SHELXL,[27] R_1 is calculated for observed data and wR_2 for all data.

CCDC-808617 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra, MS (ESI) characterization, and fluorescence titration data including fitting curves; details of the quantum chemical calculations.

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