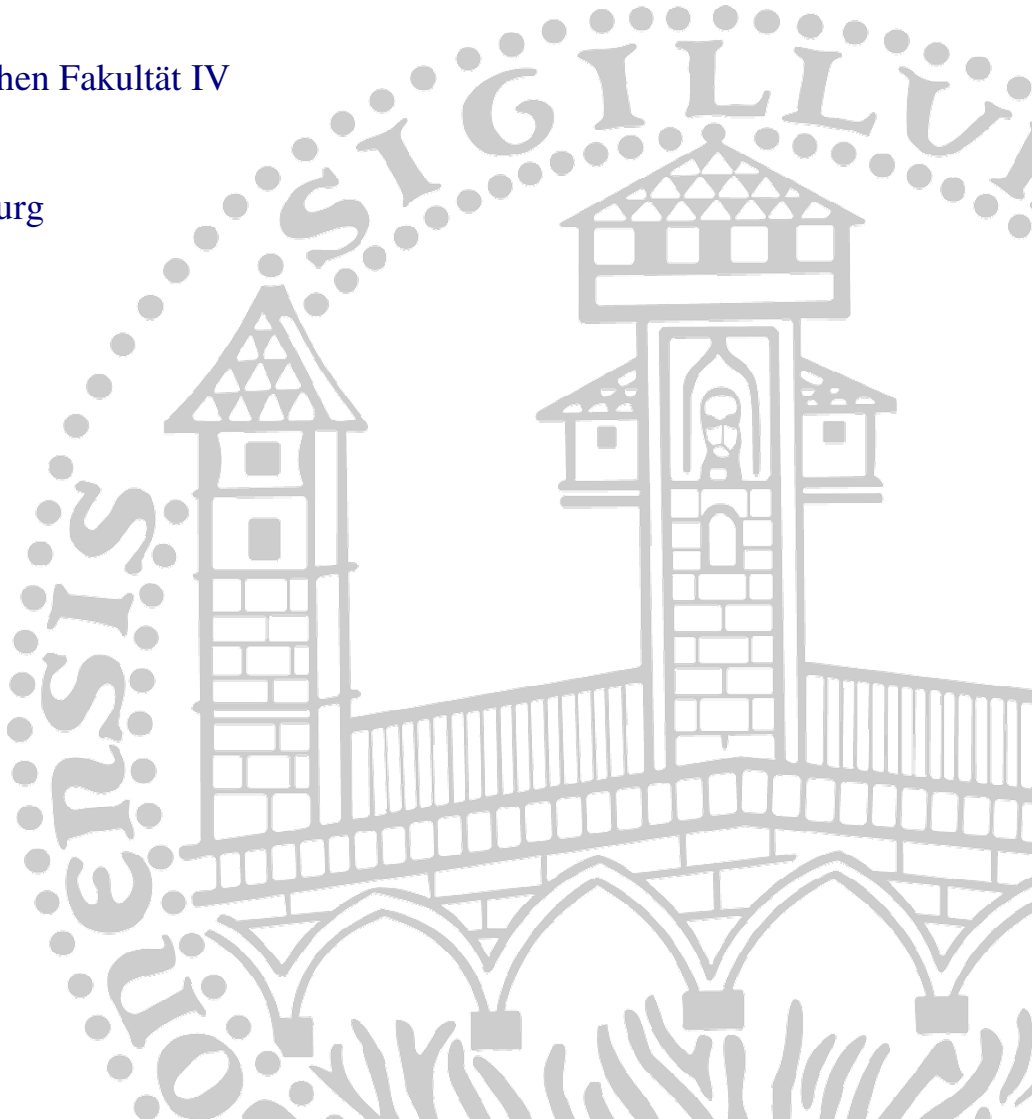
COORDINATION CHEMISTRY IN MOLECULAR RECOGNITION

Dissertation

zur Erlangung des Grades

Doktor der Naturwissenschaften (Dr. rer. nat.)

der naturwissenschaftlichen Fakultät IV
Chemie und Pharmazie
der Universität Regensburg



vorgelegt von

Stefan Stadlbauer

aus Cham

2009

Coordination Chemistry

In Molecular Recognition

Dissertation

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The experimental part of this work was carried out between Februar 2006 and March 2009 at the Institute for Organic Chemistry at the University of Regensburg under the supervision of Prof. Dr. B. König.

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Board of Examiners:	Prof. Dr. R. Winter	(Chairman)
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	Prof. Dr. O. Reiser	(2nd Referee)
	Prof. Dr. W. Kunz	(Examiner)

Für

meine Familie

Wer sich Steine zurechtlegen kann, über die er stolpert,
hat Erfolg in den Naturwissenschaften.

Erwin Chargaff
(österr. - amerik. Biochemiker u. Schriftsteller)

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Table of Contents

1. Investigation on the Binding Affinity of Nitrilotiracetato (NTA) Metal Complexes to Histidine, Imidazole and Lysozyme by Isothermal Titration Calorimetry[i]

Reversible coordinative binding of Lewis basic donors to iminodiacetato (IDA) and nitrilotriacetato (NTA) metal complexes is widely used for the design of synthetic receptors binding to peptides, proteins or enzymes at physiological conditions. However, no data on the affinity of M(II)-NTA (M = Cu, Ni, Zn) to a single histidine or imidazole moiety are available. We herein report the investigation of the binding affinity and thermodynamics of copper(II), nickel(II) and zinc(II) NTA complexes to histidine, imidazole and hen egg white lysozyme, bearing a single surface exposed histidine unit, by isothermal titration calorimetry at physiological conditions. Further, we describe a peptide-metal complex hybrid approach to enhance the binding affinity of Cu(II)-NTA to lysozyme.

[i] S. Stadlbauer, B. König *Inorg. Chim. Acta* **2009**, *submitted*.

1.1 Introduction

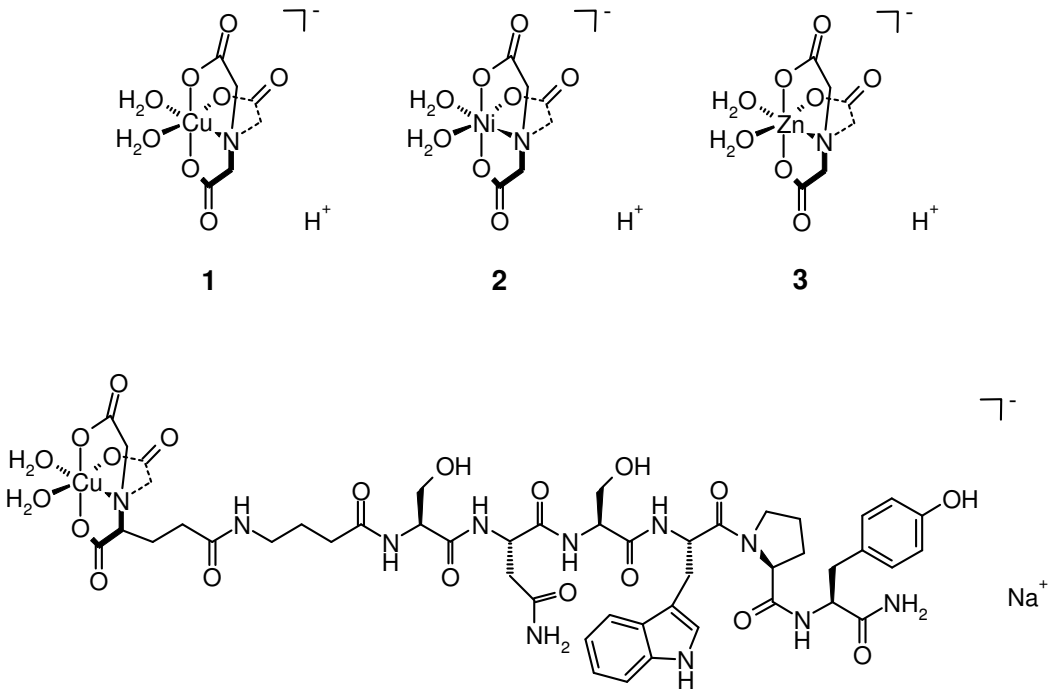
Immobilized metal (ion) affinity chromatography (IMAC) reported 1975 by Porath et al. is a common method in protein purification.[1] Typical examples for coordination compounds employed in this technique are iminodiacetato (IDA) and nitrilotriacetato (NTA) metal complexes.[2]

On the other hand, the selective and reversible binding of biomolecules to IDA and NTA complexes is used in the design of synthetic receptors binding to peptides, proteins or enzymes at physiological conditions. Several of such receptors for selective enzyme inhibition[3] or for protein/peptide sensing[4] have been reported.

We recently investigated the interaction of M(II)-cyclen (M = Zn, Cd) and M(II)-IDA / M(II)-NTA (M = Cu, Ni) complexes to imidazole by potentiometric titration.[5] Surprisingly, to the best of our knowledge, no other thermodynamic binding affinity data of imidazole or histidine to simple NTA metal complexes in aqueous solution are available in the literature.

Thus we determined the binding affinity, stoichiometry and the thermodynamics of the interaction of histidine and imidazole to M(II)-NTA (M = Cu, Ni, Zn) by isothermal titration calorimetry (ITC). Furthermore, we were interested whether the interaction of imidazole and M(II)-NTA is of sufficient strength for protein binding by a single coordinative bond. To test this hypothesis, we chose the well-established hen egg white lysozyme (HEWL),[6] which bears only one surface exposed histidine.[7]

In a previous study we demonstrated an enhanced peptide β-sheet affinity in DMSO solution using a combination of peptide β-sheet binding methoxypyrrole amino acids (MOPAS) and Zn(II)-NTA as an anchor to the histidine containing target peptide.[8] This inspired us after the evaluation of the affinity of M(II)-NTA metal complexes **1**–**3** to lysozyme to prepare a Cu(II)-NTA complex **4** modified by an amino acid sequence taken from the monoclonal HEWL antibody Hy-HEL 10 Fv. We expected that the tethered copied antibody sequence may enhance the Cu(II)-NTA affinity to lysozyme.



1 **2** **3**

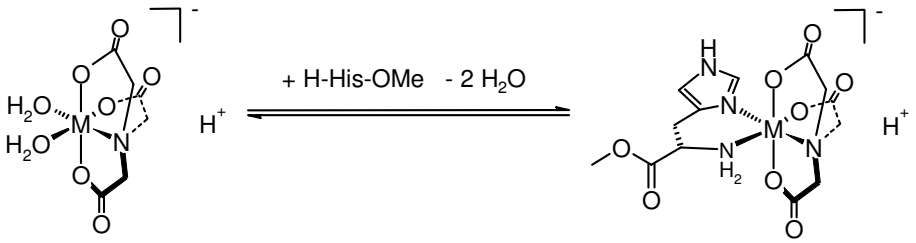
4

Figure 1. Structures of investigated NTA metal complexes in this study: Cu(II)-NTA **1**, Ni(II)-NTA **2**, Zn(II)-NTA **3** and by monoclonal antibody sequence modified Cu(II)-NTA **4**.

1.2 Results and Discussion

1.2.1 M(II)-NTA titration to histidine

The binding affinity of M(II)-NTA **1** – **3** to histidine (H-His-OMe) was investigated. The ITC data reveal a 1:1 binding process between histidine and the octahedral NTA complex: Two metal complex coordination sites are occupied by the α-amino group and the imine imidazole nitrogen atom of histidine as shown in Scheme 1.[2, 9] The binding constants of **1** – **3** to histidine exceeded the milli molar range and decreased in accordance to the Lewis acidity of the metal ions in the order Cu^{2+} (d8) > Ni^{2+} (d9) > Zn^{2+} (d10).[10] All ITC data are summarized in Table 1.



Scheme 1. Reversible bidental binding of H-His-OMe to NTA-M (M = Cu^{2+}, Ni^{2+}, Zn^{2+}).

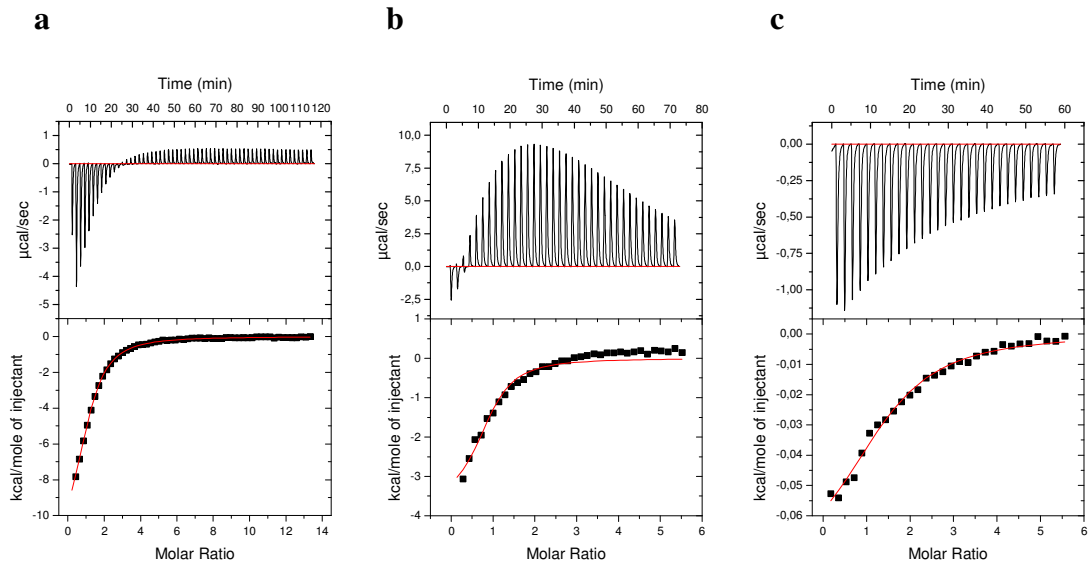
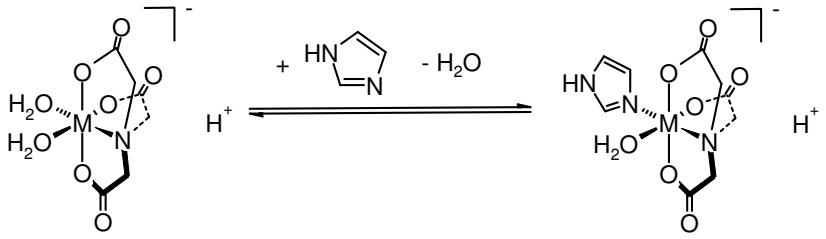


Figure 2. ITC binding isotherms for the interaction of M(II)-NTA to histidine. a) [**1**] = 60 µM, [H-His-OMe] = 3.75 mM. b) [**2**] = 1.0 mM, [H-His-OMe] = 25 mM, c) [**3**] = 1.0 mM, [H-His-OH] = 25 mM.

1.2.2 M(II)-NTA titration to imidazole

The binding of imidazole to the metal complexes was investigated analogously. Although there are two available coordinations sites of the NTA metal complex[2, 9] under the conditions of our investigation 1:1 aggregates were determined, which is in accordance with our earlier potentiometric measurements (Scheme 2).[5] Only the Cu(II)-NTA – imidazole interaction is strong enough to give good ITC data from which a binding constant of *lg* K = 4.2 was calculated (Figure 3). The interactions of imidazole with Ni(II)- and Zn(II)-NTA are too weak for ITC analysis and estimated to be smaller than milli molar (*lg* K < 3).



Scheme 2. Reversible binding of imidazole to M(II)-NTA (M = Cu^{2+}, Ni^{2+}, Zn^{2+}).

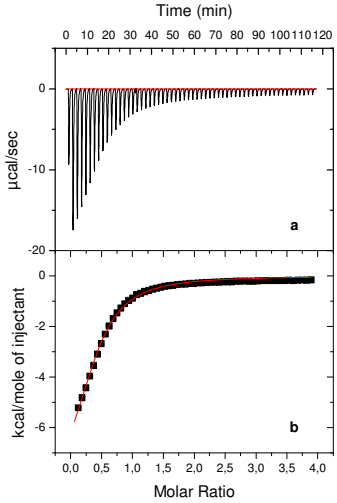


Figure 3. ITC binding isotherms for the interaction of Cu(II)-NTA to imidazole. Measurement conditions: [**1**] = 1 mM, [imidazole] = 17.5 mM.

1.2.3 M(II)-NTA titration to lysozyme

Having investigated the affinity of NTA complexes **1** – **3** to histidine and imidazole, the binding affinity to hen egg white lysozyme (HEWL) in buffered aqueous solution was determined (Figure 4). As expected, the affinity correlated with the Lewis acidity of the NTA complex and a binding to the surface exposed histidine (His15) of lysozyme was only observable with milli molar affinity (lgK = 3.3) for Cu(II)-NTA (Figure 4a). The measurements using the corresponding nickel and zinc complexes did not show a significant ITC signal and the affinity under the experimental conditions is certainly lower than milli molar (Figure 4b).[11]

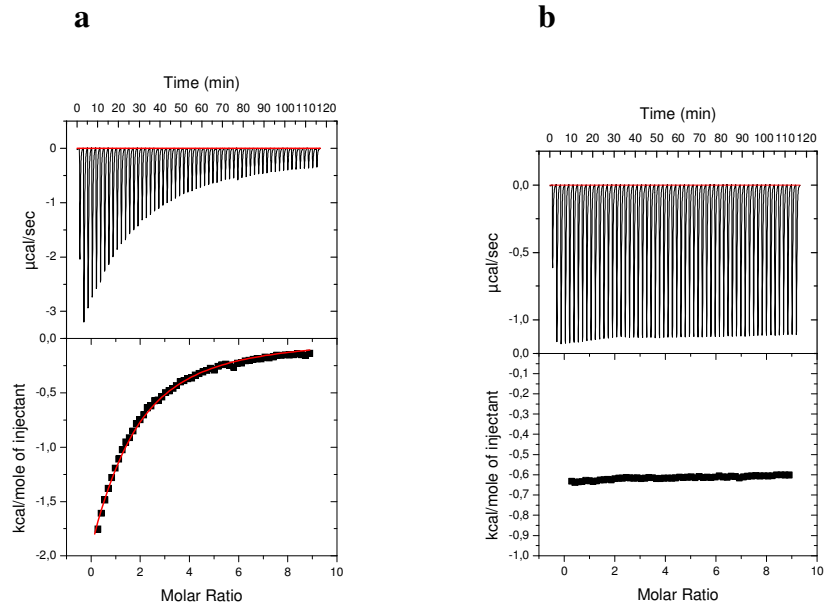


Figure 4. ITC binding isotherms for the interaction for M(II)-NTA to HEWL. Measurement conditions: a) [HEWL] = 0.25 mM, [**1**] = 10 mM. b) [HEWL] = 0.25 mM, [**2**] = 10 mM.

		Cu(II)-NTA	**Ni(II)-NTA**	**Zn(II)-NTA**[10]
histidine	n	1.11 ± 0.01	0.87 ± 0.07	1.44 ± 0.07
	*lg*K	4.59 ± 0.01	3.76 ± 0.15	3.25 ± 0.07
	ΔH	– (12.23 ± 0.14)	– (3.71 ± 0.42)	– (0.079 ± 0.001)
	TΔS	– 5.77	1.42	4.34
	ΔG	– 6.46	– 5.13	– 4.42
imidazole	n	1.16 ± 0.01		
	*lg*K	4.18 ± 0.04	< 3	< 3
	ΔH	– (6.68 ± 0.10)		
	TΔS	– 0.89		
	ΔG	– 5.79		
lysozyme	n	1		
	*lg*K	3.27 ± 0.01	< 3	< 3
	ΔH	– (5.85 ± 0.06)		
	TΔS	– 1.39		
	ΔG	– 4.46		

Table 1. Stoichiometry (n), binding constant (*lg*K), enthalpy (ΔH, kcal mol^{-1}), entropy (TΔS, kcal mol^{-1}) and free energy (ΔG, kcal mol^{-1}) for the interaction of **1** – **3** to histidine, imidazole and HEWL at physiological conditions (50 mM HEPES, pH 7.5, 25 °C).

1.2.4 Modified Cu(II)-NTA (4) titration to lysozyme

As the affinity of Cu(II)-NTA to lysozyme was found to be milli molar, we were interested in modifying **1** in order to increase its binding affinity. Figure 1 shows a crystal structure of antigen – antibody complex of HEWL and antibody Hy-HEL 10 Fv.[12] Utilizing Cu(II)-NTA as anchor group to the lysozymes surface, we reason that a tethered short amino acid sequence of the monoclonal antibody to **1** will increase the overall affinity (Figure 6a). The amino acids Ser91, Asn92, Ser93, Trp94, Pro95 and Tyr96 are located in the variable region of the monoclonal antibody with an approximate distance of 15 – 17 Å to His15 (Figure 6b). All amino acids beside proline are known to interact through hydrogen bonds, salt bridges and water mediated hydrogen bonds with lysozyme in solid state.[13] Compound **4** was prepared by solid phase peptide synthesis as described in scheme 1 (For detailed synthetic procedures see Experimental Part).

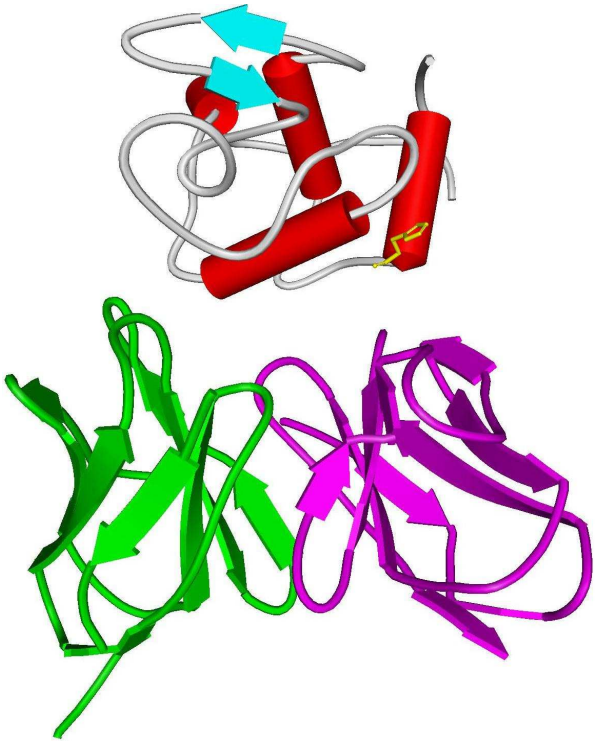


Figure 5. Schematic model of monoclonal antibody Hy-HEL 10 Fv - HEWL complex: Schematic protein structure of hen egg white lysozyme (*white, blue, red*), variable region of immunoglobulin heavy chain (VH) (*green*), variable region of immunoglobulin light chain (VL) (*magenta*), surface exposed histidine His15 (*yellow*).[12]

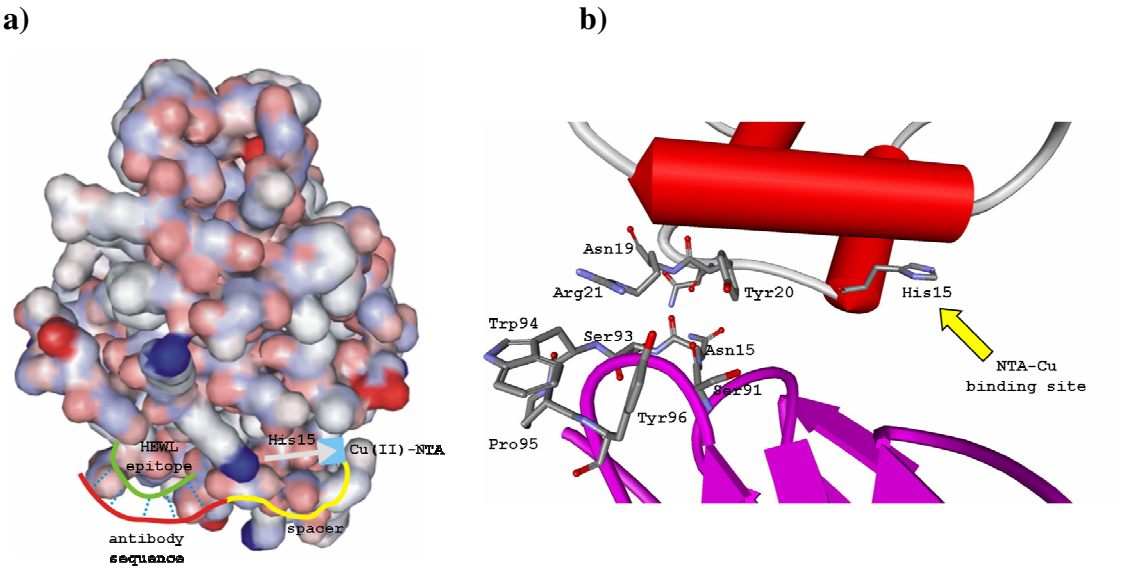


Figure 6. a) Cu(II)-NTA (*blue*) acting as an anchor recognizes lysozymes surface exposed His15 (*grey*), the spacer GABA (*yellow*) positions the antibody sequence Ser91-Asn92-Ser93-Trp94-Pro95-Tyr96 (*red*) to the lysozyme epitope (*green*) allowing hydrogen bonding or ionic interactions. b) Detail view on the supposed binding epitope of lysozyme and **4**.

ITC investigations of the binding affinity of compound **4** to lysozyme were hampered by a significant background heat of solvation of the hybrid compound. Although background corrected titration data indicate an interaction of **4** with lysozyme, the peptide appendix does certainly not enhance the binding affinity significantly if compared to the parent Cu-NTA complex **1**.

1.3 Conclusion

We have investigated the binding affinity of M(II)-NTA (M = Cu, Ni, Zn) complexes to histidine, imidazole and lysozyme by isothermal titration calorimetry. The strength of the interaction correlated to the Lewis acidity of the metal complex (Cu^{2+} > Ni^{2+} > Zn^{2+}). The NTA – histidine binding just exceeds the milli molar range for the investigated copper, nickel and zinc complexes. Only the Cu(II)-NTA complex binds to imidazole and to a surface exposed histidine residue of hen egg white lysozyme with sufficient affinity to be determined by ITC. An attempt to enhance the binding affinity of the Cu(II)-NTA complex to the lysozyme surface by covalent modification with a short lysozyme antibody sequence was not successful.

1.4 Experimental Part

1.4.1 General methods and Material

Nitrilotriacetic acid (Fluka), $Cu_2(CO)_3(OH)_2$ (Alfa Aesar), $NiCO_3 \cdot 2NiOH(OH)_2 \cdot 4H_2O$ (Alfa Aesar), $Zn(NO_3)_2 \cdot 6H_2O$ (Sigma Aldrich), imidazole (Fluka), hen egg white lysozyme E.C. 3.2.1.17 (Sigma Aldrich), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (Fluka), *N*-(9-fluorenylmethoxy-carbonyloxy)-succinimid (Fmoc-OSu) (Sigma Aldrich), rink amide resin MBHA (novabiochem), piperidine (iris biotech), dimethylformamide (DMF) (iris biotech), Fmoc amino acids (novabiochem), trifluoroacetic acid (TFA) (Fluka), triisopropylsilane (TIS) (novabiochem), 1,3–diisopropylcarbodiimide (DIC) (Sigama Aldrich), 1-hydroxybenzotriazole (HOBt) (Sigama Aldrich), 4-aminobutyric acid (GABA) (novabiochem) for preparation of **5**, H-Glu(OBzl)-OtBu · HCl (bachem) for preparation of **7** were bought and used without further purification. Analytical control of the synthesized compounds was done by common methods. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100. IR Spectra were recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. NMR spectra were recorded on Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K) or Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K) relative to external standards.

1.4.2 Isothermal calorimetry titration data

All ITC experiments were performed in buffered aqueous solution (HEPES, 50 mM, pH 7.5, ionic strength [NaCl] = 0.15 mM) at 25 °C using an ultrasensitive VP-ITC calorimeter from MicroCal (Northampton, MA, U.S.A.). The solution of titrant in the 300 µL syringe and the solution for the 1.436 mL calorimetric cell were prepared from stock buffer solution (HEPES, 50 mM, pH 7.5). Before each titration both experimental solutions (titrant, cell) were thoroughly degassed under vigorous stirring. During the ITC experiment the cell solution was stirred at 300 rpm by syringe to ensure rapid mixing and 60 x 5 µL of titrant were injected over 10 s with a spacing time between each injection of two minutes in order to allow complete equilibration. Before data

analysis the total observed heat of binding was corrected for the heat of dilution yielding the effective heat of binding. Therefore an analogue ITC experiment with the calorimeter cell filled with HEPES and titrant was carried out. The data were analyzed by non linear fitting methods using the MicroCal Origin software (Windows based). The values for the binding stoichiometry (n) and the thermodynamic parameters of interaction: enthalpy of binding (ΔH) and binding constant (*lg*K), and, consequently, free energy change (ΔG) and entropy change (TΔS) were obtained using a "one-set of site" model for the fitting routine.

ITC - binding isotherm for the interaction of compound **4** to HEWL:

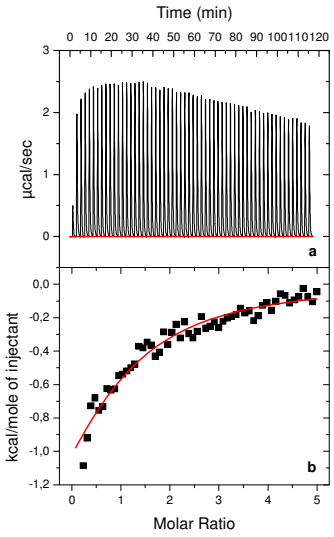


Figure 7. ITC - binding isotherm for the interaction of **4** to HEWL. Measurement conditions: [**4**] = 0.20 mM, [HEWL] = 4.5 mM. Obtained calculated data: n = 1, *lg*K= 3.57 ± 0.04, ΔH = – (2.34 ± 0.09) kcal mol^{-1}, TΔS = 2.54 kcal mol^{-1}, ΔG = – 4.88 kcal mol^{-1}.

1.4.3 Emission titration of M(II)-NTA (M = Cu, Ni, Zn) and lysozyme (HEWL)

Fluorescence measurements were performed in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Instrument Parameters

Excitation wavelength: λ_{ex} = 305 nm

Detection wavelength: λ = 320 – 600 nm

Temperature: T = 298 K

PMT voltage 600 volts

Titration conditions:

Solvent: 50 mM HEPES buffer, pH 7.5, ionic strength [NaCl] = 0.154 M

Starting volume: 2.5 mL

Concentration [metal complex]: 16.2 – 17.4 mM

Concentration [lysozyme]: 0.5 mM

Procedure:

To a cuvette with 2.5 mL of lysozyme in HEPES buffer were added 20 µL (≙ 0.3 eq) aliquots of the M(II)-NTA (M = Cu, Ni, Zn) solution. After each addition the solution was allowed to equilibrate and the emission intensity was recorded. To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

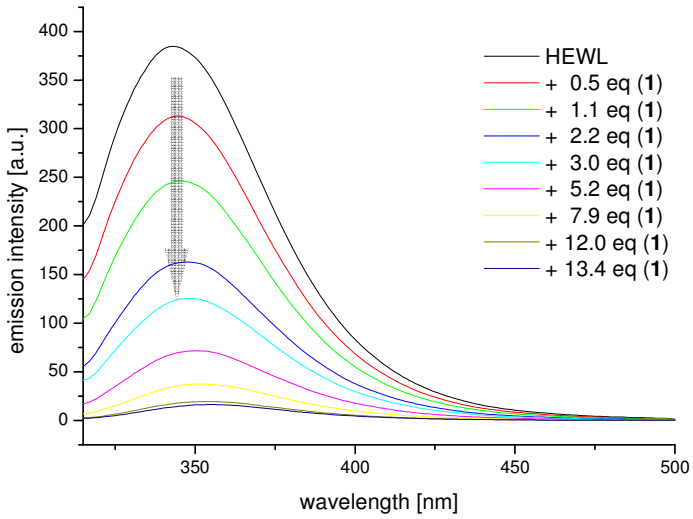


Figure 8. Emission titration of lysozyme and Cu(II)-NTA **1**: [HEWL] = 0.5 mM, [**1**] = 16.4 mM. Upon addition of **1** the emission intensity (λ = 344 nm) of lysozyme decreased.

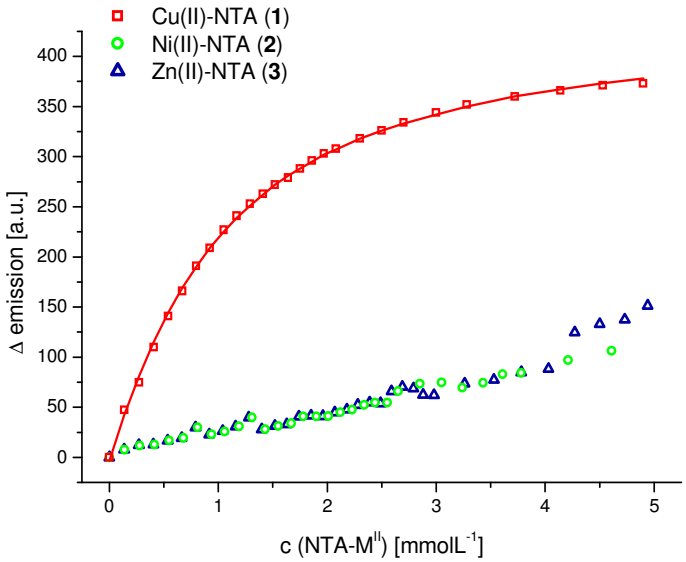


Figure 9. Emission titration of lysozyme and M(II)-NTA (M = Cu, Ni, Zn) (50 mM HEPES pH 7.0, [HEWL] = 0.5 mM, [**1**] = 17.4 mM, [**2**] = 17.7 mM, [**3**] = 17.3 mM.

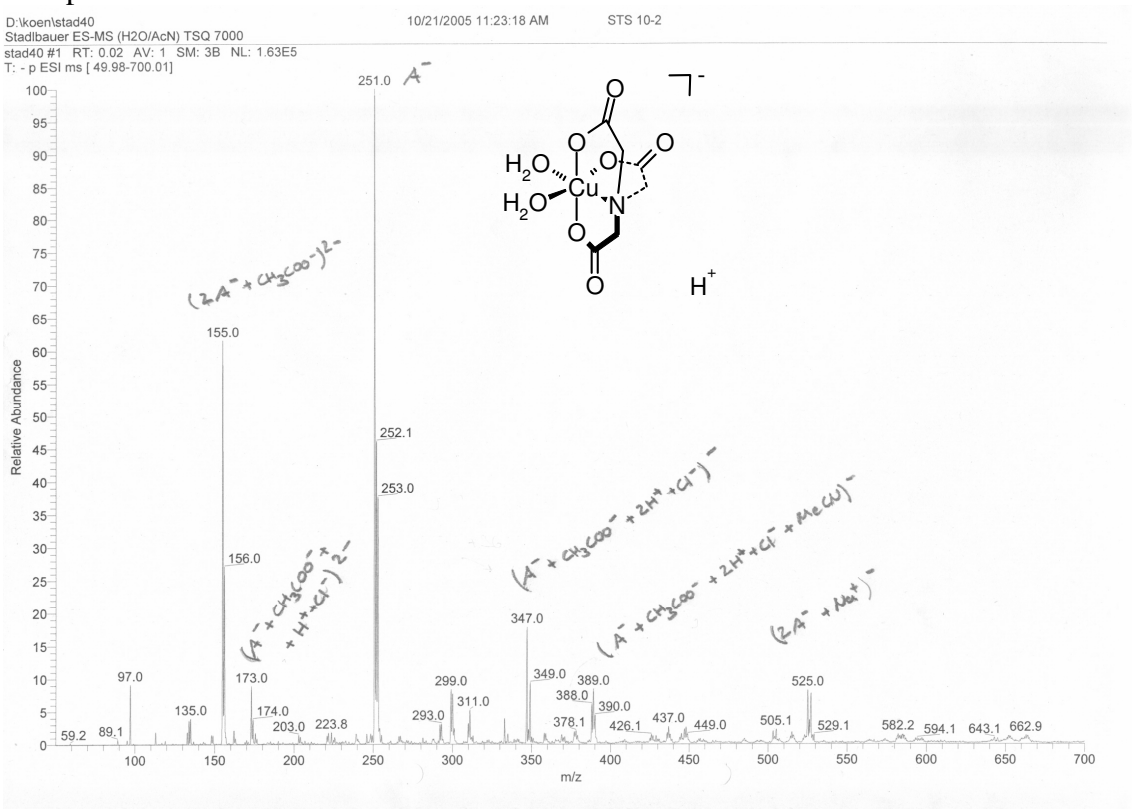
1.4.4 Synthesis

1.4.4.1 Literature known compounds

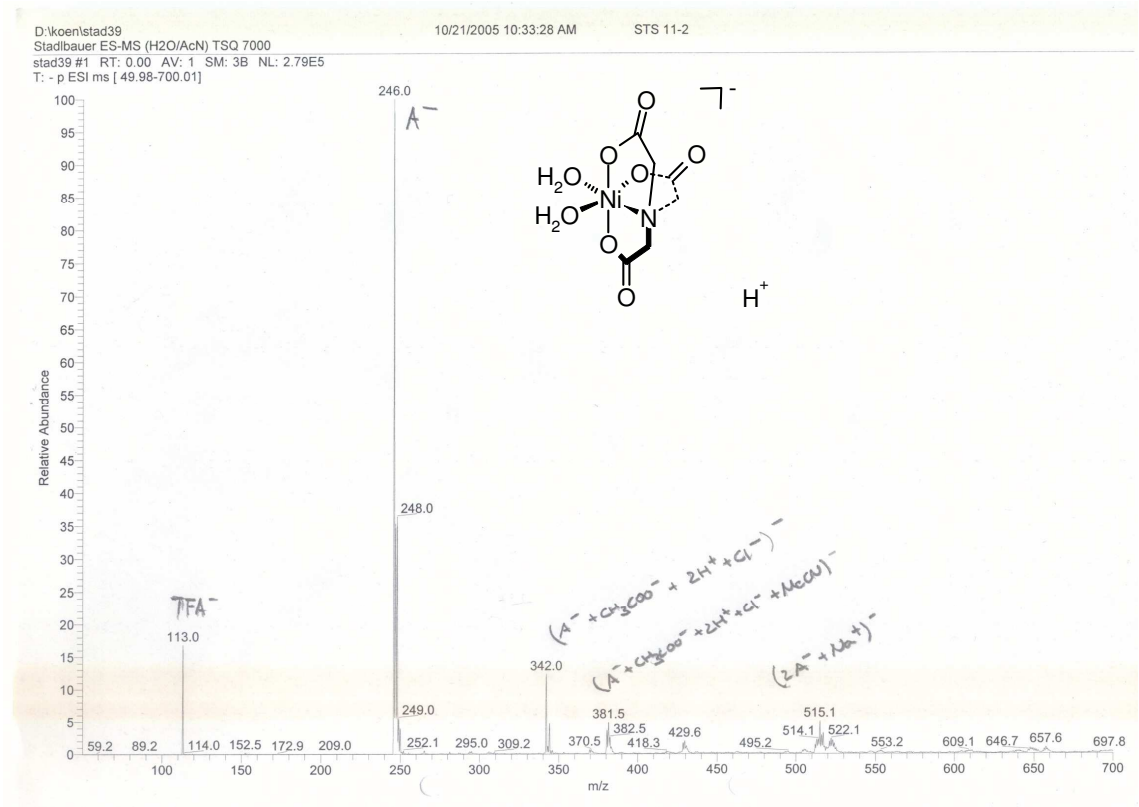
Nitrilotriacetato metal complexes **1**[5], **2**[5] and **3**[14], H-His-OMe[15] were synthesised according to literature known procedures. Fmoc-γ-amino butyric acid (Fmoc-GABA-OH) **5**[16] and 2-(bis-tert-butoxycarbonylmethyl-amino)-pentanedioic acid 1-tert-butyl ester (GluNTA-OH) **6**[17] used in the synthesis of the Cu(II)-NTA **4**, which was modified by an amino acid sequence of the lysozyme antibody, were prepared as previously reported.

Analytical control of prepared NTA complexes **1** – **4** by mass spectroscopy (ESI)

Compound **1**



Compound **2**



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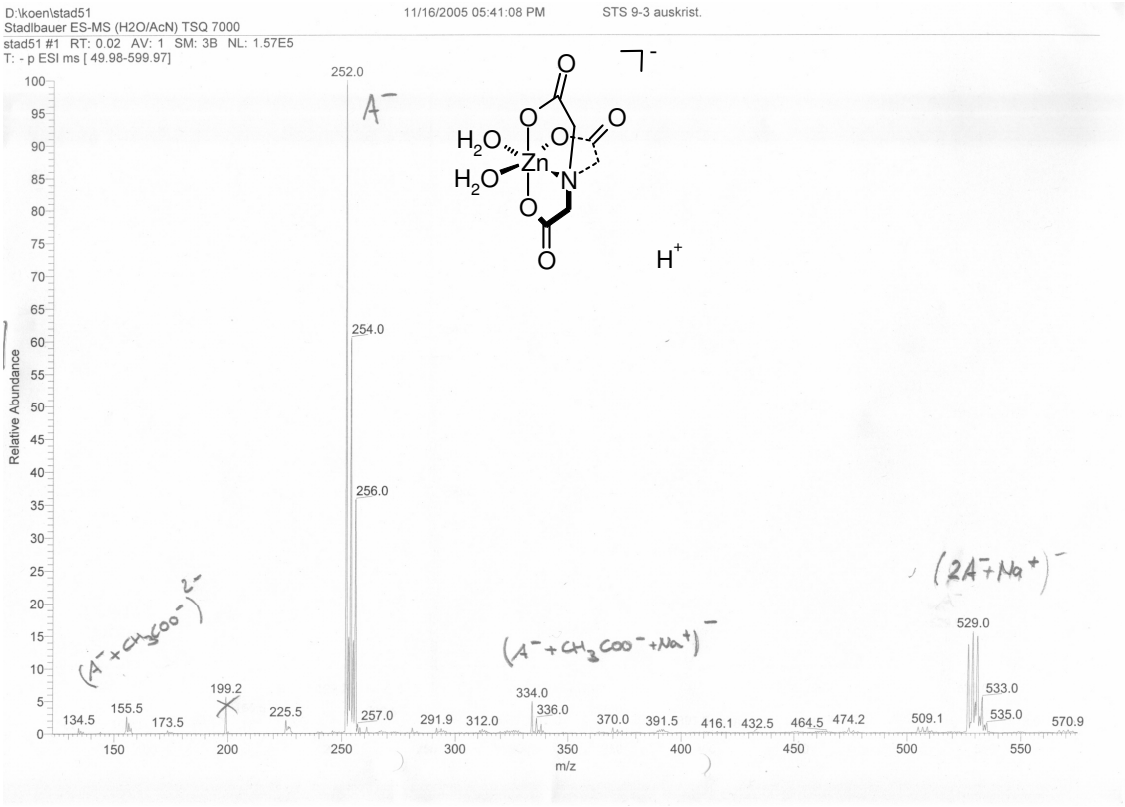

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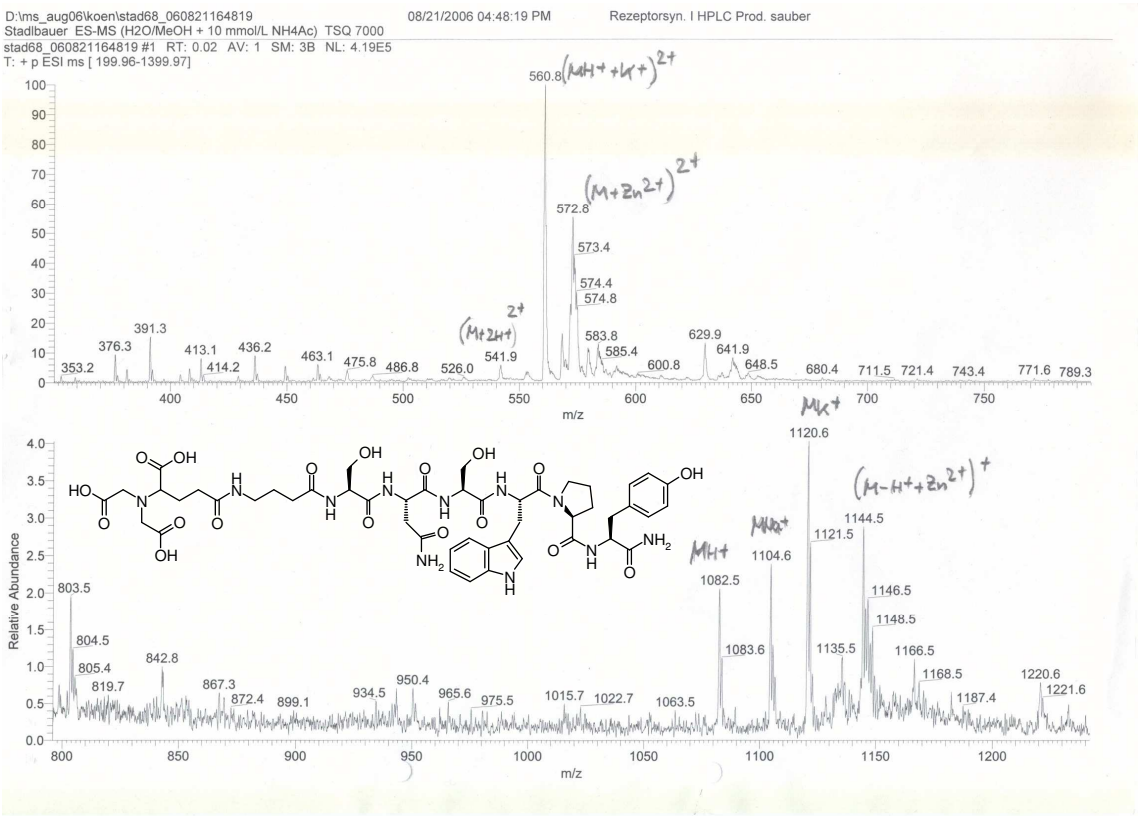

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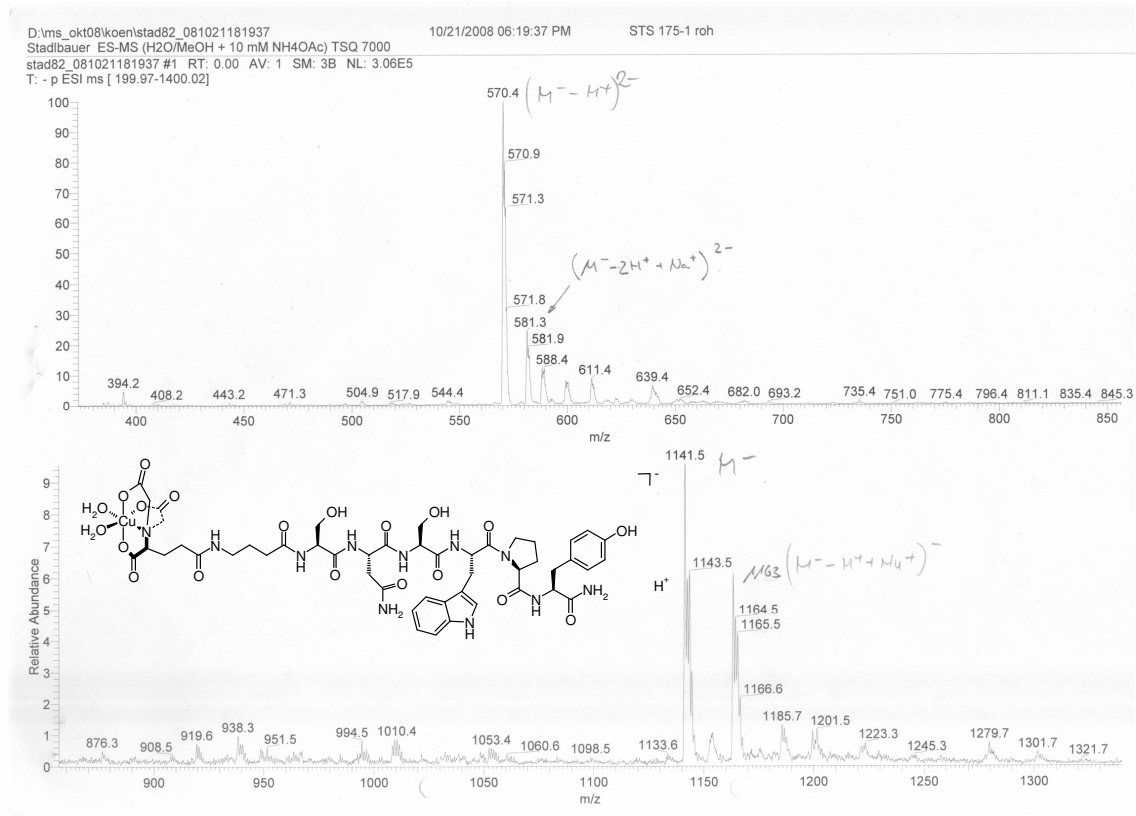
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570.4
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1201.5
1223.3
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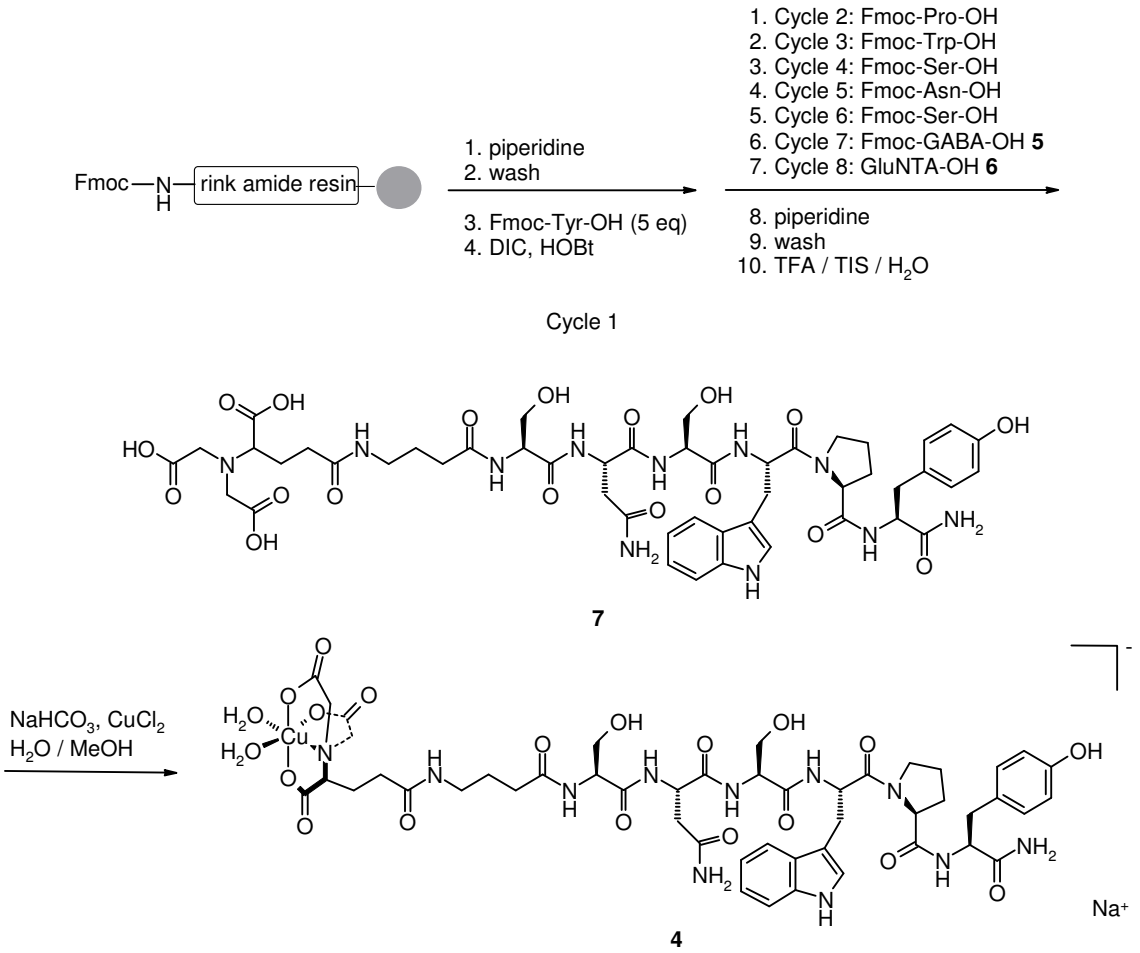
1279.7
1301.7
1321.7
876.3
908.5
919.6
938.3
951.5
994.5
1010.4
1053.4
1060.6
1098.5
1133.6
Relative Abundance
m/z

1.4.4.2 Solid phase synthesis protocol of compound **7**



Scheme 3. Solid phase peptide synthesis protocol for the preparation of modified Cu(II)-NTA **4** to enhance its binding affinity to lysozyme.

SPPS-Protocol

Loading. 400 mg of rink amide MBHA (loading 0.70 mmol/g) resin was preswollen in 3 ml CH_2Cl_2 and DMF respectively, for 1 h in a syringe, then the solution was filtered off.

Fmoc-Deprotection. The preswollen Fmoc protected resin was treated with 3 mL 20 % piperidine in DMF and the mixture was shaken for 20 min. The deprotection step was repeated two times. Then the resin was washed thoroughly with DMF. The Fmoc-deprotection of the Fmoc-protected amino acids after the coupling steps was done by the same procedure.

Coupling of Fmoc AAs. The Fmoc-AA (1.4 mmol, 5 eq), DIC (217 µL, 1.4 mmol, 5 eq) and HOBt (189 mg, 1.4 mmol, 5eq) were dissolved in 3 ml DMF. Subsequently the

coupling mixture was added to the resin (preswollen in DMF) and the syringe was shaken. After coupling step was completed the solution was filtered off and the resin was washed four times with DMF. Then Fmoc-GABA-OH **5** and GluNTA-OH **6** were coupled to the peptide sequence (Ser91-Asn92-Ser93-Trp94-Pro95-Tyr96) as described above. Compound **6** was coupled with an excess of 2 eq only. The progress of the synthesis was monitored by the Kaiser test.[18]

Cleavage. The resin placed in the syringe was washed several times successively with DMF, acetic acid and dichloromethane. In order to shrink the resin it was washed further with ether. The resin was filled in a flask and dried in high vacuum for 4 h. A mixture of 5 mL of TFA solution (95 % TFA, 2.5 % TIS, 2.5 % H_2O) was given to the dry resin and the mixture was shaken for 24 h at rt. The resin was removed by filtration and washed twice with TFA. Combined filtrates were concentrated under reduced pressure and the crude product **7** was precipitated with ice cold ether.

Purification. Crude compound **7** (concentration 10.2 mg/mL) was purified by preparative HPLC on a LabID75 Phenomenex Luna column (250 x 21.2 mm 10 um / Ser.Nr. 164449-1) at a flow rate of 22 mL/min, injection volume 400 µL with a H_2O / acetonitrile (+ 0.0059 % TFA) gradient (0 min: 3 % acetonitrile, 35 min: 58 % acetonitrile, 40 min: 95 % acetonitrile, 50 min: 95 % acetonitrile). Retention time of **7** was 20.5 - 21.3 min.

Analytical control of GluNTA-GABA-Ser-Asn-Ser-Trp-Pro-Tyr-NH_2 (**7**)

MS (ESI(+), H_2O/acetonitrile /TFA): m/z (%) = 560.9 (100) $[MH^+ + K^+]^{2+}$, 572.4 (20) $[M + K^+]^{2+}$, 1082.7 (3) $[MH^+]$, 1104.9 (2) $[MNa^+]$, 1120.6 (3) $[MK^+]$.

MF: $C_{48}H_{63}N_{11}O_{18}$ – **FW**: 1082.10 g/mol

1.4.4.3 *Glu-CuNTA-GABA-Ser-Asn-Ser-Trp-Pro-Tyr-NH_2* (**4**)

Compound **7** (13 mg, 10.9 µmol) was dissolved in water (1 mL) and a few drops MeOH and heated to 40 °C. Subsequently an aqueous solution of $NaHCO_3$ (100 mM, 435 µL, 43.5 µmol) was added and the mixture was stirred for additional 10 min. Following an aqueous solution of $CuCl_2$ (100 mM, 109 µL, 10.9 µmol) was added and the reaction mixture was stirred at 40 °C overnight (17 h). The resulting greenish-blue solution was concentrated under reduced pressure and lyophilized, yielding **4** (15.5 mg, 10.5 µmol, 96 %) as a turquoise solid.

MS (ESI(–), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 570.4 (100) $[M^- - H^+]^{2-}$, 581.3 (25) $[M^- - 2\,H^+ + Na^+]^{2-}$, 1141.5 (9) $[M^-]$, 1163.5 (6) $[M^- - H^+ + Na^+]^-$.

MF: M^-: $C_{48}H_{60}N_{11}O_{18}Cu$ – **FW**: 1142.61 g/mol

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[10] It was not possible to calculate a binding constant for interaction of Zn(II)-NTA **3** and H-His-OMe by the obtained ITC data. The binding constant reported in table 1 corresponds to the interaction of **3** and H-His-OH.

[11] ITC results were confirmed by emission titration of M(II)-NTA (M = Cu, Ni, Zn) and lysozyme in buffered aqueous solution (50 mM HEPES, pH 7.0, ionic strength [NaCl] = 0.15 M, 30 °C). Only **1** evoked a significant emission change of lysozyme (lgK = 3.0). (see Supplementary material)

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2. Utilizing Reversible Copper(II) Peptide Coordination in a Sequence Selective Luminescent Receptor[i]

Although vast information about the coordination ability of amino acids and peptides to metal ions is available, little use of this has been made to rationally design selective peptide receptors. We have combined a copper(II)nitrilotriacetato (NTA) complex with an ammonium-ion sensitive and luminescent benzocrown ether.[ii] This compound revealed a micromolar affinity and selectivity for glycine and histidine containing sequences[iii], which closely resembles that of copper(II) ion peptide binding: The two free coordination sites of the copper(II) NTA complex bind to imidazole and amido nitrogen atoms, retracing the initial coordination steps of non-complexed copper(II) ions. The benzocrown ether recognizes intramolecularly the N-terminal amino moiety and the significantly increased emission intensity signals the binding event, as only if prior coordination of the peptide has taken place, the intramolecular ammonium ion – benzocrown ether interaction is of sufficient strength in water to trigger an emission signal. Intermolecular ammonium ion – benzocrown ether binding is not observed. Isothermal titration calorimetry confirmed the binding constants derived from emission titrations. Thus, as deduced from peptide coordination studies, the combination of a truncated copper(II) coordination sphere and a luminescent benzocrown ether allows for the more rational design of sequence selective peptide receptor.

[i] S. Stadlbauer, A. Riechers, A. Späth, B. König *Chem. Eur. J.* **2008**, *14*, 2536 – 2541.
[ii] Benzocrown ether **3** was synthesized by Andreas Späth.
[iii] Parts of the peptide library were synthesized by Alexander Riechers in his *Diploma thesis*.

2.1 Introduction

The development of peptide chemosensors and the sequence-specific recognition of peptides by synthetic receptors under physiological conditions still remains a challenging task. Recently reported approaches used porphyrines,[1] helix mimics,[2] crown ethers[3] or short peptides with guanidiniocarbonyl pyrroles[4] as binding sites to specifically recognize peptidic structures. Although peptide to metal ion coordination has been studied in detail from a physicochemical and inorganic perspective,[5] applications of reversible coordination in peptide recognition are rare.[6] Recent examples are Anslyn´s[7] use of cooperative metal coordination and ion-pairing for tripeptide recognition and zinc(II)-dipicolylamine-stilbazoles, which were applied in the specific detection of phosphorylated peptides.[8]

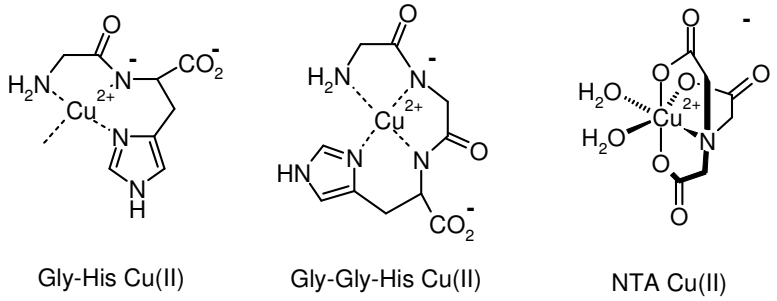
Systematic studies on peptide metal complexes date back to the 1950s and potentiometric, structural[9] and spectroscopic[10] investigation have been reported in detail.[11] Copper(II), nickel(II) and zinc(II) are by far the most studied metal ions in peptide complexes. The ability of a peptide to act as a ligand strongly depends on the amino acid sequence, with imidazole, carboxylate, deprotonated amide and amino groups as typical donor sites. The binding specificity to metal ions has been used in the development of ion selective electrodes[12] and fluorescent metal ion chemosensors for zinc[13] or copper[14] ions. Peptide metal complexes themselves have been utilized in DNA and RNA recognition[15] and cleavage.[16]

We have now set ourselves the goal of using the well studied metal ion complexation by peptides for the rational design of peptide sequence specific receptors. To this end the metal ion is replaced by a stable metal complex with some labile coordination sites available for peptide binding. We reason, that such metal complexes should selectively bind truncated sequences of peptides that are known to form stable complexes with the metal ion itself. To illustrate the feasibility of this approach, we use one of the best studied peptide metal complexes consisting of histidine- and glycine-containing peptides ligands and copper(II) as the metal ion.

2.2 Results and Discussion

2.2.1 Receptor Design

Many histidine-containing peptides form stable complexes with copper(II) ions, but the coordinating properties of the histidine residue depend significantly on the position of the residue in the peptide chain. The insertion of histidine in position two of a peptide allows for the participation of the N-terminal amine, the N-1 imidazole and the in-between amide nitrogen atoms in copper(II) ion coordination (Scheme 1).[5a] The presence of histidine in position three of a peptide chain leads to the cooperative formation of three fused chelate rings with copper (II) ions.[5a] In simple peptides with N-terminal histidine the chelate of the N-terminal amino group and the imidazole nitrogen atom can be very effective, but also complexes of higher ligand stoichiometry are discussed.[5a,17]



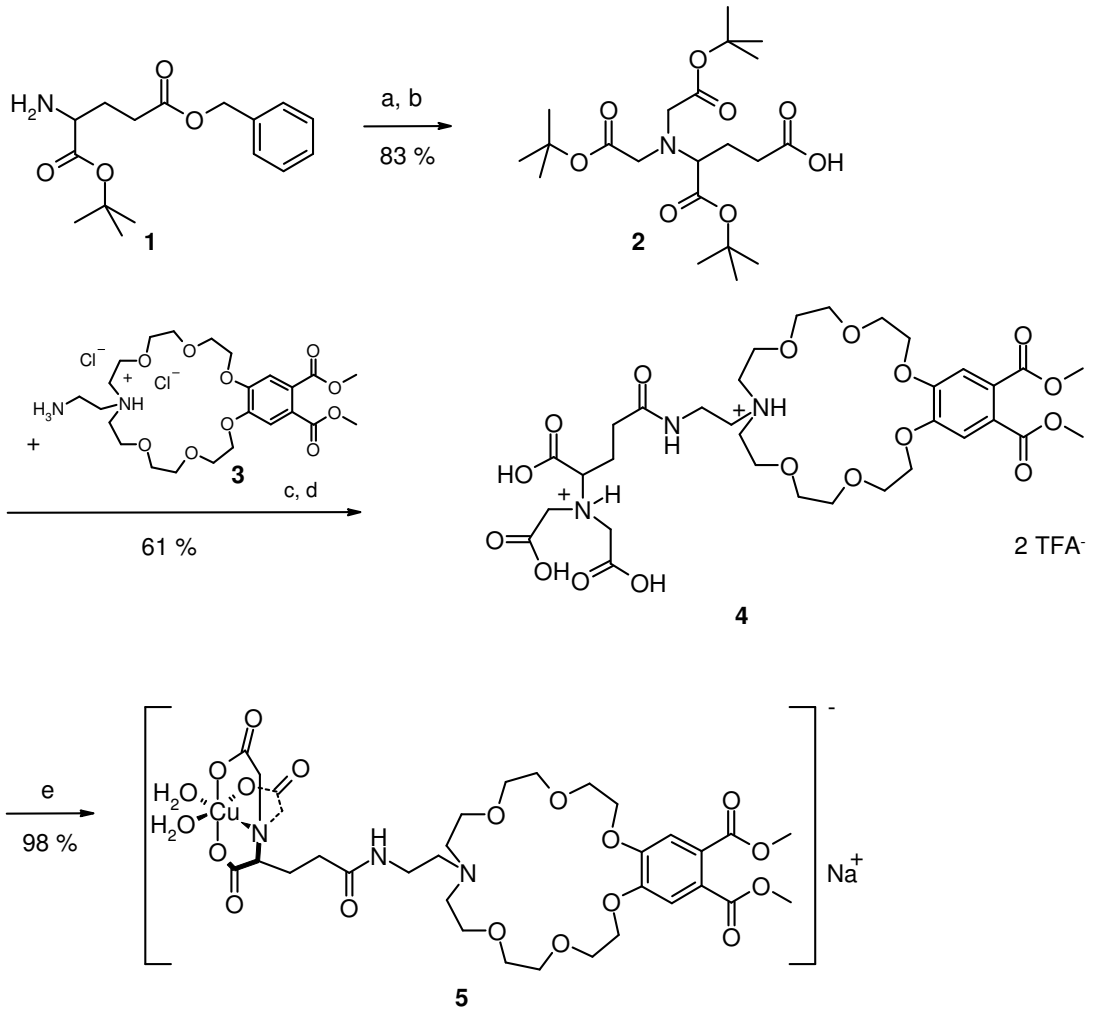
Scheme 1. Glycine-histidine copper(II) and nitrilotriacetato (NTA) copper (II) metal complexes.

Nitrilotriacetato (NTA) copper(II) complexes are stable and widely used for protein purification due to their affinity to imidazole side chains. The NTA ligand of the complexes leaves two coordination sites available for the reversible binding of donor groups.[18] The binding of a glycine-histidine peptide sequences, such as GH or GGH to a NTA-Cu^{II} complex will therefore only proceed for the N-imidazole and N-amide coordination leaving the N-terminal amino group available, if a consecutive binding starting with imidazole as the primary anchoring group is assumed.

We recently introduced luminescent benzocrown ether amino acids, which are useful molecular indicators for ammonium ions[19] and demonstrated their ability to monitor Lys side chains in coordinated peptides.[20] A combination of such luminophors with a NTA-Cu^{II} complex should lead to a peptide sequence selective receptor with predictable selectivity.

2.2.2 Synthesis

The synthesis starts from protected glutamic acid **1** that is converted into the NTA precursor **2**.[21] Peptide coupling using typical conditions with amino ester **3**[19] and *tert*-butyl ester cleavage gave compound **4**, which was converted into the NTA-Cu complex **5** (Cu-NTA CE) by treatment with copper carbonate. Peptides for the determination of binding selectivity were prepared in solution and by standard solid phase methods.



Scheme 2. Synthesis of Cu-NTA benzo crown ether **5** (Cu-NTA CE). (a) ^{t}Butylbromoacetate, DIPEA, DMF; (b) 10 % Pd/C MeOH; (c) DCC, HOBt, DIPEA; (d) TFA, quantitative; (e) $NaHCO_3$, $Cu_2(OH)_2CO_3$, H_2O.

2.2.3 Determination of peptide binding

The luminescence of the benzocrown ether moiety is largely quenched upon coordination of the NTA ligand by copper(II),[22] but partly restored if an ammonium ion binds to the crown ether. However, the crown ether binding affinity for ammonium ions in aqueous solution at physiological pH is rather weak[23] and an interaction is therefore

only observed if other parts of the peptide coordinate to the NTA-Cu^{II} complex, making the ammonium ion binding process intramolecular. Compound **5** will signal affinity to peptides, which coordinate to the two accessible NTA-Cu^{II} binding sites, but leaving an ammonium group available for crown ether binding.

The peptide binding selectivity of **5** was evaluated with a small rational library of di-, tri- and tetrapeptides, with some amino acid sequences resembling typical peptides for copper(II) complex formation. To quickly select the peptide sequences with highest affinity a screening was performed in aqueous buffered solution (HEPES, 50 mM, pH 7.5) using a microtiter array. Five equivalents of each peptide were added to a 3.75 x 10^{-5} M solution of compound **5** and the fluorescence emission intensity at 397 nm (excitation at 305 nm) was recorded. If the ratio of the observed emission intensity after and before peptide addition exceeds two, a significant interaction is expected.[24] Table 1 summarizes the results. As expected, several His (H) and Gly (G) containing peptides induced a significant emission increase.[25] However, if the distance of the imidazole moiety to the N-terminus becomes larger, the affinity drops. From peptides containing a Lys (K) residue beside His, only a di- and a tripeptide gave a weak response. Tetrapeptides bearing a His, two Gly and either Ala (A), Leu (L) or Glu (Q) as the fourth residue the common sequences XaaHGG-NH_2 (Xaa = A, L, Q) showed a strong emission increase. The exchange of His by Ser (S), Glu (Q), Gln (E) in tetrapeptides with three Gly residues leads to a complete loss of affinity to **5**. Cys containing tetrapeptides with three Gly showed a weak emission response independent of the Cys position, with the exception of the N-terminus.

Peptide sequence	$F/F_0 > 2$[a]	Peptide sequence	$F/F_0 > 2$[a]	Peptide sequence	$F/F_0 > 2$[a]
H[b]	+	CGGG[d]	-	QGGG[d]	-
HGG[c]	+	GCGG[d]	+	GQGG[d]	-
HGGG[d]	-	GGCG[d]	+	GGQG[d]	-
GHG[d]	++[e]	GGGC[d]	+	GGGQ[d]	-
GHGG[d]	++	SGGG[d]	-	AHGG[d]	++
GGHG[d]	-	GSGG[d]	-	AGHG[d]	-
GGGH[d]	-	GGSG[d]	-	AGGH[d]	-
GGH[c]	++	GGGS[d]	-	LHGG[d]	++
GH[c]	++	EGGG[d]	-	LGHG[d]	-
HK[b]	+	GEGG[d]	-	LGGH[d]	-
HKGG[d]	-	GGEG[d]	-	QHGG[d]	++
HGK[b]	+	GGGE[d]	-	QGHG[d]	-
HGKG[d]	-			QGGH[d]	-
HGGK[d]	-				

Table 1. Screening of the fluorescence response of **5** to small peptides.
[a] F/F_0 is the increase in observed emission intensity after addition of five equiv. of the peptide to compound **5**, [b] H-Xaa-OMe, [c] H-Xaa-OH, [d] H-Xaa-Xaa-Xaa-Xaa-NH_2, [e] peptide sequences marked with ++ show a particularly strong emission increase.

The peptide sequences that showed significant responses in the screening assay were investigated by emission titrations in HEPES (50 mM, pH 7.5) buffered aqueous solution. Their binding constants lg K and $K_{0.5}$ values were derived from the titration data by non linear fitting methods; the stoichiometry of the binding events was determined by Job´s plot analyses (see Experimental Part). Figure 1 shows exemplarily the emission titration curve for H-GGH-OH and **5**. Peptides GGH and GHG bind to **5** with micromolar affinity and a stoichiometry of 1:1. The binding data were confirmed by independent isothermal titration calorimetry (ITC) using the same conditions (see Experimental Part). Dipeptide GH and tetrapeptides XaaHGG-NH_2 (Xaa = A, L, Q) show lower binding affinities, but still in the order of lg$K$ = 4. Job´s plot analyses of the binding of the cysteine-containing peptides indicated a 2:1 stoichiometry. However, the fit of the titration data to mathematical binding models was not conclusive. The remaining histidine-containing peptides sequences bind weakly to compound **5** with stoichiometries deviating from 1:1 and are therefore judged as non-specific binders. The data of the peptide sequences that bind to **5** stoichiometric with high affinity are summarized in Table 2.

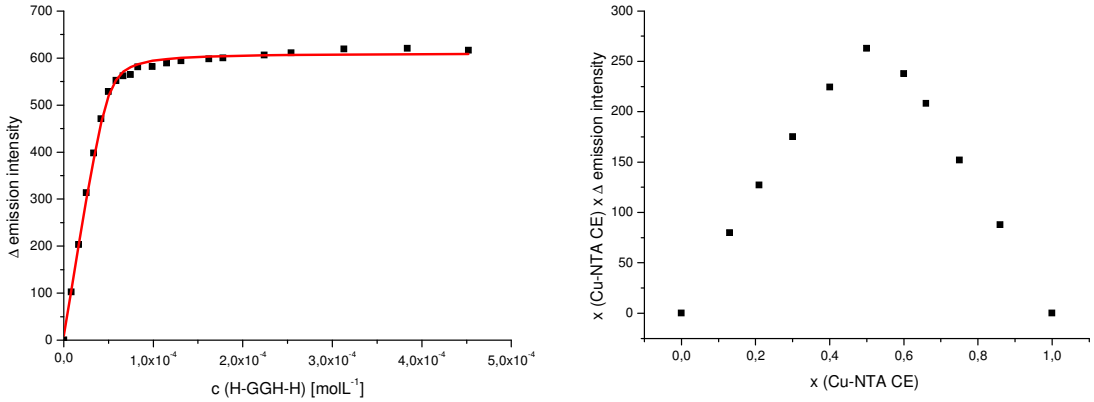


Figure 1. Emission titration (left) and Job´s plot analysis (right) of H-Gly-Gly-His-OH and **5** in aqueous buffer.

The binding data support the design concept of the receptor: Only peptide sequences that form stable copper(II) complexes, and therefore bind with part of their donor sites to the Cu-NTA complex of **5**, and, at the same time, have an N-terminal ammonium ion available, show strong and specific response. GGH and GHG (Table 2, entry 1 and 2) show the highest affinity, as they provide an optimal geometry to form a six-membered chelate with the copper ion in analogy to the initial coordination in pure copper(II) peptide complexes.

Entry	**Peptide sequence**	$K_{0.5}$ [a] [µmol]	lg K [b]
1	GGH[c]	24	5.8
2	GHG[c]	25	5.7
3	GHG-OMe	33	5.3
4	GH[c]	36	4.7
5	GHGG[d]	60	4.1
6	AHGG[d]	70	4.2
7	LHGG[d]	60	4.2
8	QHGG[d]	187	3.7
9	Ac-GHG[c]	-	-

Table 2. Binding data of peptide sequences to **5** in aqueous buffer from emission titrations. [a] Concentration of peptide needed to induce 50% of the maximal emission increase, [b] all stoichiometries determined by Job´s plot analyses as 1:1, with the exception of QHGG, which shows 2:1 [c] H-(Xaa)-OH, [d] H-(Xaa)-NH_2.

The high stability of complex **5** with GHG allows its detection from solution by electro spray mass spectroscopy. Based on the structures of peptide copper(II) complexes, we propose for the aggregate of **5** with XaaHGG peptides the arrangement shown in Figure 2. The steric bulk of residues R and R´ in the tetrapeptides (entries 5-8) may account for their somewhat reduced affinity, if compared to the GHG or GGH

sequences. The side chain of the N-terminal amino acid in these peptides does not influence the binding affinity significantly,[26] which is in accordance with the reported tolerance of an amino acid other than Gly in copper(II) complexes with XaaHG peptides.[27] The C-terminus of the peptides does not participate directly in the binding process, as the change from carboxylate anion to a methyl ester or glycylamide is possible. However, larger groups lead to a decrease in affinity, probably due to increased steric hindrance in the coordination aggregate. The N-terminal ammonium ion is essential to trigger an emission change of **5**; N-terminal acylated peptides, such as Ac-GHG (entry 9) give no emission response if added to **5**. ITC measurements revealed that Ac-GHG still shows some affinity to **5**, which is, however, significantly reduced if compared to the non acylated peptide (GHG: lg*K* = 5.1; Ac-GHG: lg*K* = 4.0; see Experimental Part).[28] Interestingly, not only the distance of the ammonium ion and the copper coordination sides within the peptide are of importance to trigger an emission output signal, but also the intervening structure. In a series of N-terminally acylated tetrapeptides the N-terminal amino acid was Lys with an ammonium ion in its side chain (Ac-KHGG-NH_2, Ac-KGHG-NH_2, Ac-KGGH-NH_2). Although the peptides have a copper ion coordination site and an ammonium ion in suitable distance, the more flexible connection of both functionalities does not lead to a stable aggregate with **5** with increased emission.

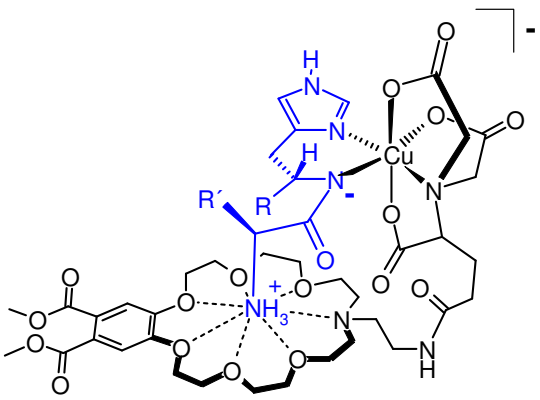


Figure 2. Proposed structure of stable peptide aggregates with compound **5** [R = CO_2^-, $CONHCH_2CO_2^-$; $CONHCH_2CO_2CH_3$, $CONHCH_2CONHCH_2CONH_2$; R´= H, CH_3, CH_2-$CH(CH_3)_2$, $CH_2CH_2CONH_2$].

While the binding of histidine containing peptides can be rationalized on the basis of known copper(II) peptide interactions, the sequence independent effect of the cysteine-containing tetrapeptides on **5** was surprising. The interaction of cysteine with copper(II) ions has been investigated, but structural information of the interaction is very limited.[29] The thiol group of cysteine strongly binds to copper(II) ions, forming polymeric species

with bridging thiolate sulphur. This coordination process is superimposed by reduction of cupric ions to Cu^{I}, which in aqueous systems undergo dismutation regenerating Cu^{II}. Analogously, the interaction of compound **5** with cysteine starts with the coordination of two thiol groups to the vacant coordination sites,[30] which explains the observed 2:1 stoichiometry of aggregates. The thiol ligands may influence the oxidation state of copper in the NTA complex,[31] but surely alter the electronic properties of the complex significantly. The UV absorption of compound **5** shows a 10 nm bathochromic shift upon addition of 5 eq. of mercaptoethanol in aqueous buffer. More significantly, in the emission spectrum a threefold increase of the emission intensity is observed (see Experimental Part). This indicates that the mechanism of emission increase in **5** by cysteine is different from the histidine peptides. While in histidine peptides the intramolecular ammonium ion binding to the luminescent benzocrown ether moiety partly restores the NTA-copper complex quenched emission of the fluorophore, cysteine thiol groups alter the NTA-copper complex itself and reduce its ability to quench the benzocrown ether emission. This explains the emission increase, which is independent from the position of the cysteine residue in the peptide sequence. Only peptides with cysteine as the N-terminal amino acid do not show any emission increase. Here, cysteine may form an S,N-chelate[32] with the NTA-copper complex, which still quenches the emission of the benzocrown ether fluorophore.

2.3 Conclusion

The combination of a copper(II)-NTA complex with an ammonium-ion sensitive and luminescent benzocrown ether yields a molecular receptor, which preferably binds to specific histidine-glycine peptide sequences under physiological conditions. Nearly micromolar affinities are observed for GGH and GHG; in tetrapeptides the recognition motif XaaHGG was identified, whereby the N-terminal amino acid residue may vary. Only the N-terminal amino group triggers an emission signal, the ammonium moiety of a lysine side chain does not. Cysteine-containing peptides trigger an emission signal of **5** as well, but due to altered electronic properties of the copper ion cancelling its quenching of the benzocrown emission. The selectivity and affinity of **5** may not be sufficient in its current state for direct practical use in peptide sensing, but the investigations clearly show that the interaction of a hybrid compound like **5** with

peptides can be rationalized on the basis of established coordination motifs of copper(II) ions to peptides. This opens the possibility for a more rational design of sequence selective peptide chemosensors tapping the extensively available knowledge of peptide to metal ion coordination reported over the last 50 years.

2.4 Experimental Part

2.4.1 Syntheses

2.4.1.1 General methods and material

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer or on a Perkin-Elmer LS55 fluorescence spectrophotometer with temperature control.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer or on a SHIMADZU UV-2550 spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck or Baker).

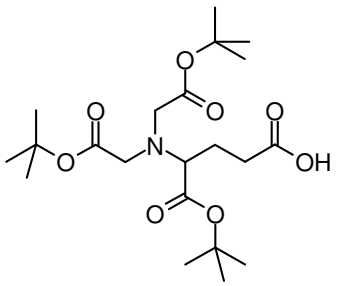
NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in 13C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

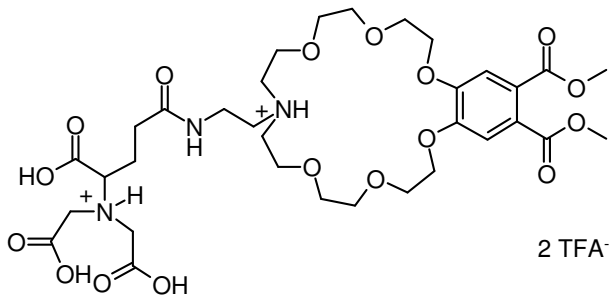
2.4.1.2 Receptor Synthesis



2-(Bis-tert-butoxycarbonylmethyl-amino)-pentanedioic acid 1-tert-butyl ester (**2**):[21] H-Glu(OBzl)-O^{t}Bu · HCl (312 mg, 0.95 mmol) was dissolved under nitrogen in 10 mL of DMF. DIPEA (0.8 mL, 4.73 mmol) and *tert*-butyl bromoacetate (0.6 mL, 3.78 mmol) were added to the stirred solution. The reaction mixture was heated to 80 °C overnight with continuous stirring and monitored by TLC (petrol ether/ethyl acetate 3:1). The solution was concentrated under reduced pressure and the residue was taken up in a small amount of ethyl acetate, filtered and washed several times with petrol ether/ethyl acetate 3:1 and ethyl acetate until the filter residue was colourless. The filtrate was evaporated and the crude product was purified using column chromatography on silica gel (petrol ether/ethyl acetate 3:1, R_f = 0.60) yielding ***2-(bis-tert-butoxycarbonylmethyl-amino)-pentanedioic acid 5-benzyl ester 1-tert-butyl ester*** (418 mg, 84 %) as a lightly yellow oil. **^{1}H-NMR** (300 MHz; $CDCl_3$): δ = 1.42 (s, 18 H, N-CH_2COO^{t}Bu), 1.45 (s, 9 H, C$^\alpha$HCOO^{t}Bu), 1.82-2.07 (m, 2 H, Glu-CH_2), 2.50-2.77 (m, 2 H, GluCH_2), 3.38 (dd, ^3J = 5.5 Hz, 9.8 Hz, 1 H, Glu-C$^\alpha$H), 3.43 (s, 4 H, N-CH_2), 5.08-5.12 (s, 2 H, Ph-CH_2), 7.26-7.38 (m, 5H, Ar-H). – **^{13}C-NMR** (75 MHz; $CDCl_3$): δ = 25.4 (–, 1 C, CH_2), 28.1 (+, 9 C, CH_3), 30.5 (–, 1 C, CH_2), 53.8 (–, 2 C, N-CH_2), 64.3 (+, 1 C, CH), 66.1 (–,

1 C, Ph-CH_2), 80.7 (C_q, 2 C, COO*tBu*), 81.3 (C_q, 1 C, COO*tBu*), 128.1 (+, 1 C, Ar-CH), 128.2 (+, 2 C, Ar-CH), 128.5 (+, 2 C, Ar-CH), 136.2 (C_q, 1 C, Ar-C), 170.5 (C_q, 2 C, NCH_2COO^tBu), 171.8 (C_q, 1 C, COO^tBu), 173.5 (C_q, 1 C, COOBn). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 522.4 (100) [MH^+], 466.3 (3) [MH^+ - C_4H_6].

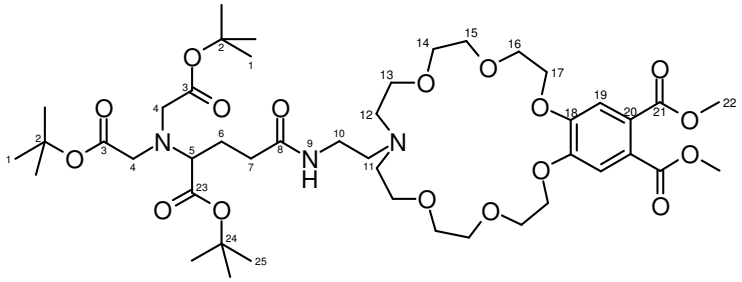
The NTA bis-ester (624 mg, 1.2 mmol) was dissolved in EtOH (20 mL), to which a spatula tip of 10 % Pd/C were added. The reaction mixture was stirred in an autoclave under 20 bar H_2 pressure for 18 h. The suspension was filtered twice and the filtrate was concentrated under reduced pressure yielding compound **2** (513 mg, 99 %) as a colourless oil. – **^{1}H-NMR** (300 MHz; $CDCl_3$): δ = 1.43 (s, 18 H, N-CH_2COO^tBu), 1.45 (s, 9 H, $C^{\alpha}HCOO^tBu$), 1.78-2.07 (m, 2 H, Glu-CH_2), 2.47-2.78 (m, 2 H, Glu-CH_2), 3.36 (dd, ^3J = 5.5 Hz, 10.1 Hz, 1 H, Glu-$C^{\alpha}H$), 3.44 (s, 4 H, N-CH_2). – **^{13}C-NMR** (75 MHz; $CDCl_3$): δ = 25.4 (–, 1 C, CH_2), 28.1 (+, 9 C, CH_3), 31.1 (–, 1 C, CH_2), 54.0 (–, 2 C, N-CH_2), 64.7 (+, 1 C, α-CH), 81.3 (C_q, 2 C, COO*tBu*), 81.7 (C_q, 1 C, COO*tBu*), 170.6 (C_q, 2 C, NCH_2COO^tBu), 171.3 (C_q, 1 C, COO*tBu*), 176.2 (C_q, 1 C, COOH). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 432.3 (100) [MH^+], 454.3 (3) [MNa^+].



4-[2-(2-{2-[{2-[4-(Bis-carboxymethyl-amino)-4-carboxy-butyrylamino]-ethyl}-(2-ethoxy-ethyl)-amino]-ethoxy}-ethoxy)-ethoxy]-5-(2-methoxy-ethoxy)-phthalic acid dimethyl ester tritriflate (**4**): Under N_2-atmosphere compound **2** (150 mg, 0.35 mmol) was dissolved in a small amount of $CHCl_3$ and mixed under ice cooling with DIPEA (0.3 mL, 1.74 mmol), DCC (86 mg, 0.42 mmol), and HOBt (56 mg, 0.42 mmol). Then crown ether-dihydrochloride **3** (245 mg, 0.42 mmol), dissolved in a small amount $CHCl_3$, was slowly added. The reaction was allowed to warm to room temp. and was stirred over night (20 h) at 40 °C. The reaction progress was monitored by TLC (ethyl acetate). After completion, the solution was diluted with ethyl acetate and the

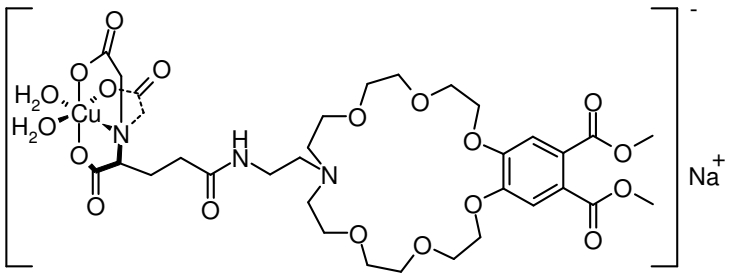
precipitated dicyclohexyl urea was filtered off over celite. The solvent was removed in vacuo and the crude product was purified using column chromatography on silica gel ($CHCl_3$/MeOH 4:1, R_f = 0.75, ethyl acetate) yielding the tris tert-butyl ester of **4** (198 mg, 60 %) as a lightly yellow oil. ***14-{2-[4-(Bis-tert-butoxycarbonylmethyl-amino)-4-tert-butoxycarbonyl-butyryl-amino]-ethyl}-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylicacid dimethylester:*** **^{1}H-NMR** (600 MHz; $CDCl_3$): δ = 1.40 (s, 18 H, HSQC: C^1H_3), 1.41 (s, 9 H, HSQC: $C^{25}H_3$), 1.79-1.88 (m, 1 H, COSY: C^6H_2), 1.98-2.07 (m, 1 H, COSY: C^6H_2), 2.33-2.45 (m, 2 H, COSY: C^7H_2), 2.63 (bs, 2 H, COSY: $C^{11}H_2$), 2.77 (bs, 4 H, $C^{12}H_2$), 3.22-3.32 (m, 3 H, COSY: C^5H, $C^{10}H_2$), 3.36 (d, ^2J = 17.2 Hz, 1 H, C^4H_2), 3.44 (d, ^2J = 17.2 Hz, 1 H, C^4H_2), 3.57 (bs, 4 H, HMBC: $C^{13}H_2$), 3.61-3.65 (m, 4 H, HMBC: $C^{14}H_2$), 3.72-3.76 (m, 4 H, HMBC: $C^{15}H_2$), 3.85 (s, 6 H, COSY: $C^{22}H_3$), 3.88- 3.91 (m, 4 H, HMBC: $C^{16}H_2$), 4.15-4.23 (m, 4 H, HMBC: $C^{17}H_2$), 6.88 (bs, 1 H, HSQC: N^9H), 7.18 (s, 2 H, HSQC: $C^{19}H$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ = 28.1 (+, 6 C, HSQC: C^1), 28.2 (+, 3 C, HSQC: C^{25}), 26.3 (–, 1 C, HSQC: C^6), 32.5 (–, 1 C, HSQC: C^7), 37.4 (–, 1 C, HSQC: C^{10}), 52.5 (+, 2 C, HSQC: C^{22}), 54.1 (–, 2 C, HMBC: C^4), 54.2 (–, 3 C, COSY: C^{11}, HSQC: C^{12}), 64.8 (+, 1 C, HSQC: C^5), 68.8 (–, 2 C, COSY: C^{13}), 69.5 (–, 2 C, HMBC: C^{16}), 69.7 (–, 2 C, HMBC: C^{17}), 70.6 (–, 2 C, HMBC: C^{14}), 71.1 (–, 2 C, HMBC: C^{15}), 80.7 (C_q, 2 C, HMBC: C^2), 81.2 (C_q, 1 C, HMBC: C^{24}), 113.7 (+, 2 C, HSQC: C^{19}), 125.4 (C_q, 2 C, HMBC: C^{20}), 150.5 (C_q, 2 C, HMBC: C^{18}), 167.7 (C_q, 2 C, HMBC: C^{21}), 170.7 (C_q, 2 C, HMBC: C^3), 171.8 (C_q, 1 C, HMBC: C^8), 172.9 (C_q, 1 C, HMBC: C^{23}). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3056, 2976, 2931, 2823, 1724, 1658, 1600, 1520, 1363, 1138, 736. – **UV** (MeOH): λ_{max} (log ε) = 202 nm (3.381), 224 (3.933), 267 (3.423). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 928.5 (100) [MH^+]. – **HRMS** Calcd for $C_{45}H_{74}N_3O_{17}$: 928.5018; Found: 928.5034 ± 0.0012. – **Elemental analysis** calcd. (%) for $C_{45}H_{73}N_3O_{17}$: C 58.24, H 7.93, N 4.53; found C 58.42, H 8.16, N 4.97.

Spectroscopic assignment for the *tert*-butyl ester of compound 4



The tris tert-butyl ester of **4** (108 mg, 0.12 mmol) was suspended in trifluoro acetic acid (TFA, 4 mL). The reaction mixture was stirred at room temp. for 20 h and the reaction progress was monitored by TLC (ethyl acetate). TFA was evaporated, the obtained triflate salt was redissolved in water and lyophilized yielding **4** (118 mg, 0.12 mmol, 100 %) as a white hygroscopic solid.

MP: 60-62°C. – **^{1}H-NMR** (300 MHz; D_2O): δ = 1.66-1.84 (m, 2 H, Glu-CH_2), 1.98-2.20 (m, 2 H, Glu-CH_2), 3.13-3.25 (m, 2 H, CH_2), 3.27-3.45 (m, 6 H, CH_2), 3.51-3.94 (m, 28 H, CH_2, OMe, N-CH_2, α-CH, NH), 3.98-4.08 (m, 2 H, CH_2), 4.0-4.25 (m, 2 H, CH_2), 7.05-7.12 (s, 2 H, CH). – **^{13}C-NMR** (75 MHz; D_2O): δ = 22.5 (–, 1 C, CH_2), 31.6 (–, 1 C, CH_2), 33.9 (–, 1 C, CH_2), 52.2 (–, 1 C, CH_2), 53.3 (+, 2 C, CH_3), 53.6 (–, 2 C, CH_2), 54.1 (–, 2 C, N-CH_2), 63.6 (–, 2 C, CH_2), 65.7 (+, 1 C, CH), 68.1 (–, 2 C, CH_2), 69.1 (–, 2 C, CH_2), 69.8 (–, 2 C, CH_2), 69.9 (–, 2 C, CH_2), 112.6 (+, 2 C, CH), 116.3 (C_q, q, $^1J_{C,F}$ = 292.1 Hz, CF_3COO^-), 124.5 (C_q, 1 C, Ar-C), 124.7 (C_q, 1 C, Ar-C), 149.58 (C_q, 1 C, Ar-C), 149.62 (C_q, 1 C, Ar-C), 162.7 (C_q, q, $^2J_{C,F}$ = 35.5 Hz, CF_3COO^-), 169.2 (C_q, 1 C, COOMe), 169.3 (C_q, 1 C, COOMe), 170.7 (C_q, 2 C, NCH_2*COOH*), 171.2 (C_q, 1 C, CONH), 174.9 (C_q, 1 C, CH*COOH*). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2957, 2914, 2360, 2341, 1732, 1642, 1528, 1428, 1351, 1200, 814, 719. – **UV** (MeOH): λ_{max} (log ε) = 202 nm (3.860), 224 (4.070), 267 (3.587). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 796.4 (100) [M – 2 H^+ + K^+]$^-$, 780.5 (10) [M – 2 H^+ + Na^+]$^-$, 758.5 (3) [M – H^+].



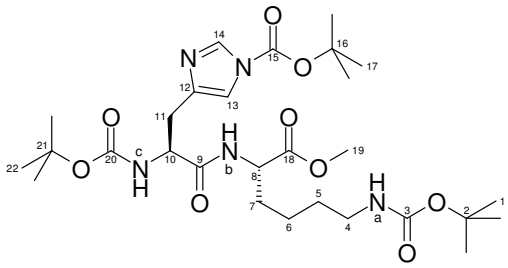
5

***Glutamic acid copper NTA crownether (Cu-NTA CE)* 5:** Compound **4** (164 mg, 0.17 mmol), $NaHCO_3$ (28 mg, 0.3 mmol) and $Cu_2(OH)_2CO_3$ (18.4 mg, 0.08 mmol) were dissolved in water (5 mL). The mixture was stirred at room temperature over night, subsequently refluxed for 3 h and was filtered immediately. The resulting greenish blue solution was concentrated under reduced pressure and lyophilized yielding **5** (185 mg, 98%) as turquoise solid.

MP: > 175°C (decomp.). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2929, 2357, 2341, 1682, 1629, 1523, 1437, 1351, 1295, 1204, 1130, 801, 721. – **MS** (ESI(+), H_2O/MeCN): m/z (%) = 819.4 (100) [M^-], 731.4 [M^- - 2 CO_2], 775.3 [M^- - CO_2], 901.4 [M^- + 2 MeCN]. The purity of the compound was determined by HPLC, diode array detection at 226 nm, and found > 97 %.

2.4.1.3 Synthesis of small peptides in solution

H-His-OMe[iv] and H-Lys(Boc)-OMe[v] were synthesised in solution according to literature known procedures.



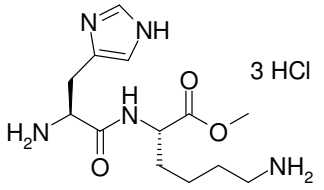
Boc-His(Boc)-Lys(Boc)-OMe

4-[(S)-2-tert-Butoxycarbonylamino-2-((S)-5-tert-butoxycarbonylamino-1-methoxy-carbonyl-pentylcarbamoyl)-ethyl]-imidazole-1-carboxylic acid tert-butyl ester: Under N_2-atmosphere Boc-His(Boc)-OH DCH salt (567 mg, 1.06 mmol), DIPEA (0.58 mL, 3.36 mmol), EDC (0.20 mL, 1.16 mmol), and HOBt (157 mg, 1.16 mmol) were dissolved in DMF (6 mL) under ice cooling. H-Lys(Boc)-OMe (250 mg, 0.96 mmol) dissolved in a little DMF was added slowly. The reaction was allowed to warm to room temp. and was stirred over night (20 h) at 40°C. The reaction progress was monitored by TLC (ethyl acetate). After completion of the reaction, water (25 mL) was added and the mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, the solvent was evaporated and the crude product was purified by flash column chromatography on silica gel (ethyl acetate / petrol ether 3:1, R_f (ethyl acetate) = 0.75) yielding Boc-His(Boc)-Lys(Boc)-OMe (59 mg, 10 %) as a colourless solid.

[iv] Z. Hongjun; H. Bangyou; S. Guangliang, Patent No. CN 1557834, 29 Dec **2004**

[v] C. Mandl, *PhD Thesis*, University of Regensburg **2004**

^{1}H-NMR (600 MHz; $CDCl_3$): δ = 1.17 (bs, 2 H, HSQC, COSY: C^6H_2), 1.42 (s, 11 H, HSQC: C^5H_2, Boc-CH_3), 1.43 (s, 9 H, HSQC: Boc-CH_3), 1.58 (s, 10 H, HSQC: Boc-CH_3, C^7H_2), 1.68-1.80 (m, 1 H, HSQC: C^7H_2), 2.94 (dd, ^3J = 5.7 Hz, ^1J = 14.8 Hz, 1 H, HSQC, COSY: $C^{11}H_2$), 3.00-3.13 (m, 3 H, HSQC, COSY: $C^{11}H_2$ diastereotope, C^4H_2), 3.68 (s, 3 H, HSQC, COSY: $C^{19}H_3$), 4.43 (bs, 1 H, COSY: $C^{10}H$), 4.49-4.56 (m, 1 H, COSY: C^8H), 4.72 (bs, 1 H, COSY: NH^a), 6.13 (bs, 1 H, COSY: NH^c), 7.16 (s, 1 H, COSY: $C^{13}H$), 7.23 (bs, 1 H, COSY: NH^b), 8.01 (s, 1 H, COSY: $C^{14}H$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ = 22.2 (-, 1 C, HSQC: C^6), 27.8 (+, 3 C, HSQC: Boc-CH_3), 28.3 (+, 3 C, HSQC: Boc-CH_3), 28.4 (+, 3 C, HSQC: Boc-CH_3), 29.6 (–, 1 C, HSQC: C^5), 30.1 (–, 1 C, COSY: C^{11}), 32.1 (–, 1 C, COSY: C^7), 40.1 (–, 1 C, HSQC: C^4), 51.8 (+, 1 C, COSY: C^8),52.2 (+, 1 C, COSY: C^{19}), 54.2 (+, 1 C, COSY: C^{10}), 79.0 (C_q, 1 C, COO*^{t}Bu*), 80.0 (C_q, 1 C, COO*^{t}Bu*), 85.7 (C_q, 1 C, COO*^{t}Bu*), 114.8 (+, 1 C, COSY: C^{13}), 136.8 (+, 1 C, COSY: C^{14}), 139.2 (C_q, 1 C, HMBC: C^{12}), 146.9 (C_q, 1 C, HMBC: C^9), 155.6 (C_q, 1 C, HMBC: C^{15}), 155.9 (C_q, 1 C, HMBC: C^3), 171.3 (C_q, 1 C, HMBC: C^{20}), 172.3 (C_q, 1 C, HMBC: C^{18}). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 598.5 (100) [MH^+], 498.5 (19) [MH^+ - Boc].

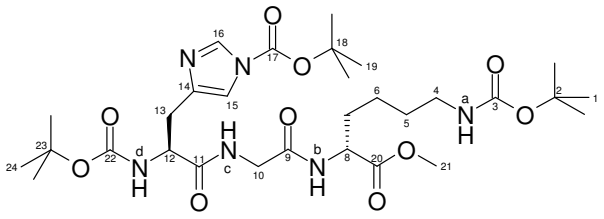


H-His-Lys-OMe

***(S)-6-Amino-2-[(S)-2-amino-3-(1H-imidazol-4-yl)-propionylamino]-hexanoic acid methyl ester*:** The protected dipeptide Boc-His(Boc)-Lys(Boc)-OMe (55 mg, 0.09 mmol) was dissolved in DCM under ice cooling and mixed with HCl/ether. The reaction was allowed to warm to room temp. and was stirred for additional 3 hours. The reaction progress was monitored by TLC (ethyl acetate), and after completion the reaction mixture was evaporated to dryness and the residue was redissolved in water and lyophilised yielding H-His-OMe · 3 HCl (36 mg, quantitative), as a colourless hygroscopic solid.

^{1}H-NMR (300 MHz; D_2O): δ = 1.39-1.53 (m, 2 H, Lys-CH_2), 1.61-2.05 (m, 4 H, Lys-CH_2), 2.93 (t, ^3J = 7.7 Hz, 2 H, Lys-CH_2), 3.34-3.50 (m, 2 H, His-CH_2), 3.79 (s, 3 H, OMe), 4.30 (dd, ^3J = 6.6 Hz, 7.7 Hz, 1 H, Lys-α-CH), 4.43 (dd, ^3J = 5.4 Hz, 8.5 Hz,1 H,

His-α-CH), 7.50 (s, 1 H, imidazole-CH), 8.78 (s, 1 H, imidazole-CH). – **^{13}C-NMR** (75 MHz; D_2O): δ = 22.0 (–, 1 C, CH_2), 26.1 (–, 1 C, CH_2), 26.3 (–, 1 C, CH_2), 30.1 (–, 1 C, CH_2), 39.2 (–, 1 C, CH_2), 52.1 (–, 1 C, CH_2), 52.9 (+, 1 C, CH), 53.1 (+, 1 C, CH), 118.8 (+, 1 C, CH), 125.7 (C_q, 1 C), 134.4 (+, 1 C, CH), 168.1 (C_q, 1 C, His C=O), 173.6 (C_q, 1 C, COOMe). – **MS** (ESI(+), H_2O/MeCN/TFA): m/z (%) = 298.2 (70) [MH^+], 190.6 (59) [M + 2 H^+ + 2 MeCN]$^{2+}$, 170.0 (100) [M + 2 H^+ + MeCN]$^{2+}$, 149.7 (13) [M + 2 H^+]$^{2+}$.

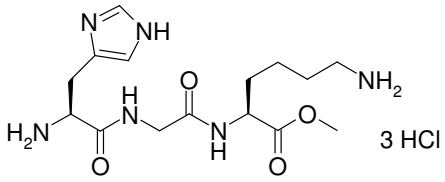


Boc-His(Boc)-Gly-Lys(Boc)-OMe

***4-((S)-2-tert-Butoxycarbonylamino-2-{[((R)-5-tert-butoxycarbonylamino-1-methoxycarbonyl-pentylcarbamoyl)-methyl]-carbamoyl}-ethyl)-imidazole-1-carboxylic acid tert-butyl ester*:** Under N_2-atmosphere Boc-His(Boc)-OH DCH salt (276 mg, 0.51 mmol), DIPEA (0.70 mL, 4.07 mmol), HBTU (215 mg, 0.57 mmol), and HOBt (76 mg, 0.57 mmol) were dissolved in DCM (10 mL) under ice cooling. H-Gly-Lys(Boc)-OMe (136 mg, 0.43 mmol) dissolved in a little DCM was added slowly. The reaction was allowed to warm to room temp. and was stirred for additional 20 h at room temperature. The reaction progress was monitored by TLC (ethyl acetate). After completion water (20 mL) was added, the organic layer was extracted with sat. $NaHCO_3$ (3x) and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash column chromatography on silica gel (dichloromethane/MeOH 97:3, R_f = 0.1) yielding Boc-His(Boc)-Gly-Lys(Boc)-OMe (55 mg, 19 %) as a colourless solid.

^{1}H-NMR (400 MHz; $CDCl_3$): δ = 1.33 (dd, ^2J = 7.3 Hz, ^3J = 14.7 Hz, 2 H, HMBC: C^7H_2), 1.41 (s, 20 H, Lys-Boc-CH_3, His-Boc-CH_3, HMBC: C^5H_2), 1.59 (s, 10 H, His-Boc-CH_3, HMBC: C^6H_2), 1.76-1.86 (m, 1 H, HMBC: C^6H_2), 2.92-3.09 (m, 3 H, HMBC: C^4H_2, $C^{13}H_2$), 3.19 (dd, ^2J = 4.2 Hz, ^3J = 14.8 Hz, 1 H, HMBC: $C^{13}H_2$), 3.70 (s, 3 H, HMBC: $C^{21}H_3$), 3.95 (bs, 2 H, COSY: $C^{10}H_2$), 4.43 (bs, 1 H, HMBC: $C^{12}H$), 4.60 (bs, 1 H, HMBC: C^8H), 4.97 (bs, 1 H, COSY: NH^a), 5.76 (bs, 1 H, COSY: NH^d),

7.18 (s, 1 H, HMBC: $C^{15}H$), 7.29 (bs, 1 H, COSY: NH^{c}), 7.75 (bs, 1 H, COSY: NH^{b}), 8.04 (s, 1 H, HMBC: $C^{16}H$). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ = 22.5 (–, 1 C, HSQC: C^{7}), 27.8 (+, 3 C, C^{Boc}), 28.3 (+, 3 C, C^{Boc}), 28.4 (+, 3 C, C^{Boc}), 29.2 (–, 1 C, HSQC: C^{5}), 29.8 (–, 1 C, HMBC: C^{13}), 31.2 (–, 1 C, HSQC: C^{6}), 39.9 (–, 1 C, HMBC: C^{4}), 43.4 (–, 1 C, HMBC: C^{10}), 51.8 (+, 1 C, HMBC: C^{8}), 52.2 (+, 1 C, HSQC: C^{21}), 52.9 (+, 1 C, HMBC: C^{12}), 78.9 (C_q, 1 C, HMBC: C^{23}), 80.3 (C_q, 1 C, HMBC: C^{2}), 85.8 (C_q, 1 C, HMBC: C^{18}), 115.2 (+, 1 C, HMBC: C^{15}), 137.4 (+, 1 C, HMBC: C^{16}), 138.3 (C_q, 1 C, HMBC: C^{14}), 146.7 (C_q, 1 C, HMBC: C^{3}), 155.5 (C_q, 1 C, HMBC: C^{17}), 156.1 (C_q, 1 C, HMBC: C^{11}), 156.8 (C_q, 1 C, HMBC: C^{9}), 171.9 (C_q, 1 C, HMBC: C^{22}), 172.8 (C_q, 1 C, HMBC: C^{20}). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 655.5 (100) [MH^+].

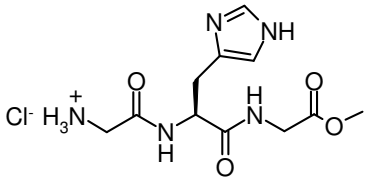


H-His-Gly-Lys-OMe

***(S)-6-Amino-2-{2-[(S)-2-amino-3-(1H-imidazol-4-yl)-propionylamino]-acetylamino}-hexanoic acid methyl ester hydrochloride*:** The protected tripeptide Boc-His(Boc)-Gly-Lys(Boc)-OMe (55 mg, 0.08 mmol) was dissolved in DCM under ice cooling and mixed with HCl/ether. The reaction was allowed to warm to room temp. and was stirred for additional 3 hours. The reaction progress was monitored by TLC (EE). The reaction mixture was evaporated to dryness and the residue was redissolved in water and lyophilised. H-His-Gly-Lys-OMe·3 HCl (37 mg, quantitative) was obtained as a colourless hygroscopic solid.

^{1}H-NMR (300 MHz; D_2O): δ = 1.31-1.53 (m, 2 H, Lys-CH_2), 1.57-2.00 (m, 4 H, Lys-CH_2), 2.95 (t, ^{3}J = 7.7 Hz, 2 H, Lys-CH_2), 3.40 (d, ^{3}J = 6.6 Hz, 2 H, His-CH_2), 3.71 (s, 3 H, OMe), 4.00 (dd, ^{2}J = 29.9 Hz, ^{3}J = 17.0 Hz, 2 H, Gly-CH_2), 4.35 (t, ^{3}J = 6.4 Hz, 1 H, Lys-α-CH), 4.40 (dd, ^{2}J = 8.8 Hz, ^{3}J = 5.2 Hz, 1 H, His-α-CH), 7.43 (s, 1 H, imidazole-CH), 8.67 (s, 1 H, imidazole-CH). – **^{13}C-NMR** (75 MHz; D_2O): δ = 22.1 (–, 1 C, CH_2), 26.1 (–, 1 C, CH_2), 26.3 (–, 1 C, CH_2), 30.0 (–, 1 C, CH_2), 39.3 (–, 1 C, CH_2), 42.1 (–, 1 C, Gly-CH_2), 52.1 (+, 1 C, CH, Gly α-CH), 52.9 (+, 1 C, CH), 53.1 (+,

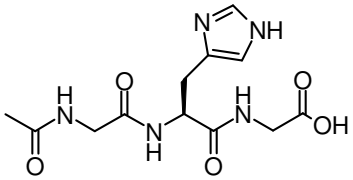
1 C, CH), 118.8 (+, 1 C, CH), 125.6 (C_q, 1 C), 134.6 (+, 1 C), 168.7 (C_q, 1 C, Gly C=O), 171.0 (C_q, 1 C, His C=O), 174.2 (C_q, 1 C, Lys C=O). – **MS** (ESI(+), H_2O/MeCN/TFA): m/z (%) = 177.9 (100) $[M + 2\ H^+]^{2+}$, 198.4 (75) $[M + 2\ H^+ + MeCN]^{2+}$, 355.0 (55) $[MH^+]$.



H-Gly-His-Gly-OMe

***[(S)-2-(2-Amino-acetylamino)-3-(1H-imidazol-4-yl)-propionylamino]-acetic acid methyl ester hydrochloride*:** H-Gly-His-Gly-OH (10.2 mg, 0.06 mmol) was suspended in 2 mL methanol and $SOCl_2$ (20 µL, 0.28 mmol) was added to the stirred solution. The reaction mixture was stirred over night, methanol was removed in vacuo and the reaction process was monitored by NMR. The crude product was dried in high vacuum obtaining H-Gly-His-Gly-OMe hydrochloride as white crystalline solid (19 mg, quantitative).

^{1}H-NMR (300 MHz; CD_3OD): δ = 3.17 (dd, ^3J = 7.0 Hz, ^2J =15.5 Hz, 1 H, CH), 3.30 (dd, ^3J = 7.0 Hz, ^2J = 15.5 Hz, 1 H, CH and solvent peak), 3.73 (s, 3 H, OCH_3), 3.76 (s, 2 H, CH_2), 3.97 (dd, ^{3}J = 17.6 Hz, 2 H, CH_2), 7.42 (s, 1 H, CH), 8.80 (s, 1 H, CH). – **^{13}C-NMR** (75 MHz; CD_3OD): δ = 28.3 (–, 1 C, CH_2), 41.7 (–, 1 C, CH_2), 41.9 (–, 1 C, CH_2), 52.8 (+, 1 C, OMe), 53.6 (+, 1 C, CH), 118.9 (C_q, 1 C, CH), 130.6 (C_q, 1 C), 135.1 (+, 1 C, CH), 167.5 (C_q, 1 C, CONH), 171.8 (C_q, 1 C, CONH), 172.2 (C_q, 1 C, CONH). – **MS** (CI - MS (NH_3)): m/z (%) = 284.2 $[MH^+]$.



Ac-Gly-His-Gly-OH

***[(S)-2-(2-Acetylamino-acetylamino)-3-(1H-imidazol-4-yl)-propionylamino]-acetic acid*:** H-Gly-His-Gly-OH (10.8 mg, 0.04 mmol) was suspended in 2 mL water and Ac_2O (40 µL, 0.40 mmol) was added to the stirred solution. The reaction mixture was

stirred for 4 h at room temp., water and excess of acetic anhydride were removed in vacuo and the reaction process was monitored by NMR. The crude product was dried in high vacuum giving Ac-Gly-His-Gly-OH as a white crystalline solid (12.5 mg, quantitative).

^{1}H-NMR (300 MHz; CD_3OD): δ = 2.00 (s, 3 H, CH_3), 3.19 (dd, ^3J = 5.3 Hz, ^2J = 15.2 Hz, 1 H, CH_2), 3.30 (dd, ^3J = 5.3 Hz, ^2J = 15.2 Hz, 1 H, CH_2 and solvent peak), 3.68 (d, ^{2}J = 17.3 Hz, 1 H, CH_2), 3.83 (d, 2 H, ^{2}J = 3.6 Hz, CH_2), 3.99 (d, ^2J = 17.1 Hz, 2 H, CH_2), 4.72 (t, ^3J = 5.3 Hz, 1 H, CH), 7.26 (s, 1 H, CH), 8.42 (s, 1 H, CH). – **^{13}C-NMR** (75 MHz; CD_3OD): δ 22.4 (+, 1 C, CH_3), 28.9 (–, 1 C, CH_2), 43.8 (–, 2 C, CH_2), 53.3 (+, 1 C, CH), 119.6 (+, 1 C, CH), 130.5 (C_q, 1 C), 135.6 (+, 1 C, CH), 171.5 (C_q, 1 C, CONH), 171.8 (C_q, 1 C, CONH), 174.2 (C_q, 1 C, CONH), 176.5 (C_q, 1 C, CONH). – **MS** (ESI(+), H_2O/MeOH + 10 mmol NH_4Ac): m/z (%) = 312.0 (100) [MH^+], 334.0 (21) [MNa^+].

2.4.1.4 Solid phase synthesis of the peptide library for screening

All peptides were synthesized on an Advanced Chemtech 496 MOS synthesizer. Rink Amide MBHA resin and Fmoc protecting group strategy were used for the entire library. Coupling was done by TBTU / HOBt / DIPEA. HOBt was used as a 0.45 M solution, TBTU as a 0.44 M solution and DIPEA as a 1.2 M solution, all in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.4 M solutions. The syntheses were carried out in a 96 well reactor block. Every peptide was synthesized on 50 mg of resin. The lot of the resin used had a loading of 0.72 mmol/g (manufacturer's claims). Before each synthesis the resin was allowed to preswell in DMF for 30 min. Each coupling was done twice using a 5 fold excess of HOBt and slightly less than 5 fold excess of TBTU. DIEA was used in 10 fold excess. Fmoc deprotection was done by shaking the resin with 40 % piperidine in DMF for 3 minutes, subsequent washing and addition of 20 % piperidine in DMF followed by shaking for 10 minutes. When the syntheses were complete, the resin was washed with MeOH and DCM (5 x 2 mL each). Where acylation was necessary, it was conducted using 5 eq of Ac_2O, 5 eq of DIPEA in DMF for 30 minutes. Cleavage from the resin was afforded by shaking the resin for 3 h after addition of 1.5 mL of TFA / TIS / H_2O (90:5:5) (v/v). Where the peptides contained cysteine, 2 % EDT were added to the cleavage mixture. After filtering off the

resin, the TFA solution was reduced in volume to about 0.5 mL. It was then transferred to a Falcon tube and precipitated with cold Et_2O. The precipitate was centrifuged at – 4 °C for 10 minutes. The solution was then carefully decanted off and the precipitate resuspended in cold Et_2O before being centrifuged again. This resuspending/centrifuging step was repeated five times. Finally, the Et_2O was decanted off again and the peptide dried under vacuum. The peptides were analysed by ES-MS, LC-MS and analytical HPLC

2.4.2 Fluorescence data

All fluorescence experiments were performed on a Varian Cary Eclipse Fluorimeter. To determine the binding constants and the $K_{0.5}$ values, fluorescence titration experiments were carried out.

2.4.2.1 Screening of binding affinity using the peptide library

The screening of the synthesized peptide library was conducted in a microtiter plate (96 wells). The fluorescence intensity was found to be constant after 15 min.

Instrument Parameters

Excitation wavelength: λ_{ex} = 305 nm

Detection wavelength: λ = 320 – 550 nm

Temperature: T = 298 K

PMT voltage 800 volts

Screening conditions

Solvent: 50 mM HEPES buffer, pH 7.5

Well volume: 400 µL

Concentration (**5**): 3.75 x 10^{-5} M

Concentration [peptide]: 1.875 x 10^{-4} M ≙ 5 eq of peptide with regard to **5**

Procedure:

To each column in the microtiter plate samples of **5** with 5 eq of the corresponding peptide and **5** without added peptide as well as **5** with 5 eq of GHG were given as reference. Emission spectra for all wells were recorded.

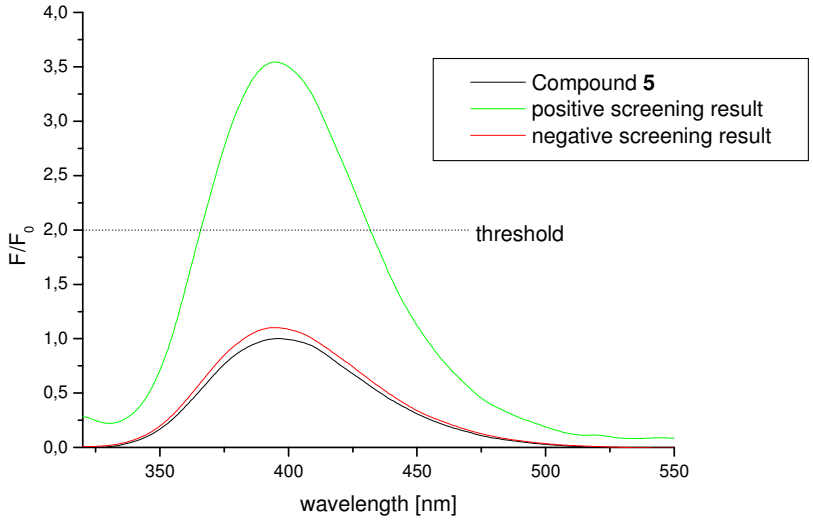


Figure 3. For peptides inducing a significant increase in emission intensity over the parent compound **5** ($F/F_0 \geq 2.0$), the binding constant and stoichiometry of the respective peptide to **5** were determined by emission titration in a cuvette.

2.4.2.2 Emission titrations

Instrument Parameters

Excitation wavelength: λ_{ex} = 305 nm

Detection wavelength: λ = 320 – 550 nm or 320 – 600 nm

Temperature: T = 298 K

PMT voltage 600 volts

Titration conditions:

Solvent: 50 mM HEPES buffer, pH 7.5

Starting volume: 1.2 mL or 2.5 mL

Concentration (**5**): 5.0 10^{-5} M

Concentration [peptide]: $7.5 \cdot 10^{-4}$ M or $4.2 – 4.3 \cdot 10^{-3}$ M

Procedure:

To a cuvette with 1.2 mL of **5** in HEPES buffer were added 40 µL (≙ 0.5 eq) aliquots of the peptide solution, while the cuvette filled with 2.5 mL of Cu-NTA CE **5** in HEPES buffer was titrated stepwise with small amounts 5 – 60 µL, depending on the initial emission response, of the substrate solution. After each addition the solution was allowed to equilibrate for 15 min before the fluorescence intensity and the UV spectrum (see Figure 18 for a representative example) were recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data.[vi] To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

H-Gly-His-Gly-Gly-NH_2

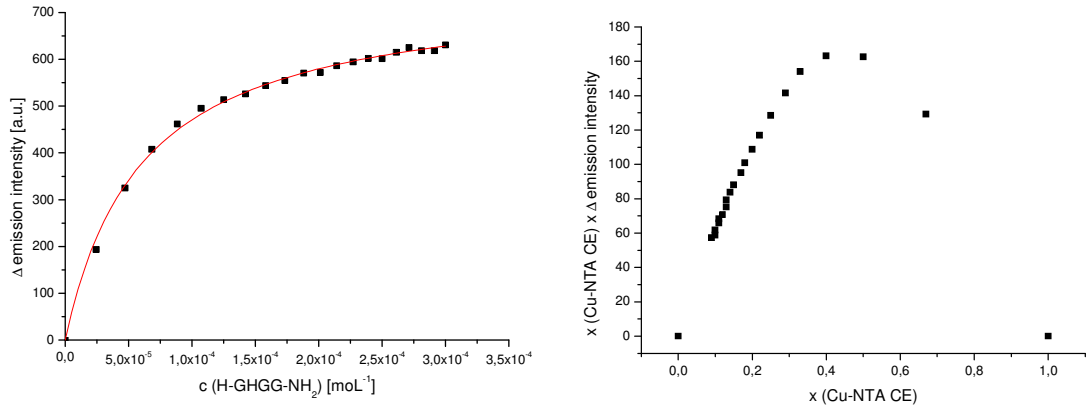


Figure 4. Emission titration of **5** with peptide H-Gly-His-Gly-Gly-NH_2.

H-Gly-His-Gly-OH

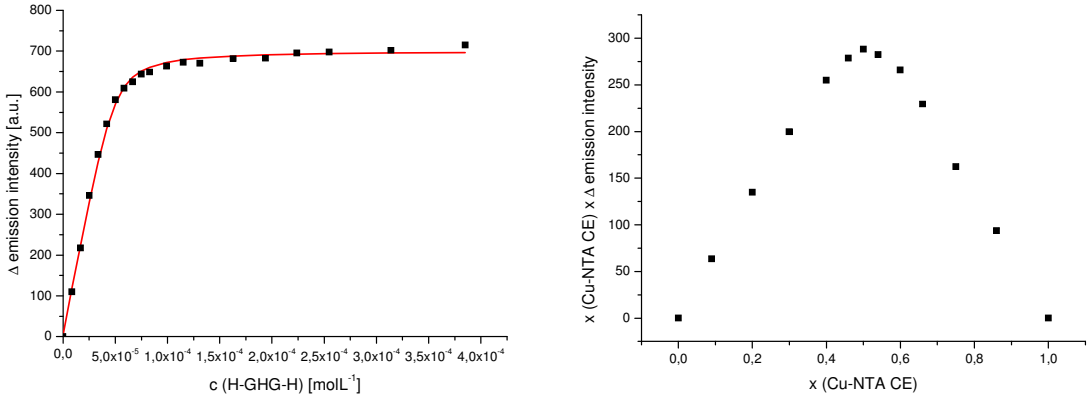


Figure 5. Emission titration of **5** with peptide H-Gly-His-Gly-OH.

[vi] (a) P. MacCarthy *Anal. Chem.* **1978**, 50, 2165. (b) C. Schmuck, P. Wich, P. *Angew. Chem. Int. Ed.* **2006**, *45*, 4277-4281.

H-Gly-His-Gly-OMe

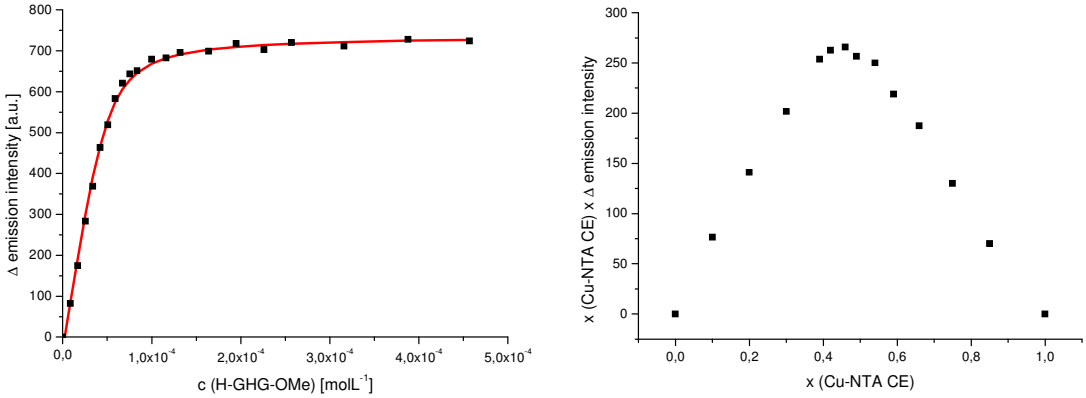


Figure 6. Emission titration of **5** with peptide H-Gly-His-Gly-OMe.

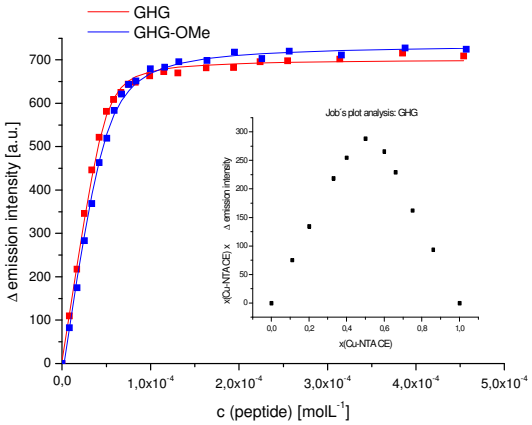


Figure 7. Comparison of emission titrations of **5** with peptides H-Gly-His-Gly-OH and H-Gly-His-Gly-OMe.

Ac-Gly-His-Gly-OH

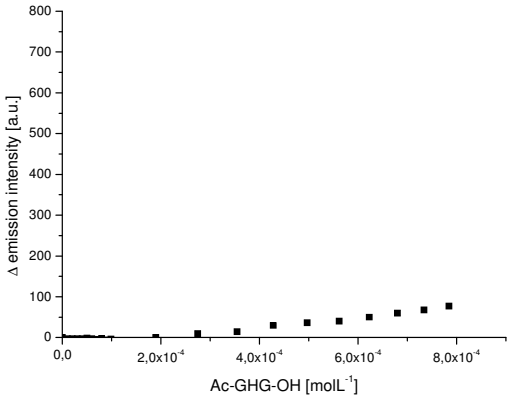


Figure 8. Emission titration of **5** with peptide Ac-Gly-His-Gly-OH.

H-Gly-Gly-His-OH

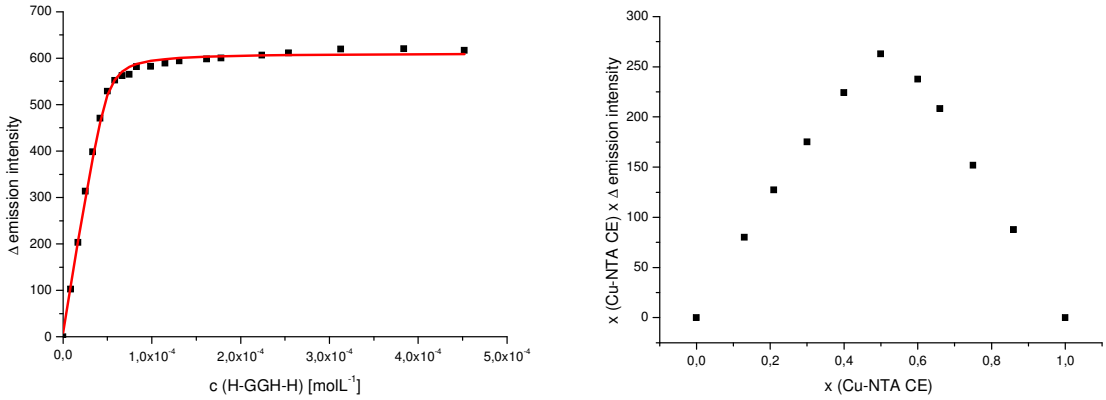


Figure 9. Emission titration of **5** with peptide H-Gly-Gly-His-OH.

H-Gly-His-OH

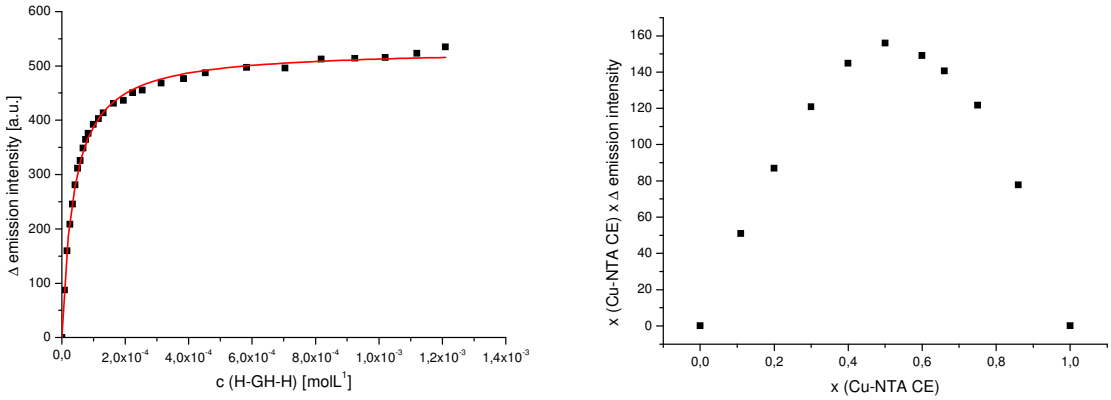


Figure 10. Emission titration of **5** with peptide H-Gly-His-OH.

H-His-OMe

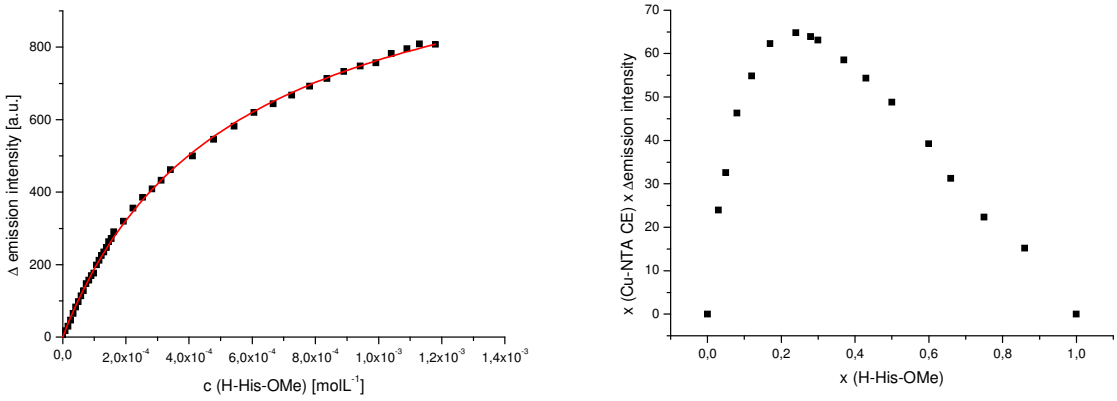


Figure 11. Emission titration of **5** with peptide H-His-OMe.

H-His-Lys-OMe

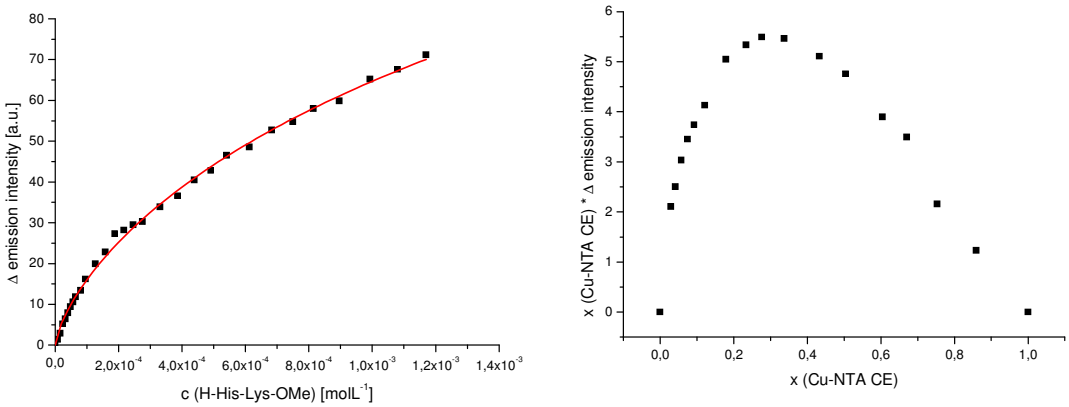


Figure 12. Emission titration of **5** with peptide H-His-Lys-OMe.

H-His-Gly-Lys-OMe

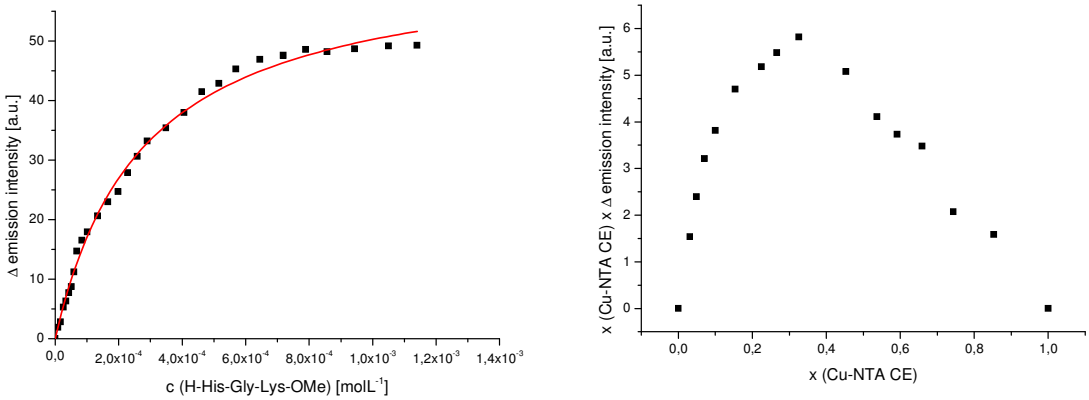


Figure 13. Emission titration of **5** with peptide H-His-Gly-Lys-OMe.

H-His-Gly-Gly-OH

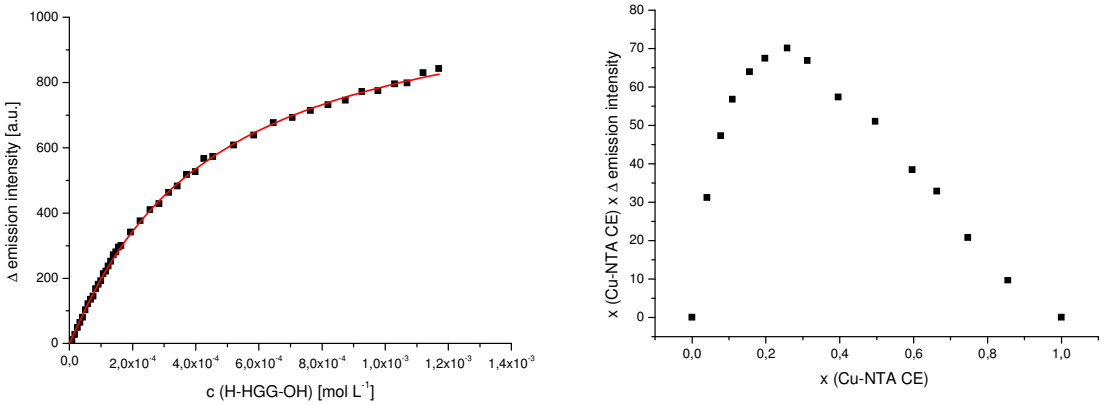


Figure 14. Emission titration of **5** with peptide H-His-Gly-Gly-OH.

H-Ala-His-Gly-Gly-NH_2

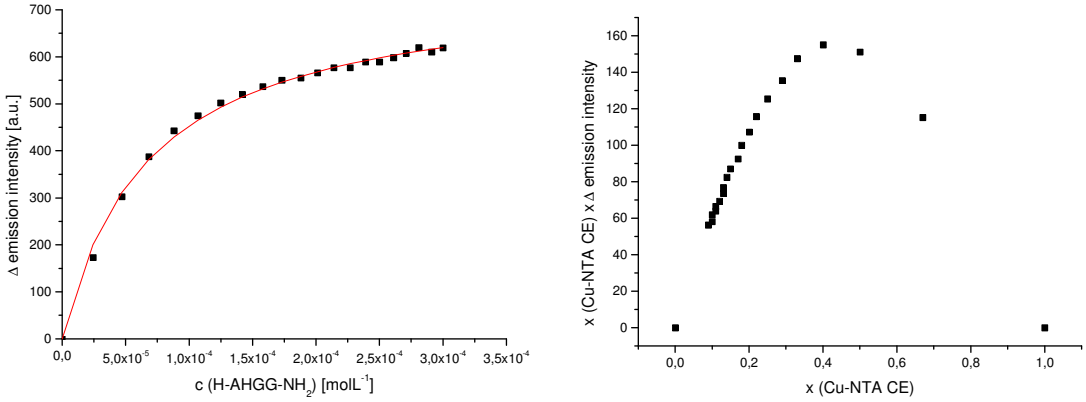


Figure 15. Emission titration of **5** with peptide H-Ala-His-Gly-Gly-NH_2.

H-Leu-His-Gly-Gly-NH_2

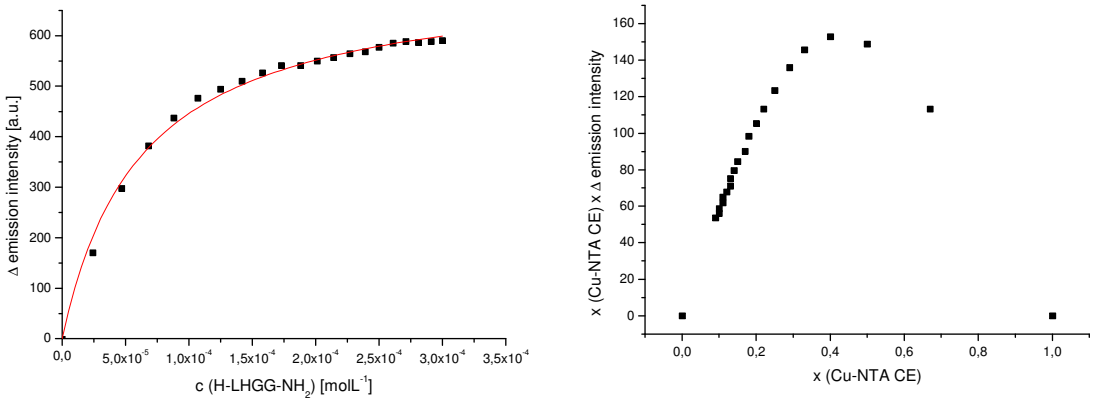


Figure 16. Emission titration of **5** with peptide H-Leu-His-Gly-Gly-NH_2.

H-Gln-His-Gly-Gly-NH_2

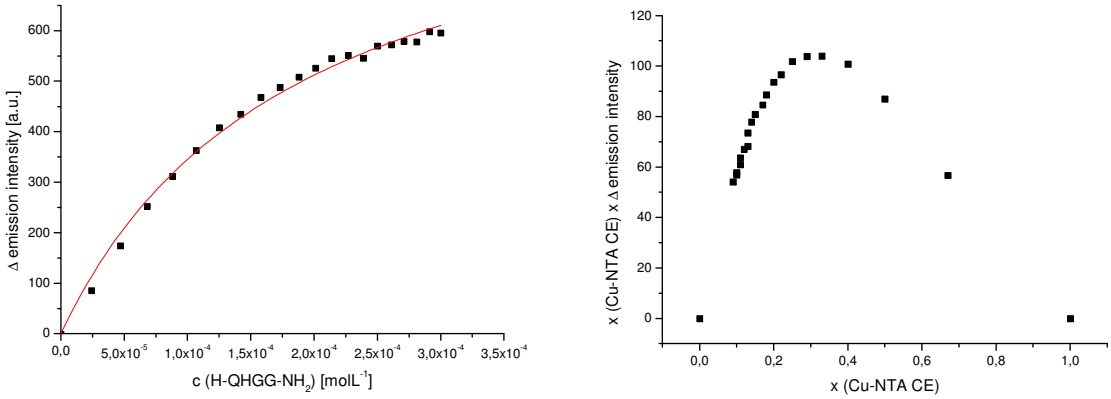


Figure 17. Emission titration of **5** with peptide H-Gln-His-Gly-Gly-NH_2.

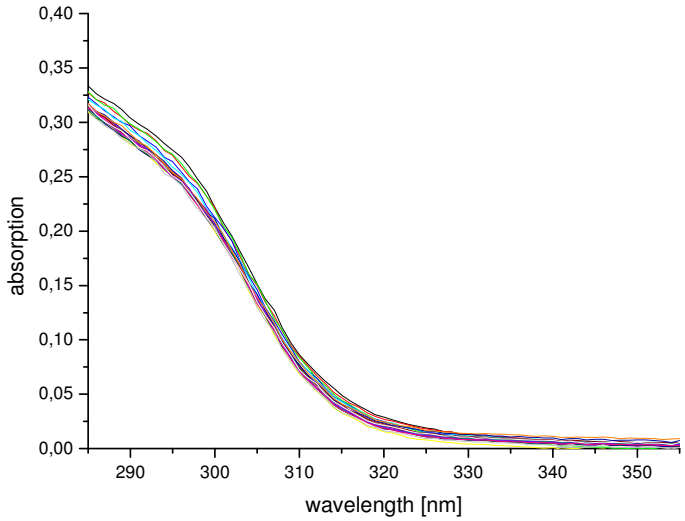


Figure 18. UV monitoring of the titration of **5** with peptide Gly-His-Gly.

2.4.3 Determination of NTA-Cu complex stability in compound 5 in the presence of peptides

a) Fluorescence study

The tripeptide GGH and the dipeptide GH themselves show affinity to copper ions as reported in literature.[vii] Therefore the stability of the copper-NTA complex of **5** in the presence of these peptides was investigated. To a HEPES (50 mM, pH 7.5) buffered solution of **5** (2.5 mL, $5.0 \cdot 10^{-5}$ M) 1.2 eq of GGH were added. After addition of GGH the fluorescence spectrum of the mixture was recorded in certain intervals (3 min, 12 min, 23, min, 53 min, 2 h 53 min). The fluorescence intensity increased if compared to the receptor due to the binding process and gave a constant output after approx. 15 min after adding. The over time recorded spectra showed no significant difference from each other thus only one is shown representatively. The sample was further kept in the fridge for 18 days and subsequently a fluorescence spectrum was collected. The emission intensity of the sample after 18 days (*blue*) was still the same as at the beginning of the experiment (*green*). This provides evidence that the fluorescence intensity enhancement during binding experiments has its origin in the binding event of

[vii] S. J. Lau, B. Sarkar, *Biochem. J.* **1981**, *199*, 649-656.

the peptides to the receptor. If decomplexation would occur, the quenching of the benzocrown ether unit by the copper ion would disappear and the fluorescence intensity would be expected to reach the same level as in the ligand **4**. Figure 19 summarizes the result. Fluorescence spectra of ligand **4** (black) and receptor **5** (red) are given for comparison.

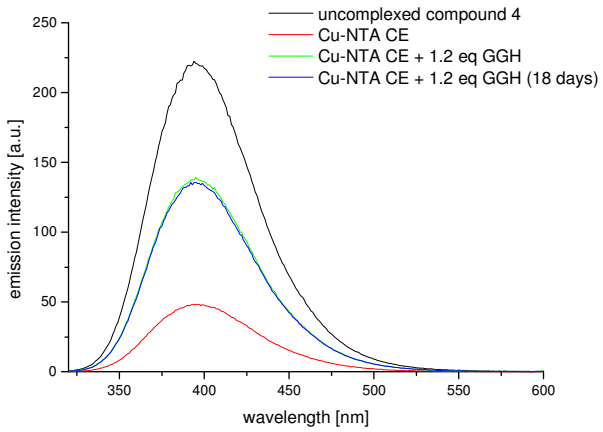


Figure 19. Determination of complex stability of Cu-NTA CE **5** in the presence of GGH (1.2 eq) in buffered aqueous solution (HEPES, pH 7.5).

b) UV absorption study

To a HEPES (50 mM, pH 7.5) buffered solution of Cu-NTA CE **5** ($5.0 \cdot 10^{-5}$ M) 5.0 eq GGH were added. Subsequently the UV absorption was measured every four minutes after addition and after 21 hours. Figure 20 shows the recorded spectra and the absorption spectra of compound **4** and **5** without peptide present for comparison.

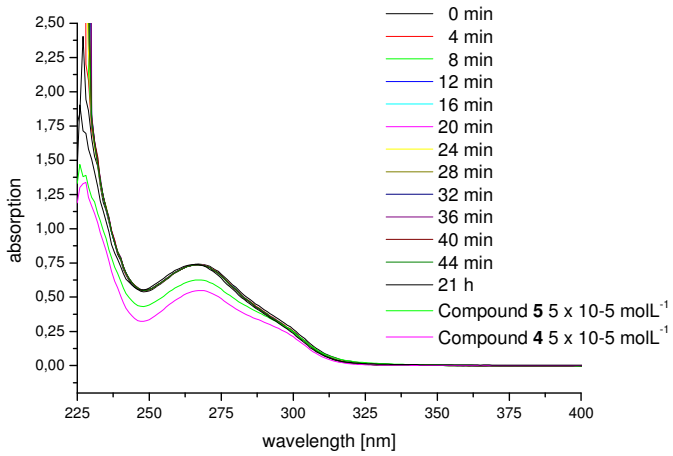


Figure 20. UV absorption spectra of Cu-NTA CE **5** with GGH (5 eq) over time. The UV absorption of the ligand **4** (pink) and Cu-NTA CE **5** (*green*) are given for comparison.

2.4.4 Change of UV- and emission spectra of NTA-Cu and 5 in the presence of mercapto ethanol

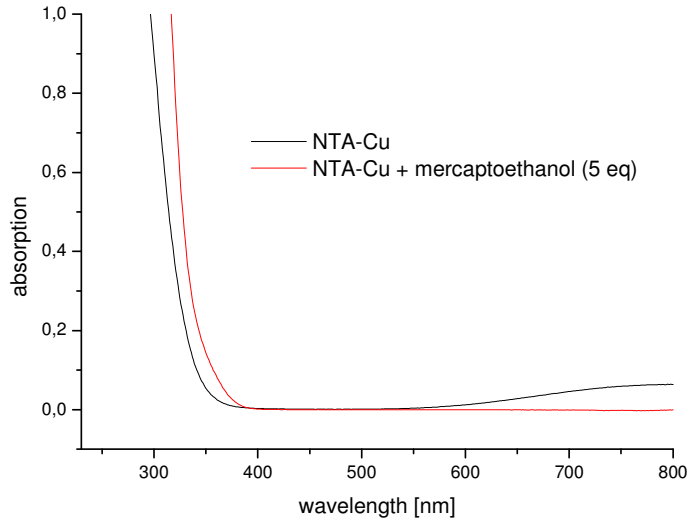


Figure 21. UV absorption spectra of NTA-Cu (black) and NTA-Cu + 5 equivalents of mercaptoethanol (red).

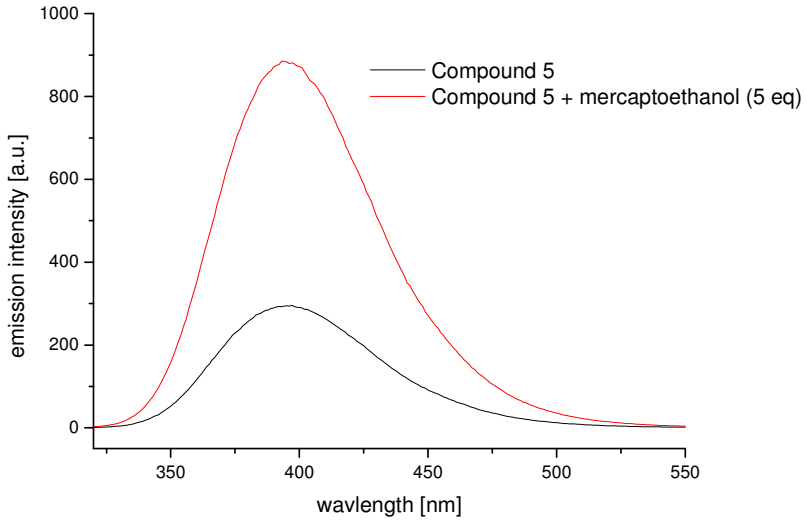


Figure 22. Fluorescence spectra of compound **5** (*black*) and compound **5** + 5 equivalents of mercaptothiol (*red*); concentration of compound **5**: c = 5.0 x 10^{-5} molL^{-1}.

2.4.5 Binding Studies by Isothermal Titration Calorimetry (ITC)

In order to verify the results of the emission titrations, the binding processes of the peptides GHG and Ac-GHG with compound **5** were determined by isothermal titration calorimetry.

All ITC experiments were performed in buffered aqueous solution (HEPES, 50 mM, pH 7.5) at 25 °C using an ultrasensitive VP-ITC calorimeter from MicroCal (Northampton, MA, U.S.A.). The sample solutions were made from stock buffer solution (HEPES, 50 mM, pH 7.5). Concentration of Cu-NTA CE **5** (titrant) in the 300 µL syringe was set to 1.2 mM, whereas the concentration of the peptide GHG or Ac-GHG in the 1.436 mL calorimetric cell was 0.05 mM. Before each titration both experimental solutions (titrant, cell) were thoroughly degassed under vigorous stirring. During the ITC experiment the cell solution was stirred at 300 rpm by syringe to ensure rapid mixing and 60 x 5 µL of titrant were injected over 10 s with a spacing time between each injection of two minutes in order to allow complete equilibration. Before data analysis the total observed heat of binding was corrected for the heat of dilution yielding the effective heat of binding. Therefore an analogue ITC experiment with the calorimeter cell filled with HEPES and Cu-NTA CE as titrant was carried out. The data were analyzed by non linear fitting methods using the MicroCal Origin software (Windows based). The values for the binding stoichiometry (n) and the thermodynamic parameters of interaction: enthalpy of binding (ΔH) and binding constant (lg K), and, consequently, free energy change (ΔG) and entropy change (ΔS) were obtained using a "one-set of site" model for the fitting routine. Table 1 summarizes the results of the ITC experiments. Figure 23 and Figure 24 show the raw ITC titration data and the processed curve.

	n	lg K [M^{-1}]	ΔH [cal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	ΔG [cal mol^{-1}]
H-GHG-OH	1.04 ± 0.01	5.10 ± 0.02	- 4168 ± 22.25	9.37	-6962
Ac-GHG-OH	1.18 ± 0.08	3.99 ± 0.04	- 4364 ± 47.40	3.61	- 5440

Table 3. Results of the ITC experiments of Cu-NTA CE vs GHG and Ac-GHG, respectively.

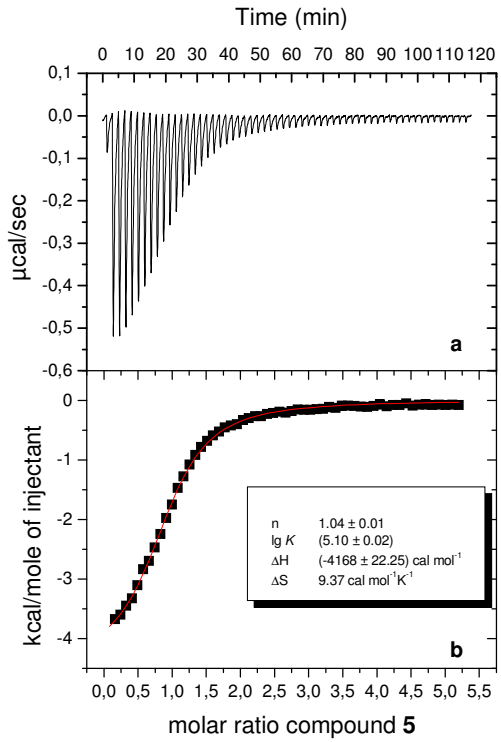


Figure 23. ITC of GHG (0.05 mM) and Cu-NTA CE (**5**) (1.20 mM) in HEPES (50 mM, pH 7) at 25 °C; (**a**) raw ITC data, (**b**) heat of dilution corrected and fitted ΔH diagram.

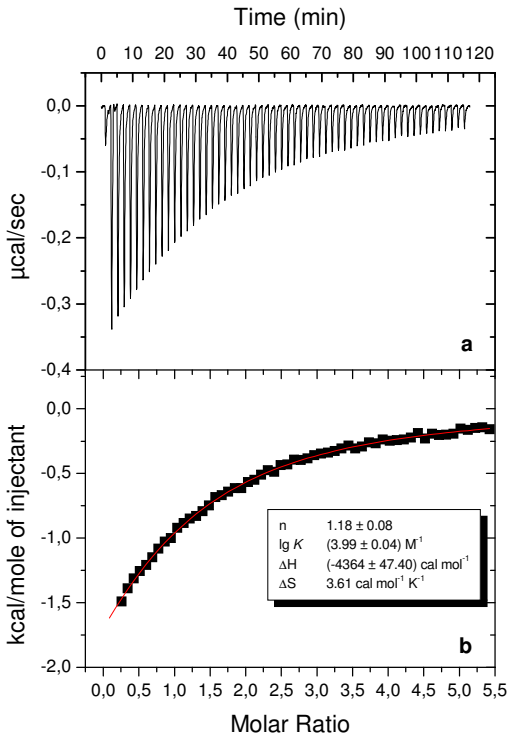
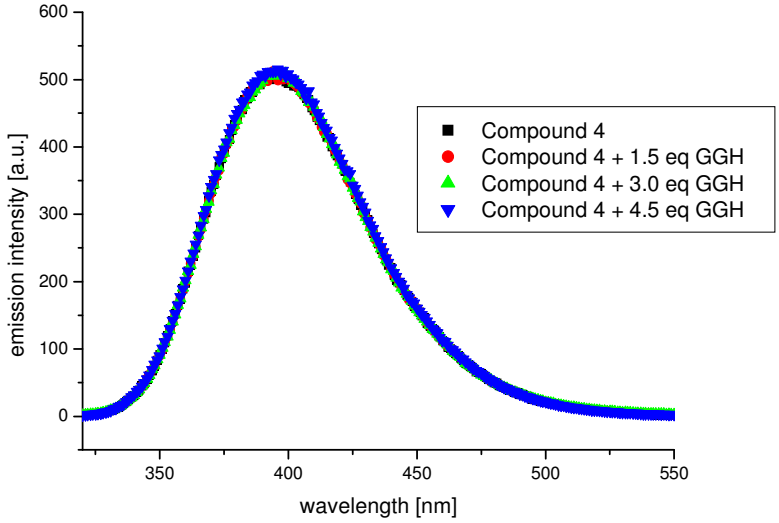


Figure 24. ITC of Ac-GHG (0.05 mM) and Cu-NTA CE (**5**) (1.20 mM) in HEPES (50 mM, pH 7.5) at 25 °C; (**a**) raw ITC data, (**b**) heat of dilution corrected and fitted ΔH diagram.

2.4.6 Change of emission spectra of ligand 4 in the presence of GGH

Compound **4** was titrated under the conditions described above with GGH to confirm no change of its emission spectrum in the presence of the target peptides.



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[25] Peptides like GGH are know to bind copper(II) ions tightly. To ensure, that the observed emission increase of **5** in the presence of such peptides is not cause by NTA-Cu decomplexation, the stability of **5** in the presence of excess GGH was investigated (see Experimental Part). If the copper ion is transferred from NTA to the peptide the emission intensity of ligand **4** should be restored, which is not the case. We therefore conclude that the NTA-Cu complex of **5** is sufficiently stable even in the presence of peptides with high copper affinity.

[26] The larger side chain in Gln starts to alter the binding motif.

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[28] The enthalphic contribution to the binding process is similar for both peptides, indicating the same metal ion coordination. The difference in the binding constant is of entropic origin, which may be explained by the desolvatisation of the crown ether unit and the ammonium ion upon aggregate formation (see Experimental Part).

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[32] Such coordination structures have been suggested for copper(II) NTA cysteine. a) B. B. Tewari, *Russ. J. Coord. Chem.* **2003**, *29*, 441 – 444, and related systems b) T. H. Cooper, M. J. Mayer, K.-H. Leung, L. A. Ochrymowycz, D. B. Rorabacher, *Inorg. Chem.* **1992**, *31*, 3796 – 3804. c) E. A. Ambundo, M.-V. Deydier, A. J. Grall, N. Aguera-Vega, L. T. Dressel, T. H. Cooper, M. J. Heeg, L. A. Ochrymowycz, D. B. Rorabacher, *Inorg. Chem.* **1999**, *38*, 4233 – 4242.

3. Fluorescent 1,4,7,10-Tetraazacyclododecane Zn(II) Complexes with high Affinity to D4 and E4 Peptide Sequences[i]

Suitable combinations of an affinity tag and an artificial probe are useful for non-covalent protein labelling. Several of such peptide tag – probe pairs have been developed and reported in the literature. The most prominent example is the His-tag – Ni(II)-NTA (nitrilotriacetic acid) pair. Recently, the Hamachi group reported a genetically encodable oligo-aspartate sequence (D4-tag) and a corresponding oligonuclear Zn(II) dipicolylamine (Zn(II)-Dpa) complex as new peptide tag – probe pair, which is orthogonal to the His-tag – Ni(II)-NTA pair. We describe now the preparation of fluorescent 1,4,7,10-tetraazacyclododecane (cyclen) Zn(II) complexes and their application as an alternative artificial probe for the D4-tag system.[ii] The binding affinities of the new complexes to the affinity tags were investigated by emission and UV-vis titration.[iii] Tetranuclear Zn(II)-cyclen complexes respond to the presence of oligo-aspartate, oligo-glutamate and oligo-aspartate dimers in aqueous solution at micromolar concentrations by a strong spectroscopic change. Based on the high binding affinities due to strong electrostatic interactions and Job´s plot analysis, we propose the formation of receptor–peptide tag aggregates. The results clearly show the potential of Zn(II)-cyclen complexes for applications as non-covalent protein markers, although their optical properties require further optimization for practical use.

[i] S. Stadlbauer, M. Bhuyan, A. Grauer, F. Schmidt, H. Nonaka, S. Uchinomiya, A. Ojida, I. Hamachi, B. König *Chem. Asian. J.* **2009**, *in preparation*.

[ii] Compounds **4** and **7** were prepared by Florian Schmidt, compound **9** was synthesized by Andreas Grauer and compound **10** by Mouchumi Bhuyan. All other compounds were prepared by Stefan Stadlbauer

[iii] Binding affinity of compounds **3** – **9** towards the peptide tags was investigated by Stefan Stadlbauer at the Kyoto University, Japan in the group of Prof. Dr. Itaru. All peptide tags were provided by the Hamachi group.The binding affinity of compound **11** was investigated at the University of Regensburg.

3.1 Introduction

The selective luminescent labelling of proteins by markers is an important quest and an ongoing challenge in molecular biology.[1] Covalent labelling uses reactive dyes or genetic fusion of fluorescent proteins e. g. green fluorescent proteins (GFPs).[2] Non-covalent labelling strategies employ antibodies or snap tags.[3] Recently, pairs of protein fused peptide tags and complementary fluorescent chemical probes were reported for applications in protein labelling[2a, 4] This combination of a peptide tag incorporated into a protein and a complementary chemical binding site is widely used in affinity chromatography for protein isolation/purification (IMAC),[5]but also in immobilization techniques and protein detection by immunoblotting.[6] The peptide tag, genetically incorporated in the protein, is specifically recognized by a complementary synthetic receptor, and serves as a selective binding epitope. The most prominent example is the His-tag – Ni(II)-NTA (nitrilotriacetic acid) pair, which is widely used in protein purification, protein immobilization,[5] and bio-imaging by fluorescent labelling of proteins.[7] For the later application, a change in the emission signal of the probe induced by tag binding is crucial. This change, either in fluorescence intensity or emission wavelength, enables a precise detection or imaging of the protein at physiological conditions.[8]

Recently the Hamachi group developed a new high affinity peptide tag – artificial probe pair, orthogonal to the His tag – Ni(II)-NTA pair, for protein labelling, employing coordination chemistry and multivalent interaction between a genetically encodable oligo-aspartate sequence (D4-tag) and a corresponding oligonuclear Zn(II) complex.[9] Since artificial zinc dipicolylamine (Zn(II)-Dpa) receptors,[5,10] and zinc 1,4,7,10-tetraazacyclododecane (Zn(II)-cyclen) complex derivatives,[11] are both known for their application as phosphate binders at physiological conditions, we considered Zn(II)-cyclen derivatives as alternative artificial probes for the developed D_4-tag system.

Herein we describe the synthesis and the investigation of new fluorescent multinuclear Zn(II)-cyclen complexes as artificial probes for oligo-aspartate (D4-tag) and oligo-glutamate (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 are therefore a suitable alternative as artificial non-covalent protein markers in bio-labelling.

3.2 Results and Discussion

3.2.1 Design of fluorescent Zn(II)-cyclen complexes

As previously shown the selective binding of fluorescent Zn(II)-Dpa tyrosine derivatives with high affinity to the triply D4 motif tethered to the porcine muscarinic 1-type acetylcholine receptor on the cell surface of mammalian cells resulted from multiple coordination chemistry as well as a multivalent effect.[9a] Although bis-Zn(II)-cyclen **1** and Zn(II)-Dpa **2** show a different coordination geometry, both binuclear metal complexes are suitable for multipoint metal–ligand interactions (figure 1). Hence the accumulated carboxylate groups of the D4-tag are able to favourably interact with the cationic cyclen complex **1**. Furthermore the non-quenchable character of zinc (II) (d^{10}) is an advantage in the development of fluorescent probes.

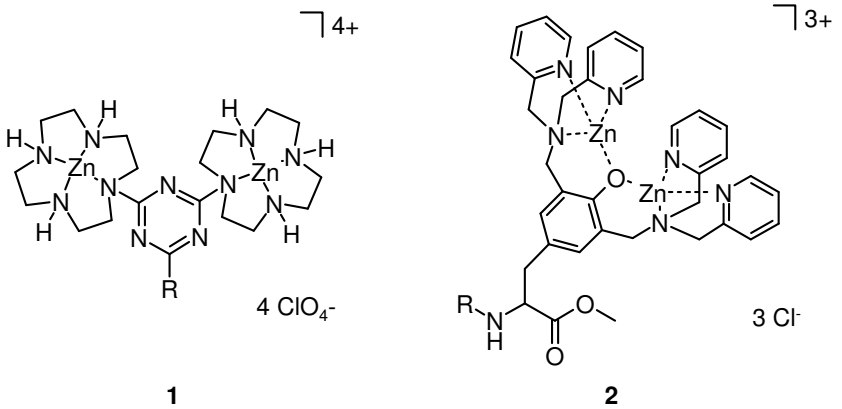


Figure 1. General structure of bis-Zn(II)-cyclen complex **1** and tyrosine based Zn(II)-Dpa complex **2**.

In this respect we designed and synthesized a set of different cyclen derivatives attached to various fluorescent dyes. Bis-Zn(II)-cyclen **1** is synthetically easily available and modified. As shown in figure 2 mono-, bi- and tetranuclear zinc (II) cyclen metal complexes were prepared according to the reported binuclear and tetranuclear Zn(II)-Dpa.[9] Several fluorescent dyes were attached to the cyclen derivatives: For the mono- and dinuclear Zn(II)-cyclen complexes coumarin and fluorescein were introduced, while the dansyl group and pyrene was used only with dinuclear Zn(II)-cyclen. Tetranuclear Zn(II)-cyclen complexes were linked by a perylene unit or a benzene moiety.

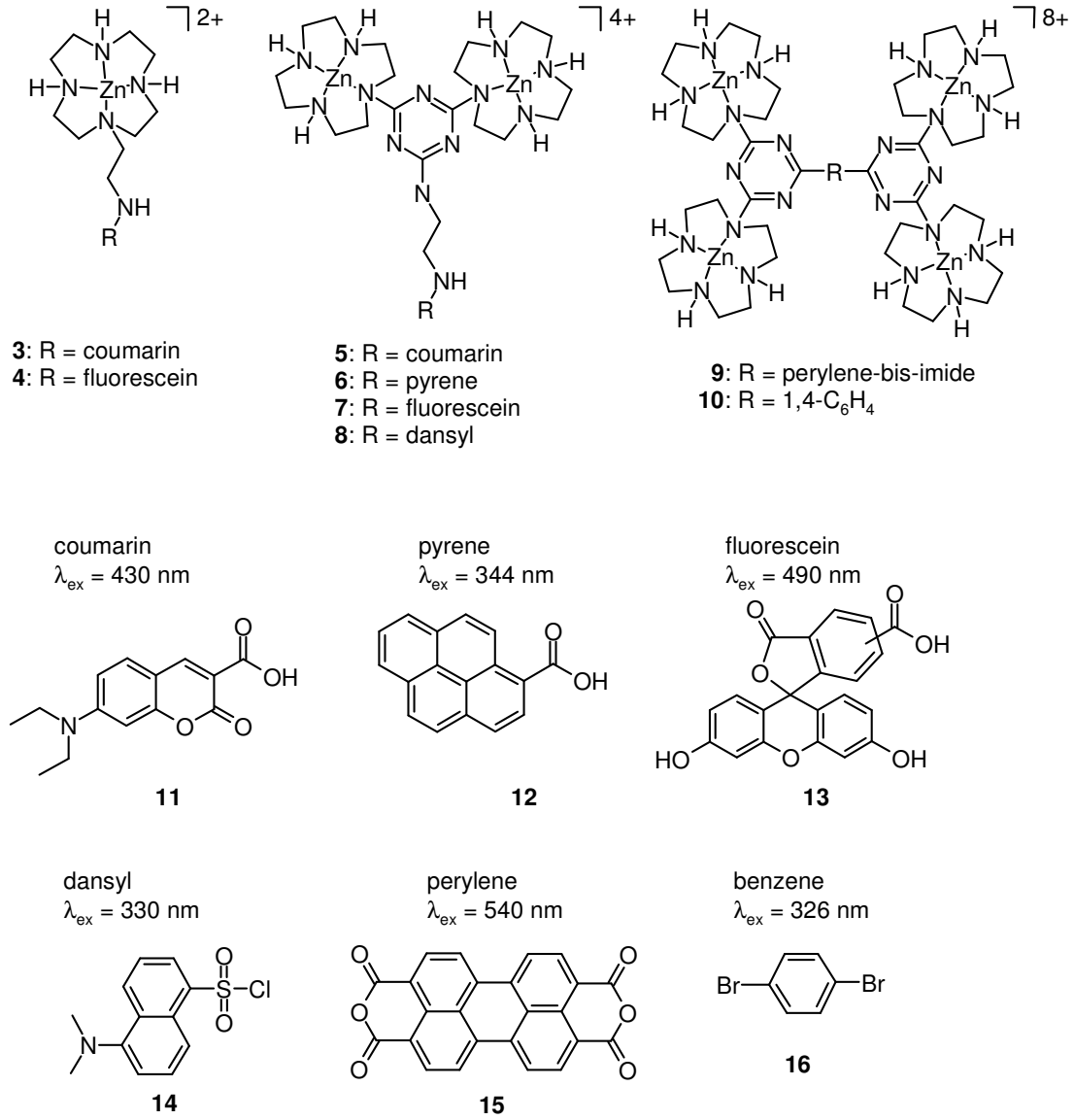


Figure 2. Dyes and linkers used in synthesis of mono-, bi- and tetranuclear Zn(II)-cyclen derivatives.

3.2.2 Syntheses

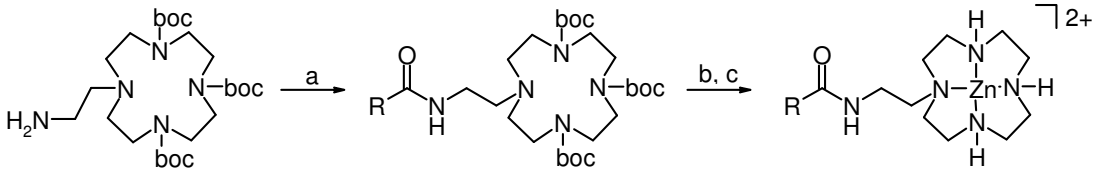
The cyclen building blocks **17**,[12] **20**[11a, g] and **26**[11b] for the synthesis of fluorescent mono-, bi- and tetranuclear Zn(II)-cyclen derivatives were prepared as previously reported. Dyes and linker were commercially available; coumarin **11**[13] and pyrene **12**[14] were prepared according to literature procedures. Detailed experimental procedures and analytical data of all prepared compounds shown in scheme 1 are provided in the Experimental Part.

3.2.2.1 Fluorescent mono and binuclear Zn(II) cyclen derivatives

Starting material for all fluorescent mono and binuclear derivatives were protected cyclen amine building blocks **17** and **20**, respectively. The key step for both synthetic routes was the amide formation between the cyclen amine **17** and **20**, respectively, and the different fluorescent dyes. The amide was formed using TBTU and HOBt and the corresponding acid yielding the Boc-protected compounds **18**, **19** and **21 – 24**. To introduce the dansyl group, dansyl chloride was allowed to react at basic conditions with cyclen amine **20**. Acidic cleavage of the Boc-groups and subsequent treatment of the generated hydrochloride salts with a basic anion exchanger resin yielded the free amine cyclen ligands. Finally, complex formation with 1 or 2 equivalents of $Zn(ClO_4)_2$ gave the labelled mono- and dinuclear Zn(II)-cyclen derivatives, respectively.

3.2.2.2 Fluorescent tetranuclear Zn(II) cyclen derivatives

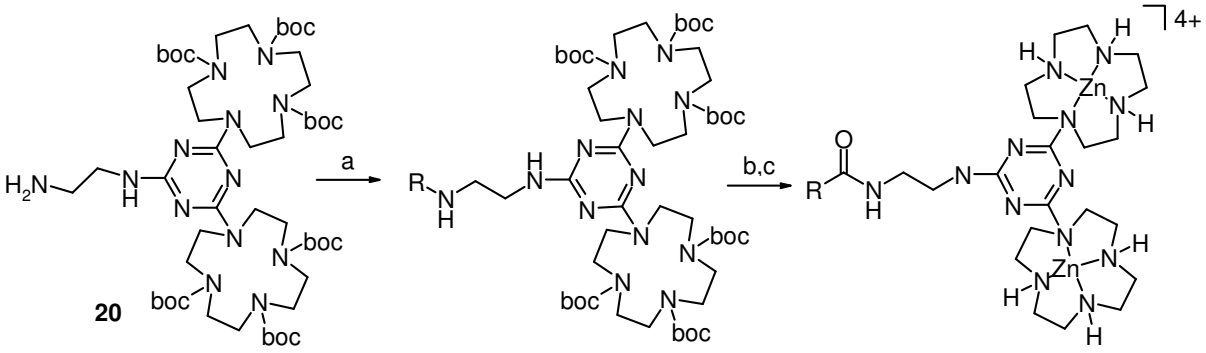
The amide formation of the cyclen building block **20** and the perylene fluorophore was achieved in an imidazole melt. Further synthetic steps to obtain the tetranuclear Zn(II) cyclen **9** were carried out by analogous procedures as described above. To achieve a more rigid structure of the tetranuclear Zn(II)-cyclen complex as in **9**, we prepared the tetranuclear Zn(II)-cyclen **10** via palladium catalyzed *Suzuki* reaction. Conversion to the final tetranuclear zinc complex was achieved by common methods as stated above. The conjugation of the fluorophore alters the photophysical properties if compared to compound **9** (vide infra).



17

18: 61 %, R = coumarin
19: 78 %, R = fluorescein

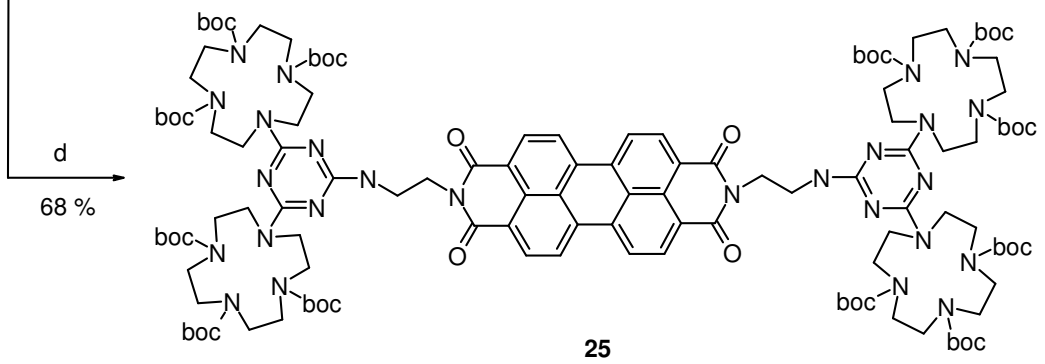
3: 100 %, R = coumarin
5: 99 %, R = fluorescein



20

21: 93 %, coumarin
22: 57 %, pyrene
23: 59 %, fluorescein
24: 62 %, dansyl

5: 93 %, coumarin
6: 83 %, pyrene
7: 75 %, fluorescein
8: 41 %, dansyl



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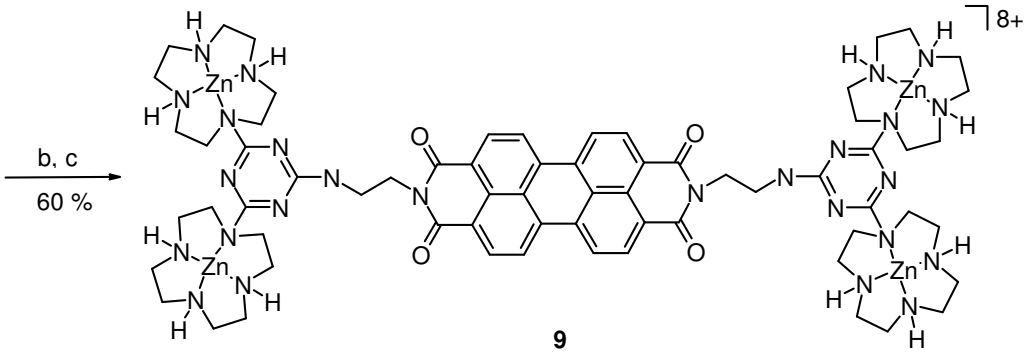

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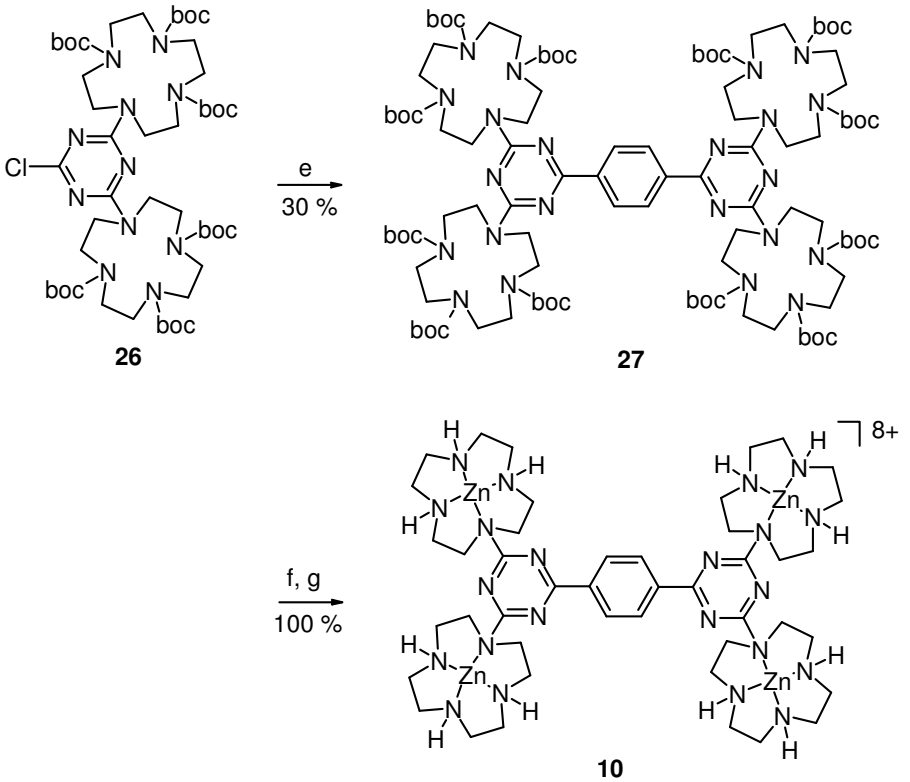


Scheme 1. (a) Appropriate acid, TBTU, HOBT, DIPEA, dry DMF, 40 °C, 2-3 h; dansyl chloride **14**, TEA, DCM, 1.5 h; (b) HCl / ether, DCM o/n, basic ion exchanger resin; (c) $Zn(ClO_4)_2$, H_2O, MeOH, 65 – 70°C; (d) 3,4,9,10-Perylene–tetracarboxylic dianhydride **15**, imidazole melt; (e) Diboronic acid of compound **16**, tetrakis*(triphenylphosphine)*-palladium, Na_2CO_3, DME, 80 °C, 48 h; (f) TFA, DCM o/n, basic ion exchanger resin; (g) $ZnCl_2$, H_2O, MeOH / MeCN, reflux, o/n. Counter ions of the complexes to yield a neutral complex are not shown for clarity.

3.2.3 Determination of the binding affinity

Our previous studies for the Zn(II)-Dpa Tyr on a series of oligo-aspartate peptides (Boc-D_n-NH_2, n = 2 – 5) and oligo-glutamate peptides (Boc-E_n-NH_2, n = 3, 4) revealed the D4-tag as the most efficient and optimal tag sequence. A further increase in length did not lead to an increase in binding affinity, while shorter tags showed lower affinity. Comparison of the binding data of oligo-aspartate and oligo-glutamate indicated ca. 6-fold higher binding affinity of the D4-tag compared to the E4-tag. Thus, we initially focused on the investigation of an affinity screening of compounds **3** – **10** to the D4-tag and E4-tag, respectively.

Emission screening experiments were performed with compounds **3** – **10** at physiological conditions in buffered aqueous solution (HEPES 25 mM, pH 7.4, 25 °C) adding in precise equivalent steps 0.5, 1.0, 5.0 and 10.0 eq. of the tag-peptide (D4, E4). The emission spectra at the appropriate excitation wavelength of the used dye were

recorded. Only the tetranuclear Zn(II)-cyclen complexes **10** and **11** responded to the addition of the tag proteins by a change in their emission spectra. For the mononuclear complexes (**3**, **4**) and for binuclear complexes (**5**, **6**, **7**, **8**) no significant change was observed. Although both tetranuclear compounds showed a change in emission their fluorescence properties responded divergent. While the emission intensity of compound **10** increased, compound **9** revealed a decrease in its emission intensity and an excimer formation could be determined. We focused on the tetranuclear Zn(II)-cyclen complexes and investigated their affinity towards the protein tags (D4-tag, E4-tag) at various concentrations using emission and UV-vis absorption titration.

Emission titration experiments of perylene complex **9** and D4-tag showed the emission intensity decreasing upon binding and aggregate induced receptor–peptide tag excimer formation at 654 nm at a 50 µM concentration of **9**, which was not observable at a 10 µM concentration. Addition of 30 % of acetonitrile to the HEPES buffered solution increased the solubility of the receptor–peptide tag complex and inhibits the aggregate and excimer formation.

An apparent binding constant in the micromolar range was derived by non-linear fitting methods from a plot of emission intensity changes against the concentration of added tag protein. The obtained values for 50 µM and 10 µM concentrations of **9** are comparable within the error limits of the experiment (table 1). Job´s plot analysis[15] gave a stoichiometric ratio of the receptor–peptide tag aggregate of 1:1. Independent UV-vis investigations confirmed the results derived from emission titration experiments. Upon binding, the absorption of **9** at 500 nm and 540 nm decreases, respectively. From these absorption changes we determined the affinity towards the tag proteins and confirmed that **9** forms a 1:1 receptor–peptide tag aggregate with nearly micromolar apparent affinity. However, no selectivity between D4 and E4 could be determined (figure 3a). The amino acids aspartic acid and glutamic acid differ only by one methylene group in their side chains, which may not effect a change in the receptor–peptide aggregate formation. Hence, a dimer of a D4-tag (H_2N-G-DDDD-G-DDDD-G-NH_2) was assumed to change the stoichiometry of the aggregate to 2:1 due to the repeated D4 motif. In fact we observed the supposed change in the stoichiometric ratio and a surprisingly high binding affinity ($\lg K_{app} = 14$) was estimated by non-linear fitting using a 2:1 binding model (figure 3b). However, the lg K value must be considered with caution, as for such high affinities, UV-vis and emission titration experiments are not suitable to determine exact values, because of the concentration limitations of the methods. [16]

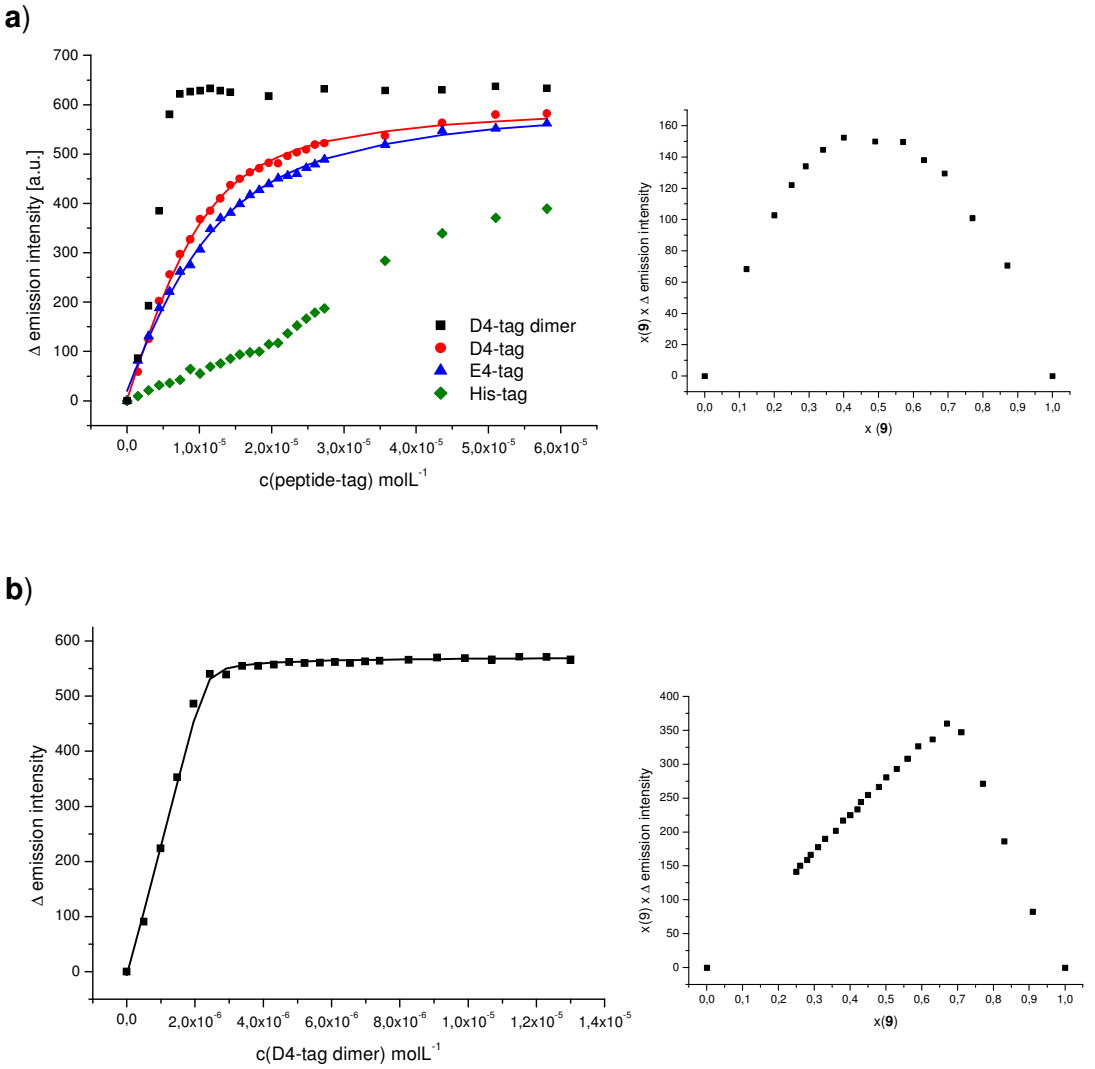


Figure 3. a) Representative binding isotherms of **9** and investigated peptide tags (left) in aqueous solution; conditions for all measurements: [**9**] = 10 µM, [peptide-tag] = 0.3 mM; representative Job´s plot analysis (right) detected by emission of compound **9** (10 µM) and D4-tag. b) Emission titration (left) and Job´s plot analysis (right) of **9** and D4-tag dimer in aqueous buffer ([**9**] = 5 µM, [D4-tag dimer] = 100 µM).

The orthogonality of compound **9** towards His-tag protein (Boc-WAHHHHHH-NH_2) was proven by measurements at the same physiological conditions: No comparable binding as seen for the D4- and E4-tag is observed between the His-tag and **9**. Only a weak unspecific interaction (K < lg 3) is indicated by the linear increase of the emission intensity (figure 3a). All obtained apparent binding constants for the tetranuclear perylene Zn(II)-cyclen complex **9** are summarized and table 1.

Next, we investigated the tetranuclear benzene Zn(II)-cyclen complex **10** in more detail. Emission titrations were performed at 50 µM and 10 µM concentrations of **10** and the D4-tag and E4-tag, respectively. For complex **10** an increase of the emission intensity upon the formation of a receptor–peptide aggregate was observed (see

Experimental Part). However, again the response of **10** to the presence of the tags was concentration dependent indicating the presence of higher aggregates of the compound.

compound	c [μM]	D4-tag lg *K*		E4-tag lg *K*		D4-dimer lg *K*
9[c]	50	4.7[a]	5.4[b]	5.3[a]	5.1[b]	–
	10	5.3[a]	5.6[b]	5.5[a]	5.2[b]	-[e]
	5	–	–	–	–	14.0
		lg K_{11}	lg K_{12}	lg K_{11}	lg K_{12}	
10[d]	50	5.8	10.6	7.3	13.3	–
	10	-[e]	-[e]	7.3	12.9	-[e]

Table 1. Binding constants for the receptor–peptide aggregate formation of complex **9** and **10** and investigated peptide. [a] Binding constant derived from emission titration. [b] Binding constant derived from UV-vis titration. [c] All stoichiometries were determined by Job´s plot analyses as 1:1, with the exception for D4-tag dimer, which shows 2:1. [d] Binding constants were derived from emission titrations and calculated using equilibria of 1:1 (K_{11}) and 1:2 (K_{12}) receptor–peptide aggregate stoichiometry as indicated by the Job´s plot analyses. [e] Binding constant could not be derived from the experimental data.

The stoichiometric ratio of the receptor–peptide tag aggregate at 50 μM concentration of **10** is 1:2, which indicates that one tetranuclear benzene Zn(II)-complex coordinates with either two D4-tag proteins or two E4-tag proteins. Assuming an equilibrium between a 1:1 and 1:2 aggregate during aggregate formation and a complete 1:2 aggregate formation at the end of the titration, allowed us to calculate apparent binding constants K_{11} and K_{12} for these processes (table 1). The titration data obtained at 10 μM (see Experimental Part) cannot be described with this binding model indicating the presence of additional equilibria at lower concentrations.

As the stoichiometry for the aggregate formation was found to be 1:2 (**10** : tag protein),[17] the findings were verified by isothermal titration calorimetry (ITC), although the binding constants are outside the typical range for ITC. The ITC measurements confirmed the 1:2 stoichiometry (for ITC results see Experimental Part).[18]

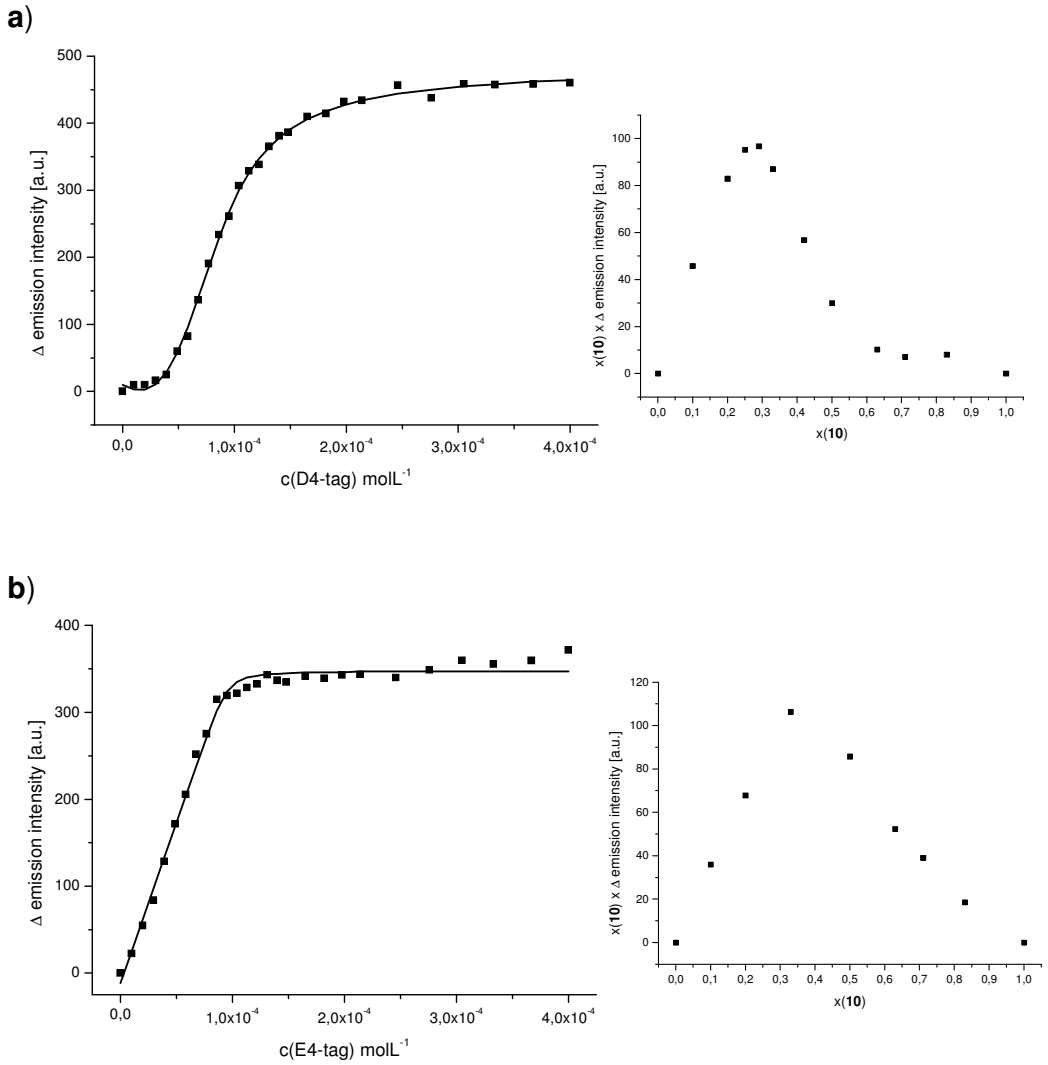


Figure 4. Binding isotherms in aqueous solution obtained by emission titration (left) of **10** and D4-tag and E4-tag, respectively, and Job´s plot analyses (right). Measurement conditions: (a) [**10**] = 50 µM, [D4] = 2 mM; (b) [**10**] = 50 µM, [E4] = 2 mM.

Finally, the orthogonality of the tetranuclear benzene Zn(II)-cyclen complex **10** to His-tags was studied. A decrease of the emission intensity of **10** upon addition of His-tag (Boc-WAHHHHHH-NH_2) clearly indicates an interaction of **10** to the His-tag (see Experimental Part). Although a strong response in emission change was observable and saturation occurred after 1.0 eq of added His-tag (~ 10 µM), no apparent binding constant could be derived from the measurements as the His-tag protein shows emission upon excitation at 326 nm because of its tryptophan residue. Thus the decreasing emission of **10** interfered with the emission increase caused by the continuous addition of His-tag protein. Nevertheless, a strong interaction of His-tag and the metal complex is assumed as a low His-tag concentration (< 10 µM) affects a strong emission decrease of **10**. Although the tetranuclear benzene Zn(II)-cyclen complex **10** is not exclusively

selective for oligo-aspartic or oligo-glutamic peptides, a distinction of His-tag peptides is possible by the different emission response.

3.2.4 Cooperativity

The cooparitivity of the supramolecular aggregate formation was investigated in more detail using Scatchard[19] and Hill plot[19] analysis (see Experimental Part). Whereas for perylene complex **9** and the tags D4 and E4 the Hill coefficient is nearly one, indicating a non-cooperative process, the Hill coefficient of the D4-tag dimer binding with a value of 2.77 indicates cooperative binding. The binding of **9** to the first D4-dimer tag may constrain the conformation of the oligo peptide facilitating the binding of another molecule of **9**. The sigmoidal shape of the emission intensity difference plots of the tetranuclear benzene Zn(II)-cyclen complex **10** suggests a positive cooperation which was verified by the Hill coefficient $n \geq 2$. The difference in the Hill coefficient of the binding of **10** to D4 and E4 is small. Scatchard plot analysis supports the conclusions: Deviation from a linear curve indicates positive cooperativity of binding. Only the binding data analysis of compound **9** to the D4-tag and E4-tag leads to a linear Scatchard plot, suggesting non-cooperative binding as already indicated by the Hill coefficient.

3.2.5 Comparison and suggested binding model

Only tetranuclear Zn(II)-cyclen complexes show a significant affinity to the oligo peptide tags (D4, E4, D4-tag dimer, His-tag) in aqueous solution based on electrostatic interactions. The different coordination geometry of Zn(II)-cyclen compared to Zn(II)-Dpa does not affect the affinity as we expected. In fact, the tetranuclear perylene Zn(II)-cyclen complex shows a similar affinity towards the tags (D4, E4) with a 1:1 stoichiometry, while its affinity significantly increases in the binding of the D4-dimer tag and the stoichiometry changes to 2:1. This change is expected due to the repeated D4 motif and the results determined for the single D4-tag. The driving force for the aggregate formation originates from electrostatic interactions, thus we proposed a binding geometry as illustrated in figure 5a: Tetranuclear perylene Zn(II)-cyclen **9** acts as a tweezer and coordinates the oligo peptide tag at the carboxylate residues on each

side of the tag resulting in a stable supramolecular aggregate. As glutamic acid and aspartic acid differ only by one methylene group in their side chain residue, we assume a similar conformation for the E4-tag and therefore an analogous aggregate formation due to the flexible cyclen moiety in **9**. Also the excimer formation at 50 µM concentration is supported by the suggested binding model (figure 5b). Although the response to peptide binding by a decreased emission of **9** is less desired for bio-imaging than an emission increase, the excimer formation of the aggregate (λ = 540 nm) possibly can be used for non-covalent protein labelling.

compound	D4-tag lg K[a]	E4-tag lg K[a]	D4-dimer lg K[a]	His-tag affinity
9	5.2[b]	5.3[b]	14.0[c]	No
10	5.8 / 10.6[b]	7.3 / 12.9[b]	–	Yes
Zn(II)-Dpa **1**[9a] (R = boc)	5.8[d]	5.1[d]	–	No
bis-Zn(II)-Dpa dimer[9a]	–	–	7.3[e]	No

Table 2. Comparison of the logarithmic binding constants of Zn(II)-Dpa to the investigated tetranuclear Zn(II)-cyclen complexes **9** and **10**.
[a] All stoichiometries determined as 1:1, with the exception of **10** and D4-dimer (2:1) and **11** and D4/E4-tag (1:2). [b] Average values for binding constant obtained by UV and/or emission titration. [c] Obtained by emission titration. [d] Obtained by ITC. [e] Obtained by ITC on a D4-tag dimer tethered to a RNase.

The more ridged tetranuclear benzene Zn(II)-cyclen complex **10** shows an emission increase upon binding of the peptide tag and higher affinities. Electrostatic interactions are considered as the main driving force for aggregate formation leading to charge neutralization in a 1:2 aggregate. Since the three dimensional structure of **10** has a flat and bar-shaped character we suppose a sequential binding due to electrostatic interaction of the D4-tag or E4-tag, forming a stable and neutral 1:2 receptor–peptide tag aggregate (figure 5c). The scaffold of the two benzene linked binuclear Zn(II)-cyclen complexes perfectly matches the oligo peptide tags (D4, E4) structure and charge distribution. In a sandwich-like aggregate of **10** and the tag protein strong electrostatic interactions are likely, resulting in the observed high apparent binding affinity. The absorption and emission properties of complex **10** lead to an overlap with protein spectra, which makes the compound unsuitable for protein labelling. However, we envision a related tetranuclear Zn(II)-cyclen complex with $\lambda_{ex} \geq 380$ nm to exclude the interference of the artificial probe and peptide tag or protein sample.

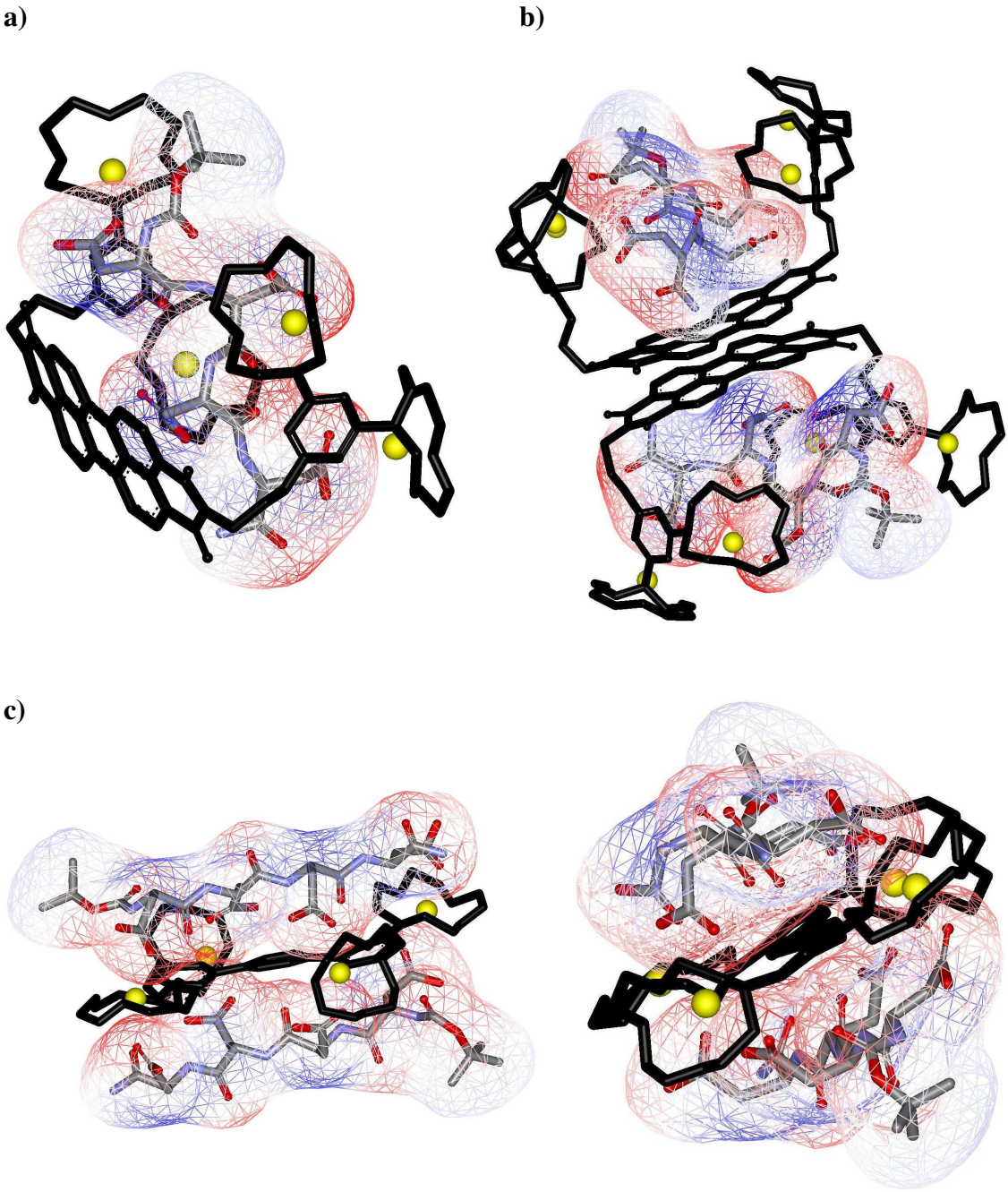


Figure 5. Proposed geometry of receptor–peptide tag aggregates of **9** or **10** and D4-tag. We expect a similar conformation for the E4-tag; therefore only binding models for the D4-tag are shown. All models were obtained by molecular modelling using the program package Spartan `06 (Wavefunction Inc.) by energy minimization (force field MMFF) of the receptor–peptide complex. Utilizing WebLabViewer Lite 4.0 an electrostatic potential surface displayed as wired mesh of the D4-tag was generated. The red colour indicates a high density of negative charges at the carboxylate residues of the tag. The cationic Zn(II)-cyclen favourably interacts with the anionic carboxylate residues. a) Compound **9** acts as a tweezer forming a stable 1:1 receptor–D4-tag peptide tag aggregate. b) Assumed geometry of an excimer formation of the aggregate shown in a). c) Sandwich-like 1:2 receptor–peptide tag aggregate formation of **10** and D4-tag.

3.3 Conclusion

In conclusion, tetranuclear Zn(II)-cyclen complexes show high affinity to D4 and E4 peptide tags in buffered aqueous solution. The perylen complex **9** binds D4 and E4 peptide tags with micromolar affinity, a D4-dimer with nanomolar affinity and shows no binding to a His-tag peptide. The emission intensity of the chromophor is quenched in the presence of the peptides, which is less suitable for bio-labelling, but the excimer formation of compound **9** may be utilized for protein imaging. The more ridged tetranuclear Zn(II)-cyclen complex **10** shows an increase in its emission intensity upon binding to D4 or E4-tags, which is advantageous for imaging of proteins. The fluorescence response of the complexes is complementary for D4, E4-tags and for His tags. However, the excitation wavelength of the currently used compound interferes with protein absorption and the development of a tetrameric Zn(II)-cyclen complex with $\lambda_{ex} \geq 380$ nm is envisaged to overcome this drawback. Overall, the investigations show a potential of tetranuclear Zn(II)-cyclen complexes as non-covalent protein markers, but their optical properties require further optimization for practical use.

3.4 Experimental Part

3.4.1 General methods and material

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer or on a Perkin-Elmer LS55 fluorescence spectrophotometer with temperature control.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer or on a SHIMADZU UV-2550 spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck or Baker).

NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in 13C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

3.4.2 Binding Studies

3.4.2.1 Screening of fluorescent Zn(II)-cyclen derivatives by emission change readout

An emission read out experiment was carried out with all compounds **3** – **10** to determine, which of the fluorescent derivatives response to the D4-tag and E4-tag. Screening experiments were done at physiological conditions in buffered aqueous solution (HEPES 25 mM, pH 7.4, 25 °C) in a cuvette. To a 50 µM (3 mL) solution of the metal complex (**3** – **10**) 10 equivalents of the peptide tag (D4 or E4) were continuously added in precise equivalent steps (0, 0.5, 1, 5 and 10 eq) and an emission spectra at the appropriated excitation wavelength of the dye was recorded.

3.4.2.2 Emission and UV-vis titrations

Procedure: To a cuvette with 400 µL of **9** and **10**, respectively, in HEPES buffer were added aliquots of a peptide tag solution (D4-tag, E4-tag, D4-tag dimer and His-tag). After each addition the solution was allowed to equilibrate before the emission intensity (λ_{ex} (**9**) = 540 nm; λ_{ex} (**10**) = 326 nm) and the UV spectrum were recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data.[20] To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of added peptide and evaluated by non linear fitting methods.

Titration conditions for 50 µM of metal complex:

Solvent: HEPES buffer 25 mM, pH 7.4

Starting volume: 400 µL

Concentration (**9** or **10**): 50 µM

Concentration [peptide tag]: 3 mM, 8 eq with regard to the metal complex **9** and 10 eq with regard to **10**

Titration conditions for 10 µM of metal complex:

Solvent: HEPES buffer 25 mM, pH 7.4

Starting volume: 400 µL

Concentration (**9** or **10**): 10 µM

Concentration [peptide tag]: 0.3 mM, 7.2 eq with regard to the metal complex **9** and 10 eq with regard to **10**

Results:

Compound **9**

a) **b)**

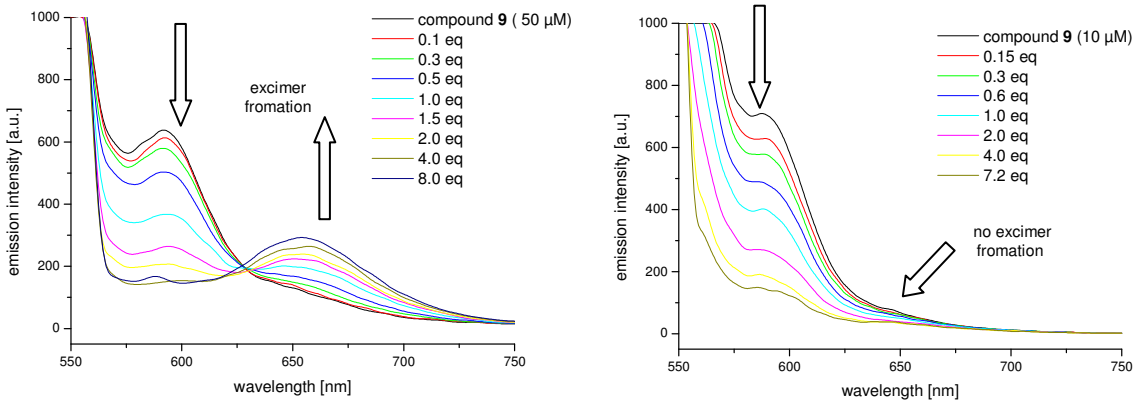


Figure 6. Emission titration of **9** and D4-tag aqueous buffered solution (λ_{ex} = 540 nm). Measurement conditions: (a) [**9**] = 50 µM, [D4] =3 mM; (b) [**9**] = 50 µM, [D4] = 0.3 mM.

a) **b)**

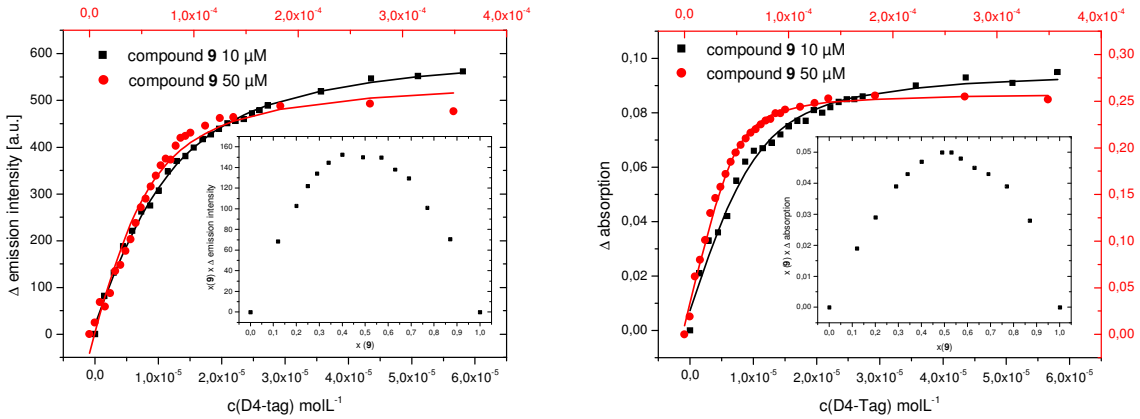


Figure 7. (a) Binding isotherm derived by emission titration of **9** and D4-tag in aqueous buffered solution; *black*: [**9**] = 10 µM, [D4-tag] = 0.3 mM; *red*: [**9**] = 50 µM, [D4-tag] = 3.0 mM; inserted: Job´s plot analysis detected by emission of compound **9** (10 µM). (b) Binding isotherm derived by UV-vis titration of **9** and D4-tag in aqueous buffered solution; *black*: [**9**] = 10 µM, [D4-tag] = 0.3 mM; *red*: [**9**] = 50 µM, [D4-tag] = 3.0 mM; *inserted*: Job´s plot analysis detected by UV-vis absorption of compound **9** (10 µM at UV maximum of 500 nm).

a) **b)**

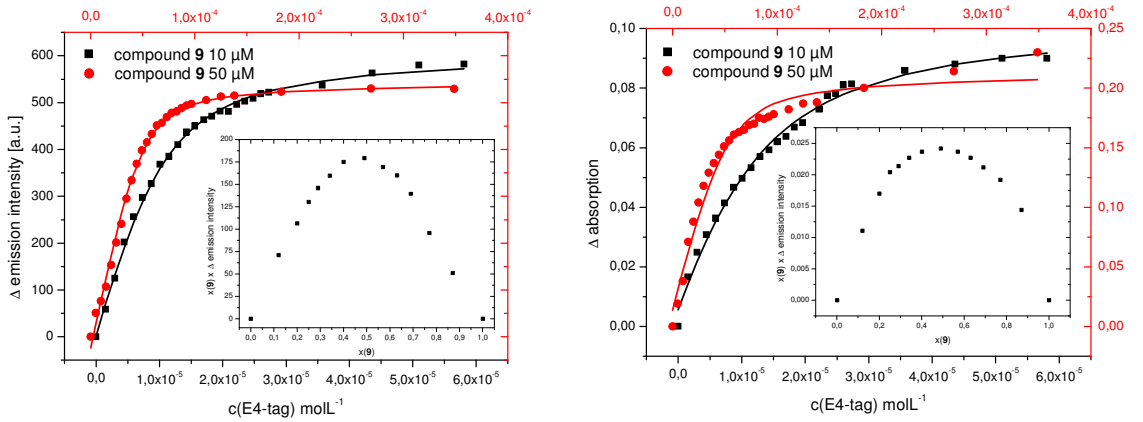


Figure 8. (a) Binding isotherm derived by emission titration of **9** and E4-tag in aqueous buffered solution; *black*: [**9**] = 10 µM, [E4-tag] = 0.3 mM; *red*: [**9**] = 50 µM, [E4-tag] = 3.0 mM; inserted: Job´s plot analysis detected by emission of compound **9** (10 µM). (b) Binding isotherm derived by UV-vis titration of **9** and E4-tag in aqueous buffered solution; *black*: [**9**] = 10 µM, [E4-tag] = 0.3 mM; *red*: [**9**] = 50 µM, [E4-tag] = 3.0 mM; *inserted*: Job´s plot analysis detected by UV-vis absorption of compound **9** (10 µM at UV maximum of 500 nm).

a) **b)**

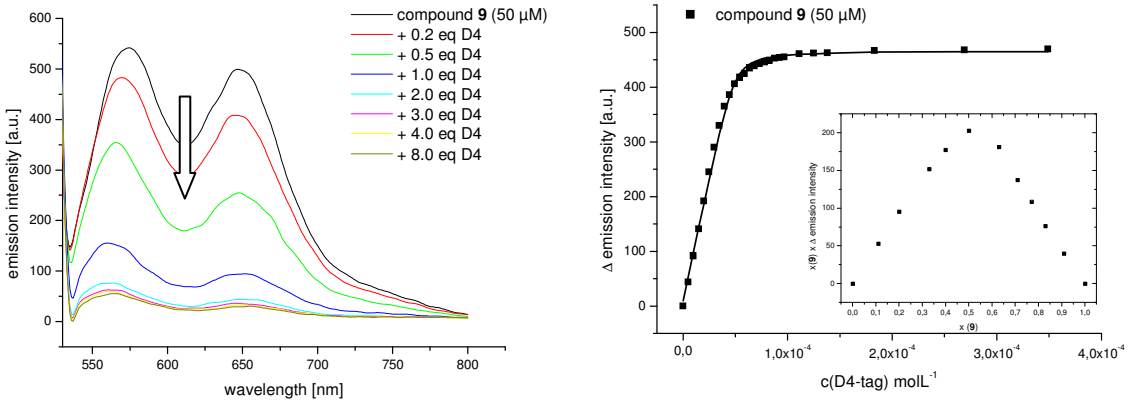


Figure 9. (a) Emission titration of **9** and D4-tag in aqueous buffered solution + 30 % MeCN in order to increase the solubility of the formed receptor–aggregate to effect the excimer formation. As can be seen no excimer formation was determined under these conditions. (b) Binding isotherm derived by emission titration of **9** and D4-tag in aqueous buffered solution + 30 % MeCN (λ_{ex} = 520 nm); [**9**] = 50 µM, [D4-tag] = 3.0 mM; inserted: Job´s plot analysis detected by emission of compound **9** (50 µM).

Compound **10**

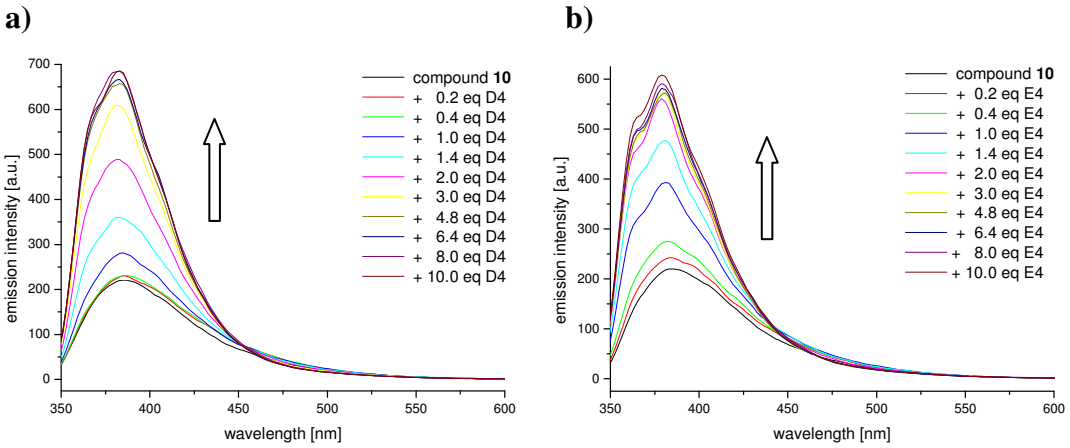


Figure 10. (a) Emission titration of **10** and D4-tag in aqueous buffered solution; [**10**] = 50 µM, [D4] = 2 mM. (b) Emission titration of **10** and E4-tag in aqueous buffered solution; [**10**] = 50 µM, [D4] = 2 mM.

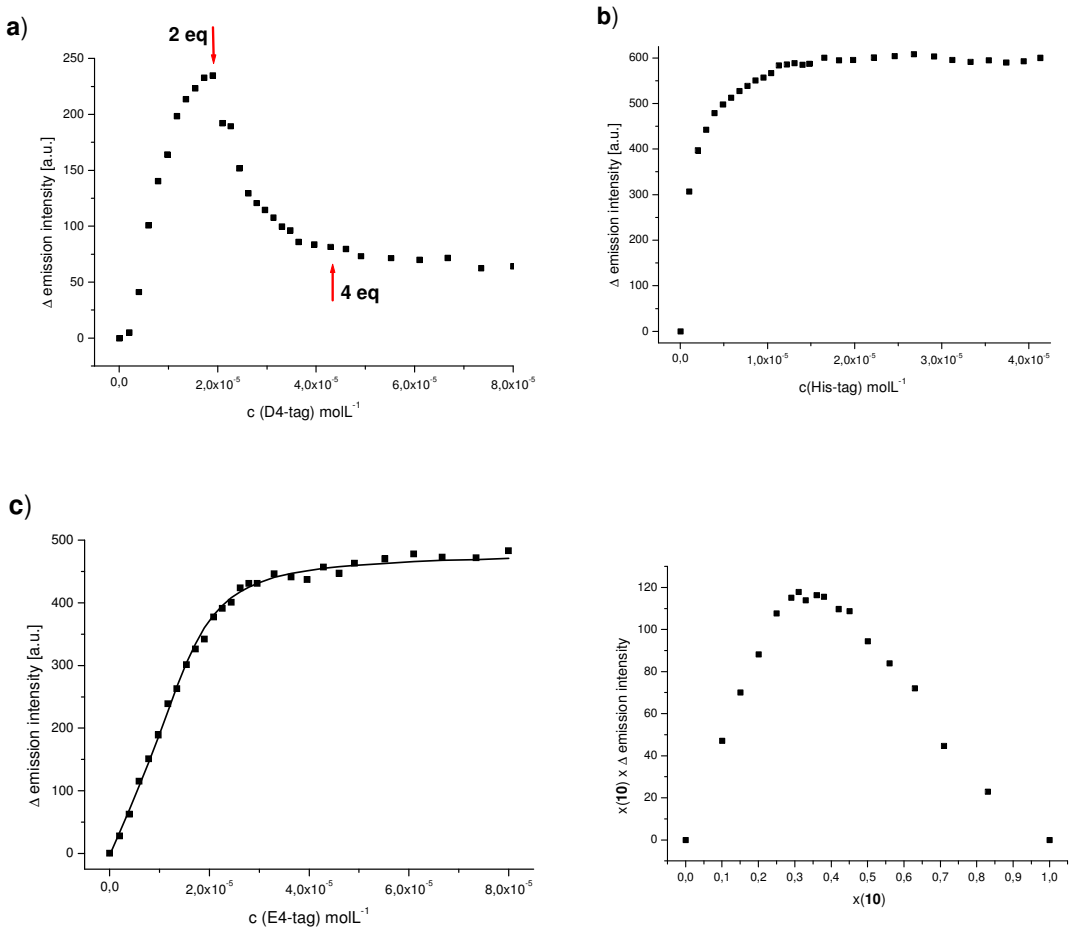


Figure 11. a) Emission titration of **10** and D4-tag: [**10**] = 10 µM, [D4] = 0.4 mM. b) Emission titration of **10** and His-tag in aqueous buffered solution, [**10**] = 10 µM, [His-tag] = 0.4 mM. c) Emission titration of **10** and E4-tag (left): [**10**] = 10 µM, [E4] = 0.4 mM; Job´s plot analysis (right).

3.4.3 Orthogonality to the His-tag

Compound **9**

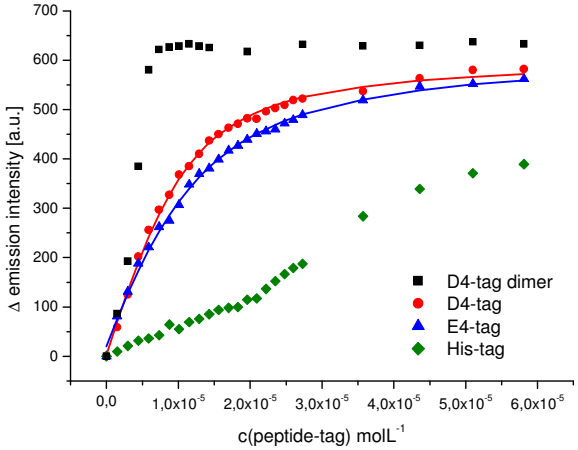


Figure 12. Representative binding isotherms of tetranuclear perylene Zn(II)-cyclen **9** and investigated peptide tags in aqueous buffered solution. For all measurements: [**9**] = 10 µM, [peptide-tag] = 0.3 mM.

Compound **10**

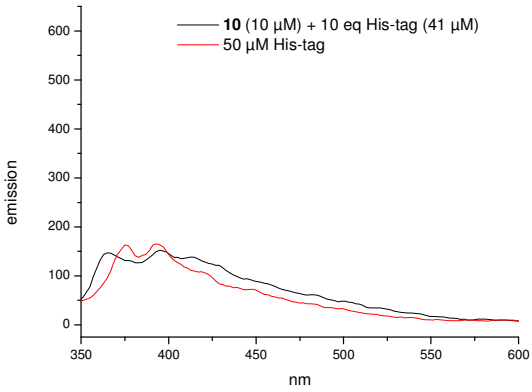


Figure 13. Comparison of a 50 µM His-tag solution (*red*) and emission at the end of titration after addition of 10 eq His-tag to **10** (10 µM) (*black*), which correspond to a 41 µM His-tag solution.

3.4.4 Isothermal titration calorimetry (ITC)

ITC experiments were performed in buffered aqueous solution (HEPES, 25 mM, pH 7.4) at 25 °C using an ultrasensitive VP-ITC calorimeter from MicroCal (Northampton, MA, U.S.A.). The sample solutions were made from stock buffer solution (HEPES, 25 mM, pH 7.4). Concentration of peptide tag (titrant) in the 300 µL syringe was set to 3.5 mM, whereas the concentration of metal complex **9** and **10**, respectively, in the 1.436 mL calorimetric cell was 97 µM. Before each titration both experimental solutions (titrant, cell) were thoroughly degassed under vigorous stirring. During the ITC experiment the cell solution was stirred at 300 rpm by syringe to ensure rapid mixing and 60 x 5 µL of titrant were injected over 10 s with a spacing time between each injection of two minutes in order to allow complete equilibration. Before data analysis the total observed heat of binding was corrected for the heat of dilution yielding the effective heat of binding. Therefore an analogue ITC experiment with the calorimeter cell filled with HEPES and peptide tag as titrant was carried out. The data could not be analyzed by non linear fitting methods using the MicroCal Origin software but they revealed that a sequential binding process occurs due to the ITC curve shape which is typical for a sequential binding model for host-guest interactions.[18]

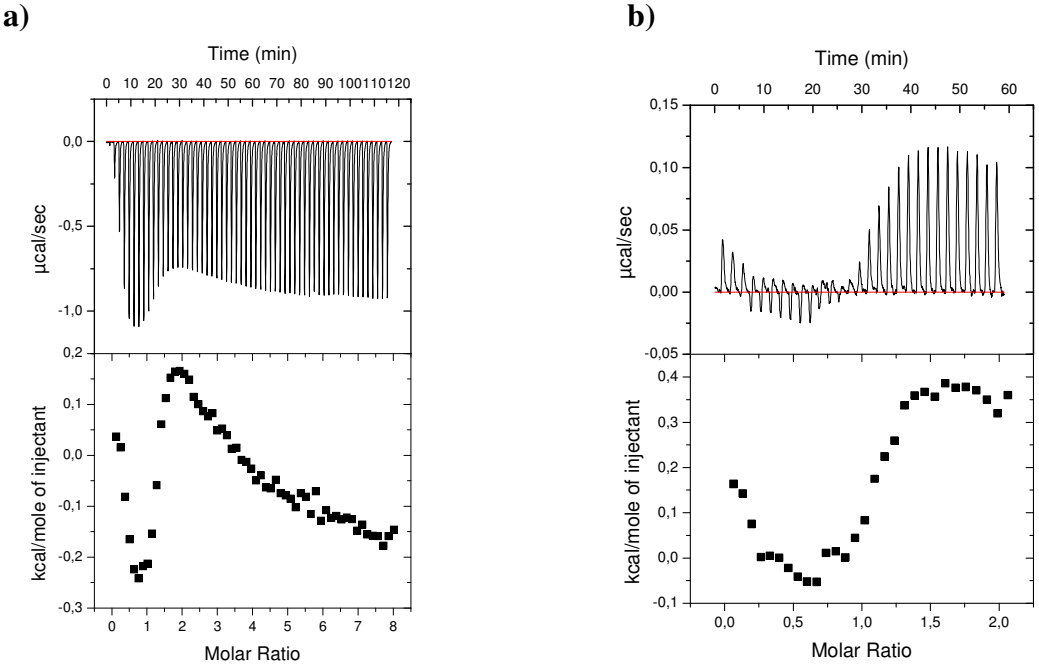


Figure 14. (a) ITC of **9** (97 µM) and D4-tag (3.5 mM) in HEPES (25 mM, pH 7.4) at 25 °C. (b) ITC of **9** (97 µM) and D4-tag (0.9 mM) in HEPES (25 mM, pH 7.4) at 25 °C. *top*: raw ITC data, *bottom*: heat of dilution corrected and fitted ΔH diagram.

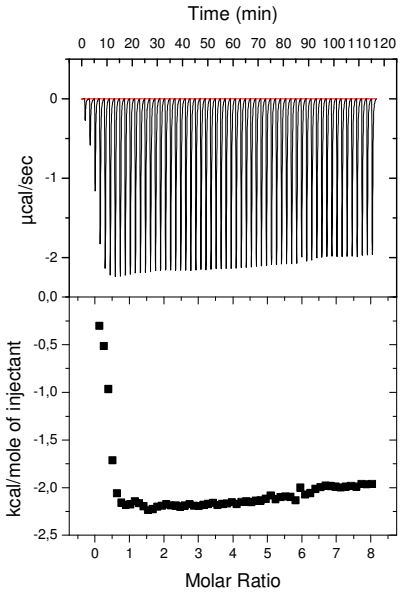


Figure 15. ITC of **9** (97 µM) and E4-tag (3.5 mM) in HEPES (25 mM, pH 7.4) at 25 °C. *top*: raw ITC data, *bottom*: heat of dilution corrected and fitted ΔH diagram.

3.4.5 Cooperativity

Hill plot

Hill Equation: $\Delta E = (E_{max} \cdot x^n) / (K_D^n + x^n)$

Compound **9**:

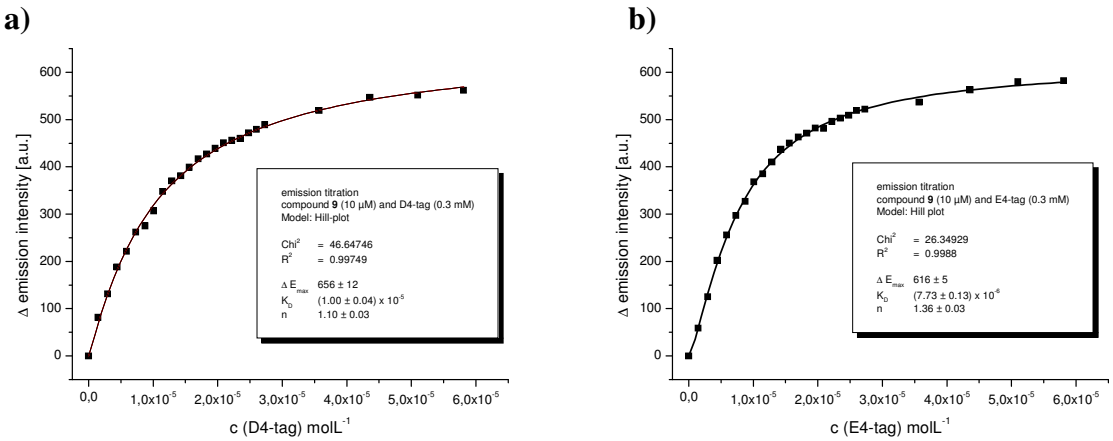


Figure 16. Analysis of the emission binding isotherms by the Hill equation. (a) Compound **9** (10 µM) and D4-tag (0.3 mM). (b) Compound **9** (10 µM) and E4-tag (0.3 mM).

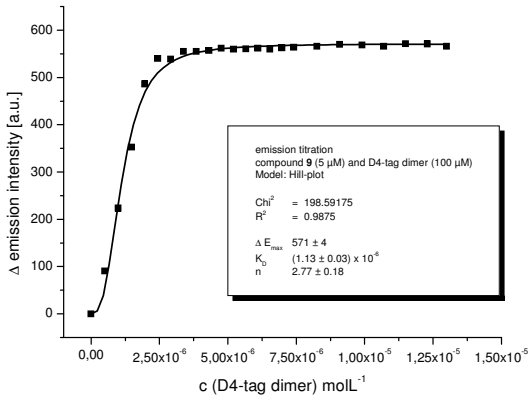


Figure 17. Analysis of the emission binding isotherms by the Hill equation, Compound **9** (5 µM) and D4-tag dimer (100 µM).

Compound **10**:

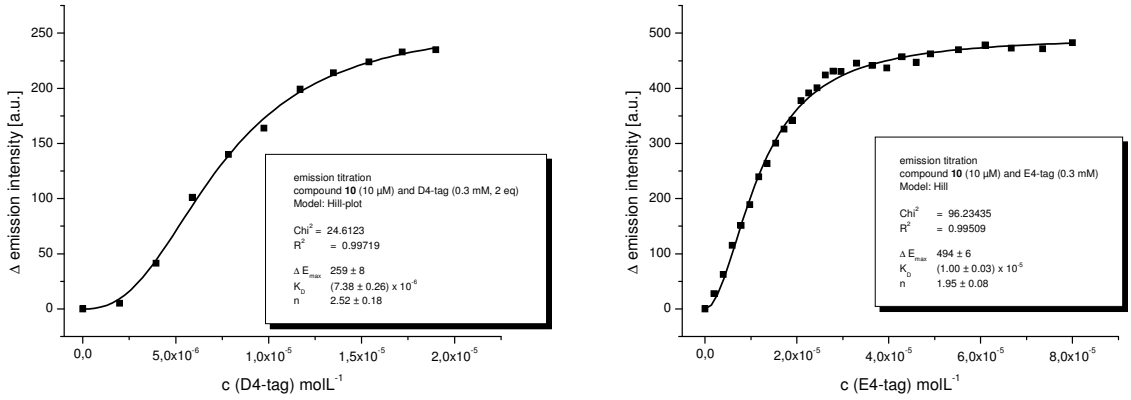


Figure 18. Analysis of the emission binding isotherms by the Hill equation. (a) Compound **10** (10 µM) and D4-tag (0.3 mM, 2 eq were added). (b) Compound **10** (10 µM) and E4-tag (0.3 mM).

Scatchard plot

a) **b)**

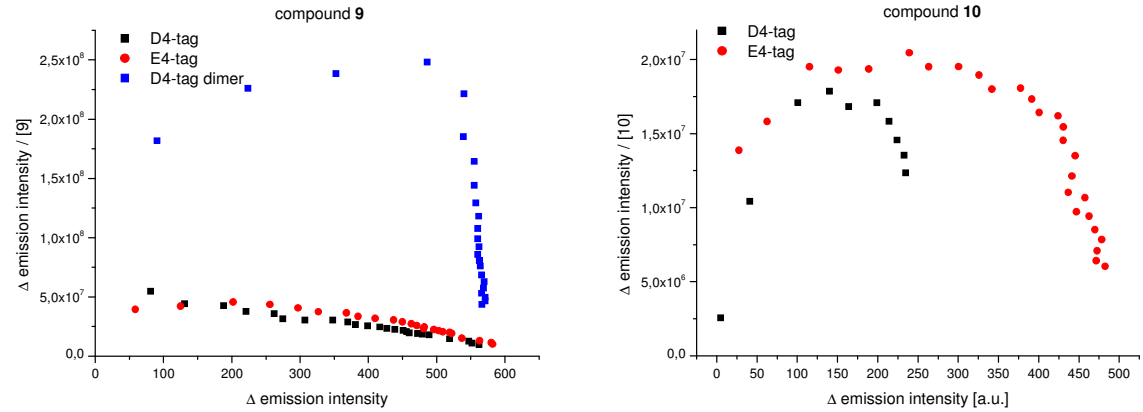


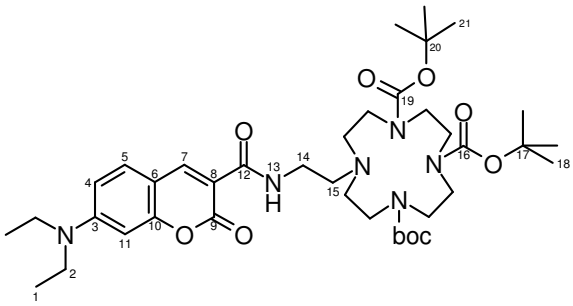
Figure 19. Scatchard plot analyses of the emission titration binding data for tetranuclear Zn(II)-cyclen complexes **9** and **10** to the investigated protein tags.

			D4-tag	*E4-tag*	*D4-tag dimer*
9	10 µM	Hill coefficient	1.10 ± 0.03	1.36 ± 0.03	2.77 ± 0.18
		Scatchard plot	no	no	yes
10	10 µM	Hill plot	2.52 ± 0.18	1.95 ± 0.08	—
		Scatchard plot	yes	yes	—

Table 3. Indicators of cooperative effects from Scatchard and Hill plot analysis during the formation of the receptor–peptide tag aggregate of tetranuclear Zn(II)-cyclen complex **9** and **10**, and the peptide tags.

3.4.6 Synthesis and characterization of new compounds

Following compounds were synthesized by literature known procedures: **5**[21], **7**[21].

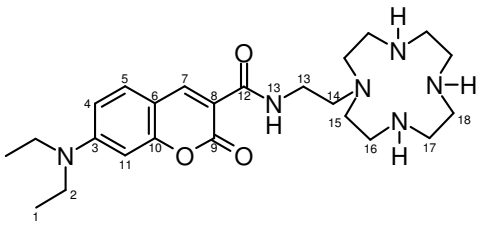


***10-{2-[(6-Diethylamino-naphthalene-2-carbonyl)-amino]-ethyl}-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester* (18):**

7-Diethylamino-2-oxo-2H-chromene-3-carboxylic acid **11** (170 mg, 0.63 mmol), DIPEA (437 µL, 2.54 mmol), TBTU (203 mg, 0.63 mmol), and HOBt (97 mg, 0.63 mmol) were dissolved under nitrogen atmosphere in dry DMF (4 mL) under ice cooling and stirred for 1 h. Subsequently compound **17** (327 mg, 0.63 mmol) was added. The reaction was allowed to warm to room temperature and stirred 3 h at 40 °C. The reaction progress was monitored by TLC (EE). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (EE; R_f = 0.50) yielding compound **18** (290 mg, 0.38 mmol, 61 %) as a yellow solid.

MP: 200–202 °C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 1.21 (t, ^{3}J = 7.1 Hz, 6 H, HSQC, NOESY: C^1H_3), 1.42 (s, 18 H, HSQC, HMBC: $C^{21}H_3$), 1.43 (s, 9 H, HSQC, HMBC: $C^{18}H_3$), 2.65-2.91 (m, 6 H, HMBC, NOESY: $C^{14}H_2$; HSQC, HMBC: cyclen-CH_2), 3.29 (bs, 4 H, HSQC, HMBC: cyclen-CH_2), 3.36-3.51 (m, 6 H, HSQC, HMBC: cyclen-CH_2), 3.42 (q, ^{3}J = 7.1 Hz, 4 H, HSQC, NOESY: C^2H_2), 3.51-3.62 (m, 4 H, HSQC, NOESY: $C^{15}H_2$; HSQC, HMBC: cyclen-CH_2), 6.47 (d, ^{4}J = 2.3 Hz, 1 H, HSQC, NOESY: $C^{11}H$), 6.62 (d, ^{4}J = 2.3 Hz, ^{3}J = 9.0 Hz, 1 H, HSQC, NOESY: C^4H), 7.39 (d, ^{3}J = 9.0 Hz, 1 H, HSQC, HMBC, NOESY: C^5H), 8.65 (s, 1 H, HSQC, HMBC, NOESY: C^7H), 8.81 (t, ^{3}J = 5.2 Hz, 1 H, HSQC, NOESY: NH^{13}). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 12.4 (+, 2 C, HSQC, NOESY: C^1H_3), 28.5 (+, 6 C, HSQC: $C^{21}H_3$), 28.6 (+, 3 C, HSQC: $C^{18}H_3$), 35.2 (–, 1 C, HSQC, NOESY: $C^{15}H_2$), 45.0 (–, 2 C, HSQC, HMBC: C^2H_2), 47.6 (–, 1 C, HSQC, HMBC: cyclen-CH_2), 47.9 (–, 3 C, HSQC, HMBC: cyclen-CH_2), 49.9 (–, 2 C, HSQC, HMBC: cyclen-CH_2), 51.4 (–, 1 C, HSQC, HMBC,

NOESY: $C^{14}H_2$), 53.9 (–, 1 C, HSQC, HMBC: cyclen-CH_2), 55.3 (–, 1 C, HSQC, HMBC: cyclen-CH_2), 96.5 (+, 1 C, HSQC, NOESY: $C^{11}H$), 109.9 (+, 1 C, HSQC, NOESY: C^4H), 131.1 (+, 1 C, HSQC, HMBC, NOESY: C^5H), 147.9 (+, 1 C, HSQC, NOESY: C^7H), 79.1, 79.4 (C_q, 3 C, HSQC, HMBC: C^{17}, C^{20}), 108.3 (C_q, 1 C, HSQC, HMBC: C^6), 110.1 (C_q, 1 C, HSQC, HMBC: C^8), 152.6 (C_q, 1 C, HSQC, HMBC: C^3), 155.3, 155.6, 156.0 (C_q, 3 C, HSQC, HMBC: C^{16}, C^{19}), 157.6 (C_q, 1 C, HSQC, HMBC: C^{10}), 162.7 (C_q, 1 C, HSQC, HMBC: C^9), 163.4 (C_q, 1 C, HSQC, HMBC: C^{12}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2974, 2925, 1686, 1617, 1582, 1511, 1413, 1362, 1245, 1154, 975, 824, 772. – **UV** ($CHCl_3$): λ_{max} (log ε) = 262 (4.396), 421 (4.862). – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 759.5 (100) [MH^+], 781.5 (15) [MNa^+], 797.5 (5) [MK^+], 659.4 (7) [MH^+ - boc]. – **HRMS** Calcd for $C_{39}H_{62}N_6O_9$ 758.4578; Found: 758.4567. – **MF**: $C_{39}H_{62}N_6O_9$ – **FW**: 758.96 g/mol



***7-Diethylamino-2-oxo-2H-chromene-3-carboxylic acid [2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethyl]-amide* (28)**

Compound **18** (100 mg, 0.13 mmol) was dissolved in dry DCM (2 mL) and cooled to 0 °C. Subsequently 4 mL HCl saturated diethyl ether were added. The solution was stirred 15 min at 0 °C and additionally 20 h at room temperature. The solvent was removed in vacuo yielding quantitatively the protonated hydrochloride of compound **28** as a yellow solid (78 mg, 0.13 mmol).

MF: $C_{24}H_{38}N_6O_3$ x 2 HCl – **FW**: 531.52 g/mol

X-Ray structure and crystal data of protonated **28**:

Monoclinic; Space group: C c; unit cell dimensions: a = 15.260 Å, α = 90°, b = 28.512 Å, β = 116.46°, c = 8.7960 Å, γ = 90 °; V = 3426.2 $Å^3$; Z = 4, D_x = 1.358 Mg/m^3; μ = 0.254 mm^{-1}; F(000) = 1480. Data collection: T = 123 K; graphite monochromator. A thin rod yellow crystal with dimensions of 0.410 x 0.100 x 0.050 mm was used to measure 7477 reflections (3600 unique reflections, R_{int} = 0.0201)

from 1.65° to 25.03° on a STOE-IPDS diffractometer with the rotation method. Structure refinement: The F^2 value was refined using the full-matrix least squares refinement method, with a goodness-of-fit 0f 1.018 for all reflections and 434 parameters.

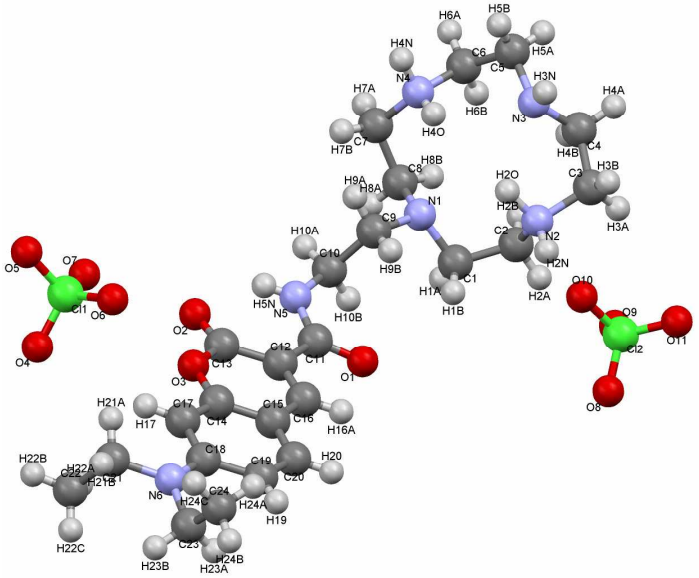


Table of atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for protonated **28**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor:

atom	x	y	z	U(eq)
O(1)	-761(2)	1173(1)	-1266(3)	35(1)
O(2)	-963(2)	-269(1)	-445(4)	39(1)
O(3)	240(2)	-427(1)	-1077(3)	30(1)
N(1)	-1543(2)	1714(1)	2313(4)	28(1)
N(2)	-465(2)	2584(1)	3060(4)	31(1)
N(3)	-351(2)	2489(1)	6318(4)	32(1)
N(4)	-1406(2)	1628(1)	5704(4)	33(1)
N(5)	-1333(2)	645(1)	-23(4)	33(1)
N(6)	2710(2)	-915(1)	-2617(4)	35(1)
C(1)	-1697(3)	2062(1)	982(5)	33(1)
C(2)	-1498(3)	2552(1)	1713(5)	33(1)
C(3)	-262(3)	3002(1)	4196(5)	38(1)
C(4)	-686(3)	2937(1)	5449(5)	37(1)
C(5)	-832(3)	2346(2)	7352(5)	37(1)
C(6)	-1732(3)	2057(1)	6286(5)	35(1)
C(7)	-2190(3)	1383(1)	4207(5)	35(1)
C(8)	-2426(3)	1649(1)	2574(5)	32(1)
C(9)	-1152(3)	1269(1)	2020(5)	30(1)
C(10)	-1865(3)	999(1)	437(5)	36(1)
C(11)	-801(3)	760(1)	-827(5)	30(1)
C(12)	-219(2)	386(1)	-1125(5)	29(1)
C(13)	-370(3)	-105(1)	-861(5)	30(1)
C(14)	943(2)	-305(1)	-1574(5)	29(1)
C(15)	1073(3)	169(1)	-1865(5)	29(1)
C(16)	472(3)	506(1)	-1625(5)	31(1)

C(17)	1471(2)	-664(1)	-1776(5)	28(1)
C(18)	2189(2)	-568(1)	-2342(4)	31(1)
C(19)	2350(3)	-88(1)	-2603(5)	33(1)
C(20)	1807(3)	264(1)	-2389(5)	32(1)
C(21)	2571(3)	-1408(1)	-2278(6)	39(1)
C(22)	1725(4)	-1642(2)	-3724(6)	51(2)
C(23)	3346(4)	-828(2)	-3424(7)	55(2)
C(24)	4387(4)	-717(2)	-2212(11)	86(3)
N(7)	392(10)	-4221(4)	-73(11)	164(5)
C(25)	-802(9)	-4278(4)	-3278(12)	129(4)
C(26)	-119(7)	-4266(3)	-1466(12)	92(3)
Cl(1)	1476(1)	-1732(1)	1396(1)	29(1)
O(4)	1182(2)	-1912(1)	-274(4)	48(1)
O(5)	1718(2)	-2118(1)	2596(4)	41(1)
O(6)	2327(2)	-1440(1)	1890(4)	40(1)
O(7)	682(2)	-1467(1)	1445(4)	42(1)
Cl(2)	776(1)	3182(1)	949(1)	33(1)
O(8)	875(2)	3084(1)	-545(4)	54(1)
O(9)	-234(2)	3265(1)	531(4)	47(1)
O(10)	1096(2)	2786(1)	2093(4)	52(1)
O(11)	1357(3)	3580(1)	1782(5)	63(1)

Table of bond lengths [Å] and angles [deg] for protonated **28**:

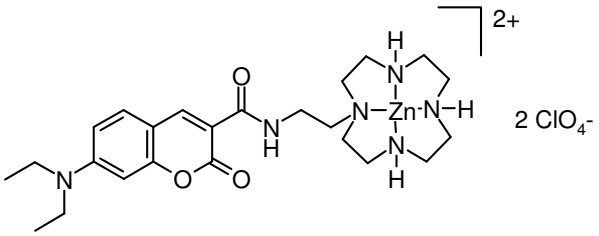
bond	Length	bond	angle	bond	angle
Cl(1)-O(5)	1.455(3)	O(5)-Cl(1)-O(6)	108.45(19)	C(3)-C(4)-H(4A)	110.00
Cl(1)-O(6)	1.438(3)	O(5)-Cl(1)-O(7)	108.63(19)	C(3)-C(4)-H(4B)	110.00
Cl(1)-O(4)	1.427(3)	O(6)-Cl(1)-O(7)	110.18(18)	H(4A)-C(4)-H(4B)	108.00
Cl(1)-O(7)	1.444(3)	O(4)-Cl(1)-O(7)	109.6(2)	C(6)-C(5)-H(5A)	110.00
Cl(2)-O(9)	1.436(4)	O(4)-Cl(1)-O(5)	109.62(19)	C(6)-C(5)-H(5B)	110.00
Cl(2)-O(10)	1.444(3)	O(4)-Cl(1)-O(6)	110.3(2)	N(3)-C(5)-H(5B)	110.00
Cl(2)-O(8)	1.416(3)	O(8)-Cl(2)-O(9)	110.0(2)	N(3)-C(5)-H(5A)	110.00
Cl(2)-O(11)	1.427(4)	O(10)-Cl(2)-O(11)	108.9(2)	H(5A)-C(5)-H(5B)	108.00
O(1)-C(11)	1.249(5)	O(8)-Cl(2)-O(10)	110.5(2)	N(4)-C(6)-H(6B)	110.00
O(2)-C(13)	1.211(6)	O(8)-Cl(2)-O(11)	109.5(2)	C(5)-C(6)-H(6A)	110.00
O(3)-C(13)	1.378(5)	O(9)-Cl(2)-O(10)	107.4(2)	H(6A)-C(6)-H(6B)	108.00
O(3)-C(14)	1.371(5)	O(9)-Cl(2)-O(11)	110.5(2)	C(5)-C(6)-H(6B)	110.00
N(1)-C(1)	1.471(5)	C(13)-O(3)-C(14)	123.3(3)	N(4)-C(6)-H(6A)	110.00
N(1)-C(8)	1.476(6)	C(1)-N(1)-C(9)	112.0(3)	N(4)-C(7)-H(7A)	109.00
N(1)-C(9)	1.473(5)	C(8)-N(1)-C(9)	112.8(3)	H(7A)-C(7)-H(7B)	108.00
N(2)-C(2)	1.494(6)	C(1)-N(1)-C(8)	112.1(3)	C(8)-C(7)-H(7B)	109.00
N(2)-C(3)	1.496(5)	C(2)-N(2)-C(3)	114.2(3)	N(4)-C(7)-H(7B)	109.00
N(3)-C(5)	1.459(6)	C(4)-N(3)-C(5)	114.5(3)	C(8)-C(7)-H(7A)	109.00
N(3)-C(4)	1.459(5)	C(6)-N(4)-C(7)	115.2(3)	C(7)-C(8)-H(8A)	109.00
N(4)-C(7)	1.500(5)	C(10)-N(5)-C(11)	121.3(3)	C(7)-C(8)-H(8B)	109.00
N(4)-C(6)	1.493(5)	C(18)-N(6)-C(23)	122.2(3)	H(8A)-C(8)-H(8B)	108.00
N(5)-C(10)	1.463(6)	C(21)-N(6)-C(23)	116.7(4)	N(1)-C(8)-H(8B)	109.00
N(5)-C(11)	1.334(6)	C(18)-N(6)-C(21)	120.8(3)	N(1)-C(8)-H(8A)	109.00
N(6)-C(18)	1.357(5)	C(3)-N(2)-H(2N)	105(3)	N(1)-C(9)-H(9A)	109.00
N(6)-C(21)	1.473(5)	C(3)-N(2)-H(2O)	104(3)	N(1)-C(9)-H(9B)	109.00
N(6)-C(23)	1.457(7)	H(2N)-N(2)-H(2O)	117(4)	C(10)-C(9)-H(9A)	109.00
N(2)-H(2N)	0.95(5)	C(2)-N(2)-H(2N)	108(3)	C(10)-C(9)-H(9B)	109.00
N(2)-H(2O)	1.06(5)	C(2)-N(2)-H(2O)	109(3)	H(9A)-C(9)-H(9B)	108.00
N(3)-H(3N)	0.83(6)	C(4)-N(3)-H(3N)	111(3)	N(5)-C(10)-H(10B)	110.00
N(4)-H(4O)	0.81(6)	C(5)-N(3)-H(3N)	105(4)	C(9)-C(10)-H(10A)	110.00

N(4)-H(4N)	0.96(5)	H(4N)-N(4)-H(4O)	117(5)	N(5)-C(10)-H(10A)	110.00
N(5)-H(5N)	0.80(5)	C(6)-N(4)-H(4O)	106(3)	C(9)-C(10)-H(10B)	110.00
N(7)-C(26)	1.129(13)	C(7)-N(4)-H(4N)	112(3)	H(10A)-C(10)-H(10B)	108.00
C(1)-C(2)	1.510(5)	C(7)-N(4)-H(4O)	105(3)	C(12)-C(16)-H(16A)	119.00
C(3)-C(4)	1.518(6)	C(6)-N(4)-H(4N)	102(3)	C(15)-C(16)-H(16A)	119.00
C(5)-C(6)	1.516(6)	C(11)-N(5)-H(5N)	121(4)	C(14)-C(17)-H(17)	120.00
C(7)-C(8)	1.519(6)	C(10)-N(5)-H(5N)	117(4)	C(18)-C(17)-H(17)	120.00
C(9)-C(10)	1.538(6)	N(1)-C(1)-C(2)	110.7(3)	C(20)-C(19)-H(19)	119.00
C(11)-C(12)	1.484(6)	N(2)-C(2)-C(1)	109.6(3)	C(18)-C(19)-H(19)	119.00
C(12)-C(16)	1.355(6)	N(2)-C(3)-C(4)	111.1(3)	C(15)-C(20)-H(20)	119.00
C(12)-C(13)	1.454(5)	N(3)-C(4)-C(3)	108.9(4)	C(19)-C(20)-H(20)	119.00
C(14)-C(17)	1.362(5)	N(3)-C(5)-C(6)	109.6(3)	N(6)-C(21)-H(21B)	109.00
C(14)-C(15)	1.407(5)	N(4)-C(6)-C(5)	108.0(4)	H(21A)-C(21)-H(21B)	108.00
C(15)-C(16)	1.408(6)	N(4)-C(7)-C(8)	111.0(3)	C(22)-C(21)-H(21A)	109.00
C(15)-C(20)	1.415(7)	N(1)-C(8)-C(7)	111.2(4)	C(22)-C(21)-H(21B)	109.00
C(17)-C(18)	1.418(5)	N(1)-C(9)-C(10)	114.3(4)	N(6)-C(21)-H(21A)	109.00
C(18)-C(19)	1.427(5)	N(5)-C(10)-C(9)	109.9(4)	H(22B)-C(22)-H(22C)	110.00
C(19)-C(20)	1.368(6)	N(5)-C(11)-C(12)	118.1(3)	H(22A)-C(22)-H(22C)	109.00
C(21)-C(22)	1.504(7)	O(1)-C(11)-N(5)	121.4(4)	C(21)-C(22)-H(22B)	109.00
C(23)-C(24)	1.500(10)	O(1)-C(11)-C(12)	120.5(4)	C(21)-C(22)-H(22C)	110.00
C(1)-H(1B)	0.9900	C(13)-C(12)-C(16)	119.8(4)	C(21)-C(22)-H(22A)	110.00
C(1)-H(1A)	0.9900	C(11)-C(12)-C(13)	120.9(4)	H(22A)-C(22)-H(22B)	109.00
C(2)-H(2B)	0.9900	C(11)-C(12)-C(16)	119.3(3)	N(6)-C(23)-H(23B)	109.00
C(2)-H(2A)	0.9900	O(3)-C(13)-C(12)	117.0(4)	C(24)-C(23)-H(23A)	109.00
C(3)-H(3A)	0.9900	O(2)-C(13)-C(12)	127.7(4)	N(6)-C(23)-H(23A)	109.00
C(3)-H(3B)	0.9900	O(2)-C(13)-O(3)	115.3(3)	C(24)-C(23)-H(23B)	109.00
C(4)-H(4B)	0.9900	O(3)-C(14)-C(15)	119.6(3)	H(23A)-C(23)-H(23B)	108.00
C(4)-H(4A)	0.9900	O(3)-C(14)-C(17)	116.4(3)	H(24A)-C(24)-H(24C)	110.00
C(5)-H(5A)	0.9900	C(15)-C(14)-C(17)	124.0(4)	C(23)-C(24)-H(24C)	109.00
C(5)-H(5B)	0.9900	C(14)-C(15)-C(20)	116.1(4)	H(24A)-C(24)-H(24B)	110.00
C(6)-H(6B)	0.9900	C(16)-C(15)-C(20)	125.5(4)	C(23)-C(24)-H(24A)	109.00
C(6)-H(6A)	0.9900	C(14)-C(15)-C(16)	118.4(4)	C(23)-C(24)-H(24B)	109.00
C(7)-H(7B)	0.9900	C(12)-C(16)-C(15)	122.0(4)	H(24B)-C(24)-H(24C)	109.00
C(7)-H(7A)	0.9900	C(14)-C(17)-C(18)	119.7(3)	N(7)-C(26)-C(25)	174.7(11)
C(8)-H(8B)	0.9900	C(17)-C(18)-C(19)	117.3(3)	H(25B)-C(25)-H(25C)	110.00
C(8)-H(8A)	0.9900	N(6)-C(18)-C(17)	121.8(3)	H(25A)-C(25)-H(25B)	109.00
C(9)-H(9B)	0.9900	N(6)-C(18)-C(19)	120.9(3)	H(25A)-C(25)-H(25C)	109.00
C(9)-H(9A)	0.9900	C(18)-C(19)-C(20)	121.6(4)	C(26)-C(25)-H(25A)	110.00
C(10)-H(10B)	0.9900	C(15)-C(20)-C(19)	121.3(4)	C(26)-C(25)-H(25B)	109.00
C(10)-H(10A)	0.9900	N(6)-C(21)-C(22)	113.4(4)	C(26)-C(25)-H(25C)	109.00
C(16)-H(16A)	0.9500	N(6)-C(23)-C(24)	114.5(5)		
C(17)-H(17)	0.9500	H(1A)-C(1)-H(1B)	108.00		
C(19)-H(19)	0.9500	N(1)-C(1)-H(1A)	109.00		
C(20)-H(20)	0.9500	N(1)-C(1)-H(1B)	110.00		
C(21)-H(21A)	0.9900	C(2)-C(1)-H(1A)	110.00		
C(21)-H(21B)	0.9900	C(2)-C(1)-H(1B)	109.00		
C(22)-H(22B)	0.9800	C(1)-C(2)-H(2A)	110.00		
C(22)-H(22C)	0.9800	C(1)-C(2)-H(2B)	110.00		
C(22)-H(22A)	0.9800	N(2)-C(2)-H(2B)	110.00		
C(23)-H(23A)	0.9900	N(2)-C(2)-H(2A)	110.00		
C(23)-H(23B)	0.9900	H(2A)-C(2)-H(2B)	108.00		
C(24)-H(24B)	0.9800	H(3A)-C(3)-H(3B)	108.00		
C(24)-H(24C)	0.9800	N(2)-C(3)-H(3B)	109.00		
C(24)-H(24A)	0.9800	C(4)-C(3)-H(3B)	109.00		
C(25)-C(26)	1.465(14)	N(2)-C(3)-H(3A)	109.00		
C(25)-H(25B)	0.9800	C(4)-C(3)-H(3A)	110.00		

C(25)-H(25C)	0.9800	N(3)-C(4)-H(4A)	110.00
C(25)-H(25A)	0.9800	N(3)-C(4)-H(4B)	110.00

To obtain the free base of compound **28** a weak basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (538 mg, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The hydrochloride salt (65 mg, 0.11 mmol) was dissolved in water, put onto the column and eluated with water. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 50 mg (0.11 mol, 100 %) of free base **28**, as yellow solid.

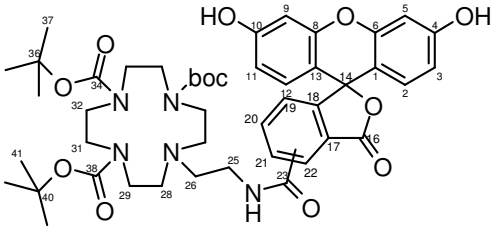
MP: 200–202 °C. – **^{1}H-NMR** (600 MHz Kryo; D_2O): δ (ppm) = 1.15 (t, ^{3}J = 7.1 Hz, 6 H, HMBC: $C^{1}H_3$), 2.86 (t, ^{3}J = 5.8 Hz, 2 H, HMBC: $C^{14}H_2$), 2.93 (bs, 4 H, HMBC: $C^{18}H_2$), 2.99 (bs, 4 H, HMBC: $C^{15}H_2$), 3.12-3.32 (m, 8 H, HMBC: $C^{16}H_2$, $C^{17}H_2$), 3.38 (q, ^{3}J = 7.1 Hz, 4 H, $C^{2}H_2$), 3.54 (t, ^{3}J = 5.8 Hz, 2 H, HMBC: $C^{13}H_2$), 6.31 (s, 1 H, ROESY: $C^{11}H$), 6.67 (d, ^{3}J = 8.9 Hz, 1 H, ROESY: $C^{4}H$), 7.31 (d, ^{3}J = 9.0 Hz, 1 H, ROESY: $C^{5}H$), 8.26 (s, 1 H, HMBC: $C^{7}H$). – **^{13}C-NMR** (100 MHz; D_2O): δ (ppm) = 11.7 (+, 2 C, HSQC, HMBC: $C^{1}H_3$), 36.2 (–, 1 C, HSQC, HMBC: $C^{13}H_2$), 41.5 (–, 2 C, HSQC, HMBC: $C^{18}H_2$), 42.0 (–, 2 C, HSQC, HMBC: $C^{17}H_2$), 44.2 (–, 2 C, HSQC, HMBC: $C^{16}H_2$), 45.7 (–, 2 C, HSQC, HMBC: $C^{2}H_2$), 48.1 (–, 2 C, HSQC, HMBC: $C^{15}H_2$), 52.6 (–, 1 C, HSQC, HMBC: $C^{14}H_2$), 96.2 (+, 1 C, HSQC, ROESY: $C^{11}H$), 107.1 (C_q, 1 C, HSQC, HMBC: C^{8}), 108.8 (C_q, 1 C, HSQC, HMBC: C^{6}), 111.7 (+, 1 C, HSQC, ROESY: $C^{4}H$), 131.7 (+, 1 C, HSQC, ROESY: $C^{5}H$), 148.1 (+, 1 C, HSQC, HMBC: $C^{7}H$), 152.5 (C_q, 1 C, HSQC, HMBC: C^{3}), 156.8 (C_q, 1 C, HSQC, HMBC: C^{10}), 163.2 (C_q, 1 C, HSQC, HMBC: C^{9}), 165.1 (C_q, 1 C, HSQC, HMBC: C^{12}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3402, 3321, 2992, 2850, 1694, 1614, 1506, 1417, 1349, 1230, 1188, 1133, 1073, 792. – **UV** ($CHCl_3$): λ_{max} (log ε) = 262 nm (4.298), 424 (4.845). – **MS** (ESI(+), TFA/MeOH): m/z (%) = 229.9 (100) $[M + 2\ H^+]^{2+}$, 459.1 (43) $[MH^+]$. – **HRMS** Calcd for $[MH^+]$ $C_{24}H_{39}N_6O_3$ 459.3084; Found: 459.3085. – **MF**: $C_{24}H_{38}N_6O_3$ – **FW**: 458.61 g/mol



***Mono-Zn(II)-cyclen coumarin* (3):**

Mono-cyclen coumarin **28** (55 mg, 0.12 mmol) was dissolved in 1 mL water and heated to 70 °C to get a clear yellow solution. Subsequently zinc(II)-perchlorate (44 mg, 0.12 mmol) dissolved in 1 ml water was added slowly. The reaction mixture was stirred for additional 20 h at 70 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized. Mono-Zn(II) cyclen coumarin **3** (86 mg, 0.12 mmol, 100 %) was obtained as a yellow solid.

MP: 194 °C. – **^{1}H-NMR** (300 MHz; CD_3CN): δ (ppm) = 1.17 (t, ^3J = 6.3 Hz, 6 H, ethyl-CH_3), 2.50-3.53 (m, 24 H, ethyl-CH_2, cyclen-CH_2, ED-CH_2), 5.39-8.27 (m, 6 H, cyclen-NH, coumarin-CH), 8.51-8.92 (m, 1 H, coumarin-CH), 9.17 (bs, 1 H, coumarin-CH). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3480, 2950, 1107, 1614, 1542, 1508, 1351, 1239, 1064, 927, 794. – **UV** (HEPES pH 7.4, 25 mM): λ_{ex} (log ε) = 430 nm (4.015), 264 (3.465). – **MS** (ESI(+), H_2O/MeOH): m/z (%) = 521.1 (100) $[M^{2+} - H^+]^+$, 557.2 (10) $[M^{2+} + Cl^-]^+$. – **HRMS** Calcd for $[M^{2+} - H^+]$ $C_{24}H_{37}N_6O_3Zn$ 521.2219; Found: 521.2209. – **MF**: $C_{24}H_{38}N_6O_3Zn(ClO_4)_2$ – **FW**: 722.89 g/mol



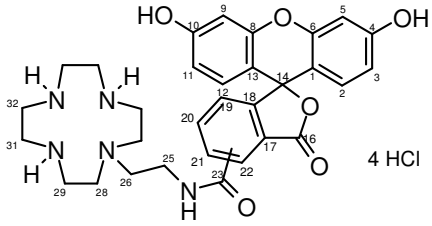
***Regioisomeric tri-tert-butyl 10-(2-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5/6-ylcarboxamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate* (19):**

A regioisomeric mixture of 5/6-carboxyfluorescein **13** (100 mg, 0.27 mmol) were filled in a nitrogen flushed round bottom flask and dissolved in a mixture of DCM/DMF (2:1, 5 mL). Then, DIPEA (164 µL, 1.20 mmol) and HOBt monohydrate (50 mg, 0.32 mmol) were added and the mixture was cooled to 0°C. After addition of TBTU (100 mg,

0.32 mmol) and stirring for 30 minutes, compound **17** (210 mg, 0.40 mmol) was added in small portions to the reaction mixture.

Further the mixture was stirred for 2 h at 40 °C. Subsequently the solvent was evaporated and the crude product was purified by column chromatography on flash-silica-gel ($CHCl_3$/MeOH 11:1) to obtain **19** as yellow amorphous solid (0.19 g, 0.21 mmol, 78%).

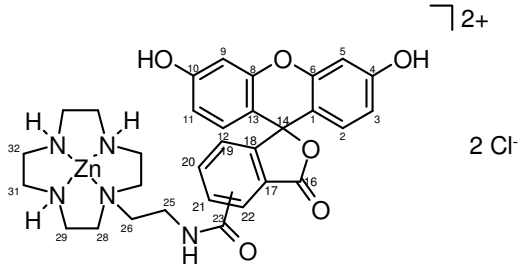
MP: 214°C – **^{1}H-NMR** (400 MHz, MeOD, COSY, ROESY, HSQC, HMBC): δ (ppm) = 1.37-1.49 (m, 27 H, C^{37}, C^{42}), 2.64-2.82 (m, 4.8 H, C^{26b}, C^{28}), 2.91 (t, ^{3}J = 6.6 Hz, 1.2 H, C^{26a}), 3.30-3.73 (m, 14 H, $C^{25a/b}$, C^{29}, C^{31}, C^{32}), 6.52 (dt, ^{3}J = 8.7 Hz, ^{4}J = 1.7 Hz, 2 H, C^3, C^{11}), 6.59 (dd, ^{4}J = 8.7 Hz, ^{5}J = 4.8 Hz, 2 H, C^5, C^9), 6.68 (d, ^{3}J = 2.2 Hz, 2 H, C^2, C^{12}), 7.28 (d, ^{3}J = 8.1 Hz, 0.6 H, C^{19a}), 7.59 (s, 0.4 H, C^{19b}), 8.05 (d, ^{3}J = 8.0 Hz, 0.4 H, C^{22b}), 8.11 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.1 Hz, 0.4 H, C^{21b}), 8.18 (dd, ^{3}J = 8.1 Hz, ^{4}J = 1.5 Hz, 0.6 H, C^{20a}), 8.41 (s, 0.6 H, C^{22a}). – **^{13}C-NMR** (100 MHz, MeOD, COSY, ROESY, HSQC, HMBC): δ (ppm) = 28.8 (+, 6 C, C^{41}), 29.1 (+, 3 C, C^{37}), 36.1 (–, 0.4 C, C^{25b}), 36.5 (–, 0.6 C, C^{25a}), 48.6-48.2 (–, 6 C, C^{29}, C^{31}, C^{32}), 50.4 (–, 1 C, $C^{26a/b}$), 55.5 (–, 2 C, C^{28}), 79.4 (C_q, 1 C, C^{14}), 81.0 (C_q, 1 C, C^{36}), 81.1 (C_q, 2 C, C^{40}), 103.6 (+, 2 C, C^2, C^{12}), 110.7 (C_q, 2 C, C^1, C^{13}), 113.8 (+, 2 C, C3, C^{11}), 123.9 (+, 0.4 C, C^{19b}), 124.8 (+, 0.6 C, C^{22a}), 125.7 (+, 0.6 C, C^{19a}), 126.2 (+, 0.4 C, C^{22b}), 128.7 (C_q, 1 C, $C^{17a/b}$), 130.1 (+, 2 C, C^5, C^9), 130.3 (+, 0.4 C, C^{21b}), 135.4 (+, 0.6 C, C^{20a}), 137.7 (C_q, 0.6 C, C^{21a}), 142.1 (C_q, 0.4 C, C^{20b}), 154.0 (C_q, 2 C, C^6, C^8), 154.6 (C_q, 0.4 C, C^{18b}), 156.5 (C_q, 0.6 C, C^{18a}), 157.2 (C_q, 1 C, C^{34}), 157.5 (C_q, 2 C, C^{38}), 161.5 (C_q, 2 C, C^4, C^{10}), 168.0 (C_q, 0.4 C, C^{23b}), 168.2 (C_q, 0.6 C, C^{23a}), 170.5 (C_q, 1 C, $C^{16a/b}$). – **IR** (ATR) [cm^{-1}]: ν = 2976, 2935, 2830, 1760, 1657, 1611, 1456, 1417, 1365, 1246, 1155, 1109, 993, 849, 761, 664, 605 – **ES-MS** (H_2O/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 874.5 (100) [MH^+], 774.4 (12) [MH^+ - boc], 437.7 (32) [$M + 2H^+]^{2+}$, 287.6 (64) [$M + 2H^+$ - 3boc]$^{2+}$ – **HR-MS** Calcd for [MH^+] $C_{46}H_{60}N_5O_{12}$ 874.4238; Found 874.4225 – **UV** (MeCN): λ_{max} (log ε) = 454 nm (2.703), 480 (2.670). – **MF**: $C_{46}H_{59}N_5O_{12}$. – **MW**: 874.01 g/mol.



***Regioisomeric N-(2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl)-3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5/6-carboxamide tetrahydrochloride* (29):**

Compound **19** (120 mg, 0.13 mmol) was dissolved in a small amount of DCM and cooled to 0°C. Subsequently HCl saturated diethyl ether (2.65 mL), the mixture was allowed to reach rt and stirred for 16 h, while a yellowish precipitate was formed. To get the desired product, the solvents were removed under reduced pressure yielding hydrochloride **29** as an orange amorphous solid (0.10 g, 0.13 mmol, quant.). Characterization of **29** was done by NMR. Before proceeding the next reaction step quantitative deprotonation of hydrochloride **29** was achieved by a weakly basic ion exchanger resin.

^{1}H-NMR (400 MHz, D_2O): δ (ppm) = 2.75-3.73 (m, 21 H, CH_2 + amide-NH), 6.80-8.71- (m, 9 H, aryl-CH). – **^{13}C-NMR** (100 MHz, D_2O, DEPT135): δ (ppm) = 36.4 (–, 0.4 C, C^{25b}) 36.8 (–, 0.6 C, C^{25a}), 41.5-44.2 (–, 6 C, C^{29}, C^{31}, C^{32}), 47.9 (–, 1 C, C^{26b}), 48.1 (–, 1 C, C^{26a}), 52.5 (–, 2 C, C^{28}), 102.5 (+, 2 C, C^2, C^{12}), 118.6 (+, 2 C, C^3, C^{11}), 132.0 (+, 2 C, C^5, C^9). *further signals could not be detected* – **ES-MS** (MeCN/TFA): *m/z* (%) = 287.5 (100) $[M + 2\,H^+]^{2+}$, 574.2 (26) $[MH^+]$.– **MF**: $C_{31}H_{35}N_5O_6$. – **MW**: 573.65 g/mol.

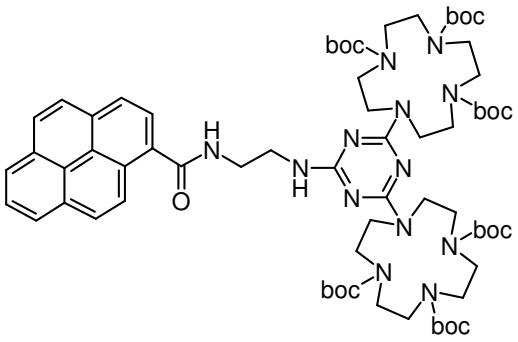


***Mono-Zn(II)-cyclen 5/6-carboxyfluorescein* (4):**

Compound **29** (92 mg, 0.16 mmol) was dissolved in 2 mL of warm water and zinc(II)-chloride (23 mg, 0.17 mmol) was added. The pH value was adjusted to 7-8 by addition of 1 M LiOH and the reaction mixture was heated up to reflux for 4 h. Lyophilization obtained the crude product as an orange solid, which was purified by precipitation from a mixture of amixture of 2-propanol/methanol with diethyl ether. The precipitate was

gained by centrifugation, redissolved in water and freeze-dried again to obtain **4** (110 mg, 0.16 mmol, 99 %) as an orange amorphous solid.

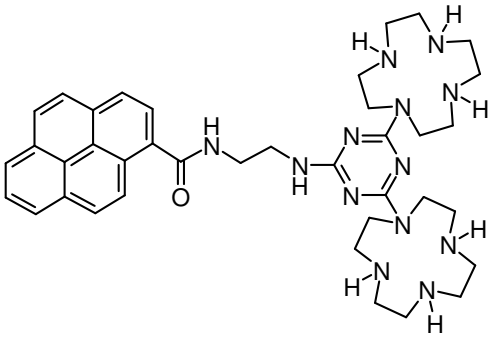
^{1}H-NMR (400 MHz, D_2O): δ (ppm) = 2.74-3.73 (m, 21 H, CH_2 + amide-NH), 6.47-6.76 (m, 4 H, C^3, C^5, C^9, C^{11}), 6.88 (dd, ^4J = 8.7 Hz, ^5J = 4.8 Hz, 2 H, C^2, C^{12}), 7.28 (s, 0.6 H, C^{19a}), 7.59 (s, 0.4H, C^{19b}), 7.98-8.20 (m, 1.4 H, C^{20a}, C^{21b}, C^{22b}), 8.43 (s, 0.6 H, C^{22a}). – **^{13}C-NMR** (100 MHz, D_2O, HSQC): δ (ppm) = 36.5-36.8 (–, 0.4 C+0.6 C, $C^{25'a/b}$), 41.5-44.3 (–, 6 C, C^{29}, C^{31}, C^{32}), 46.7 (–, 2 C, C^{28}), 47.9 (–, 0.4 C, C^{26b}), 48.1 (–, 0.6 C, C^{26a}), 51.9-52.5 (–, 0.4 C+0.6 C, $C^{25a/b}$), 102.8 (+, 2 C, C^5, C^9), 112.1-112.5 (C_q, 2 C, C^1, C^{13}), 115.8-116.5 (+, 2 C, C^3, C^{11}), 125.3 (+, 0.4 C, C^{19b}), 126.4 (+, 0.6 C, C^{22a}), 127.7 (+, 0.6 C, C^{19a}), 127.9 (+, 0.4 C, C^{22b}), 129.0 (+, 0.4 C, C^{21b}), 130.5-130.7 (+, 2 C, C^2, C^{12}), 132.2 (+, 0.6 C, C^{20a}), 135.1 (C_q, 0.6 C, C^{21a}), 138.1 (C_q, 0.4 C, C^{20b}), 144.2 (C_q, 0.6 C, C^{17b}), 145.5 (C_q, 0.4 C, C^{17a}), 154.7 (C_q, 2 C, C^6, C^8), 155.2 (C_q, 0.4 C, C^{18b}), 163.9 (C_q, 0.6 C, C^{18a}), 165.0 (C_q, 2 C, C^4, C^{10}), 168.3 (C_q, 0.4 C, C^{23b}), 168.5 (C_q, 0.6 C, C^{23a}), 170.6 (C_q, 1 C, $C^{16a/b}$). – **ES-MS** (M): *m/z* (%) = 318.6 (100) [M^{2+}], 636.2 (52) [M^{2+} - H^+], 672.2 (35) [M^{2+} + Cl^-]$^+$. – **MF**: $[C_{31}H_{35}N_5O_6Zn]Cl_2$. – **MW**: 709.93 g/mol.



***Pyrene-1-carboxylic acid {2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-ethyl}-1,4,7-tricarboxylic acid tri-tert-butyl ester* (22):**

Pyrene-1-carboxylic acid **12** (148 mg, 0.60 mmol), DIPEA (622 µL, 3.61 mmol), TBTU (213 mg, 0.66 mmol), and HOBt (101 mg, 0.66 mmol) were dissolved under nitrogen atmosphere in dry DMF (4 mL) under ice cooling and stirred for 1 h. Subsequently amine **20** (500 mg, 0.46 mmol) was added. The reaction was allowed to warm to room temperature and was stirred 30 min at rt and 7 h at 40 °C. The reaction progress was monitored by TLC (ethyl acetate/petrol ether 4:1). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate/petrol ether 3:2; R_f = 0.30) yielding compound **22** (337 mg, 0.26 mmol, 57 %) as a lightly yellow solid.

MP: 210 °C. – **^{1}H-NMR** (600 MHz Kryo; $CDCl_3$): δ (ppm) = 0.65 (bs, 4 H, boc-CH_3), 1.35, 1.41 (s, 50 H, boc-CH_3), 2.83-3.96 (m, 36 H, cyclen-CH_2, ED-CH_2), 5.14 (bs, 1H, NH), 7.92-7.98 (m, 2 H, pyrene-CH_2), 7.98-8.06 (m, 4 H, pyrene-CH_2), 8.08-8.20 (m, 2 H, pyrene-CH_2), 8.43 (m, 1 H, pyrene-CH_2). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 28.4 (+, 18 C, boc-CH_3), 40.0 (–, 1 C, ED-CH_2), 40.5 (–, 1 C, ED-CH_2), 50.1 (–, 16 C, cyclen-CH_2), 79.6 (C_q, 4 C, HSQC: boc), 79.9 (C_q, 2 C, HSQC: boc), 124.2 (+, 1 C, pyrene-CH), 124.3 (C_q, 1 C, pyrene), 124.5 (C_q, 2 C, pyrene), 124.5 (+, 1 C, pyrene-CH), 124.7 (+, 1 C, pyrene-CH), 125.32 (+, 1 C, pyrene-CH), 125.35 (+, 1 C, pyrene-CH), 126.0 (+, 1 C, pyrene-CH), 126.9 (+, 1 C, pyrene-CH), 128.1 (+, 2 C, pyrene-CH), 128.2 (C_q, 1 C, pyrene), 130.6 (C_q, 1 C, pyrene), 130.9 (C_q, 1 C, pyrene), 132.0 (C_q, 1 C, pyrene), 155.3, 155.7 (C_q, 6 C, -NR_2COO^tBu), 165.1 (C_q, 1 C, triazine), 166.1 (C_q, 2 C, triazine), 170.6 (C_q, 1 C, -NHCOR). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2976, 1686, 1539, 1496, 1474, 1410, 1364, 1246, 1161, 1109, 973, 851, 777, 759. – **UV** ($CHCl_3$): λ_{max} (log ε) = 242 nm (4.890), 267 (4.368), 278 (4.572), 329 (4.349), 343 (4.494). – **MS** (ESI(+), DCM / MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1308.9 (100) [MH^+], 655.1 (14) [M + 2 H^+]$^{2+}$, 627.0 (10) [M – C_4H_8 + 2 H^+]$^{2+}$, 605.0 (15) [M – boc + 2 H^+]$^{2+}$, 577.0 (30) [M – C_4H_8 – boc + 2 H^+]$^{2+}$, 548.9 [M – 2 C_4H_8 – boc + 2 H^+]$^{2+}$. – **MF**: $C_{68}H_{101}N_{13}O_{13}$ – **FW**: 1308.64 g/mol



***Pyrene-1-carboxylic acid {2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-amide* (30):**

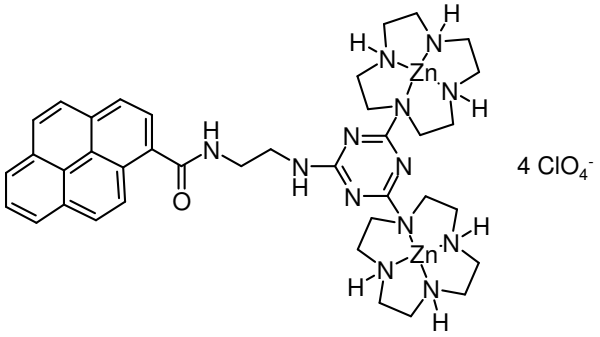
Compound **22** (150 mg, 0.12 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently 4.6 mL HCl saturated diethyl ether were added. The solution was stirred 15 min at 0 °C and additionally 18 h at room temperature. The solvent was removed in vacuo yielding quantitatively the protonated hydrochloride of compound **30** as a colourless solid.

^{1}H-NMR (400 MHz; CD_3OD): δ (ppm) = 2.67-4.00 (m, 36 H, cyclen-CH_2, ED-CH_2, solvent signal), 8.03-8.09 (m, 1 H, pyrene-CH), 8.10-8.18 (m, 2 H, pyrene-CH), 8.19-8.38 (m, 5 H, pyrene-CH), 8.56 (d, ^{3}J = 8.7 Hz, 1 H, pyrene-CH). – **^{13}C-NMR** (100 MHz; CD_3OD): δ (ppm) = 40.3, 41.5, 44.9, 45.6, 47.0 (–, 18 C, cyclen-CH_2, ED-CH_2), 125.4 (C_q, 1 C), 125.70 (C_q, 2 C), 125.70 (+, 1 C, pyrene-CH), 126.8 (+, 1 C, pyrene-CH), 127.11 (+, 1 C, pyrene-CH), 127.27 (+, 1 C, pyrene-CH), 127.84 (+, 1 C, pyrene-CH), 128.4 (+, 1 C, pyrene-CH), 129.79 (+, 1 C, pyrene-CH), 129.87 (+, 1 C, pyrene-CH), 130.0 (+, 1 C, pyrene-CH), 131.85 (C_q, 1 C), 132.0 (C_q, 1 C), 132.6 (C_q, 1 C), 134.1 (C_q, 1 C), 156.8 (C_q, 2 C), 164.7 (C_q, 1 C), 173.0 (C_q, 1 C). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 354.7 (100) $[M + 2\ H^{2+}]^{2+}$, 708.4 (10) $[MH^+]$, 822.5 (4) $[MH^+ + TFA]$. – **MF**: $C_{38}H_{53}N_{13}O$ 6 x HCl – **FW**: 963.15 g/mol

To obtain the free base of compound **30** a weak basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (805 mg, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The hydrochloride salt (115 mg, 0.12 mmol) was dissolved in water, put onto the column and eluated with water. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 69 mg (0.10 mol, 83 %) of **30**, as lightly yellow solid.

MP: 192 °C. – **^{1}H-NMR** (600 MHz; CH_3CD / $CDCl_3$ 1:1): δ (ppm) = 2.93 (m, 8 H, cyclen-CH_2), 2.97 (m, 2 H, cyclen-CH_2), 3.01 (m, 6 H, cyclen-CH_2), 3.16 (m, 8 H, cyclen-CH_2), 3.49-4.20 (m, 12 H, cyclen-CH_2, ED-CH_2), 7.97-8.03 (m, 5 H, pyrene-CH), 8.15 (d, ^{3}J = 8.4 Hz, 1 H, pyrene-CH), 8.20 (d, ^{3}J = 7.0 Hz, 2 H, pyrene-CH), 8.39 (d, ^{3}J = 9.3 Hz, 1 H, pyrene-CH). – **^{13}C-NMR** (150 MHz; CH_3CD / $CDCl_3$ 1:1): δ (ppm) = 39.1 (–, 1 C, ED-CH_2), 39.7 (–, 1 C, ED-CH_2), 42.2 (–, 2 C, cyclen-CH_2), 42.6 (–, 2 C, cyclen-CH_2), 42.8 (–, 1 C, cyclen-CH_2), 44.9 (–, 6 C, cyclen-CH_2), 45.9 (–, 2 C, cyclen-CH_2), 46.3 (–, 3 C, cyclen-CH_2), 123.4 (+, 1 C, pyrene-CH), 123.6 (C_q, 1 C, pyrene), 123.7 (+, 1 C, pyrene-CH), 123.9 (C_q, 1 C, pyrene), 124.3 (+, 1 C, pyrene-CH), 125.1 (+, 1 C, pyrene-CH), 125.4 (+, 1 C, pyrene-CH), 125.8 (+, 1 C, pyrene-CH), 126.4 (+, 1 C, pyrene-CH), 127.7 (C_q, 1 C, pyrene), 127.9 (+, 1 C, pyrene-CH), 128.1 (+, 1 C, pyrene-CH), 129.92 (C_q, 1 C, pyrene), 129.94 (C_q, 1 C, pyrene), 130.6 (C_q, 1 C, pyrene), 132.0 (C_q, 1 C, pyrene), 165.5 (C_q, 1 C, triazine-NH-ED), 166.3 (C_q, 1 C, cyclen-triazine), 166.5 (C_q, 1 C, cyclen-triazine), 171.2 (C_q, 1 C, -NHCO). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3372, 3266, 2935, 2764, 2697, 1623, 1536, 1480,

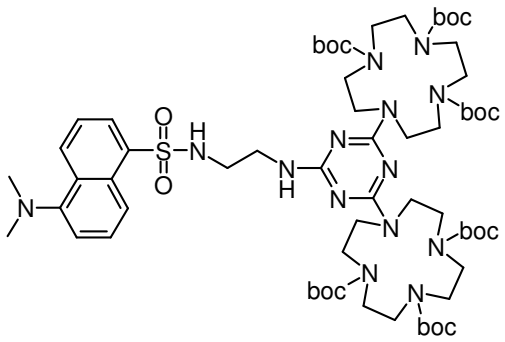
1417, 1355, 1284, 1230, 1145, 1081, 972, 852. – **UV** (MeOH): λ_{max} (log ε) = 227 nm (4.501), 242 (4.459), 266 (4.002), 276 (4.191), 327 (3.957), 341 (4.091). – **MS** (ESI(+), EE/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 354.7 (100) $[M + 2\ H^{2+}]^{2+}$, 708.4 (8) $[MH^+]$, 822.5 (4) $[MH^+ + TFA]$. – **HRMS** Calcd for $[MH^+]$ $C_{38}H_{54}N_{13}O$ 708.4574; Found: 708.4567. – **MF**: $C_{38}H_{53}N_{13}O$ – **FW**: 707.93 g/mol



***Bis-Zn(II)-cyclen pyrene* (6):**

Bis-cyclen-pyrene **30** (58 mg, 82 µmol) were dissolved in 1 mL of MeOH and heated to 65 °C to give a clear yellow solution. Subsequently, zinc(II)-perchlorate (64 mg, 172 µmol) dissolved in 1 ml of MeOH was added slowly. The reaction mixture was stirred for additional 20 h at 65 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized. Bis-Zn(II)-cyclen pyrene **6** (101 mg, 82 µmol, 100 %) was obtained as a colourless solid.

MP: 193 °C. – **^{1}H-NMR** (300 MHz; MeCN): δ (ppm) = 2.40-4.54 (m, 36 H, cyclen-CH_2, ED-CH_2), 5.63-7.05 (m, 6 H, cyclen-NH_2, 7.13-7.65 (bs, 1 H, NH), 7.68-7.95 (m, 1 H, pyrene-CH), 7.99-8.33 (m, 7 H, pyrene-CH_2), 8.34-8.51 (m, 1 H, pyrene-CH_2). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3487, 3182, 1605, 1530, 1427, 1342, 1285, 1048, 975, 931,858, 810, 775, 621. – **UV** (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 344 (4.177), 330 (4.039), 277 (4.222), 267 (4.051), 243 (4.437), 230 (4.561). – **MS** (ESI(+), TFA/MeCN): m/z (%) = 476.7 (100) $[M^{4+} + 2\ CH_3COO^-]^{2+}$, 447.2 (62) $[M^{4+} + CH_3COO^-]^{2+}$. – **MF**: $C_{38}H_{53}N_{13}OZn_2(ClO_4)_4$ – **FW**: 1236.49 g/mol

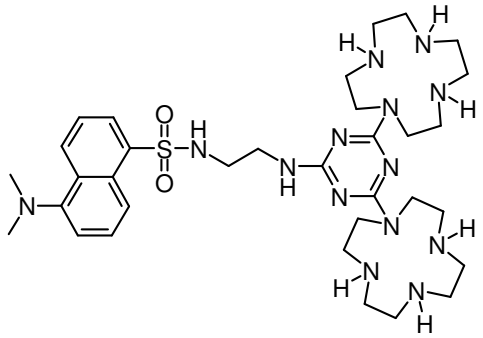


***5-Dimethylamino-naphthalene-1-sulfonic acid {2-[4,6-bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1,4,7-tricarboxylic acid tri-tert-butyl ester* (24):**

Literature known compound, improved procedure[11e]

To a solution of compound **20** (700 mg, 0.65 mmol) in dry DCM (15 mL) triethylamine (234 µL, 1.68 mmol) was added and subsequently the reaction mixture was cooled to 0 °C. After dropwise addition of a solution of dansyl chloride **14** (227 mg, 0.84 mmol) in dry DCM (5 mL) to the vigorous stirred reaction mixture, the reaction was kept at 0 °C for four additional hours under vigorous stirring. The reaction progress was monitored by TLC (ethyl acetate/petrol ether 1:1). After completion of the reaction, the reaction was quenched with water and extracted with DCM (4x). Subsequently, the organic layer was washed with sat NH_4Cl solution (3x) and dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate/petrol ether 1:1; $R_f = 0.50$) yielding compound **24** (667 mg, 0.51 mmol, 79 %) as a yellow solid.

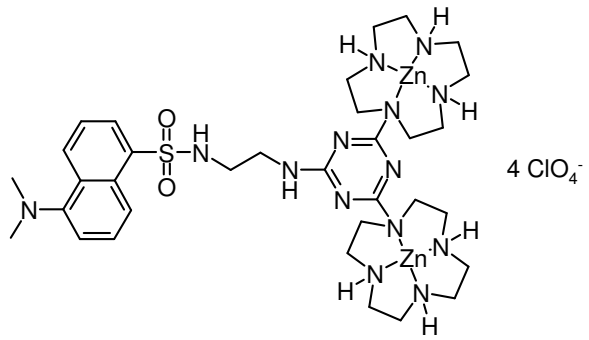
^{1}H-NMR (300 MHz; $CDCl_3$): δ (ppm) = 1.40 (bs, 54 H, boc-CH_3), 2.84 (s, 6 H, N-Me_2), 3.01-3.89 (m, 36 H, cyclen-CH_2, ED-CH_2), 4.76 (bs, 1 H, NH), 7.12 (d, ^{3}J = 7.4 Hz, 1 H, dansyl-CH), 7.40-7.54 (m, 2 H, dansyl-CH), 8.20 (dd, ^{3}J = 6.7 Hz, ^{3}J = 6.7 Hz, 1 H, dansyl-CH), 8.35 (d, ^{3}J = 7.7 Hz, 1 H, dansyl-CH), 8.47 (d, ^{3}J = 8.5 Hz, 1 H, dansyl-CH). – **MF**: $C_{63}H_{104}N_{14}O_{14}S$ – **FW**: 1313.66 g/mol



5-Dimethylamino-naphthalene-1-sulfonic acid {2-[4,6-bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-amide **(31):** *Literature known compound, improved procedure.*[11e]

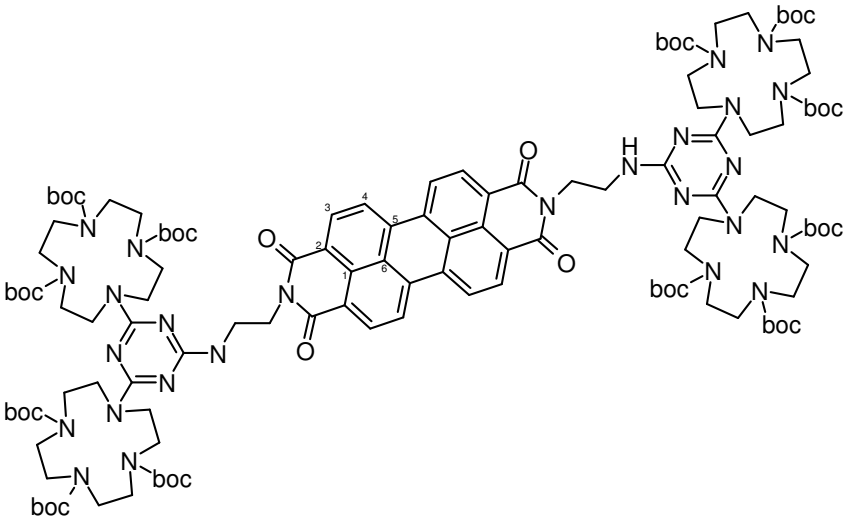
Compound **24** (600 mg, 0.46 mmol) was dissolved in DCM (8 mL) and cooled to 0 °C. Subsequently 14 mL HCl saturated diethyl ether were added. The solution was stirred 15 min at 0 °C and additionally 20 h at room temperature. The solvent was removed in vacuo, the residue was redissolved in water and after lyophilisation the protonated hydrochloride of compound **31** was obtained quantitative as a lightly yellow solid. To obtain the free base of compound **31** a weak basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (3.44 g, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The hydrochloride salt (440 mg, 0.46 mmol) was dissolved in water, put onto the column and eluated with water. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 326 mg (0.46 mol, 99 %) of **31**, as yellow solid.

^{1}H-NMR (300 MHz; D_2O): δ (ppm) = 3.08-3.30 (m, 26 H, cyclen-CH_2, ED-CH_2), 3.33 (s, 6 H, N-Me_2), 3.45 (t, ^{3}J = 5.0 Hz, 2 H, ED-CH_2), 3.86 (bs, 8 H, cyclen-CH_2), 7.70-7.85 (m, 2 H, dansyl-CH), 7.90 (d, ^{3}J = 7.7 Hz, 1 H, dansyl-CH), 8.26 (d, ^{3}J = 7.1 Hz, 1 H, dansyl-CH), 8.38 (d, ^{3}J = 8.8 Hz, 1 H, dansyl-CH), 8.56 (d, ^{3}J = 8.8 Hz, 1 H, dansyl-CH). – **MF**: $C_{33}H_{56}N_{14}O_2S$ – **FW**: 712.96 g/mol



***Bis-Zn(II)-cyclen dansyl* (8):** *Literature known compound, improved procedure.*[11e]
Compound **31** (120 mg, 0.17 mmol) was dissolved in 1 mL of water and heated to 65 °C to give a clear yellow solution. Subsequently zinc(II)-perchlorate (64 mg, 172 µmol) dissolved in 1 ml of water was added slowly. The pH was adjusted by addition of 1 M NaOH (approx. 2 mL) to pH 7. The reaction mixture was stirred for additional 23 h at 70 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized. The crude product (200 mg) was recrystallized from a EtOH / H_2O (4:1) mixture as a yellow solid (89 mg, 0.07 mmol, 41 %).

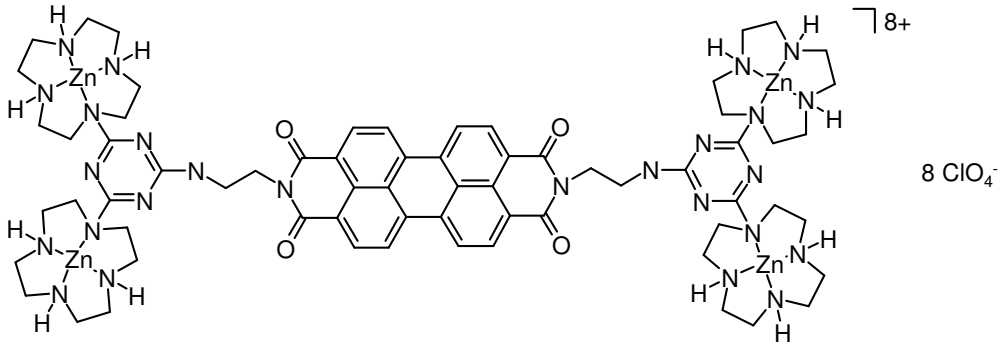
^{1}H-NMR (300 MHz; CD_3CN): δ (ppm) = 2.51 (t, ^3J = 5.2 Hz, 2 H, CH_2), 2.61-2.75 (m, 4 H, CH_2), 2.78-2.92 (m, 18 H, CH_2), 2.93-3.11 (m, 10 H, CH_2), 3.27-3.52 (m, 4 H, CH_2), 3.97-4.61 (m, 4 H, CH_2), 5.99 (t, ^3J = 6.0 Hz, 1 H, NH), 6.13 (t, ^3J = 5.8 Hz, 1 H, NH), 7.26 (d, ^3J = 7.7 Hz, 1 H, CH), 7.57 (d, ^3J = 8.5 Hz, 1 H, CH), 7.62 (d, ^3J = 9.1 Hz, 1 H, CH), 8.16 (dd, ^3J = 7.3 Hz, 1.0 Hz, 1 H, CH), 8.26 (d, ^3J = 8.8 Hz, 1 H, CH), 8.54 (d, ^3J = 8.5 Hz, 1 H, CH). – **UV** (HEPES pH 7.4, 25 mM): λ_{ex} (log ε) = 330 nm (3.575), 227 (4.637). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 479.1 (100) $[M^{4+} + 2\,CH_3COO^-]^{2+}$, 449.1 (82) $[M^{4+} - H^+ + CH_3COO^-]^{2+}$, 420.1 (20) $[M^{4+} - 2\,H^+]^{2+}$. – **MF**: $C_{33}H_{56}N_{14}O_2SZn_2(ClO_4)_4$ – **FW**: 1241.52 g/mol



Compound 25:

Compounds **20** (991 mg, 0.92 mmol) and 3,4,9,10-Perylene–tetracarboxylic dianhydride **15** (150 mg, 0.38 mmol) were mixed with 4 g of imidazole and filled into a Schlenk tube. Under an atmosphere of nitrogen the mixture was melted with a heat gun and then put into an oil bath where it was stirred at 120 °C for one day. The hot melt was poured into 20 mL of water and the product was extracted three times with 15 ml EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash-chromatography on silica gel (DCM / MeOH 97:3, (R_f = 0.28) as eluent to give **25** as dark red solid (1.53 g, 0.71 mmol, 68 %).

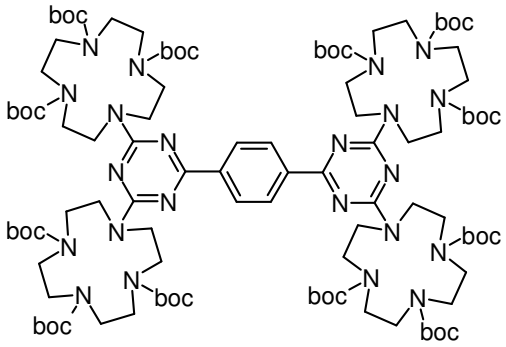
MP: 175-176 °C. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 1.23-1.53 (m, 108 H, Boc-CH_3), 2.66-3.92 (m, 68 H, Cyclen-CH_2 + CH_2), 4.39 (bs, 4 H, CH_2), 5.30 (bs, 2 H, NH), 8.30 (d, ^{3}J = 7.41, 4 H, 2), 8.43 (d, ^{3}J = 7.68, 4 H, 3). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 28.5 (+, 36 C, Boc-CH_3), 39.5 (–, 2 C, CH_2), 40.1 (–, 2 C, CH_2), 49.5-51.5 (–, 32 C, cyclen-CH_2), 79.7 (C_q, 12 C, O*C*$(CH_3)_3$), 122.9 (C_q, 2 C, C^6), 123.2 (+, 4 C, C^3), 125.9 (C_q, 2 C, C^5), 129.0 (C_q, 4 C, C^1), 131.2 (+, 4 C, C^2), 134.2 (C_q, 4 C, C^4), 155.5-157.4 (C_q, 12 C, Boc-CO), 163.3 (C_q, 4 C, C^5), 166.0 (C_q, 6 C, triazene-C). – **MS** (ES, DCM/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 1259.6 (100) [M + 2 H^+]. – **Elemental analysis** calcd. (%) for $C_{126}H_{190}N_{26}O_{28}$ (2517.01) + 2 H_2O: C 59.28, H 7.66, N 14.26; found: C 59.32, H 7.67, N 14.01. – **IR** (neat) [cm^{-1}]: $\tilde{\nu}$ = 3297, 2986, 2942, 2928, 2898, 2366, 2336, 1697, 1655, 1542, 1476, 1419, 1349, 1061, 1049, 965, 811, 738, 620. – **UV** (MeCN): λ_{max} (log ε) = 456 nm (3.873), 484 nm (4.355), 521 nm (4.497). – **MF**: $C_{126}H_{190}N_{26}O_{28}$ – **MW**: 2517.01 g/mol



***Tetranuclear Zn(II)-cyclen perylene complex* (9):**

Compound **25** (230 mg, 0.09 mmol) was dissolved in methanol and cooled to 0 °C in an ice bath. To the solution HCl saturated diethyl ether (0.7 ml/mmol Boc-group) was added. The mixture was allowed to warm to room temperature and was stirred over night. The solvent was evaporated and the crude product was dissolved in water and purified over an ion exchanger column using a strongly basic ion exchange resin (OH^--form, loading 0.9 mmol/mL, 4 eq. per protonated nitrogen) to give a colorless solid after freeze drying. Two solutions one containing the obtained colorless solid from the column in water and a second containing $Zn(ClO_4)_2 \cdot 6\ H_2O$ (125 mg, 0.33 mmol, 4.4 eq. per deprotected receptor) in absolute ethanol were prepared. These two solutions were simultaneously added drop wise to 80 °C hot water under vigorous stirring. The resulting mixture was stirred at 90 °C over night. The solution was freeze dried and the product was recrystallized from a water methanol mixture to give the complex **9** as violet red solid in 60 % yield. (118 mg, 0.05 mmol).

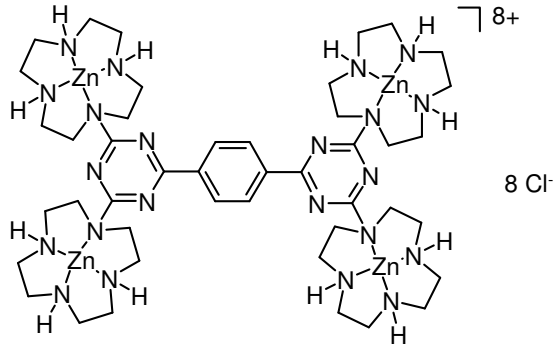
MP > 180 °C. – **MS** (ES, H_2O/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 436.3 (35) [K^{8+} - H^+ + 3 CH_3COO^-], 451.8 (100) [K^{8+} + 4 CH_3COO^-], 602 (48) [K^{8+} - H^+ + 4 CH_3COO^-], 608.0 (32) [K^{8+} + OH^- + 4 CH_3COO^-], 621.5 (36) [K^{8+} + 5 CH_3COO^-]. – **IR** (neat) [cm^{-1}]: $\tilde{\nu}$ = 3276, 2926, 1692, 1654, 1558, 1438, 1361, 1347, 1089, 964, 810, 746. – **UV** (HEPES pH 7.4): λ_{max} (log ε) = 500 nm (3.733), 538 nm (3.844). – **MF**: $[C_{66}H_{94}N_{26}O_4Zn_4]^{8+}(ClO_4)_8$. – **MW**: 2207.88 g/mol.



Compound 27:

To a mixture of Boc protected bis-cyclen **26** (200 mg, 0.18 mmol), and $Pd(PPh_3)_4$ (15 mg, 0.013 mmol) in DME, Benzene-1, 4-diboronic acid of **16** (16 mg, 0.09 mmol) was added which was immediately followed by aqueous Na_2CO_3 (2 M, 2 mL). The mixture was refluxed for 48 h under N_2 atmosphere. After cooling the solvent was evaporated under reduced pressure to dryness. THF was added and the suspension was placed in ultra sonication bath for few minutes. 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 / petrol ether 1:1, $R_f = 0.68$) to afford **27** as a colourless solid (128 mg, 0.06 mmol, 31 %)

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 1.39 (s, 108 H, boc-CH_3), 3.37-3.59 (m, 64 H, cyclen-CH_2), 8.37 (s, 4 H, Ar-H). – **^{13}C NMR** (300 MHz, $CDCl_3$) δ (ppm) = 28.5 (+, boc-CH_3), 50.24 (–, cyclen-CH_2), 79.9 (C_q, Boc), 128.1 (+, Ar-CH), 156.6 (C_q, Boc), 169.4 (C_q, triazene). – **MS** (ESI(+), TFA/MeCN): m/z (%) = 476.7 (100) $[M^{4+} + 2\ CH_3COO^-]^{2+}$, 1060.0 (100) $[M + 2\ H^+]^{2+}$, 1010.0 (45) $[M + 2\ H^+ - boc]^{2+}$, 2119.2 (15) $[MH^+]$. – **MF**: $C_{104}H_{176}N_{22}O_{24}$ – **FW**: 2118.70 g/mol



***Tetranuclear Zn(II)-cyclen benzene* (10):**

Boc-protected compound **27** (128 mg, 61 µmol) dissolved in DCM was treated with trifluoroacetic acid (1.5 mL, 19 mmol). Subsequently DCM was evaporated and the obtained TFA salt was dissolved in water and passed through a basic ion exchanger column resin. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 53 mg (58 µmol, 96 %) of deprotected **27** as colourless solid. Further it was dissolved in 3 mL acetonitrile and anhydrous $ZnCl_2$ (32 mg, 23 mmol) dissolved in methanol was added slowly. The reaction mixture refluxed overnight under vigorously stirring. The hot reaction mixture was decanted and upon cooling compound **10** precipitated as a colourless solid. After filtration and drying in vacuo 82 mg (56 µmol, 92 %) tetranuclear Zn(II)-cyclen **10** were obtained.

MP: 255-257°C. – **^{1}H NMR** (600 MHz, DMSO) δ (ppm) = 2.8-3.4 (bs, 64 H, cyclen-CH_2), 3.9 (bs, 6 H, NH), 4.4 (m, 6 H, NH). – **^{13}C NMR** (150 MHz, DMSO) δ (ppm) = 44.6, 46.4, 47.3, 50.3 (–, cyclen-CH_2), 130.5 (+, Ar-CH), 140.6 (C_q, benzene), 163.5, 171.5 (C_q, triazene). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3398, 2933, 1680, 1524, 1347, 1193, 1132, 1087, 971, 813, 723. – **UV** (HEPES pH 7.4, 25 mM): λ_{max} (log ε) = 285 nm (4.530). – **MS** (ESI(+),H_2O/MeOH +10mM NH_4OAc)): m/z (%) = 490.7 (100) (M^{8+} +5 CH_3COO^-] $^{3+}$, 471.3 (80) (M^{8+} +- H^+ + 4 CH_3COO^-] $^{3+}$, 353.7 (50) (M^{8+} + 4 CH_3COO^-] $^{4+}$. – **MF**: $[C_{44}H_{92}N_{22}Zn_4]^{8+}Cl_8$. – **FW**: 1474.50 g/mol

3.5 References

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[16] The concentration limit is [**9**] = 10 µM for UV-vis measurements and [**9**] = 5 µM for emission measurements. While binding isotherms obtained by UV and emission for

10 µM concentration of **9** cannot be described by a 2:1 binding model, a curve fit to this binding model is possible for 5 µM concentrations. Still, the value should be considered as an estimate and requires verification by a suitable independent method.

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4. Synthesis of New Amphiphilic 1,4,7,10-Tetraazacyclododecane Zn(II) Complexes for a Template Guided Cooperative Self-Assembly of Nucleotides at Interfaces

This chapter deals with the synthesis of new amphiphilic 1,4,7,10-tetraazacyclododecane Zn(II) complexes for a template guided cooperative self-assembly of nucleotides at interfaces fabricated by combination of self-assembly monolayer technique and Langmuir Blodgett technique.

All compounds were prepared at the University of Regensburg by Stefan Stadlbauer. The investigations at interfaces are in progress at the Institute of Physical Chemistry and Electrochemistry of the Russian Academy of Sciences in Moscow in the group of Prof. M. A. Kalinina.

4.1 Introduction

Complex biomolecules such as DNA, RNA or proteins are of enormous importance for our life as they carry significant biological information and regulate fundamental biological processes in living cells. The biosynthesis of proteins proceeds via several essential steps: amino acid biosynthesis, transcription, translation, post-translation modification and protein folding, in order to transfer the information of the structural design of a protein stored in DNA to RNA and then to protein structure.[1] Since in 1966 the standard genetic code was fully deciphered we understand how protein sequences consisting of the 20 amino acids along with several unnatural amino acids are enciphered.[1a, 2] The protein amino acid sequence is assigned to a special nucleotide triplet sequence called codon (XXX, X = nucleotide base) of purine bases (adenine (A), guanine (G)) and pyrimidine bases (cytosine (C), thymine (T)) in the DNA and translated by mRNA and tRNA using the rules of codon–anticodon pairing of the standard genetic code.[1a, 3] Although there is an obvious degeneracy of the genetic code as the known 64 nucleotide triplet codons, including three stop codons, encode only 20 amino acids, no ambiguity in the assignment between the codons and the cognate amino acids is observable.[3, 4] The driving forces for the codon–anticodon pairing is hydrogen bonding by means of base pairing which follows the rules of Watson-Crick base pairing and non-Watson-Crick base pairing like wobble base pairing.[1c, 5]

To date, several strategies were developed to take advance of base pairing for planar molecular-recognition systems in the fields of supramolecular and material chemistry at the interface.[6] The modification of self-assembled monolayers (SAMs)[7] or Langmuir monolayers[8] with nucleotide bases or their synthetic derivatives was demonstrated. Although the self-assembled monolayers[9] and the Langmuir–Blodgett (LB) films,[10] respectively, are both well developed applications they show some limitations. As the LB technique provides the possibility of precise adjustment of structure, properties and sterical pattern of the prepared monolayers at the air / water interface[11] employing various kinds of substrates and chemicals,[12] several drawbacks exist: Instability of the LB films, thermodynamic restrictions due to the layer structure[9] and especially the preparation of so called “head-up” layers, where the polar groups are assembled at the air, is not possible.[10c] Due to the fact, that most of the chemically and biologically significant binding processes for analytical detection and bio-like self-

assembly proceed in polar media and interfaces,[13] covalent to surfaces attached SAMs, with a "head-up" orientation of their polar functional moiety are assumed to be most suitable for the modification of surface properties.[6a, 7a] However, the sterical hindrance[14] in modified SAMs caused by the head groups of neighbouring molecules may create problems.

Previously, we demonstrated a novel surface design methodology, which combines the advantages of SAM-based and LB monolayer approaches.[15] The technique allows the fabrication of double layers modified by amphiphilic Zn(II)-cyclen metal complexes from self-assembled monolayers and LB films, which provides a high control of the in-plane steric arrangement. Embedding an amphiphilic binuclear Zn(II)-cyclen, which binds specific and with high affinity to various biologically relevant phosphates,[16] we showed the sterically induced, cooperative lateral self-assembly of two complementary nucleotides in a planar film.

To extend the scope of this methodology and to expand the level of cooperativity, we herein report the syntheses of a set of new amphiphilic Zn(II)-cyclen metal complexes for use in the guided assembly of complementary nucleotides in 2D systems.

4.2 Development of new amphiphilic Zn(II)-cyclen derivatives

Utilizing metal complexes as recognition unit on self-assembled layers is of interest, as the charged metal complexes act as a polar bulky head group of the amphiphiles. Further they provide a Lewis acidic binding site with high specifity and affinity for various kinds of Lewis basic donors, e.g. phosphor anions, biologically important phosphates or phosphorylated peptides and proteins.[17] Such reversible coordination to Lewis acidic metal complexes typically occurs with large enthalpies compared to other non-covalent interactions as e. g. hydrogen bonding, allowing a binding in aqueous media.[18] As previously demonstrated by surface plasmon resonance technique (SPR) amphiphilic Zn(II)-cyclen complex **1**[15a] and **2**[15b] (hereafter Zn(II)-BC) enable a template guided 2D arrangement of the complimentary nucleotides adenosine and uridine as shown in Figure 2.

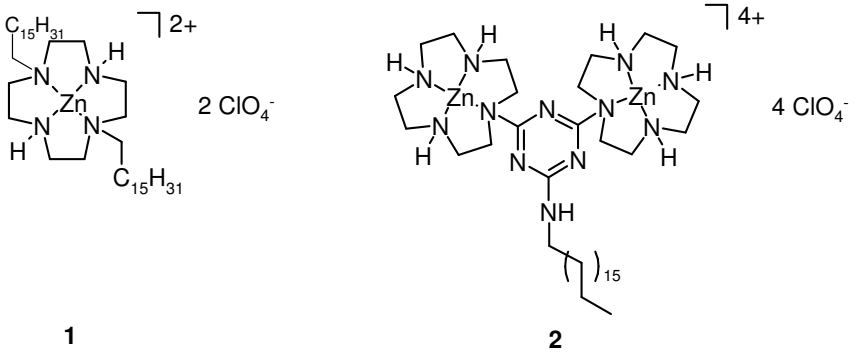


Figure 1. Amphiphilic mononuclear and binuclear Zn(II)-cyclen complexes.

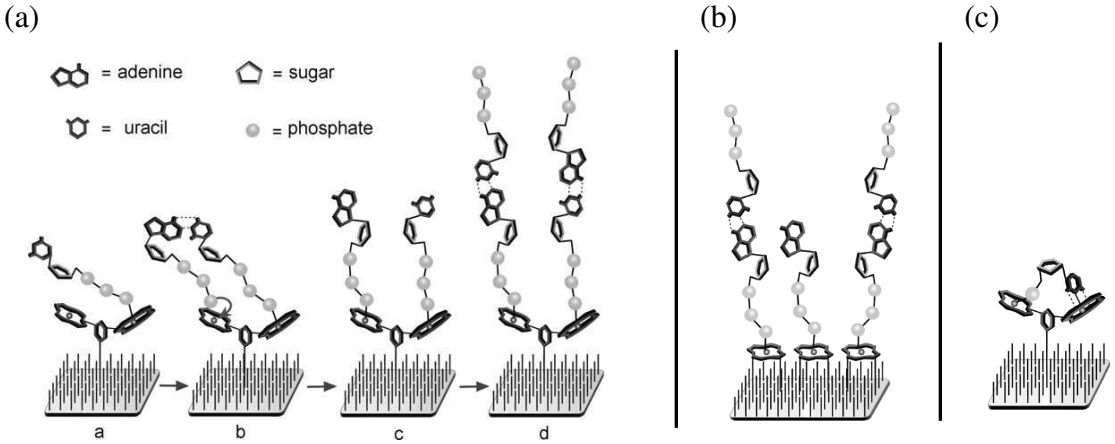


Figure 2. Proposed mechanism of template guided self-assembly of nucleotide bilayers. (a) Complementary nucleotide bilayer induced by complex **2**. (b) Complementary nucleotide bilayer induced by complex **1**. (c) 5`UMP binding to complex **2**.

Based on SPR results we suggested a stepwise self-assembly mechanism of complementary nucleotides (5´-AXP, 5´-UXP, X = mono-, di- or triphospate) on the prepared SAM-Zn(II)-cyclen template (Figure 2.). Complex **1** and **2** form complimentary nucleotide bilayers (Figure 2a and b) except for 5´-UMP where Zn(II)-BC binds the phosphate and the nucleobase moiety simultaneous (Figure 2c).

We report here the synthesis of additional amphiphilic mono- and binuclear Zn(II)-cyclen derivatives and an amphiphilic tris Zn(II)-cyclen complex (Figure 3) to expand the variation of surface binding sites.

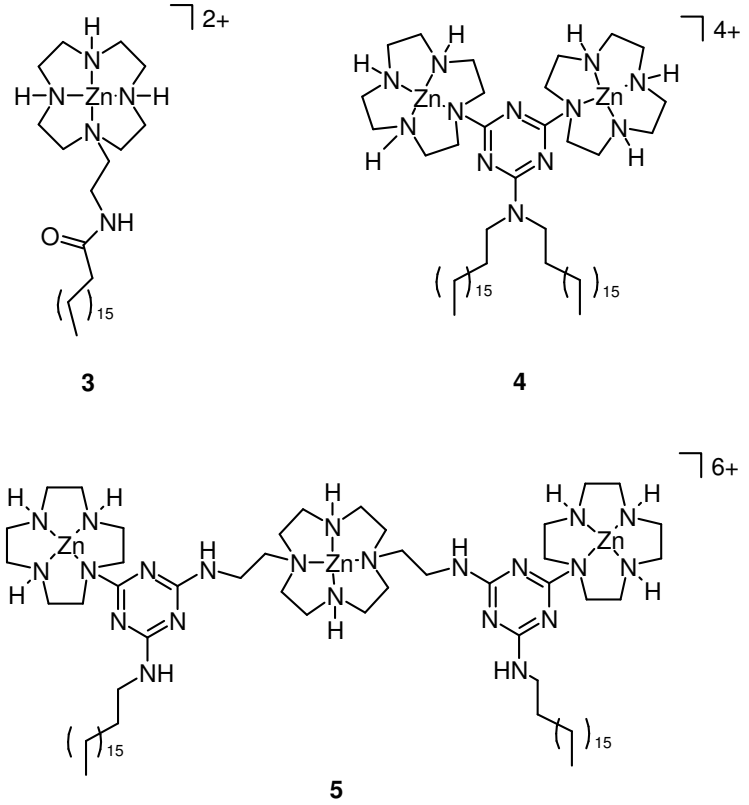


Figure 3. New amphiphilic Zn(II)-cyclen derivatives synthesized in this work: Mononuclear Zn(II)-cyclen complex (Zn(II)-MC) **3**, binuclear Zn(II)-cylen complex bearing two C_{18} alkyl chains **4** (Zn(II)-BC $C18_2$) and trinuclear Zn(II)-cyclen complex (Zn(II)-TC) **5**. Perchlorate counter ions (ClO_4^-) are not shown.

Complex **1** offers two alkyl chains affecting a higher hydrophobicity and therefore a tighter anchoring to the SAMs compared to **3**. The single hydrophobic chain of **3** therefore may lead to a different behaviour towards nucleotides. Additionally, compound **3** embedded in the SAM may evoke a supposed arrangement as shown in Figure 4 whereas two Zn(II)-MC are able to act as pseudo binuclear Zn(II)-cyclen complex.

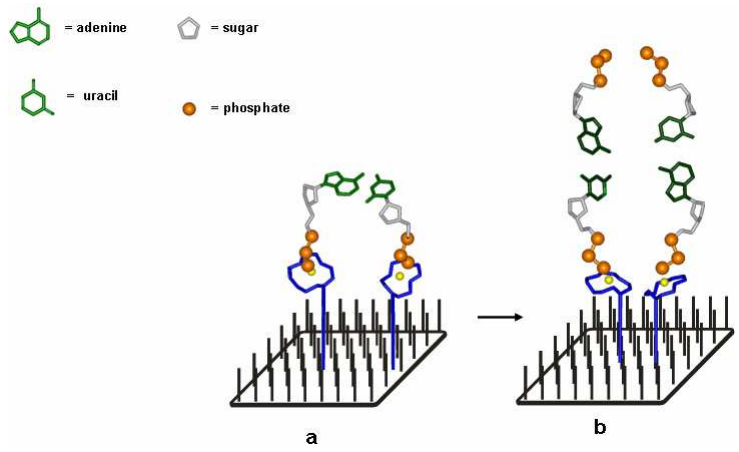


Figure 4. Assumed arrangement of Zn(II)-MC as a pseudo binuclear Zn(II)-cyclen complex. (a) Adenine and uracil nucleotide base pairing to the Zn(II)-MC template, if a pre-arrangement of Zn(II)-MC to a pseudo Zn(II)-BC proceeds. (b) Treating with complementary nucleotides effects a nucleotide bilayer as described in Figure 2.

Introduction of an additional chain increases the hydrophobicity of derivative **4** and may lead to a modification of the response of the prepared layers to substrates or change their stability.

A SAM based on complex **5** may permit the formation of a complementary bilayer system comprising of three different nucleotides by stepwise self-assembly of complementary nucleotides. Mimicing of a codon on the surface and therefore a specific readout of biologic information may become possible. Figure 5 shows a proposed codon assembly and anticodon recognition on Zn(II)-cyclen derivate **5** as template.

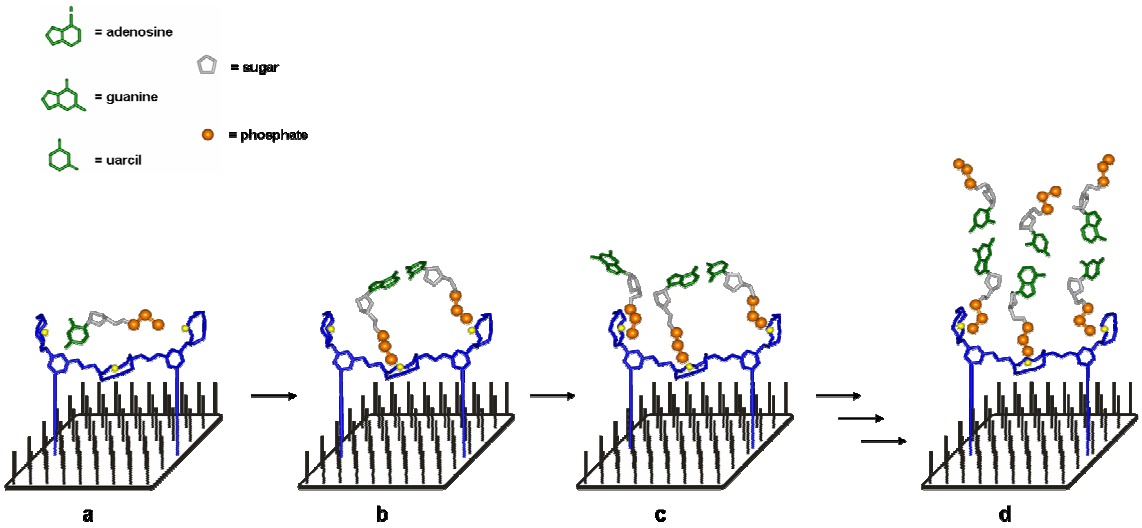
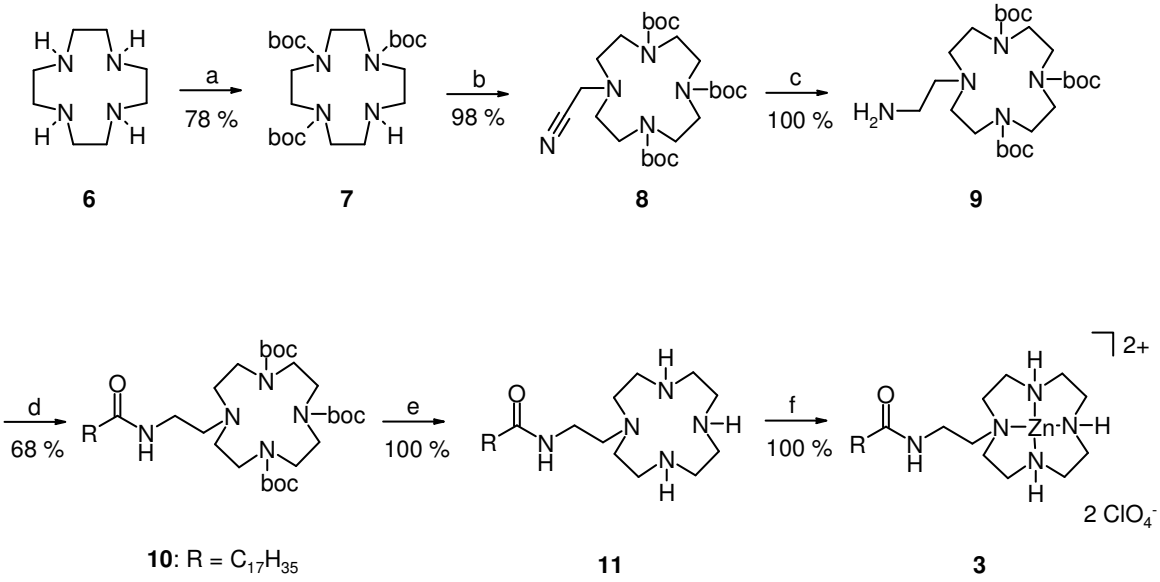


Figure 5. Proposed stepwise self-assembly of complementary nucleotides on a SAM-Zn(II)-TC template.

4.3 Syntheses

4.3.1 Synthesis of amphiphilic mononuclear Zn(II)-cyclen C18

The protected mono-cyclen amine **9**[19] was prepared according to literature known procedures and allowed to form the amide with stearic acid using TBTU and HOBt as activation reagents in a dry DMF / THF solvent mixture. Cleavage of the Boc-groups by HCl saturated ether, subsequent deprotonation by basic anion exchanger resin and complex formation by treatment with one equivalent $Zn(ClO_4)_2$ gave the amphiphilic mononuclear Zn(II)-cyclen C18 **3** (Zn(II)-MC).

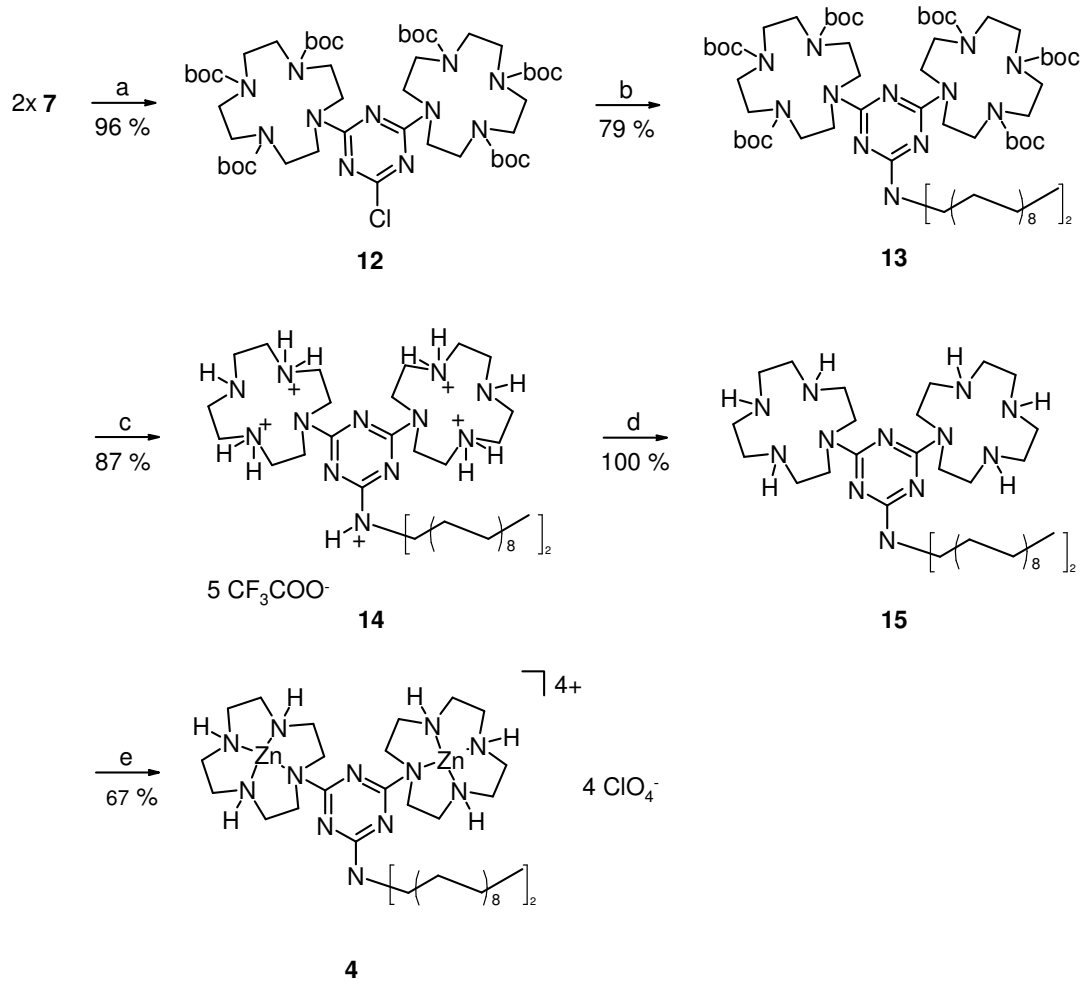


Scheme 1. Synthesis of mononuclear Zn(II)-cyclen C18 (Zn(II)-MC) **3**. (a) Boc_2O, DCM, RT, 3 h; (b) bromoacetonitrile, K_2CO_3, MeCN, 80 °C, 20 h; (c) H_2/Raney Ni, EtOH/NH_3, RT, 20 bar, 48 h; (d) stearic acid, TBTU, HOBt, DIPEA, DMF / THF, 40 °C, 3 h; (e) HCl / ether, DCM o/n, basic ion exchanger resin; (f) $Zn(ClO_4)_2$, MeOH, 65 °C, 19 h.

4.3.2 Synthesis of amphiphilic binuclear Zn(II)-cyclen bis-C18

The binuclear Zn(II)-cyclen complex **4** was synthesized analogously to the reported procedure for complex **2**. To enhance the hydrophobicity of compound **5** and therefore effect a higher and tighter interaction between **5** and the SAM, a secondary amine with two alkyl chains (C18) was tethered to the bis-cyclen moiety **12**,[15] which was available by a two step literature known synthesis. The remaining chloride residue of template **12**

was allowed to react with di-octadecylamine in a nucleophilic aromatic substituion at similar conditions as used for the precursor of Zn(II)-BC.



Scheme 2. Synthesis of binuclear Zn(II)-cyclen complex **4**. (a) Cyanuric chloride, K_2CO_3, acetone, reflux, 18 h; (b) di-octadecylamine, K_2CO_3, dioxane, 90 °C, 24 h; (c) trifluoroacetic acid, DCM, RT, o/n; (d) basic ion exchanger resin; (e) $Zn(ClO_4)_2$, MeOH, 65 °C, 16 h.

The overall reaction yield of compound **13** over 3 steps was 59% and a scale up to a multiple gram scale preparation of compound **4** was possible. Treating **13** with TFA over night and subsequent passing through a basic ion exchanger resin gave the free amine of ligand **15**. Finally, complex formation of the amine ligand with two equivalents of $Zn(ClO_4)_2$ gave after recrystallisation analytically pure Zn(II)-BC $C18_2$ **4** in 67 % yield.

4.3.3 Synthesis of amphiphilic trinuclear Zn(II)-cyclen C18

The trinuclear Zn(II)-cyclen complex 5 was prepared from two building blocks: cyclen modified triazine **16** and 1,7-bis-boc-4,10-diaminoethyl cyclen **17** (Figure 6).

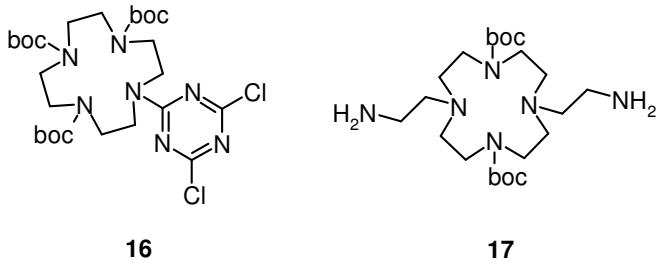
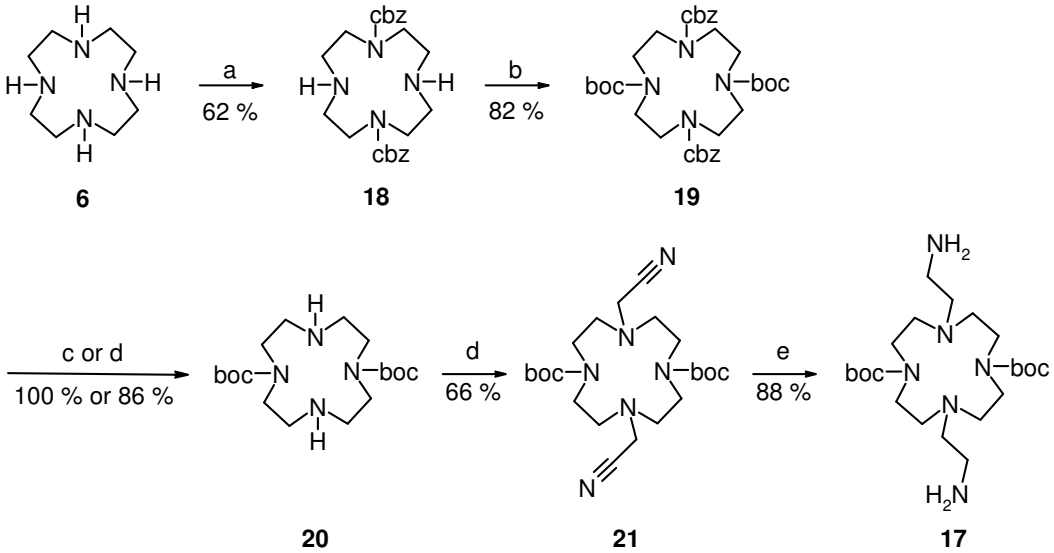


Figure 6. Building blocks for the synthesis of Zn(II)-TC: cyclen modified triazine **16** and the 1,7-bis-boc-4,10-diaminoethyl cyclen **17**.

Compound **16** was synthesized as previously reported.[15] The synthesis of compound **17** is shown in Scheme 3.

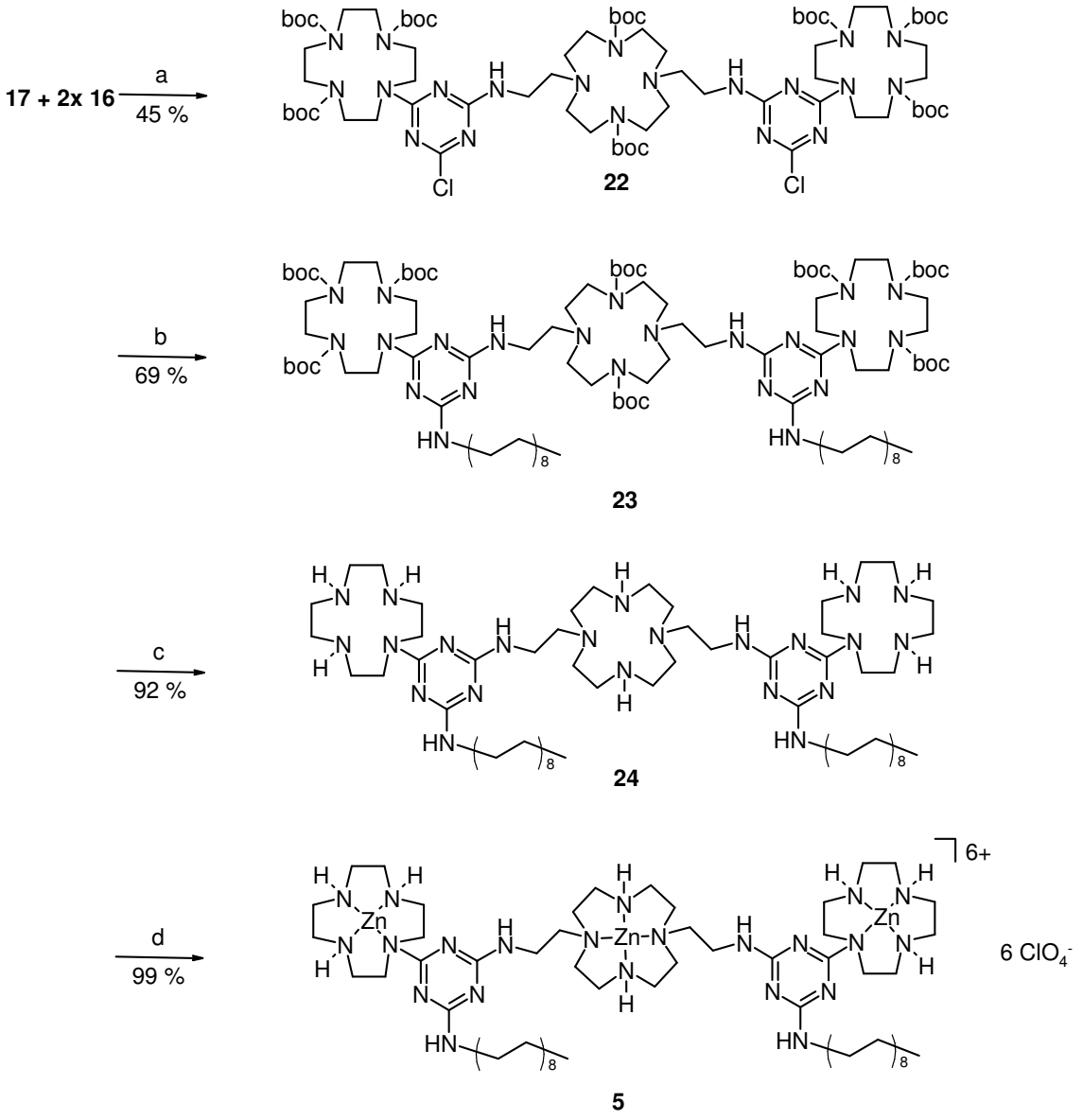


Scheme 3. Synthesis of 11,7-bis-boc-4,10-diaminoethyl cyclen **17**. (a) Cbz-Cl, dioxane, water, pH 2–3; (b) Boc_2O, DCM, 3 h; (c) H_2, Pd/C, ethanol, RT, 48 h, quantitative; (d) Pd/C, ethanol / cyclohexane (2:1), reflux, o/n, 86 %; (e) bromoacteonitrile, MeCN, 50 °C, 18 h; (f) H_2, Raney-Ni, NH_3 saturated ethanol, 25 bar, 4 d.

Starting from cyclen **6**, the Cbz protection group was selectively introduced at controlled pH using the method of *Kovacs* and *Sherry*.[20] Treatment with Boc_2O gave the orthogonally trans-protected cyclen derivative **19**. The Cbz groups were removed, depending on the reaction scale, by catalytic amounts of palladium on charcoal and hydrogen atmosphere of 25 bar in a autoclave (up to 500 mg scale) or transfer

hydrogenation with 50 % (w/w) palladium on charcoal as reported by Anantharamaiah *et al*[21] (up to 2.5 g scale), yielding 1,7–Boc protected cyclen **20**. In the next step twofold alkylation with bromo acetonitrile followed by reduction of the nitrile by Raney nickel gave **17**.

Efforts to develop a shorter synthesis by introduction of the alkyl amine linker of **17** directly to cyclen by the method of *Kovacs* and *Sherry* or to **16** by nucleophilic aromatic substitution were not successful.



Scheme 4. Synthesis of the amphiphilic trinuclear Zn(II)-cyclen complex **5**. (a) K_2CO_3, dry MeCN, RT, 3 h; (b) octadecylamine, K_2CO_3, dry MeCN, reflux, 24 h; (c) HCl / ether, DCM, RT, 21 h; basic ion exchange resin H_2O / MeOH (8:2); (d)) $Zn(ClO_4)_2$, MeOH, 65 °C, 22 h.

The combination of cyclen building block **16** and **17** at mild reaction conditions gave the tris cyclen **22**. Hydrophobic alkyl chains were introduced by a nucleophilic aromatic substitution. Subsequent treatment by HCl saturated ether and basic ion exchanger resin gave tris cyclen **24**, which upon treatment with three equivalents of $Zn(ClO_4)_2$ yielded the amphiphilic tris Zn(II)-cyclen complex **5** (Scheme 4).

4.4 Conclusion and Outlook

In summary, we prepared three amphiphilic Zn(II)-cyclen complexes as binding sites at interfaces prepared by a combination of SAM and LB film approaches. Detailed investigations of the binding properties of surfaces incorporating the new amphiphilic complexes are in progress.

4.5 Experimental Part

4.5.1 General methods and material

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck or Baker).

NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in 13C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

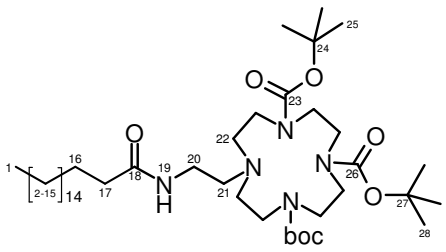
Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

General. Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Detection via UV light at 254 nm / 326 nm or through staining with ninhydrin in EtOH. Column chromatography was performed on silica gel (70–230 mesh) from Merck. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Commercially

available solvents of standard quality were used. Dry THF, which was prepared by distillation from potassium. If otherwise stated, purification and drying was done according to accepted general procedures.[22] Elemental analyses were carried out by the center for Chemical Analysis of the Faculty of Natural Sciences of the University Regensburg.

4.5.2 Synthesis

All compounds synthesized by literature known procedures are pointed in the text and are referred to the citation.

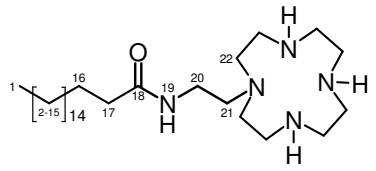


***10-(2-Octadecanoylamino-ethyl)-1,4,7,10 tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester* (10)**

Stearic acid (170 mg, 0.60 mmol), DIPEA (412 µL, 2.39 mmol), TBTU (211 mg, 0.66 mmol), and HOBt (101 mg, 0.66 mmol) were dissolved under nitrogen atmosphere in dry DMF (4 mL) under ice cooling and stirred for 1 h. Subsequently amine **9** (308 mg, 0.60 mmol) dissolved in DMF was added. The reaction was allowed to warm to room temperature and was 3 h at 40 °C. The reaction progress was monitored by TLC (EE). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (EE; R_f = 0.45) yielding compound **10** (320 mg, 0.41 mmol, 68 %) as a colourless oil.

^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 0.86 (t, ^3J = 7.0 Hz, 3 H, HSQC, COSY: C^1H_3), 1.18-1.31 (m, 28 H, HSQC, COSY: C^2H_2-$C^{15}H_2$), 1.43 (s, 9 H, HSQC: $C^{28}H_3$), 1.45 (s, 18 H, HSQC: $C^{25}H_3$), 1.54-1.66 (m, 2 H, HSQC, COSY: $C^{16}H_2$), *rotation isomers:* 2.16^a, 2.29^b (t, $^3J^a$ = 8.0 Hz, $^3J^b$ = 7.6 Hz, 2 H, HSQC, COSY: $C^{17}H_2$), 2.63 (bs, 6 H, HSQC, COSY: $C^{22}H_2$; HSQC, COSY: $C^{21}H_2$), 3.24-3.59 (m, 14 H, HSQC, COSY: $C^{22}H_2$; HSQC, COSY: $C^{20}H_2$), 6.28 (bs, 1 H, HSQC: NH^{19}). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, HSQC, COSY: C^1H_3), *assignment:* 22.6 (–, 1 C),

29.30 (–, 1 C), 29.37 (–, 1 C), 29.50 (–, 1 C), 29.60 (–, 2 C), 29.62 (–, 1 C), 29.65 (–, 6 C), 31.9 (–, 1 C), HSQC, COSY: C^2H_2-$C^{15}H_2$), 25.7 (–, 1 C), HSQC, COSY: C^2H_2-$C^{15}H_2$), 28.5 (+, 6 C, HSQC, COSY: $C^{16}H_2$), 28.6 (+, 3 C, HSQC: $C^{28}H_3$), *rotation isomers:* 33.9 [b], 36.5[a] (–, 1 C, HSQC, COSY: $C^{17}H_2$), 36.5 (–, 1 C, HSQC, COSY: $C^{21}H_2$), 48.1 (–, 4 C, HSQC, COSY: $C^{16}H_2$), 49.9 (–, 2 C, HSQC, COSY: $C^{16}H_2$), 52.7 (–, 1 C, HSQC, COSY: $C^{20}H_2$), 55.0 (–, 1 C, HSQC, COSY: $C^{16}H_2$), 55.6 (–, 1 C, HSQC, COSY: $C^{16}H_2$), 79.6, 79.8 (C_q, 3 C, HSQC, HMBC: C^{24}, C^{27}), 155.4 (C_q, 1 C, HSQC, HMBC: C^{26}), 156.2 (C_q, 2 C, HSQC, HMBC: C^{23}), *rotation isomers:* 173.5[a], 175.9[b] (C_q, 1 C, HSQC, HMBC: C^{18}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2922, 2850, 1689, 1460, 1416, 1365, 1248, 1156, 1019, 772. – **MS** (ESI(+), EE/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 782.7 (100) [MH^+]. – **HRMS** Calcd for $C_{43}H_{83}N_5O_7$ 779.6136; Found: 779.6123. – **MF**: $C_{43}H_{83}N_5O_7$ – **FW**: 782.16 g/mol

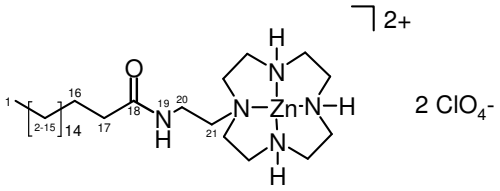


Octadecanoic acid [2-(1,4,7,10 tetraaza-cyclododec-1-yl)-ethyl]-amide **(11)**

Compound **10** (246 mg, 0.31 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently 6.3 mL HCl / ether were added and the solution was stirred for 20 h at room temperature. The solvent was removed in vacuo yielding the protonated hydrochloride of compound **10** quantitative as a colourless solid. To obtain the free base of compound **10** a strong basic ion exchange resin was swollen for 15 min in water/MeOH (8:2) and washed neutral with water. A column was charged with resin (3.5 ml, 40.0 mmol hydroxy equivalents at a given capacity of 0.9 mmol/mL). The hydrochloride salt was dissolved in a mixture of MeOH/water (8:2), put onto the column and eluated with the same solvent mixture. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilized to yield quantitatively 149 mg (0.31 mmol) of free base **10**, as colourless hygroscopic solid.

MP: 148 – 150 °C. – **^{1}H-NMR** (300 MHz; $CDCl_3$): δ (ppm) = 0.84 (t, ^3J = 6.7 Hz, 3 H, C^1H_3), 1.22 (m, 28 H, C^2H_2-$C^{15}H_2$), 1.59 (m, 2 H, $C^{16}H_2$), 2.17 (t, ^3J = 7.4 Hz, 2 H, $C^{17}H_2$), 2.36-3.67 (m, 20 H, $C^{20}H_2$, $C^{21}H_2$, $C^{22}H_2$), 6.78 (bs, 1 H, NH^{19}). – **^{13}C-NMR** (75 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, CH_3), 22.7, 25.8, 29.35, 29.49, 29.56,

29.66, 29.70, 31.9 (–, 14 C, CH_2), 36.7 (–, 1 C, CH_2), 37.5 (–, 1 C, CH_2), 45.4 (–, 2 C, CH_2), 46.1 (–, 2 C, CH_2), 47.2 (–, 2 C, CH_2), 51.5 (–, 2 C, CH_2), 53.8 (–, 2 C, CH_2), 173.6 (C_q, 1 C, R*C*ONHR`). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2916, 2850, 1644, 1546, 1466, 1350, 1276, 812, 718. – **MS** (ESI(+), EE/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 482.4 (100) [MH^+], 262.1 (50) [M + 2 H^+ + MeCN]$^{2+}$, 241.1 (50) [M + 2 H^+]$^{2+}$. – **HRMS** Calcd for $C_{28}H_{59}N_5O$ 482.4798; Found: 482.4785. – **MF**: $C_{28}H_{59}N_5O$ – **FW**: 481.82 g/mol

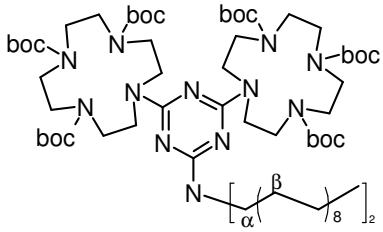


***Mononuclear Zn(II)-cyclen C18 (Zn(II)-MC)* (3)**

Compound **11** (50 mg, 0.10 mmol) was dissolved in 1 mL MeOH and heated to 65 °C to get a clear solution. Subsequently zinc(II)-perchlorate (44 mg, 117 µmol) dissolved in 1 ml MeOH was added slowly to the stirred reaction mixture. The reaction mixture was stirred for additional 19 h at 65 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized to yield 77 mg (0.10 mmol, 100 %) of Zn(II)-MC as a lightly brownish solid.

MP: 161 °C. – **^{1}H-NMR** (600 MHz; $CDCl_3$ / CD_3OD 1:1): δ (ppm) = 0.58 (t, ^{3}J = 7.1 Hz, 3 H, HSQC, COSY: C^1H_3), 0.93-1.06 (m, 28 H, HSQC, COSY: C^2H_2-$C^{15}H_2$), 1.26-1.36 (m, 2 H, HSQC, COSY: $C^{16}H_2$), 2.03 (t, ^{3}J = 7.8 Hz, 2 H, HSQC, COSY: $C^{17}H_2$), 2.45-2.58 (m, 8 H, HSQC, COSY: cyclen-CH_2), 2.59-2.73 (m, 8 H, HSQC, COSY: cyclen-CH_2), 2.75-2.87 (m, 2 H, HSQC, COSY: $C^{20}H_2$), 3.15 (t, ^{3}J = 5.0 Hz, 2 H, HSQC, COSY: $C^{21}H_2$), 3.65 (bs, 1 H, HSQC: NH), 4.24 (bs, 2 H, HSQC: NH). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 13.2 (+, 1 C, HSQC, COSY: C^1H_3), 22.1, 28.65, 28.73, 28.94, 29.08, 31.3(–, 14 C, HSQC, COSY: C^2H_2-$C^{15}H_2$), 25.0 (–, 1 C, HSQC, COSY: $C^{16}H_2$), 35.6 (–, 1 C, HSQC, COSY: $C^{17}H_2$), 36.4 (–, 1 C, HSQC, COSY: $C^{21}H_2$), 42.0 (–, 1 C, HSQC, COSY: cyclen-CH_2), 43.4 (–, 2 C, HSQC, COSY: cyclen-CH_2), 44.0 (–, 1 C, HSQC, COSY: $C^{20}H_2$), 44.1 (–, 1 C, HSQC, COSY: cyclen-CH_2), 50.9 (–, 2 C, HSQC, COSY: cyclen-CH_2), 51.0 (–, 1 C, HSQC, COSY: cyclen-CH_2), 53.7 (–, 1 C, HSQC, COSY: cyclen-CH_2), 178.2 (C_q, 1 C, C^{18}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2917, 2850, 1647, 1625, 1467, 1076, 720, 622. –

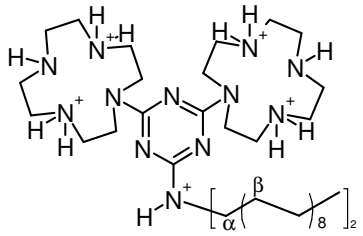
MS (ESI(+),MeOH + 10 mmol/L NH_4Ac): m/z (%) = 544.4 (100) $[M^{2+} - H^+]^+$. – **HRMS** Calcd for $C_{28}H_{58}N_5O$ $[M^{2+} - H^+]^+$ 544.3933; Found: 544.3921. – **MF**: $[C_{28}H_{59}N_5OZn]\ (ClO_4)_2$ – **FW**: 746.09 g/mol



10-[4-(4-tert-Butoxycarbonyl-7-tert-butoxycarbonylmethyl-10-ethoxycarbonyl-1,4,7,10-tetraaza-cyclododec-1-yl)-6-dioctadecylamino-[1,3,5]triazin-2-yl]-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester **(12)**

Protected bis-cyclen **12** (400 mg, 0.38 mmol), di-octadecylamine (494 mg, 0.95 mmol) and K_2CO_3 (157 mg, 1.14 mmol) were dissolved in dioxane (10 mL). The reaction mixture was heated to 90 °C for 24 h. After cooling to rt the solution solidified which further was dissolved in PE and K_2CO_3 was filtered off. The organic layer was washed twice with H_2O and dried over $MgSO_4$. After removing the solvent the crude mixture was chromatographed on flash silica gel (LM: PE/EE 2:1, R_f = 0.4) obtaining compound **13** (468 mg, 0.30 mmol) in 79 % yield as a colourless hygroscopic solid.

^{1}H-NMR (600 MHz; $CDCl_3$): δ (ppm) = 0.85 (t, ^3J = 7.0 Hz, 6 H, HSQC: alkyl-CH_3), 1.23 (s, 60 H, HSQC: alkyl-CH_2), 1.41, 1,43 (s, 54 H, HSQC: boc-CH_3), 1.49 (m, 4 H, β-CH_2), 3.02-3.91 (m, 36 H, cyclen-CH_2, α-CH_2). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 2 C, alkyl-CH_3), 22.7 (–, 2 C, alkyl-CH_2), 27.1 (–, 2 alkyl-C, CH_2), 28.5 (+, 18 C, boc-CH_3), 28.8 (–, 2 alkyl-CH_2), 29.3 (–, 2 C, alkyl-CH_2), 29.7 (–, 22 C, alkyl-CH_2), 31.9 (–, 2 C, alkyl-CH_2), 46.8 (–, 2 C, α-CH_2), 50.3 (–, 16 C, cyclen-CH_2), 79.6 (C_q, 6 C, boc), 155.9 (C_q, 6 C, $NHCOO^tBu$), 164.5 (C_q, 3 C, triazine). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2974, 2923, 2853, 1690, 1533, 1494, 1466, 1407, 1364,1247, 1160, 1103, 1026, 971, 859, 812 , 776. – **MS** (ESI(+),MeOH/DCM + 10 mmol NH_4Ac): m/z (%) = 1542.6 (100) $[MH^+]$, 721.8 (9) $[M + 2\ H^+ - boc]^{2+}$, 693.7 (10) $[M + 2\ H^+ - boc - C_4H_8]^{2+}$, 665.7 (7) $[M + 2\ H^+ - boc - 2\ C_4H_8]^{2+}$, 637.7 (4) $[M + 2\ H^+ - boc - 3\ C_4H_8]^{2+}$. – **MF**: $C_{85}H_{160}N_{12}O_{12}$ – **FW**: 1542.28 g/mol

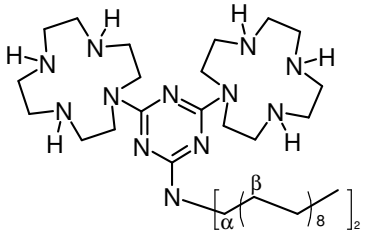


5 CF_3COO^-

[4,6-Bis-(1-aza-4,7,10-azonia-cyclododec-1-yl)-[1,3,5]-triazin-2-yl]-dioctadecyl-amine-heptakis-trifluoroacetate **(14)**

Compound **13** (318 mg, 0.21 mmol) was dissolved in DCM (10 ml) and cooled to 0 °C. To this solution 1.3 mL (1.97 g, 17.3 mmol) TFA was added. The reaction mixture was allowed to warm to rt and stirred for 21.5 h. The solvent was removed in vacuo yielding deprotected product **14** (318 mg, 0.18 mmol, 86 %) as pale yellow oil.

^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 0.85 (t, ^{3}J = 6.8 Hz, 6 H, HSQC: CH_3), 1.23 (s, 60 H, HSQC: alkyl-CH_2 chain), 1.47-1.53 (m, 4 H, β-CH_2), 2.75-2.98 (m, 4 H, cyclen-CH_2), 3.00-3.29 (m, 20 H, cyclen-CH_2), 3.38 (t, ^{3}J = 7.2 Hz, 4 H α-CH_2), 3.59-3.99 (m, 20 H, cyclen-CH_2), 6.66-7.66 (bs, 6 H, NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 2 C, HSQC: CH_3), 22.6 (–, 2 C, alkyl-CH_2), 27.2 (–, 2 C, alkyl-CH_2), 28.1 (–, 2 C, HMBC, COSY: β-CH_2), 29.3 (–, 2 C, alkyl-CH_2), 29.7 (–, 22 C, alkyl-CH_2), 31.9 (–, 2 C, alkyl-CH_2), 45.7, 47.3, 47.7, 48.2, 48.5, 48.8 (–, 16 C, cyclen-CH_2), 47.4 (–, 2 C, HMBC, COSY: α-CH_2), 164.1 (C_q, 1 C, HMBC: C-triazine alkyl chain), 165.5 (C_q, 2 C, HMBC: C-triazine cyclen). – **MS** (ESI(+), MeCN/TFA): m/z (%) = 471.6 (100) $[M + 2\ H^+]^{2+}$, 1056.0 (12) $[MH^+\ TFA]^+$, 521.7 (12) $[M + boc + 2\ H^+]^{2+}$, 1042.1 (6.5) $[MH^+ + boc]^+$, 942 (4) $[MH^+]$. – **MF**: $C_{55}H_{112}N_{12}$ 5 · CF_3COOH – **FW**: 1511.69 g/mol (without TFA: 941.57 g/mol)

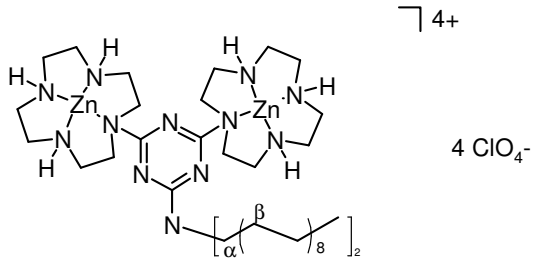


[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-dioctadecyl-amine **(15)**

To obtain the free base of compound **14** (245 mg, 0.14 mmol) a strong basic ion exchange resin was swollen for 15 min in water/MeOH (8:2) and washed with water until a neutral pH was reached. A column was charged with resin (4.7 ml, 4.22 mmol

hydroxy equivalents at a given capacity of 0.9 mmol/mL). The TFA salt **14** was dissolved in water/MeOH (8:2), and passed through column with same solvent mixture. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilized to yield 132 mg (0.14 mmol, 100 %) of free base **15**, as colourless semi-solid.

^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 0.85 (t, ^3J = 6.8 Hz, 6 H, HSQC: CH_3), 1.23 (s, 60 H, HSQC: alkyl-CH_2 chain), 1.47-1.53 (m, 4 H, β-CH_2), 2.75-2.98 (m, 4 H, cyclen-CH_2), 3.00-3.29 (m, 20 H, cyclen-CH_2), 3.38 (t, ^3J = 7.2 Hz, 4 H α-CH_2), 3.59-3.99 (m, 8 H, cyclen-CH_2), 6.66-7.66 (bs, 6 H, NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ(ppm) = 14.0 (+, 2 C, HSQC: CH_3), 22.6 (–, 2 C, alkyl-CH_2), 27.2 (–, 2 C, alkyl-CH_2), 28.1 (–, 2 C, HMBC, COSY: β-CH_2), 29.3 (–, 2 C, alkyl-CH_2), 29.7 (–, 22 C, alkyl-CH_2), 31.9 (–, 2 C, alkyl-CH_2), 45.7, 47.3, 47.7, 48.2, 48.5, 48.8 (–, 16 C, cyclen-CH_2), 47.4 (–, 2 C, HMBC, COSY: α-CH_2), 164.1 (C_q, 1 C, HMBC: C-triazine alkyl chain), 165.5 (C_q, 2 C, HMBC: C-triazine cyclen). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2919, 2850, 1672, 1536, 1490, 1419, 1359, 1174, 1125, 832, 798, 720. – **MS** (ESI(+), MeCN/TFA): m/z (%) = 471.6 (100) $[M + 2\ H^+]^{2+}$, 1056.0 (12) $[MH^+\ TFA]^+$, 521.7 (12) $[M + boc + 2\ H^+]^{2+}$, 1042.1 (6.5) $[MH^+ + boc]^+$, 942 (4) $[MH^+]$. – **HRMS** Calcd for $C_{55}H_{113}N_{12}$: 941.9211; Found: 941.9192. – **MF**: $C_{55}H_{112}N_{12}$ – **FW**: 941.57 g/mol

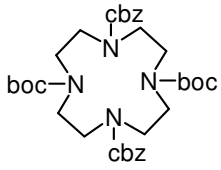


bis-Zn-cyclen bis-octadecylamine (Zn^{2+}-BC bis-C18) (3)

Compound **15** (160 mg, 017 mmol) was dissolved in 12 mL MeOH. To this solution zinc(II)-perchlorate (139 mg, 0.37 mmol) in 3 ml MeOH was slowly added. The reaction mixture was stirred for 7 h at room temperature and then heated to reflux for a further 16 h period. Subsequently the solvent was removed in vacuo, the residue was redissolved by heating in MeOH (1.1 mL) and chloroform was added dropwise till there was a clear solution. The solution was first cooled slowly to room temperature and further put to the fridge. The overlaying methanol solution was removed by decantation

and the colourless gelatine like product was washed with cold MeOH. After drying in vacuo compound **3** (162 mg, 0.11 mmol, 67 %) was obtained as a colourless solid.

MP: 153 °C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 0.87 (t, ^{3}J = 7.0 Hz, 6 H, HSQC: alkyl-CH_3), 1.26 (s, 50 H, HSQC: alkyl-CH_2 chain), 1.30 (bs, 10 H, HSQC: alkyl-CH_2 chain), 1.57 (bs, 4 H, HMBC, COSY: β-CH_2), 2.63-4.43 (m, 42 H, HSQC: cyclen-CH_2, HMBC, COSY: α-CH_2, NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.4 (+, 2 C, HSQC: alkyl-CH_3), 23.4 (–, 2 C, alkyl-CH_2), 27.66 (–, 1 C, alkyl-CH_2), 27.73 (–, 1 C, alkyl-CH_2), 28.7 (–, 2 C, alkyl-CH_2), 30.08, 30.15,30.28, 30.32, 30.36, 30.40 (–, 24 C, alkyl-CH_2), 32.65 (–, 2 C, alkyl-CH_2), 44.2, 44.3, 44.6, 44.7, 45.7, 45.9, 46.2, 46.3, 46.6, 47.1, 47.3, 47.5, 47.6, 48.1, 48.4, 48.6, 49.6 (–, 18 C, HSQC, COSY: cyclen-CH_2, HMBC, COSY: α-CH_2), 165.3 (C_q, 1 C, HMBC: C-triazine alkyl chain), 168.1 (C_q, 1 C, HMBC: C-triazine cyclen), 168.3 (C_q, 1 C, HMBC: C-triazine cyclen). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2921, 2852, 1693, 1561, 1525, 1465, 1420, 1346, 1282, 1085, 812, 622. – **MS** (ESI(+), H_2O/MeOH + 10 mM NH_4Ac): m/z (%) = 595.5 $[M^{4+} + 2\ CH_3COO^-]^{2+}$, 1189.9 (5) $[M^{4+} - H^+ + 2\ CH_3COO^-]^{3+}$, 1065.9 (4) $[MH^+]$. – **MF**: $[C_{55}H_{112}N_{12}Zn_2]\ (ClO_4)_4$ – **FW**: 1470.14 g/mol

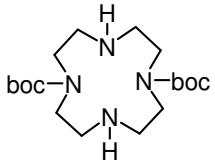


1,7-Cbz-4,10-Boc-cyclen

***10-Phenylacetyl-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid 4-benzyl ester 1,7-di-tert-butyl ester* (19)**

Compound **18** (2.24 g, 5.1 mmol) was dissolved in 300 mL of DCM and a solution of Boc_2O (3.1 g, 14.2 mmol) in DCM (40 mL) was added dropwise in a time period of 15 min. The reaction mixture was stirred for additional 20 h over night at room temperature. Solvent was removed under reduced pressure and the residue was taken up in sat. aq. $NaHCO_3$ and then extracted with DCM (3x). The organic layer was again washed with sat. aq. $NaHCO_3$ (3x), dried over $MgSO_4$ and the solvent was removed. The crude product was purified using flash chromatography (PE/EE 2:1, R_f = 0.2) yielding 1,7-z-4,10-boc-cyclen **19** (2.66 g, 4.2 mmol, 83 %) as a colourless semi-solid.

^{1}H-NMR (300 MHz; $CDCl_3$): δ (ppm) = 1.38 (s, 18 H, boc-CH_3), 3.36 (bs, 16 H, cyclen-CH_2), 5.11 (s, 4 H, Ph-CH_2), 7.17-7.42 (m, 10 H, Ar-H). – **^{13}C-NMR** (75 MHz; $CDCl_3$): δ (ppm) = 28.3 (+, 6 C, boc-CH_3), 50.1 (–, 4 C, cyclen-CH_2), 50.4 (–, 4 C, cyclen-CH_2), 67.2 (–, 2 C, Ph-CH_2), 80.0 (C_q, 2 C, boc), 128.2 (+, 2 C, Ar-CH), 128.4 (+, 4 C, Ar-CH), 128.5 (+, 4 C, Ar-CH), 136.4 (C_q, 2 C, Ar), 156.3 (C_q, 2 C, NHCOO), 156.8 (C_q, 2 C, NHCOO). – **IR** (KBr): $\tilde{\nu}$ [cm^{-1}] = 2974, 2933, 1693, 1470, 1415, 1366, 1246, 1164, 1099, 992, 774, 698. – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 641.3 (36) [MH^+], 658.3 [$M + NH_4^+$], 1299.0 [$2 M + NH_4^+$]. – **Elemental analysis** calcd. (%) for $C_{34}H_{48}N_4O_8$: C 63.73, H 7.55, N 8.74; found C 63.55, H 7.59, N 8.59. – **MF**: $C_{34}H_{48}N_4O_8$ – **FW**: 640.89 g/mol



1,7-bis-Boc-cyclen

***1,4,7,10-Tetraaza-cyclododecane-1,7-dicarboxylic acid di-tert-butyl ester* (20)**

Literature known compound, but different experimental procedure. [23]

Method A: 50 % (w/w) Pd / C

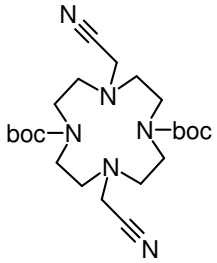
1,7-Cbz-4,10-Boc-cyclene **19** (2.56 g, 4.0 mmol) was dissolved in a mixture of ethanol/cyclohexene (114 mL / 57 mL), palladium on activated charcoal [10 %, 50 % (w/w)] was added to the solution and reaction mixture was refluxed for 17 h. The reaction progress was monitored by TLC (EE/PE 1:1). Palladium on charcoal was filtered of through a pad of basic celite. The solvent was removed under reduced pressure giving 1,7-Boc-cyclene **20** (1.49 g, 4.0 mmol, 100 %) as a white solid.

Method B: catalytic Pd / C

1,7-Cbz-4,10-Boc-Cyclene **19** (1.19 g, 1.9 mmol) was dissolved in EtOH (20 mL), to which a spatula tip 10 % Pd/C were added. The reaction mixture was stirred 7 days in an autoclave under 20 bar H_2 pressure. The suspension was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. **20** (595 mg, 86 %) was isolated as colourless oil without further purification.

MP: 187 °C. – **^{1}H-NMR** (300 MHz; $CDCl_3$): δ (ppm) = 1.40 (s, 18 H, boc-CH_3), 2.64-3.18 (m, 8 H, cyclen-CH_2), 3.22-3.91 (m, 8 H, cyclen-CH_2). – **^{13}C-NMR** (75 MHz;

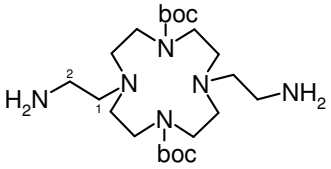
$CDCl_3$): δ (ppm) 28.4 (+, 6 C, boc-CH_3), 48.8, 49.2, 49.6, 49.7, 49.8, 50.5. 50.6 (–, 8 C, cyclen-CH_2), 80.8, 81.0 (C_q, 2 C, boc), 155.4 (C_q, 2 C, $NHCOO^tBu$). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ [cm] = 3189, 2976, 2936, 2773, 1693, 1473, 1414, 1366, 1276, 1251, 1164, 775. – **MS** (ESI(+), DCM/MeCN/TFA): m/z (%) = 373.2 (100) [MH^+]. – **HRMS** Calcd for $C_{18}H_{36}N_4O_4$ 373.2815; Found: 373.2813. – **MF**: $C_{18}H_{36}N_4O_4$ – **FW**: 372.51 g/mol



***4,10-Bis-cyanomethyl-1,4,7,10tetraaza-cyclododecane-1,7-dicarboxylic acid di-tert-butyl ester* (21)**

Compound **20** (1.98 g, 5.3 mmol) was dissolved in MeCN (40 mL), K_2CO_3 (1.76 g, 12,8 mmol) and bromoacetonitrile (812 µL, 11.7 mmol) were added. The reaction mixture was stirred at 50 °C for 18 h and the reaction progress was monitored by TLC. K_2CO_3 was filtered off and the filtrate was concentrated. The crude product was purified using flash chromatography (EE/PE 2:1, R_f = 0.48) obtaining **21** (1.58 g, 3.5 mmol, 66 %) as a white solid.

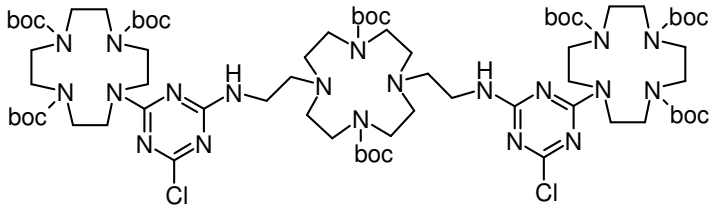
MP: 106 °C. – **^{1}H-NMR** (600 MHz; $CDCl_3$): δ (ppm) = 1.38 (s, 18 H, boc-CH_3), 2.69 (bs, 8 H, cyclen-CH_2), 3.10-3.44 (m, 8 H, cyclen-CH_2), 3.69 (bs, 4 H, spacer-CH_2). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) 28.5 (+, 6 C, HSQC: boc-CH_3), 42.3 (–, 2 C, HSQC, COSY: CH_2CN), 45.9 (–, 2 C, HSQC: cyclen-CH_2), 46.3 (–, 2 C, HSQC: cyclen-CH_2), 54.2 (–, 2 C, HSQC: cyclen-CH_2), 55.2 (–, 2 C, HSQC: cyclen-CH_2), 80.2 (C_q, 2 C, HSQC: boc), 115.0 (C_q, 2 C, HSQC: CN), 155.7 (C_q, 2 C, HSQC: $NHCOO^tBu$). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2974, 2826, 2229, 1690, 1454, 1413, 1365, 1275, 1244, 1157, 1014, 858, 769. – **MS** (ESI(+), MeOH/DCM + 10 mmol NH_4Ac): m/z (%) = 451.3 (100) [MH^+], 395.2 (30) [MH^+ - C_4H_8], 351.2 (18) [MH^+ - boc], 473.3 (17) [MNa^+], 251.1 (9) [MH^+ - 2 boc], 489.3 (8) [MK^+]. – **Elemental analysis** calcd. (%) for $C_{22}H_{38}N_6O_4$: C 58.64, H 8.50, N 18.65; found C 58.13, H 8.79, N 17.96. – **HRMS** Calcd for $C_{22}H_{38}N_6O_4$ 450.2954; Found: 450.2950. – **MF**: $C_{22}H_{38}N_6O_4$ – **FW**: 450.59 g/mol



***4,10-Bis-(2-amino-ethyl)-1,4,7,10 tetraaza-cyclododecane-1,7-dicarboxylic acid di-tert-butyl ester* (17)**

Nitrile **21** (400 mg, 0.89 mmol) was dissolved in NH_3 saturated ethanol and catalytic amounts of Raney Ni was added to the solution. The reaction mixture was stirred in autoclave at rt at 25 bar H_2 pressure for 4 days. The catalyst was filtered off through a pad of basic celite. Reaction control was done by TLC (EE/PE 2:1) and NMR. After removal of the solvent under reduced pressure the amine **17** (358 mg, 0.78 mmol, 88 %) was obtained as a colourless oil.

^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 1.41 (s, 18 H, boc-CH_3), 2.46 (t, ^{3}J = 6.2 Hz, 4 H, HMBC: C^1H_2), 2.65 (m, 8 H, cyclen-CH_2), 2.72 (t, ^{3}J = 6.2 Hz, 4 H, HMBC: C^2H_2), 3.33 (bs, 8 H, cyclen-CH_2). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) 28.4 (+, 6 C, HSQC: boc-CH_3), 39.5 (–, 2 C, HMBC: C^2H_2), 47.3 (–, 4 C, HSQC: cyclen-CH_2), 54.7 (–, 4 C, HSQC: cyclen-CH_2), 58.1 (–, 2 C, HMBC: C^2H_2), 79.4 (C_q, 2 C, HMBC, HSQC: boc), 155.9 (C_q, 2 C, HMBC, HSQC: NHCOO^{t}Bu). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3349, 2972, 2925, 2816, 1680, 1570, 1460, 1411, 1364, 1300, 1281, 1250, 1155, 965, 860, 819, 753. – **MS** (ESI(+), DCM/MeCN/TFA): m/z (%) = 459.4 (100) [MH^+], 230.1 (46) [M + 2 H^+]$^{2+}$, 250.6 (40) [M + 2 H^+ MeCN] $^{2+}$. – **HRMS** Calcd for $C_{22}H_{47}N_6O_4$: 459.3659; Found: 459.3648. – **MF**: $C_{22}H_{46}N_6O_4$ – **FW**: 458.65 g/mol

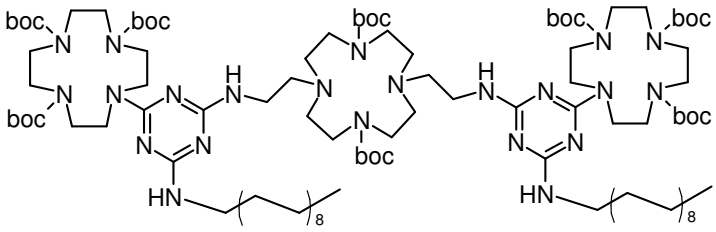


***6-[2-(7-{2-[4-Chloro-6-(1,4,7,10-tetraaza-cyclododec-1-yl)-1,6-tricarboxyl acid tri-tert-butyl-[1,3,5]triazin-2-ylamino]-ethyl}-1,4,7,10-tetraaza-cyclododec-1-yl-1,6tricarboxyl acid tri-tert-butyl-)-ethylamino]-[1,3,5]triazin-2-yl}-1,3,6,9-tetraaza-cycloundecane-1,1´,6,6´,9,9´-tricarboxylic acid tri-tert-butyl ester* (22)**

Compound **16** (298 mg, 0.48 mmol) and K_2CO_3 (151 mg, 1.09 mmol) were suspended in MeCN (2 mL) and vigorously stirred. A solution of free amine **17** (100 mg,

0.22 mmol) in MeCN (4 mL) was added to the stirred mixture. Reaction progress was monitored by TLC. The reaction solution was stirred 3 days at rt. K_2CO_3 was filtered off and the filtrate was concentrated. The crude product was purified by flash column chromatography using flash silica gel (EE/PE 8:2 → EE/MeOH 9:1; R_f = 0.12) yielding the skeletal structure **22** (157 mg, 0.10 mmol, 45 %) as colorless solid.

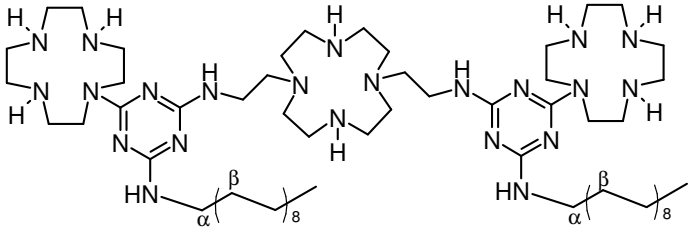
MP: 153 °C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 1.39 (s, 54 H, boc-CH_3), 1.42 (s, 18 H, boc-CH_3), 2.47-2.90 (m, 12 H, cyclen-CH_2, spacer-CH_2), 3.11-3.83 (m, 44 H, CH_2, cyclen-CH_2, spacer-CH_2). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ(ppm) = 28.34, 28.39 (+, 24 C, boc-CH_3), 38.1 (–, 2 C, spacer-CH_2), 47.8, 49.8, 50.4, 51.0 (–, 20 C, cyclen-CH_2), 53.4 (–, 2 C, spacer-CH_2), 54.5 (–, 4 C, cyclen-CH_2), 79.9, 80.2 (C_q, 8 C, boc), 156.0, 156.2, 157.1 (C_q, 8 C, boc), 165.2, 165.7, 168.5, 169.3 (C_q, 6 C, triazine). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2975, 2931, 2818, 2361, 1693, 1571, 1529, 1466, 1366, 1249, 1163, 1029, 972, 859, 775. – **MS** (ESI(+),MeOH/DCM + 10 mmol NH_4Ac): m/z (%) = 1626.4 (100) [MH^+], 813.7 (61) [$M + 2\ H^+]^{2+}$, 764.6 (39) [$M + 2\ H^+$ - boc]$^{2+}$. – **MF**: $C_{74}H_{130}N_{20}O_{16}$ – **FW**: 1626,89 g/mol



***N-Octadecyl-6-[2-(7-{2-[4-chloro-6-(1,4,7,10-tetraaza-cyclododec-1-yl)-1,6-tricarboxyl acid tri-tert-butyl-[1,3,5]triazin-2-ylamino]-ethyl}-1,4,7,10-tetraaza-cyclododec-1-yl-1,6tricarboxyl acid tri-tert-butyl-)-ethylamino]-n-octadecyl-6-[1,3,5]triazin-2-yl}-1,3,6,9tetraaza-cycloundecane-1,1´, 6,6´, 9,9´-tricarboxylic acid tri-tert-butyl ester* (23)**

Compound **22** (273 mg, 0.17 mmol), K_2CO_3 (70 mg, 0.50 mmol) and octadecylamine (117 mg, 0.44 mmol) were suspended in dry MeCN (8 mL) and vigorously stirred. The solution was heated to reflux for 24 h and reaction progress was monitored by TLC. Subsequently K_2CO_3 was filtered off and the filtrate was concentrated. The crude product was purified by flash column chromatography using flash silica gel ($CHCl_3$/MeOH 97.5 : 2.5; R_f = 0.3) yielding compound **23** (244 mg, 0.12 mmol, 69 %) as colourless oil.

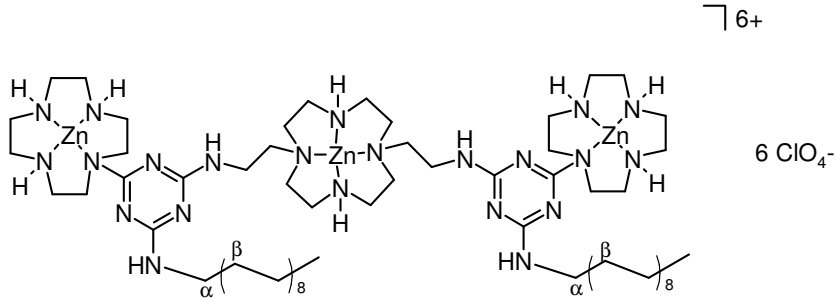
^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 0.86 (t, ^3J = 7.0 Hz, 6 H, alkyl-CH_3), 1.23 (s, 60 H, alkyl-CH_2), 1.41 (s, 76 H, boc-CH_3, β-CH_2), 2.20-2.92 (m, 12 H, cyclen-CH_2, spacer-CH_2), 3.13-3.87 (m, 48 H, cyclen-CH_2, spacer-CH_2, α-CH_2). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 2 C, alkyl-CH_3), 22.6 (–, 2 C, CH_2), 27.0 (–, 2 C, CH_2), 28.47, 28.50 (–, 24 C, boc-CH_3), 29.32 (–, 2 C, CH_2), 29.53, 29.61, 29.66 (–, 22 C, alkyl-CH_2), 30.1 (–, 2 C, CH_2), 31.8 (–, 2 C, CH_2), 40.6 (–, 2 C, alkyl-CH_2), 47.7 (–, 4 C, CH_2), 50.1, 50.4 (–, 18 C, CH_2), 54.2, 54.6, 55.1 (–, 6 C, CH_2,), 79.51, 79.59, 79.8 (C_q, 8 C, boc), 156.0, 156.3 (C_q, 8 C, boc), 165.7 (C_q, 6 C, triazine). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2973, 2925, 2853, 1688, 1549, 1465, 1408, 1363, 1247, 1157, 1104, 972, 860, 812, 775. – **MS** (ESI(+),MeOH/DCM + 10 mmol NH_4Ac): m/z (%) = 1047.2 (100) $[M + 2\ H^+]^{2+}$, 2093.3 (45) $[MH^+]$. – **MF**: $C_{110}H_{206}N_{22}O_{16}$ – **FW**: 2093.01 g/mol



2N-Octadecyl-6-(1,4,7,10-tetraaza-cyclododec-1-yl)-N'-octadecyl-[2-(7-{2-[4-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-[1,3,5]triazine-2,4-diamine **(24)**

Compound **23** (236 mg, 0.113 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently 10 mL HCl saturated ether were added and the solution was stirred for 21 h at room temperature. The solvent was removed in vacuo yielding the protonated hydrochloride of compound **24** as a colourless solid (179 mg, 0.113 mmol). To obtain the free base of compound **24** a strong basic ion exchange resin was swollen for 15 min in water/MeOH (8:2) and washed neutral with water. A column was charged with resin (5.4 ml, 40.0 mmol hydroxy equivalents at a given capacity of 0.9 mmol/mL). The hydrochloride salt was dissolved in water/MeOH (8:2), loaded onto the column and eluated with the same solvent mixture. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilized to yield 136 mg (0.105 mmol, 92 %) of free base **24** as colourless solid.

MP: 62 °C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 0.80 (t, ^{3}J = 6.9 Hz, 6 H, alkyl-CH_3), 1.18 (s, 60 H, alkyl-CH_2), 1.42 (m, 4 H, β-CH_2), 2.52-3.75 (m, 60 H, α-CH_2, spacer-CH_2, cyclen-CH_2). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 2 C, alkyl-CH_3), 22.7 (–, 2 C, alkyl-CH_2), 27.1 (–, 2 C, alkyl-CH_2), 29.3 (–, 2 C, alkyl-CH_2), 29.5 (–, 2 C, alkyl-CH_2), 29.63, 29.69 (–, 20 C, alkyl-CH_2), 30.0 (–, 2 C, alkyl-CH_2), 31.9 (–, 2 C, alkyl-CH_2), 39.6, 40.7, 40.8, 45.3, 46.6, 48.2, 50.8, 52.0, 54.0 (–, 30 C, α-CH_2, spacer-CH_2, cyclen-CH_2), 165.7 (C_q, 6 C, triazine). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3274, 2921, 2851, 1547, 1497, 1463, 1419, 1352, 1308, 1119, 1062, 916, 810, 719, 648. – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 431.6 (100) $[M + 3\ H^+]^{3+}$, 664.8 (19) $[M + 2\ H^+ + HCl]^{2+}$, 647.3 (4) $[M + 2\ H^+]^{2+}$, 1328.5 (1.4) $[MH^+ + HCl]^+$, 1364.5 $[MH^+ + 2\ HCl]^+$, 1292.3 (0.7) $[MH^+]^+$. – **HRMS** Calcd for $C_{70}H_{143}N_{22}$: 1292.1866; Found: 1292.1818. – **MF**: $C_{70}H_{142}N_{22}$ – **FW**: 1292.06 g/mol



Tris-Zn(II)-cyclen C18 (Zn^{2+}-TC) (5)

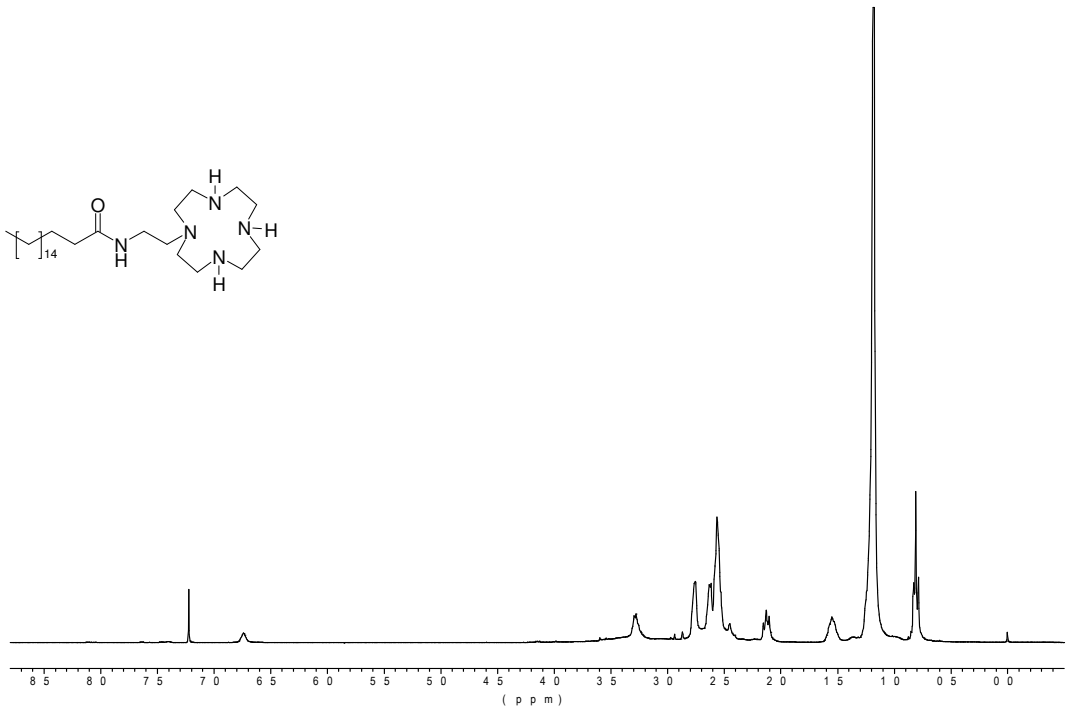
Compound **24** (142 mg, 0.11 mmol) was dissolved in 10 ml MeOH and $Zn(ClO_4)_2 \cdot 6\ H_2O$ (123 mg, 0.33 mmol) in 3 mL MeOH were added slowly under vigorous stirring. The reaction mixture was heated to reflux for 22 h at room. The solvent is removed in vacuo. The residue was redissolved in water and lyophilized obtaining the tris-Zn-cyclen-bis-octadecylamine complex as a pale ochre solid (227 mg, 0.11 mmol, 99 %).

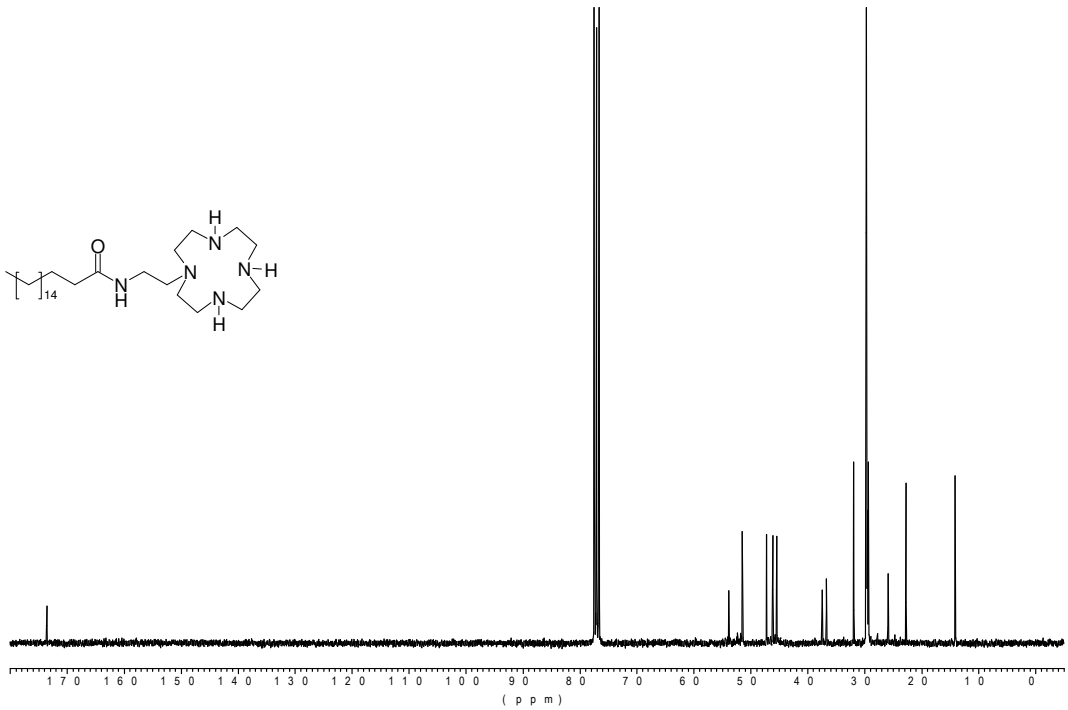
MP: >200 °C. – **^{1}H-NMR** (400 MHz; CD_2Cl_2 / CD_3OD 1:1): δ (ppm) = 0.86 (t, ^{3}J = 7.0 Hz, 6 H, alkyl-CH_3), 1.25 (s, 60 H, alkyl-CH_2), 1.49-1.65 (m, 4 H, β-CH_2), 2.53-4.49 (m, 60 H, α-CH_2, spacer-CH_2, cyclen-CH_2). – **^{13}C-NMR** (100 MHz; CD_2Cl_2 / CD_3OD 1:1): δ (ppm) = 14.3 (+, 2 C, alkyl-CH_3), 23.3 (–, 2 C, alkyl-CH_2), 27.7 (–, 2 C, alkyl-CH_2), 30.18, 30.34, 30.39 (–, 26 C, alkyl-CH_2), 32.6 (–, 2 C, alkyl-CH_2), 40.2, 41.6, 41.8, 44.3, 44.8, 46.2, 46.3, 47.0, 47.3, 49.0, 50.6, 53.1 (–, 30 C, α-CH_2, spacer-CH_2, cyclen-CH_2, solvent peak: CD_3OD), 166.6, 171.0 (C_q, 6 C, triazine). –

IR (ATR) [cm^{-1}]: $\tilde{\nu}$ [cm] = 3532, 3288, 2924, 2852, 1638, 1561, 1458, 1431, 1351, 1288, 1064, 969, 813, 784. – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 534.9 (100) $[M^{6+} - H^+ + 2\ CH_3COO^-]^{3+}$, 555.0 (49) $[M^{6+} + 3\ CH_3COO^-]^{3+}$, 801.8 (36) $[M^{6+} - 2\ H^+ + 2\ CH_3COO^-]^{2+}$, 851.8 (45) $[M^{6+} - H^+ + 2\ CH_3COO^- + ClO_4]^{2+}$. – **MF**: $[C_{70}H_{142}N_{22}Zn_3]\ (ClO_4)_6$ – **FW**: 2084.89 g/mol

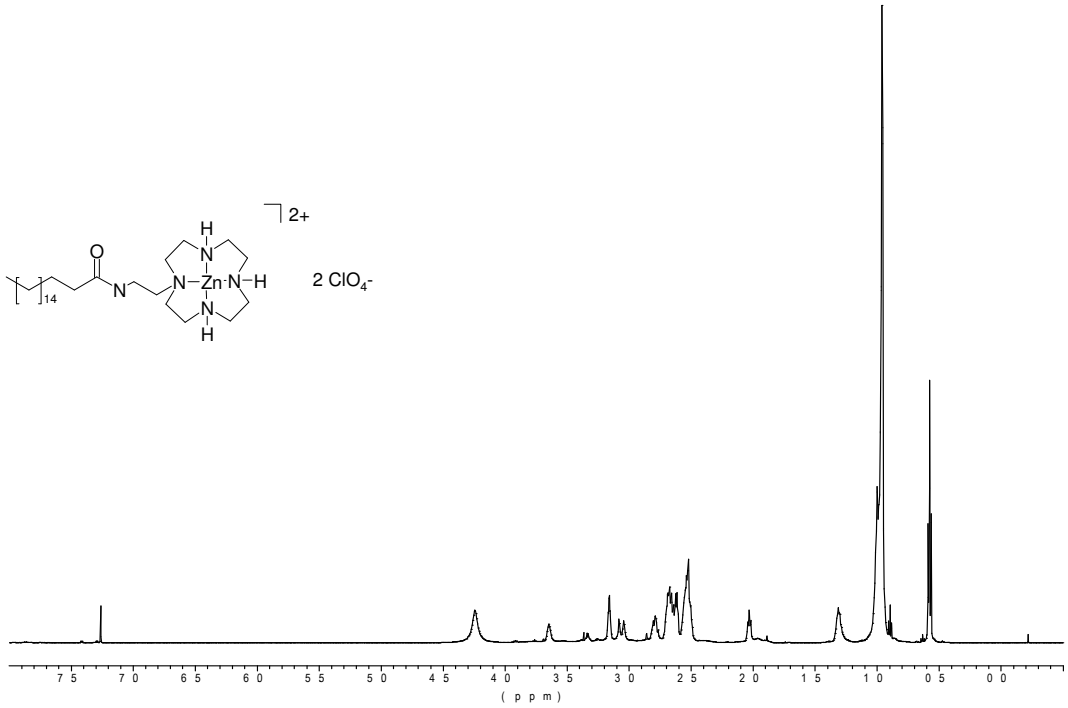
4.5.3 ^{1}H and ^{13}C spectra of prepared compounds

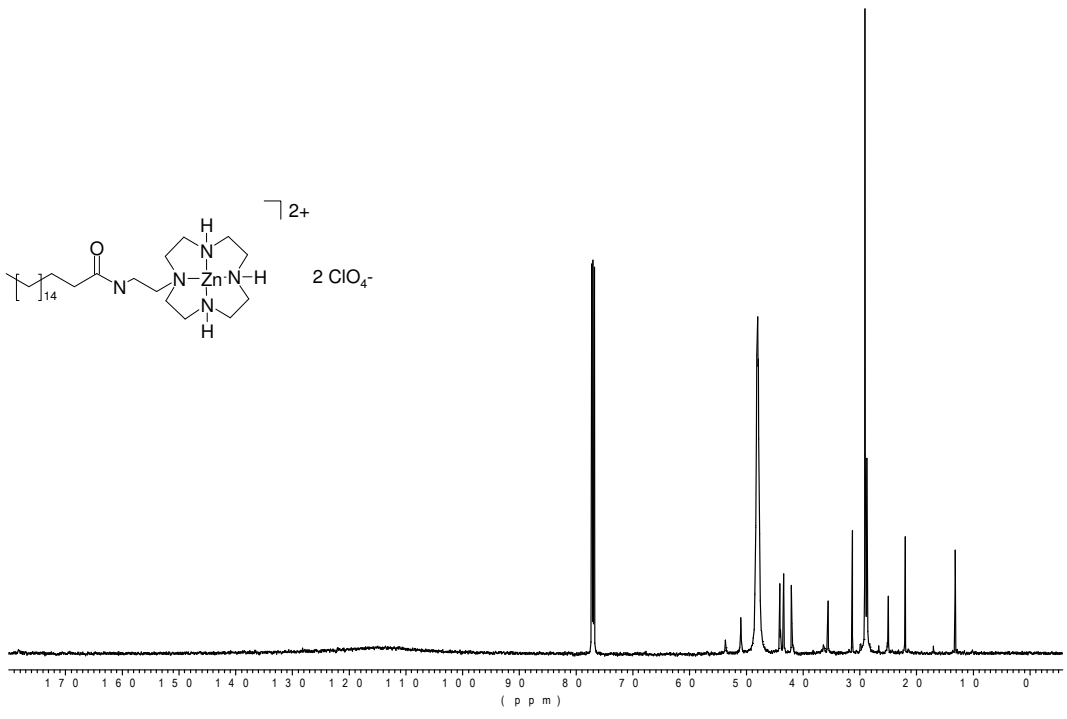
Compound **11**



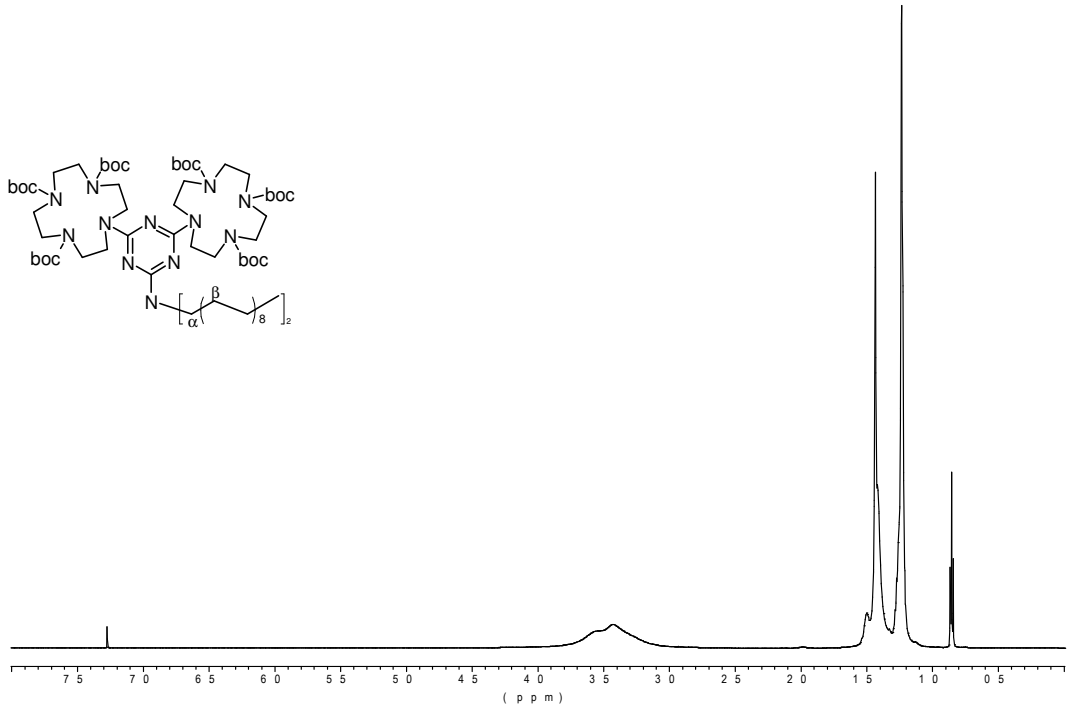


Compound **3**





Compound **13**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N
β
α
8
2
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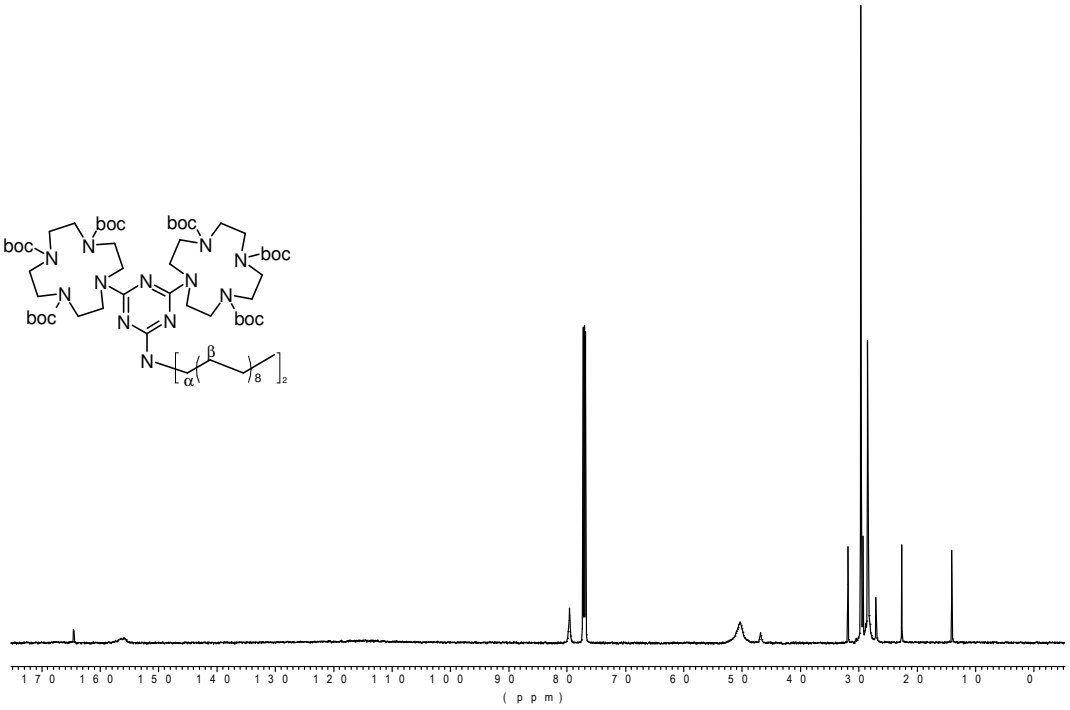
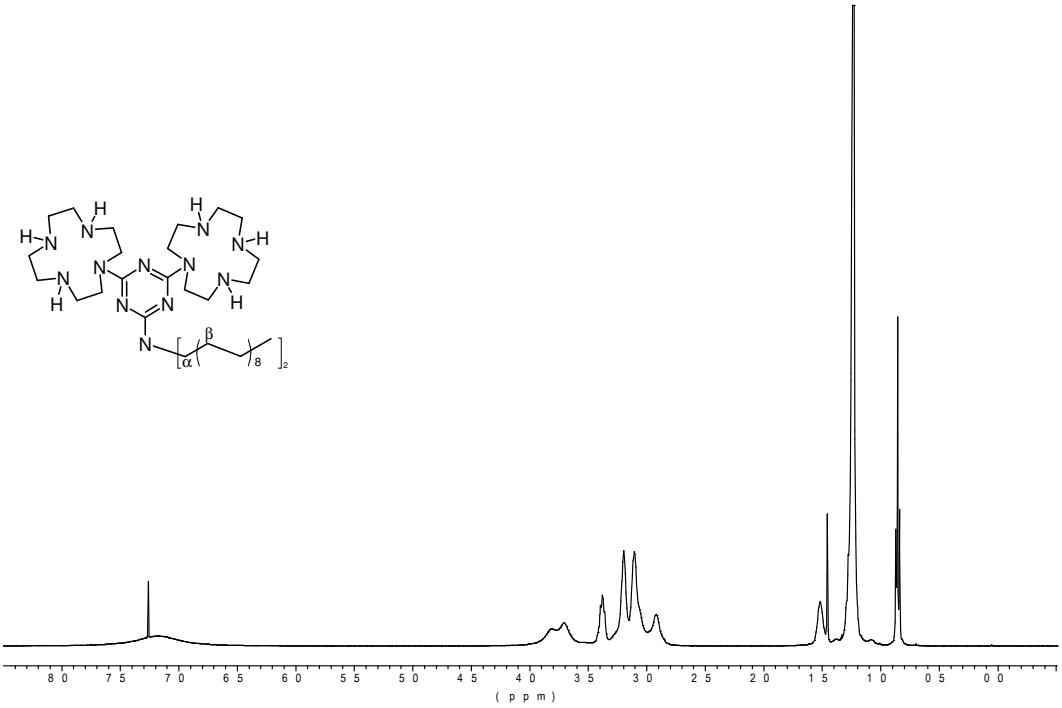
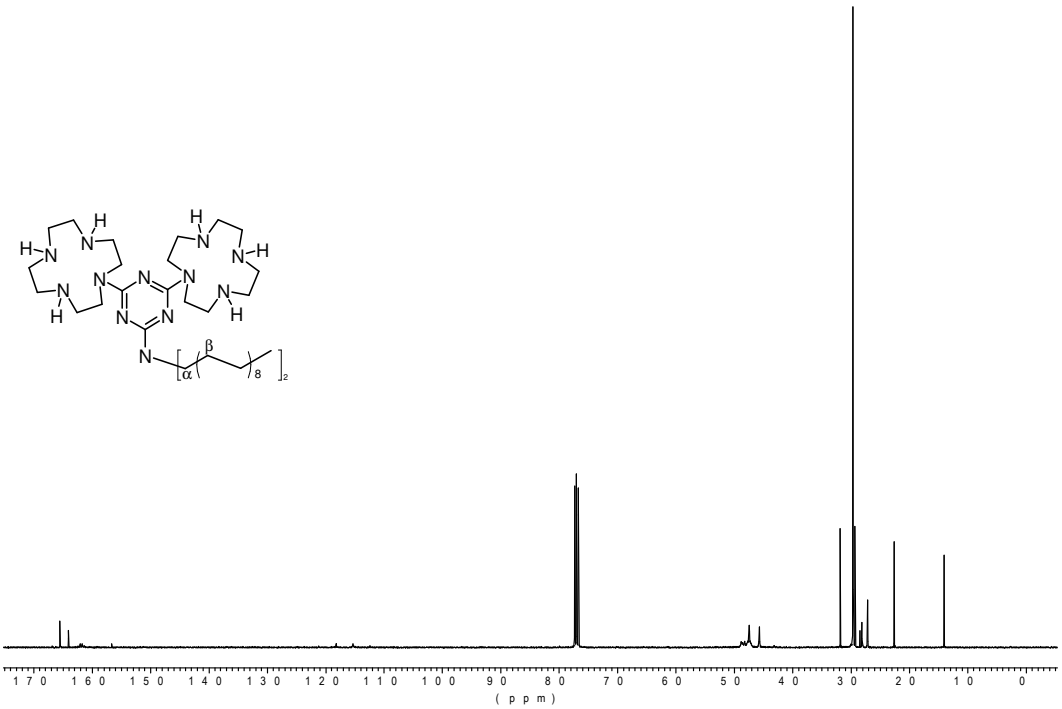
(ppm)

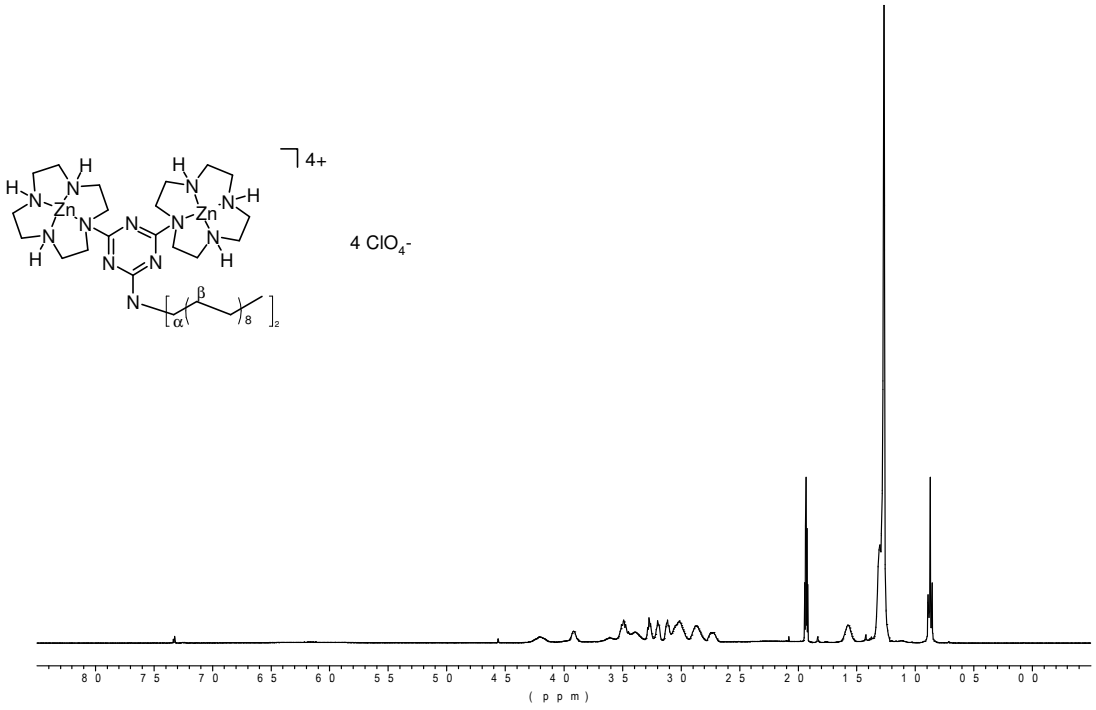

boc
N
β
α
8
2
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

Compound **15**





Compound **4**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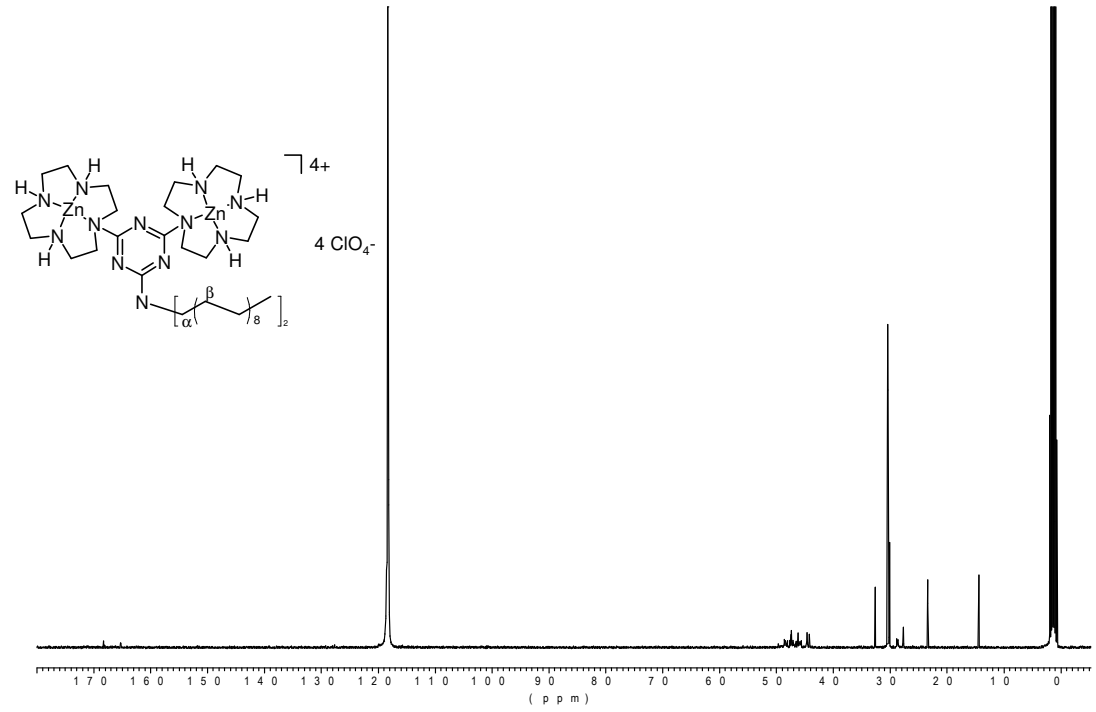
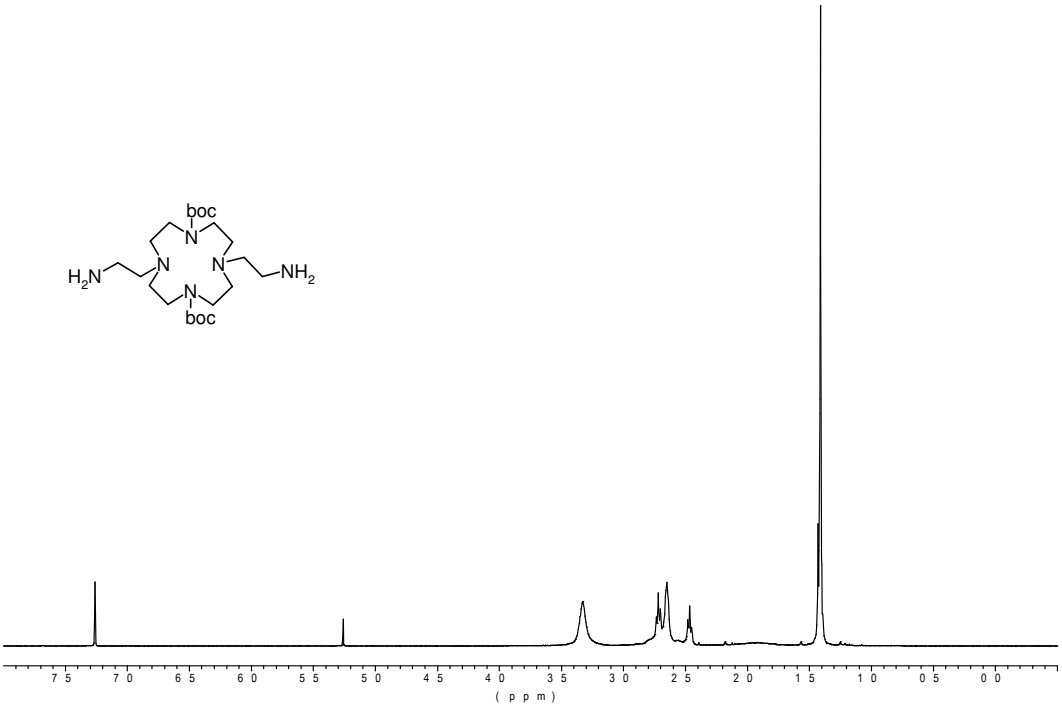
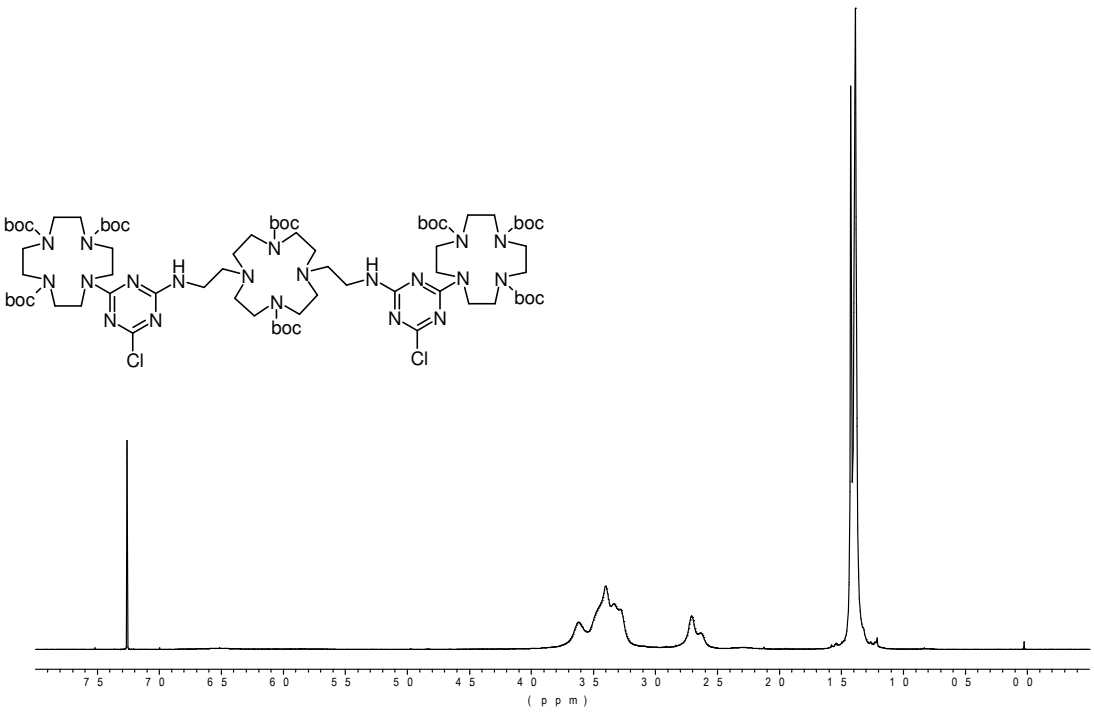
4 ClO4-
80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 05 00
(ppm)


4+
4 ClO4-
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

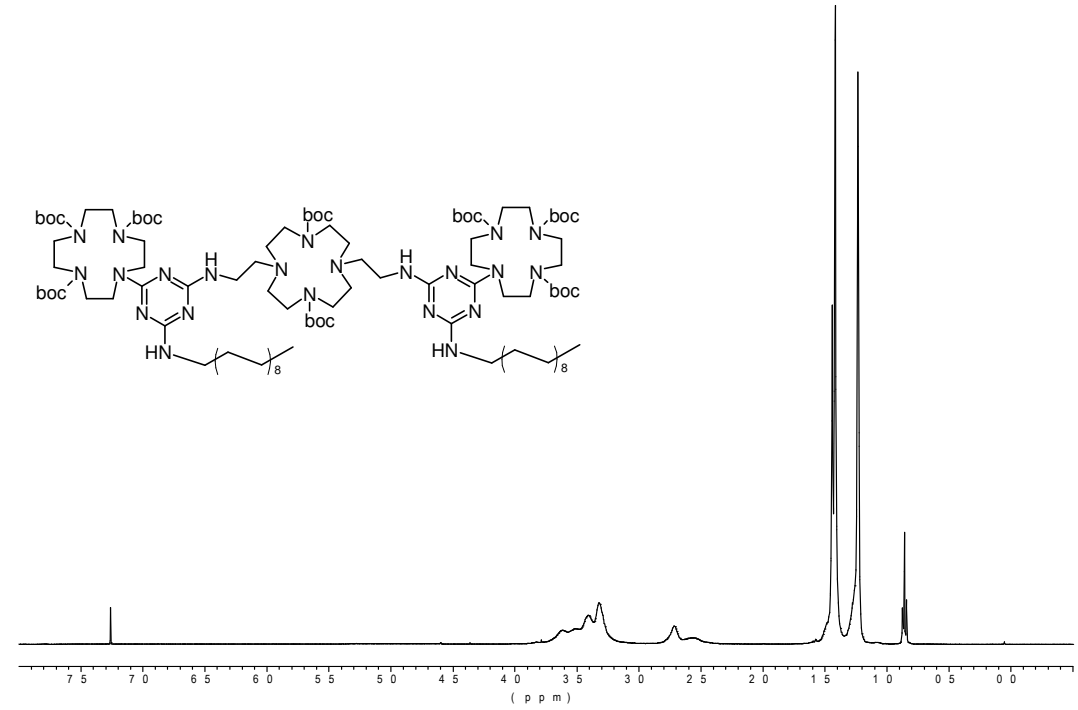
Compound **17**

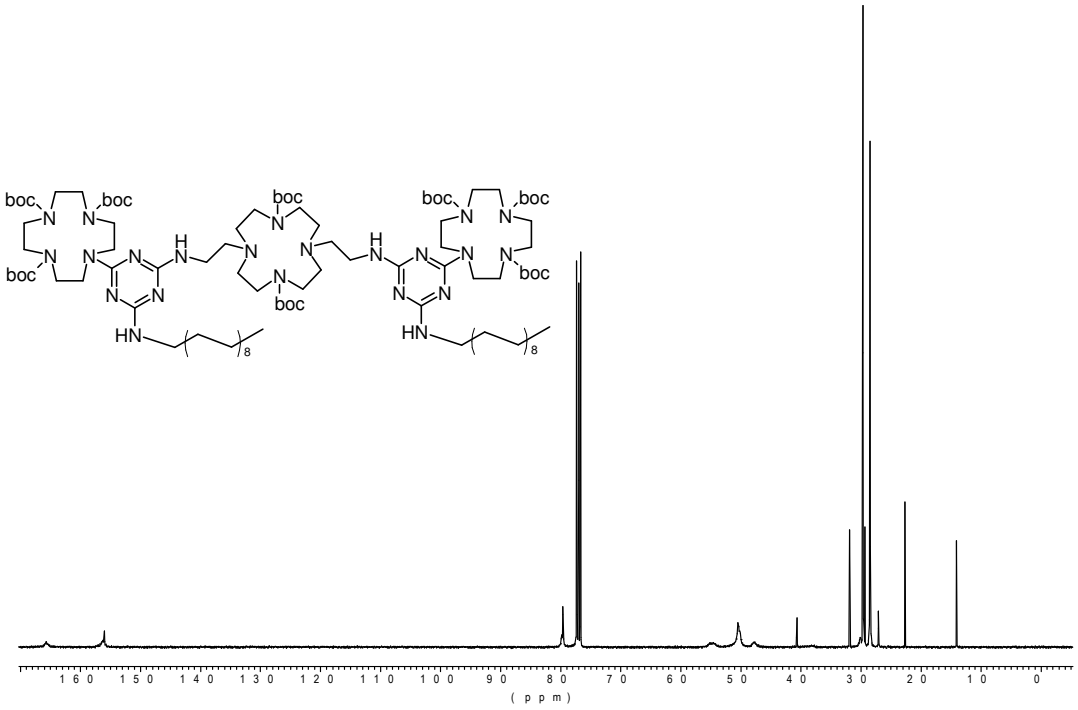


Compound **22**

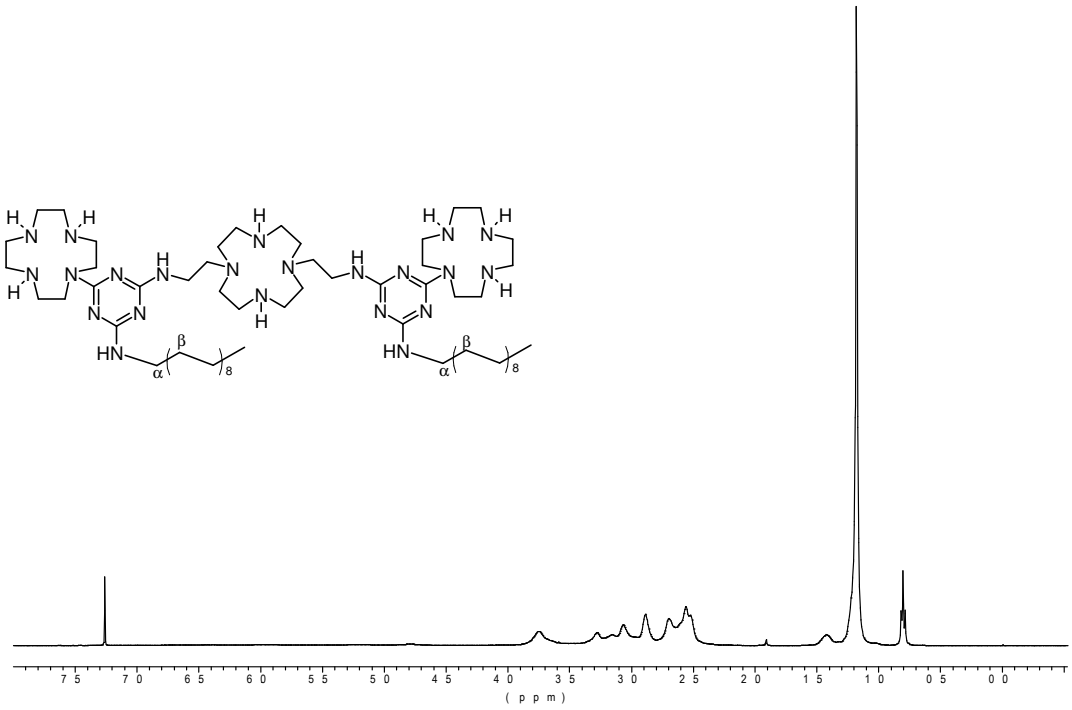


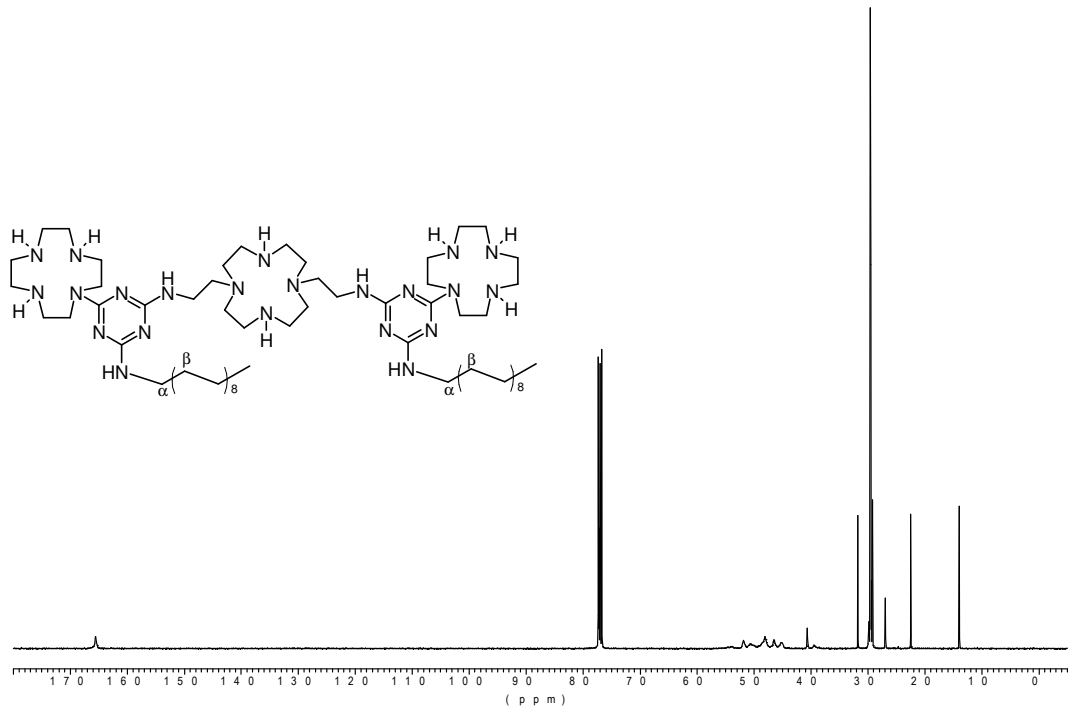
Compound **23**


boc
N
H
HN
8
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
(ppm)

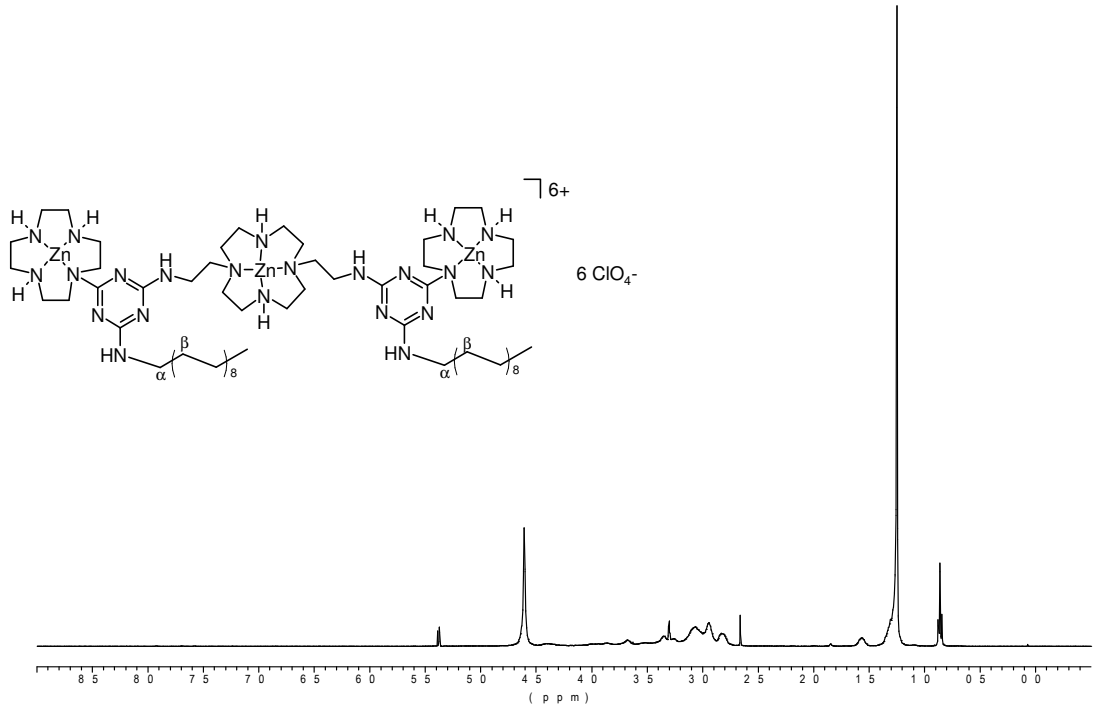

boc
N
H
HN
8
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

Compound **24**


7 5 7 0 6 5 6 0 5 5 5 0 4 5 4 0 3 5 3 0 2 5 2 0 1 5 1 0 0 5 0 0
(p p m)


1 7 0 1 6 0 1 5 0 1 4 0 1 3 0 1 2 0 1 1 0 1 0 0 9 0 8 0 7 0 6 0 5 0 4 0 3 0 2 0 1 0 0
(p p m)

Compound **5**


6+
6 ClO4-
HN
α
β
8
85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 05 00
(ppm)

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5. Polydiacetylene Based Colorimetric Self Assembled Vesicular Receptors for Biological Phosphate Ion Recognition[i]

This chapter deals with the preparation of self assembled vesicular polydiacetylene (PDA) particles with embedded metal complex receptor sites. The particles respond to the presence of ATP and PPi (pyrophosphate) in buffered aqueous solution by visible changes of their colour and emission properties. Blue PDA vesicles of uniform size of about 200 nm were obtained upon UV irradiation from mono- and dinuclear zinc(II)-cyclen and iminodiacetato copper [Cu(II)-IDA] modified diacetylenes, embedded in amphiphilic diacetylene monomers.[ii] Addition of ATP and PPi to the PDA vesicle solution induces a colour change from blue to red observable by the naked eye. The binding of ATP and PPi changes the emission intensity. Other anions like ADP, AMP, $H_2PO_4^-$, CH_3COO^-, F^-, Cl^-, Br^- and I^- failed to induce any spectral changes.[iii] The zinc(II)-cyclen nanoparticles are useful for the facile detection of PPi and ATP in millimolar concentrations in neutral aqueous solutions, while Cu(II)-IDA modified vesicular PDA receptors are able to selectively discriminate between ATP and PPi.

[i] D. A. Jose, S. Stadlbauer, B. König *Chem. Eur. J.* **2009**, *in print*.
[ii] Compound **8-Zn** was prepared by S. Stadlbauer, all other compounds including vesicles were prepared by Dr. D. A. Jose.
[iii] All analytical measurements were performed by Dr. D. A. Jose.

5.1 Introduction

The development of chemical sensors continues to be of great interest in modern analytical chemistry.[1] Demands arise from clinical diagnostics, environmental and food analysis or in the detection of illicit drugs, explosives and chemical warfare agents. A molecular chemical sensor typically consists of the analyte binding site and a signaling unit, which transduces the binding event into a macroscopically observable output, e.g. a colour change or a light emission. For the development of devices it is often useful to immobilize the sensor molecule on surfaces, in membranes, gels or on nanoparticles. Immobilization can be achieved, among other methods,[2] by the incorporation of receptors into self-assembled monolayers or bilayer membranes.[3] Polydiacetylene (PDA) is a particular interesting material in this respect, as diacetylene surfactants will self-assemble in water to form vesicles that can be photopolymerized to generate PDA in situ.[4,5,6] The polymers conjugated backbone provides absorbance and fluorescence properties which are useful for the transduction of the analytical signal.[7] Functionalized polydiacetylene films and vesicles have been used as chemosensors to monitor metal ions,[7a] glucose,[8] proteins[9] and for the colorimetric detection of the influenza virus.[10]

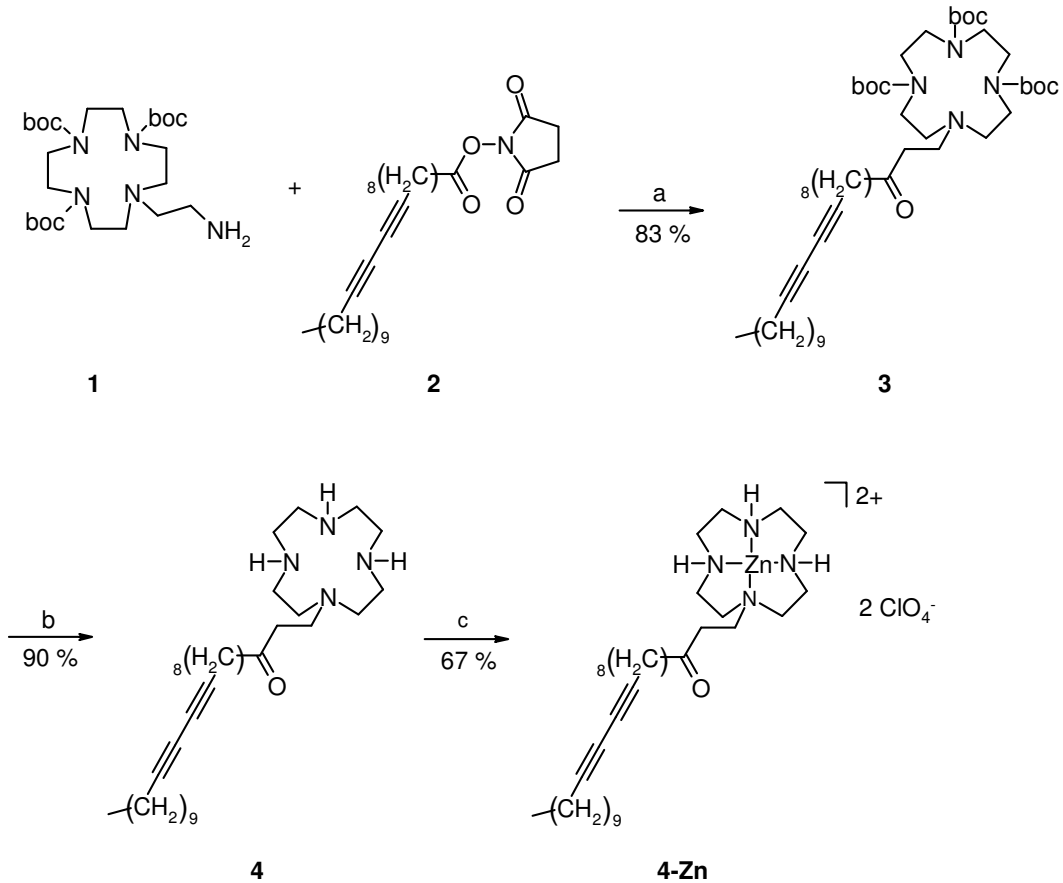
We have recently reported the use of amphiphilic metal complex binding sites for molecular recognition on ordered surface bilayers.[11] Lewis-acidic metal complexes, such as zinc(II) cyclen (cyclen = 1,4,7,10-tetraazacyclododecane) complexes, can reversibly coordinate oxoanions and other Lewis basic guest molecules with high affinity and selectivity.[12, 13] The binding is even possible in the presence of competing solvent molecules, such as water. This renders these metal complexes particular suitable as binding sites for anionic analytes[14] of biological origin under physiological conditions.

We herein describe the preparation of self-assembled PDA vesicles with embedded amphiphilic metal zinc(II) cyclen and Cu(II)-IDA complexes. The PDA polymer nanoparticles act as solid support for the receptor sites and as the chromophore of the signaling unit.

5.2 Result and discussion

5.2.1 Synthesis of amphiphilic diacetylene zinc(II) cyclen and copper(II)-IDA complexes

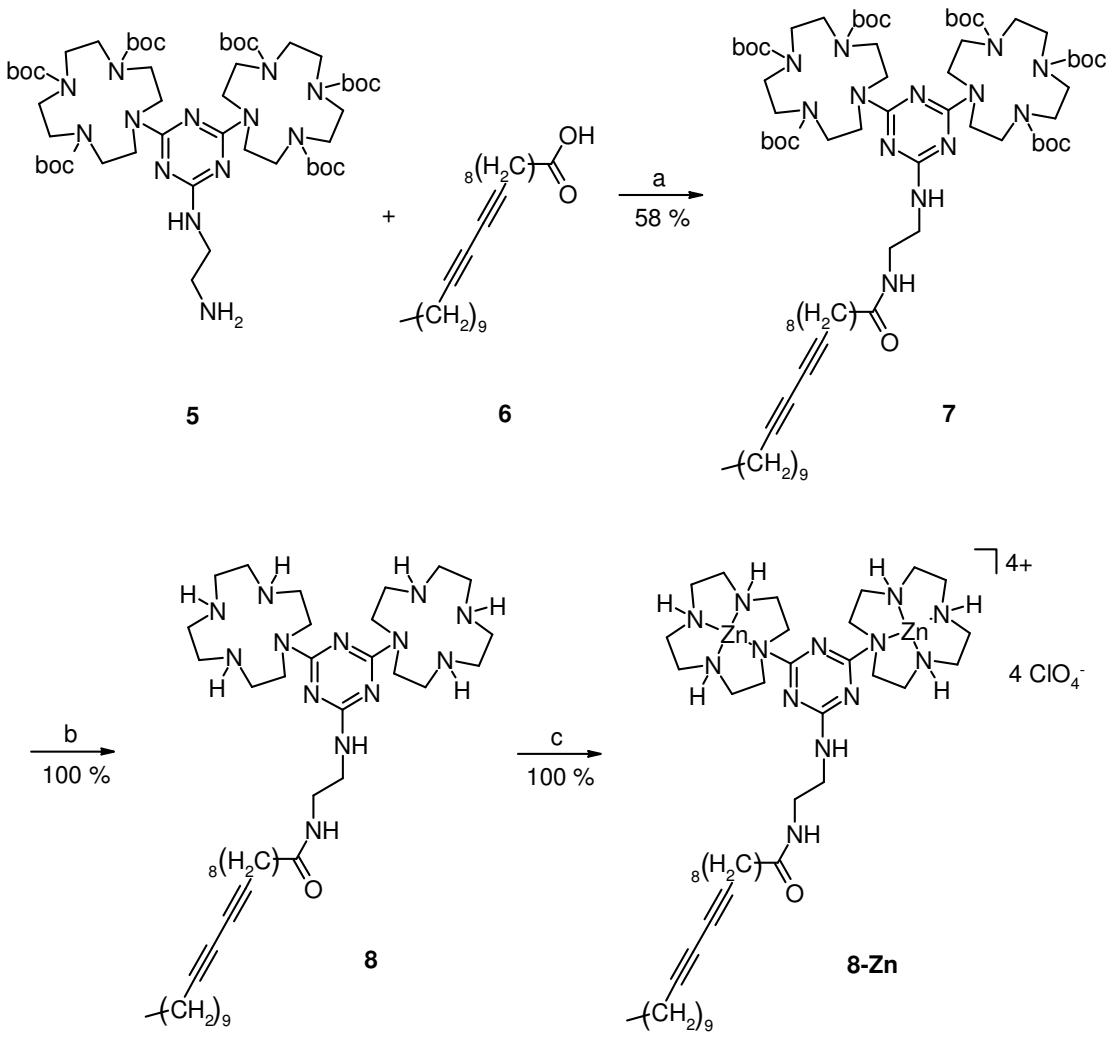
The cyclen, bis-cyclen and iminodiacetato (IDA) modified diacetylene monomers **4**, **8** and **11** were prepared by amide formation as shown in scheme 1 – 3. A methanolic solution of **4** and **8** was reacted with an aqueous solution of $Zn(ClO_4)_2$ to yield the amphiphilic receptor molecule **4**-**Zn** (scheme 1) and **8**-**Zn** (scheme 2), respectively, in good yields. Receptor molecule **11**-**Cu** (scheme 3) was prepared by treating a methanolic solution of **11** with an aqueous solution of $CuCl_2$ at room temperature and subsequently refluxed at 70 °C. All newly prepared monomeric diacetylene complexes **4**-**Zn, 8**-**Zn** and **11**-**Cu** were characterized by standard analytical and spectroscopic techniques and are very stable when stored under nitrogen in the dark. Detailed experimental procedures and analytical data of the compounds are provided in the Experimental Part.



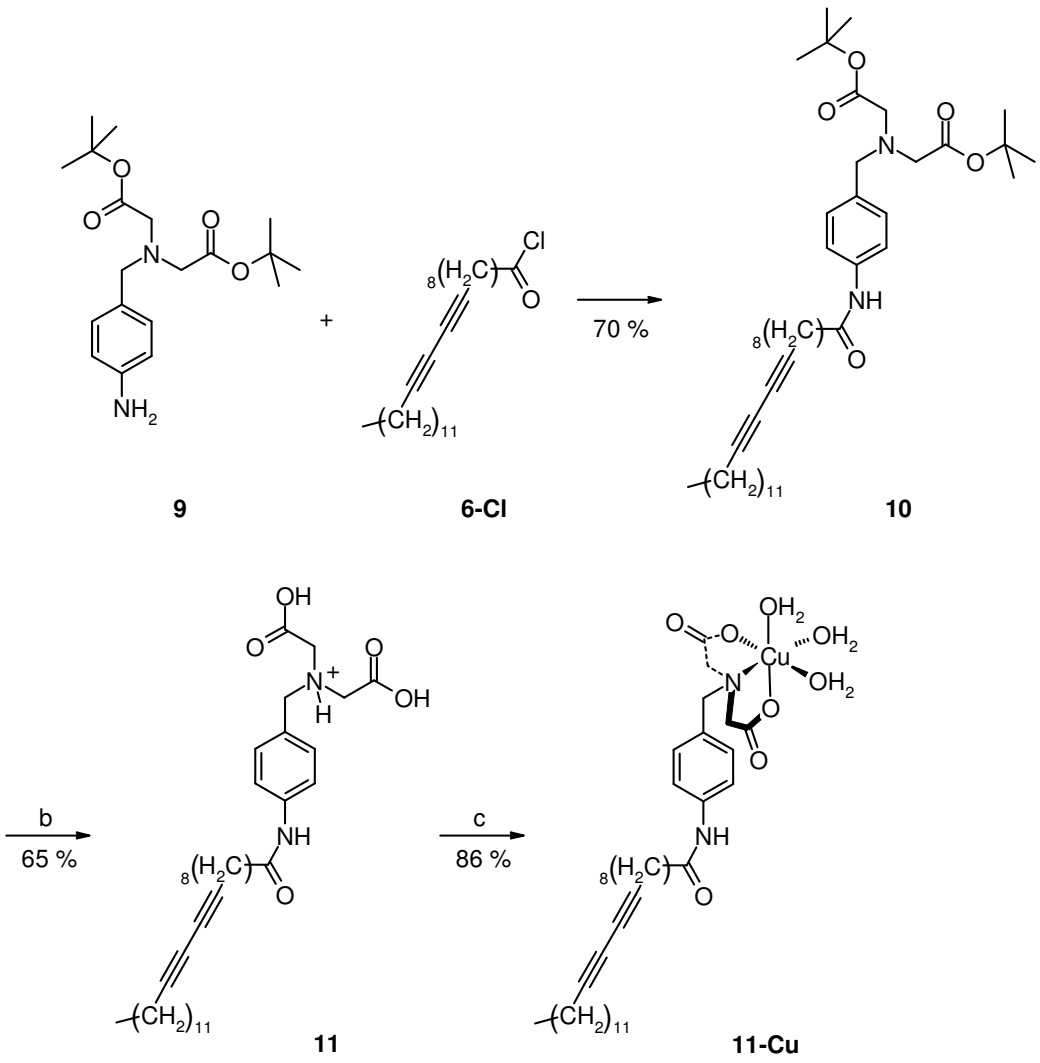
1 **2** **3**

4 **4-Zn**

Scheme 1. Synthesis of Zn(II)-cyclen complex modified 10, 12-tricosodiyonic acid (TCDA, **6**) **4-Zn**. (a) NEt_3, CH_2Cl_2, RT, 10 h; (b) TFA, CH_2Cl_2, RT, o/n; (c) $Zn(ClO_4)_2$, MeOH, RT, 12 h → reflux, 5 h.



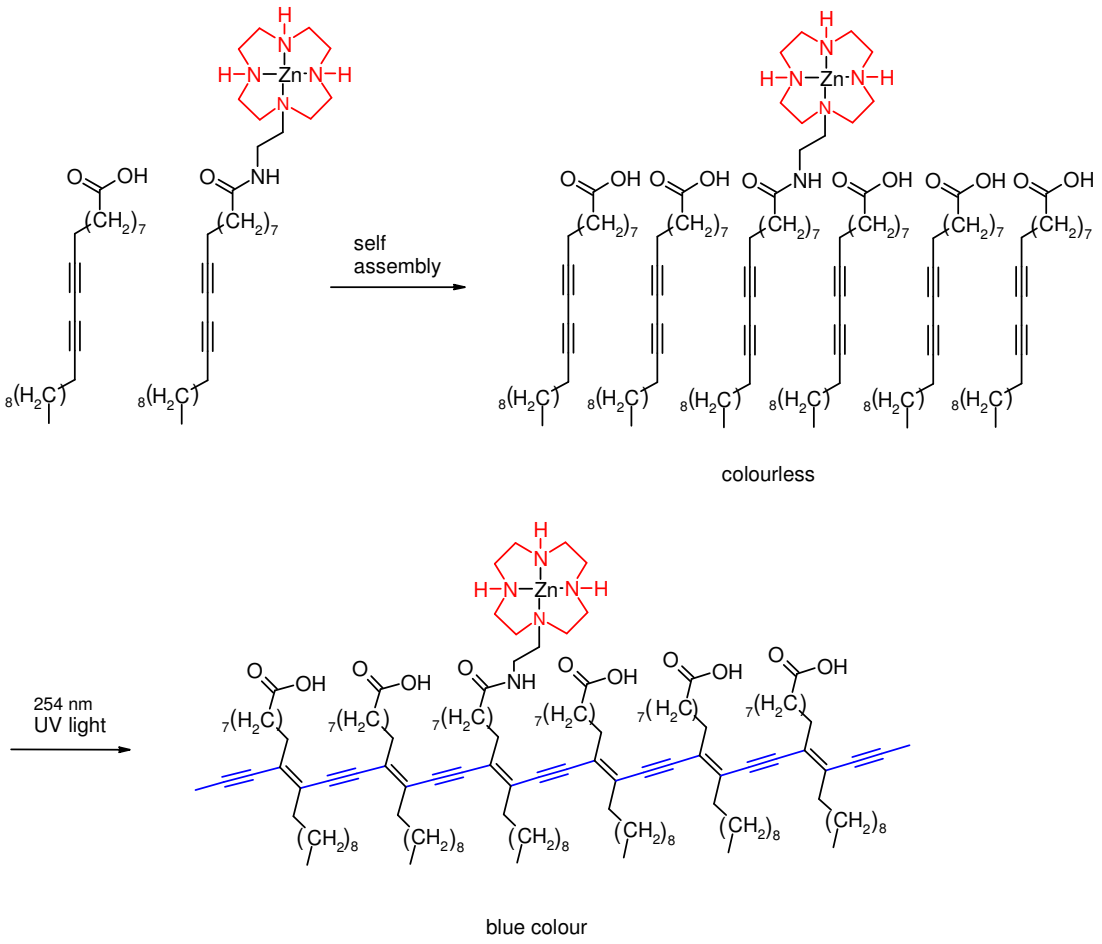
Scheme 2. Synthesis of bis-Zn(II)-cyclen complex modified 10, 12-tricosodiyonic acid (TCDA, **6**) **8-Zn**. (a) DIPEA, TBTU, HOBt, DMF, RT, 2 h; (b) 1.) HCl/ether, CH_2Cl_2, RT, 20 h, 2.) basic ion exchanger resin; (c) $Zn(ClO_4)_2$, MeOH, reflux, 20 h.



Scheme 3. Synthesis of copper(II)-IDA modified 10, 12-pentacosadiynoic acid (PCDA) **11-Cu**. (a) NEt_3, THF, RT, 12 h; (b) TFA, CH_2Cl_2, RT, 2 h; (c) $CuCl_2$, H_2O, MeOH, RT, 10 h.

5.2.2 Synthesis of vesicular PDA receptors

The liposomes were prepared according to known literature methods.[15] A mixture containing the modified diacetylene monomer **4-Zn**, **8-Zn** or **11-Cu** and the unmodified diacetylene monomer 10, 12-tricosayonic acid (TCDA) or 10, 12-pentacosadiynoic acid (PCDA) in a molar ratio of 10 : 90, respectively, was dissolved in dichloromethane in a 25 mL round bottom flask. The solvent was evaporated by a stream of N_2 gas and an appropriate amount of buffered aqueous solution (HEPES 10 mmol, pH = 7.2) was added to the round bottom flask to give the desired concentration of the lipid (1 x 10^{-3} M).



Scheme 4. Representative mixed diacetylene liposome system of TCDA and **4-Zn** after mixing and upon polymerization induced by irradiation with light of 254 nm wavelength; counter ions are not shown.

This solution was sonicated at 80 °C for 40 min. The resulting milky solution was filtered through a syringe filter while hot and the filtrate was cooled and stored at 0 °C overnight. The self assembled bilayer vesicles were polymerized at room temperature by irradiating the solutions with light of 254 nm wavelength for 5 to 10 min, whereby the colourless vesicle solution turned blue (scheme 4). The average size of the liposomes was 160 – 180 nm as determined by dynamic light scattering (DLS). Liposomes containing **4-Zn** are named **LP-4-Zn**; liposomes containing **8-Zn** are named **LP-8-Zn**; and liposomes containing **11-Cu** are named **LP-11-Cu**, respectively.

5.2.3 Particle Size measurement

Dynamic light scattering (DLS) particle sizing measurements were preformed by a Zetasizer 3000 from Malvern instruments Ltd. Malvern, UK.

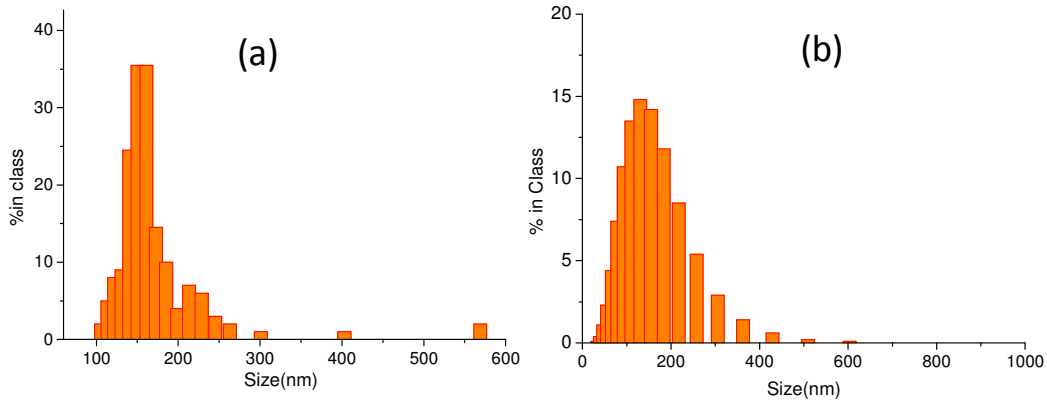


Figure 1. Particle size distribution curves of (a) **LP-4-Zn** and (b) **LP-8-Zn**.

The vesicle solutions were diluted 5 to 6-fold and measured at room temperature by keeping the typical count rate at 30-50 kcps. Each diameter value was an average result of continuous measurements in 5 min. At least three measurements were preformed for each solution. Table 1 summarizes the results. The obtained values are in good agreement with comparable liposomes that have been reported earlier in literature.[16] The two-component vesicles have an average size of 230 nm. Upon UV irradiation, the vesicle contract to 160-180 nm, likely due to the covalent cross linking that reduces the distance between the lipid molecules (Figure 1 and scheme 4).

	before irradiation	after irradiation
LP-4-Zn	230 ± 20 nm	180 ± 15 nm
LP-8-Zn	220 ± 25 nm	160 ± 10 nm
LP-11-Cu	205 ± 25 nm	145 ± 10 nm

Table 1. Results of DLS particle size measurement for **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu** vesicles.

5.2.4 Binding studies of LP-4-Zn, LP-8-Zn and LP-11-Cu with different anions

Zinc (II) complexes of macrocyclic polyamines, such as Zn(II)-cyclen show affinity to phosphate anions and nucleotides.[17,18] We therefore expected a response of the Zn(II)-cyclen modified PDA vesicles to phosphorylated anions. Figure 2 depicts schematically the assembly and analyte response of the vesicular metal complex sensor system.

Although there is still no clear evidence for the molecular origin of the colour change of PDAs upon analyte binding, it has widely been accepted that the colour change is associated with a conformational change of the polydiacetylene backbone.[19] Accordingly, in the blue coloured polymerized vesicular receptor an extended conjugation of the *p*-orbitals in the main chain of the polydiacetylene polymers is present. Upon analyte binding to the embedded receptor sites the conjugated *p*-orbitals undergo distortion, leading to a partial twist of the *p*-orbitals. Thus, the dark blue colour of the polymers gradually shifts to a red colour depending on the amount of the stress induced.

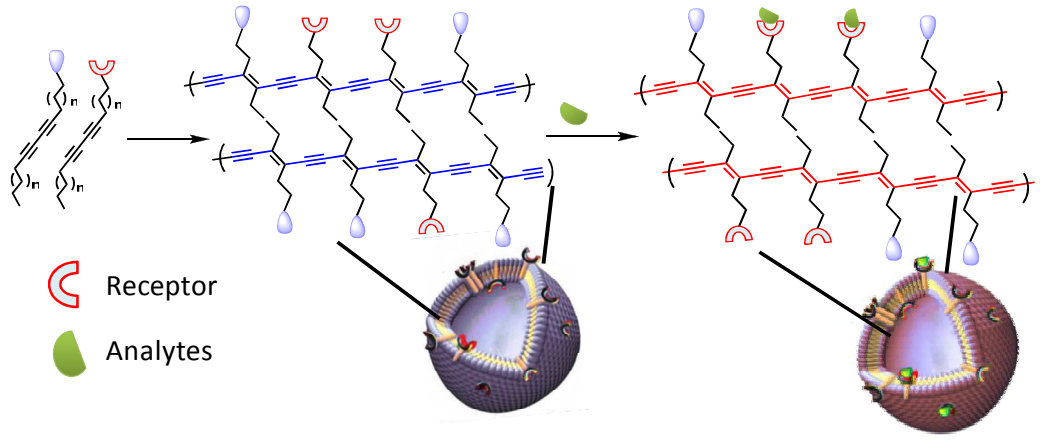


Figure 2. Schematic representation of the preparation and the analyte response of self assembled polydiacetylene vesicles with embedded metal complex binding sites for anions.

Initially, electronic absorption spectra for a 5 x 10^{-5} M solution of the blue coloured **LP-4-Zn** vesicles were recorded in the absence and presence of various anions (Figure 3). The absorption spectra of **LP-4-Zn** in buffered aqueous solution (HEPES 10 mmol, pH 7.2) at room temperature shows before the addition of analytes distinct absorption bands at λ_{max} = 640, 589 and 543 nm. Upon addition of an excess amount of ATP, PPi or CN^- ions the absorption band at 640 nm disappeared completely and an intense absorption band at 489 and 543 nm is observed. The colour of the solution turns red. No changes in the absorption spectra or colour is observed upon addition of other anions

like F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, CH_3COO^-, AMP or ADP under similar conditions (Figure 3b).

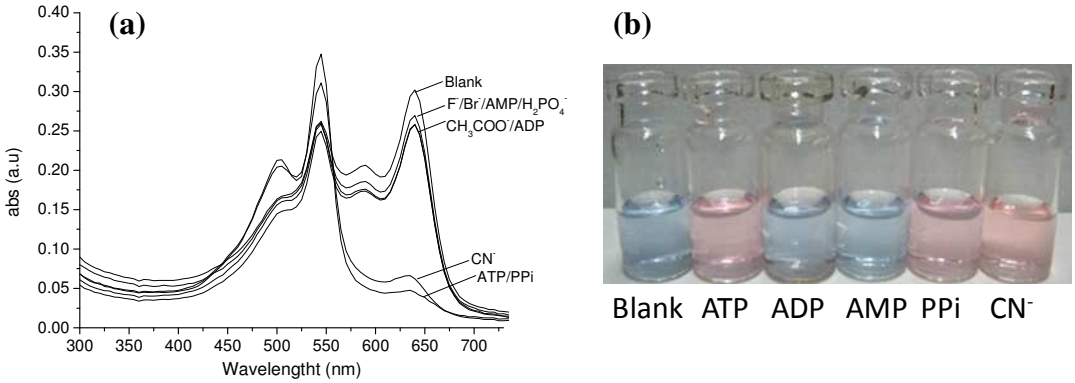


Figure 3. (a) UV-visible spectra of **LP-4-Zn** (5 x 10^{-5} M) in the presence of different anions (aqueous solution, HEPES 10 mmol, pH 7.2, 100 equiv. of the anion salt added). (b) Colour change of the blank **LP-4-Zn** with different anions.

The colour change of the liposomes from blue to red was quantified by calculating the colorimetric response (CR) using equation 1. The CR value is derived from the change in the ratio of absorbance at 640 nm and 550 nm in the absence (A_0) and presence (A_X) of different analytes. The absorption ratio before analyte addition is calculated as $A_0 = I_{620} / (I_{620} + I_{490})$ and the absorption ratio after analyte addition follows from $A_x = I_{620} /(I_{620} + I_{490})$, respectively.

$$\% \ \mathbf{CR} = [(A_0 - A_x)/A_0] \cdot 100. \quad \text{(Equation 1)}$$

Figure 4a and b show the induced changes in the **LP-4-Zn** absorption spectrum upon addition of ATP and PPi.

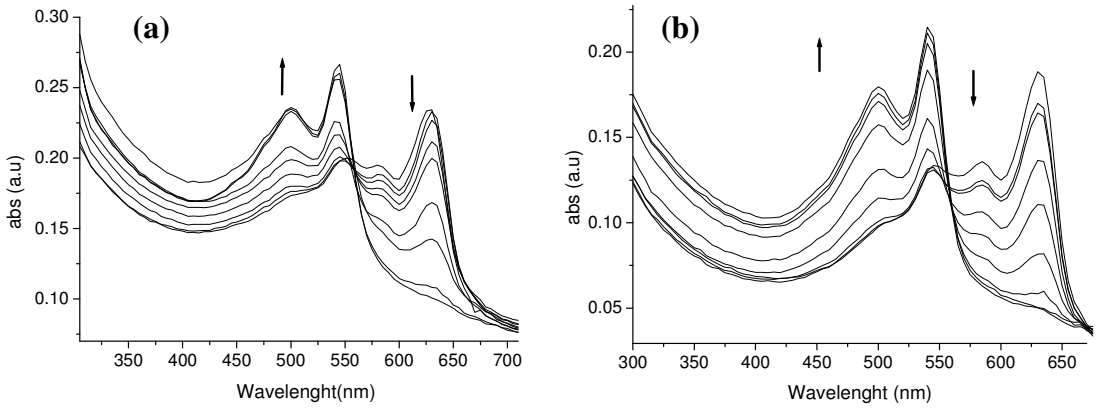


Figure 4. UV-visible absorption titration of **LP-4-Zn** (5 x 10^{-5} M) (a) Upon addition of ATP (5 x 10^{-6} – 3.5 x 10^{-3} M) (b) Upon addition of PPi (5 x 10^{-6} – 3.5 x 10^{-3} M).

Recent studies[20] from our group demonstrate that dinuclear Zn(II)-cyclen complexes show an even higher affinity to ATP and PPi ions than mononuclear Zn(II)-cyclen complexes. The vesicular receptors containing **8-Zn** were therefore expected to have an increased affinity to ATP and PPi ions. Titration experiments revealed that approximately 20 equivalents of ATP or PPi were sufficient to achieve complete saturation. This is considerably less as compared to **LP-4-Zn**. Other anions like F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, CH_3COO^-, AMP and ADP did not induce any spectral changes of the liposome solution. Table 2 and figure 5 summarize the results.

	none	ATP	PPi	ADP	AMP	$H_2PO_4^-$	F^-	CN^-
LP-4-Zn	-	54.0	67.2	21.7	-	0.9	12.6	46.1
LP-8-Zn	-	73.7	81.8	20.8	-	2.4	2.5	79.8
LP-11-Cu	-	9.7	86.4	6.5	-	-	-	51.6

Table 2. Calculated colorimetric response (% CR) values of **LP-4-Zn, LP-8-Zn** and **LP-11-Cu** upon addition of various anions.

Liposome containing a mixture of both metal complexes **4-Zn** and **8-Zn** behave in their response towards anions like the liposomes containing only **8-Zn**. This shows that only the presence of the dinuclear Zn(II)-cyclen metal complex binding sites evokes an increased affinity of the vesicular receptors to the anion.

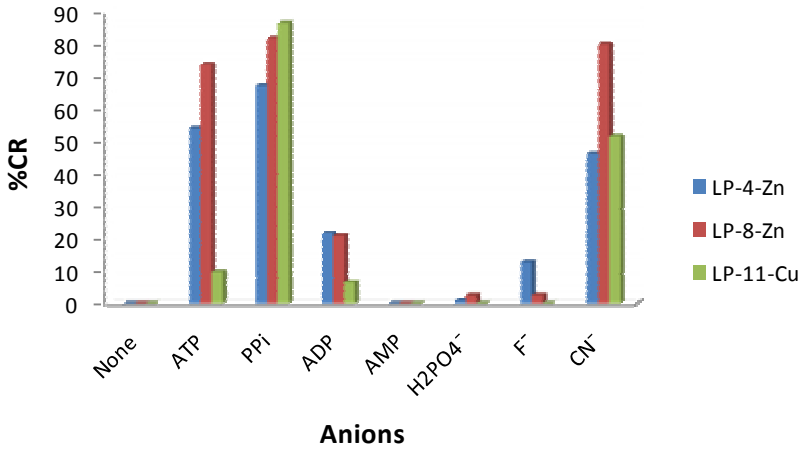


Figure 5. Colorimetric response (% CR) to different anions with liposomes **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu**.

While the binding of ATP and PPi as highly charged oxoanions to the bis-Zn(II)-cyclen binding sites was expected from previous studies, the response to the presence of

cyanide ions is surprising. We suspected a pH effect or a dissociation of the zinc ion from the metal complex. To investigate if the colorimetric response towards cyanide ions is induced by decomplexation of the cyclen complexes, the liposome solution was treated with the strong complexing agent EDTA. The addition of EDTA did not lead to a colour change on the solution. Displacement of zinc ions from the vesicular receptor can therefore not be attributed as the origin of the colorimetric change. Carboxy-terminated polydiacetylene vesicles are known to undergo a blue to red chromic transition in the pH range 9.0 - 10.1.[21] The pH of the aqueous buffered vesicles solution (HEPES 10 mM, pH 7.2) was determined at the end of each titration. In the case of added basic cyanide ions the pH of the solution considerably increased to 10.0 - 10.5, in all other cases the pH value remained between 7.2 - 8.0. This confirms that the pH change of the solution causes the colour change upon addition of a large excess of cyanide ions.

Apparent binding constants of the liposome–anion complex were derived from the change in UV-visible absorbance at 490 nm with respect to the concentration of specific anions using non-linear regression analysis (Figure 7). The calculated binding constant (lg K_{app}) values are given in Table 3. CR values and lg K_{app} values reveal that ATP and PPi ions strongly interact with the interface of the vesicle receptor, while other phosphates and halide ions do not induce a significant colour and spectral change.

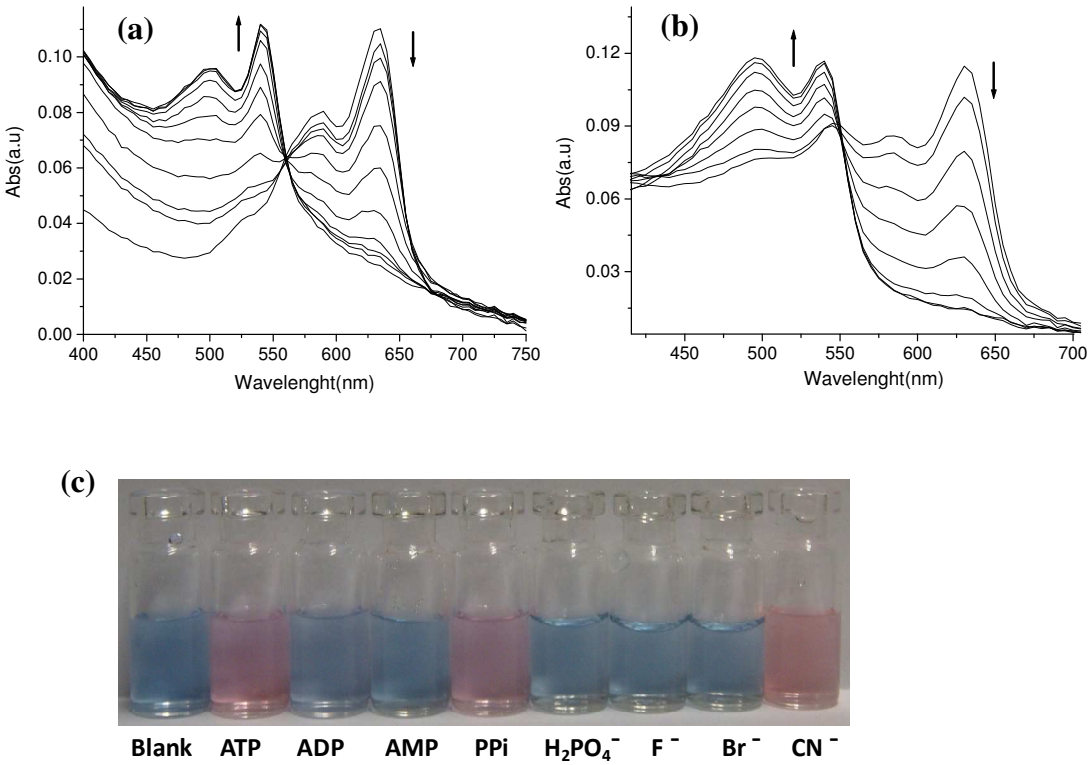


Figure 6. UV-visible absorption titration of **LP-8-Zn** (8.2 x 10^{-5} M) (a) Upon addition of ATP (5 x 10^{-6} – 3.5 x 10^{-3} M) (b) Upon addition of PPi (5 x 10^{-6} – 3.5 x 10^{-3} M) (c) Colour change of **LP-8-Zn** with 100 equivalents of different anions.

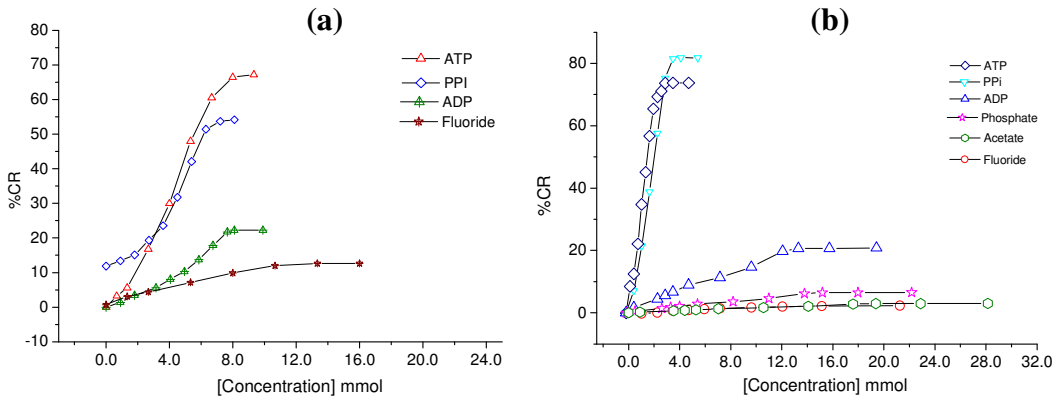


Figure 7. UV-visible titration profile of (a) **LP-4-Zn** (5 x 10^{-5} M) and (b) **LP-8-Zn** (8.2 x 10^{-5} M) with different anion in aqueous buffered solution (HEPES 10 mmol, pH 7.2,) at RT.

Receptor liposomes	Anion affinity [lg K_{app}]						
	ATP	**PPi**	**ADP**	**AMP**	**$H_2PO_4^-$**	**F^-**	**CH_3COO^-**
LP-4-Zn	2.2	2.5	< 1.0	–	–	< 1.0	–
LP-8-Zn	2.7	3.2	< 1.0	–	–	–	–
LP-11-Cu	–	2.6	–	–	–	–	–

Table 3. Calculated apparent binding constant (lg K_{app}) of **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu** with different analytes.

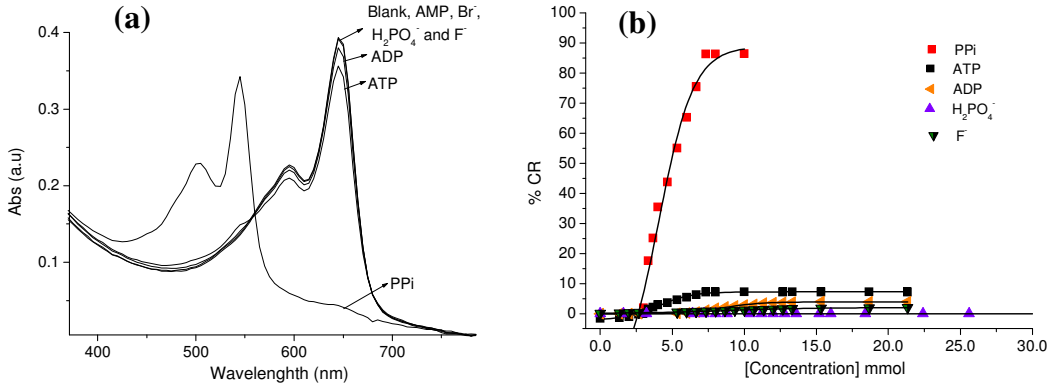


Figure 8. UV-visible absorption titration of **LP-11-Cu** (6.0 x 10^{-5} M) (a) Upon addition of different anions (6.0 x 10^{-3} M) (b) UV-visible titration profile with different anion in aqueous buffered solution (HEPES 10 mmol, pH 7.2,) at RT.

After knowing the binding properties of Zn(II)-cyclen based receptor **LP-4-Zn** and **LP-8-Zn**, we investigated the binding of Cu(II)-IDA based receptor **LP-11-Cu** to different anions in more detail. Upon addition of an excess amount of PPi (100 equivalent) the absorption band at 640 nm disappeared completely and an intense absorption band at 489 and 543 nm is observed (Figure 8). The colour of the solution turned red. However, no changes in the absorption spectra or colour could be observed with other anions like F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, CH_3COO^-, AMP, ADP and ATP under similar conditions. It is interesting to note that Cu(II)-IDA complex modified vesicles behave differently as compared to Zn(II)-cyclen modified vesicles with phosphates. Among different phosphates **LP-11-Cu** responded only to PPi with an apparent binding constant lg K_{app} = 2.7 and did not show any spectral and colour changes with ATP as **LP-4-Zn** and **LP-8-Zn** do (Figure 8). Thus **LP-11-Cu** is suitable for the selective detection of PPi

over ATP in aqueous media. Coordinatively unsaturated Cu(II) complexes exhibit strong binding tendencies towards anionic substrates due to the d^9 electronic configuration of the metal center, which ensures high ligand field stabilization effects. As a result, the anionic substrate PPi is effectively bound even in the strongly solvating aqueous conditions. Very few synthetic receptors have been reported so far that allow a discrimination of PPi and ATP in aqueous media.[22]

5.2.5 Emission studies with different anions

The room temperature emission spectra (Figure 9) of **LP-4-Zn** (5 x 10^{-5} M), **LP-8-Zn** (8.2 x 10^{-4} M) and **LP-11-Cu** (6 x 10^{-5} M) recorded in aqueous buffered solution (HEPES 10 mmol, pH 7.2) show a very weak emission band centered at 625 nm upon excitation at 510 nm. The intensity of **LP-4-Zn** and **LP-8-Zn** emission at 570 nm and 640 nm increases significantly upon addition of an excess of ATP and PPi (Figure 9b and 10a).

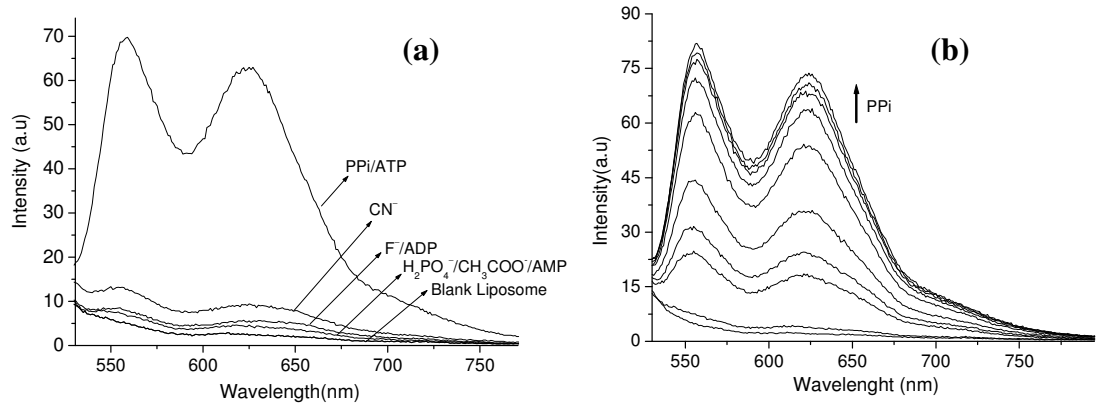


Figure 9. Emission titration of **LP-4-Zn** (5 x 10^{-5} M) (a) with different anions (100 equivalents) (b) titration with increasing amounts of PPi (5x10^{-6} M – 3.5 x10^{-3} M).

Other anions like ADP, AMP, CN^-, Br^-, $H_2PO_4^-$ or F^- induce only very little or no change in the emission intensity (Figure 9a). Although CN^- ions induce changes in the absorption spectrum of the liposomes due to pH increase, the effect on the emission spectrum is small (Figure 10b). In the case of **LP-11-Cu** among different phosphorylated ions only PPi induced an emission enhancement. Other phosphates like ATP, ADP, AMP and H_2PO_4 did not show any significant change in the emission spectra (Figure 11).

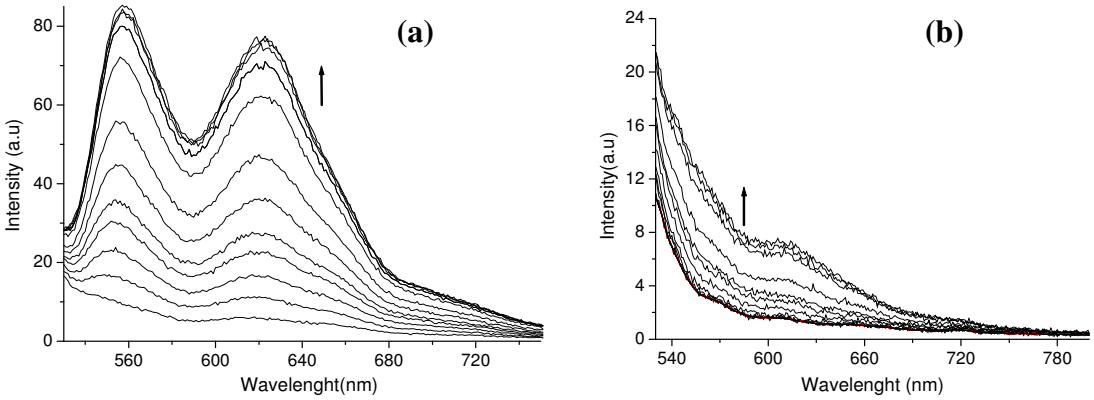


Figure 10. Emission titration spectra of **LP-8-Zn** (8.2 x 10^{-5} M) (a) titration with varying concentrations of ATP (1 x 10^{-5} - 3.2 x 10^{-3} M) (b) titration with varying concentrations of CN^- (1 x 10^{-5} - 3.2 x 10^{-3} M).

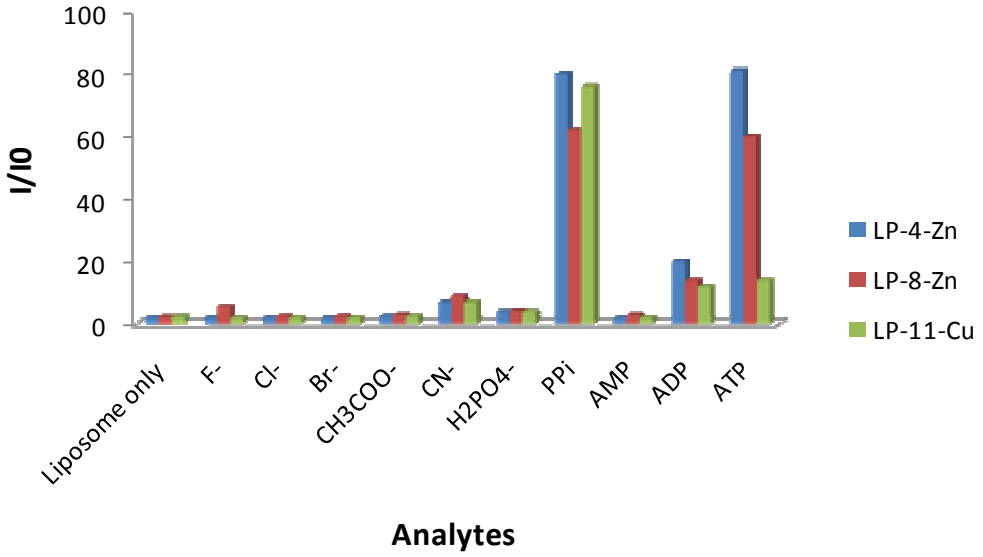


Figure 11. Relative changes in emission intensity (λ_{ex} = 510 nm) upon treatment with different anions in aqueous buffered solution (HEPES 10 mmol, pH 7.2) of **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu**, respectively.

5.2.6 Test paper analysis

The response in a colour change of **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu** in the presence of ATP and PPi was used to prepare a test paper stripe, which allows the simple detection of ATP and PPi at millimolar concentrations in water.

Arrays of unpolymerized **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu** vesicles containing test papers were prepared by soaking filter papers in HEPES buffered aqueous solution of the vesicles (pH 7.2) and drying them in air. These colourless test papers were stored in the dark and irradiated with light of 254 nm before further experiments. For detecting the analyte, the blue coloured test paper was immersed in the aqueous analyte solution for several seconds and then air-dried. As shown in Figure 12, in the case of **LP-4-Zn** and **LP-8-Zn** a colour change of the test papers is observed for aqueous solutions containing ATP and PPi ions. However, in the case of **LP-11-Cu** only with PPi a colour change was obtained. Other anions did not induce any colour change at equal concentrations. Based on this colour change of the test paper ATP or PPi concentration levels in aqueous media may be estimated (Figure 13).

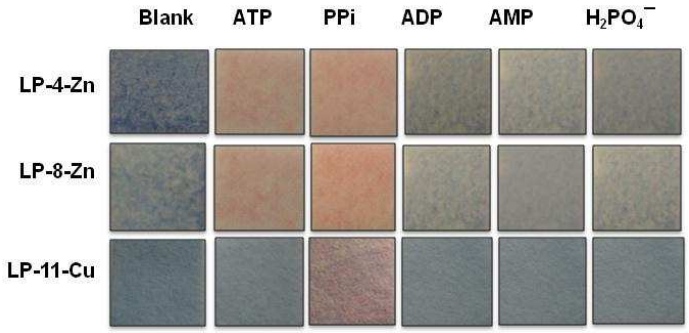


Figure 12. Colour changes of **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu** (0.35 mmol) test papers treated with different anions (15 mmol) at room temp.

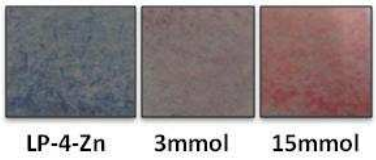


Figure 13. LP-4-Zn test paper dipped with two different concentration of aqueous PPi solution.

5.2.7 Light microscopy images of the vesicles

To monitor the changes of the morphology of the vesicles upon analyte binding they were investigated under a light microscope (Leica, Wetzlar, Germany). Freshly prepared vesicles solutions **LP-4-Zn, LP-8-Zn** and **LP-11-Cu** were dropped on the glass slide and viewed using normal light microscopy. Figure 14 shows the appearance of the PDA vesicles **LP-4-Zn, LP-8-Zn** and **LP-11-Cu** without any anionic analyte. The images reveal that they were well separated and spherical in shape.

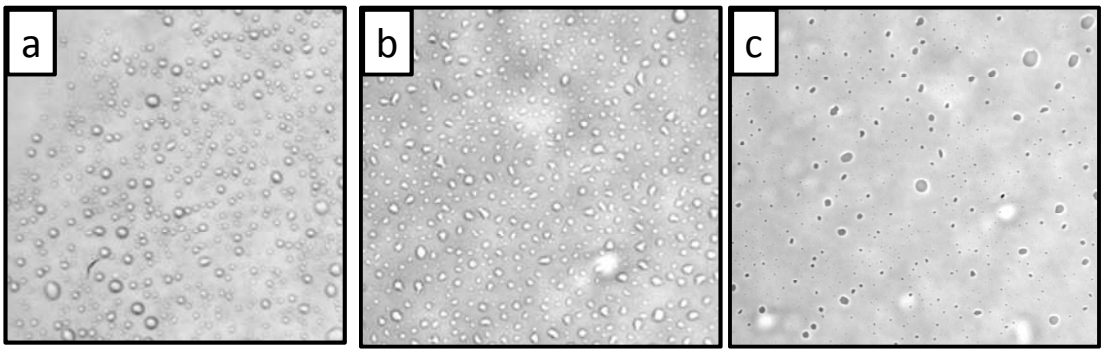


Figure 14. Light microscopy images (20 X objective lens) of (a) **LP-4-Zn** (b) **LP-8-Zn** and (c) **LP-11-Cu**.

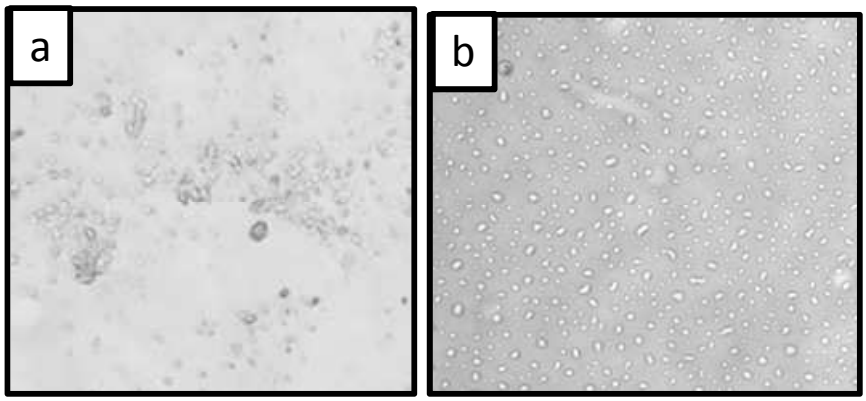


Figure 15. Light microscopy images (20 X, objective lens) of (a) **LP-8-Zn** with **PPi** (b) **LP-8-Zn** with **$H_2PO_4^-$**.

Next, the liposomes particles morphology of **LP-8-Zn** was investigated in the presence of PPi, ATP or $H_2PO_4^-$. Blank blue colour **LP-8-Zn** vesicles were exposed to the PPi, ATP or $H_2PO_4^-$ for a few minutes and then viewed under the light microscope with identical magnification. The images of the PPi and ATP exposed liposomes show that the binding of these anions induces aggregation of the liposomes[23] (Figure 15a). However, in the case of $H_2PO_4^-$ this was not observed due to the weaker binding of this

ion (Figure 15b). In ongoing more detailed investigations of the morphology changes using high resolution electron microcopy we will try to understand the mechanism of the surface anion binding induced rapid liposome aggregation.

5.3 Conclusion

In conclusion, we have shown that nanometer sized polymerized vesicular receptors **LP-4-Zn**, **LP-8-Zn** and **LP-11-Cu** can be prepared from amphiphilic diacetylene metal complexes. The vesicular receptors obtained from Zn(II)-cyclen functionalized polydiacetylene monomers respond to the presence of ATP and PPi at neutral pH at millimolar concentrations with visible changes of their colour and emission. Other anions, such as halides, do not induce an analytic response. The Cu(II)-IDA modified vesicular PDA receptors show a selective response to PPi, which allows a simple colorimetric and visible discrimination between ATP and PPi. The induced aggregation of the liposomes by the specific binding of ATP or PPi analytes to the surface receptors is the likely origin of the intensive changes in their optical properties. Polymerized vesicular receptors with metal complex binding sites may find applications for simple analytic tasks, as shown with anion test papers. However, the development of more complex vesicular receptors with increased affinity and specificity may be envisaged.

5.4 Experimental Part

5.4.1 General methods and material

Emission Spectroscopy. Fluorescence measurements were performed with aqueous buffered solution (HEPES 10 mmol, pH = 7.2,) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and aqueous buffered solution (HEPES 10 mmol, pH = 7.2,).

NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analysed by first order, the coupling constants are given in Hertz [Hz]. Characterisation of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in 13C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (-) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

Light microscopy. Light microscopy was done with a simple light microscope (Leica, Wetzlar, Germany).

Dynamic light scattering (DLS). Dynamic light scattering (DLS) for particle size measurements were preformed by using Zetasizer 3000 from Malvern instruments Ltd. Malvern, UK using 1 cm UV-visible cuvettes. Vesicle solutions were diluted 5 to 6-fold and measured at RT by keeping the count rate at 80-100 kcps. Each diameter value was an average result of continuous measurements over 5 min. At least three measurements were preformed for each solution.

5.4.2 Binding studies

General. All binding studies were conducted in buffered aqueous solution (HEPES 10 mmol, pH 7.2). The cuvette with 3000 µL of liposomes in HEPES buffered solution was titrated stepwise with small amounts (beginning with 0.2 equiv) of the anionic analyte solution. After each addition the solution was allowed to equilibrate for 3 – 5 min before the fluorescence and the UV spectrum (where permitted by the concentration range) were recorded. The total amount of anion required for complete binding was determined by plotting the graph between the changes in absorbance at 540 nm against the concentration of added anions. To determine the binding constants, the obtained absorbance at 540 nm was volume corrected, plotted against the anion concentration and evaluated by nonlinear curve fitting.

UV-absorption titration. Stock solutions (20 mL) of the liposomes **LP-4-Zn** (1.0×10^{-3} M), **LP-8-Zn** (8.2×10^{-4} M) and **LP-11-Cu** (1.0×10^{-3} M) were prepared in buffered aqueous solution (HEPES 10 mmol, pH 7.2), respectively, and stored in dark. These solutions were appropriately diluted and irradiated by UV light before the spectroscopic studies. Solutions of the sodium salt (200 mmol) of the respective anions were prepared analogue and were stored in the dark. All titration experiments were performed using 5.0×10^{-5} M solutions of **LP-4-Zn**, 8.2×10^{-5} M solutions of **LP-8-Zn** and 6.0×10^{-5} M solution of **LP-11-Cu** in water (HEPES 10 mmol, pH 7.2) and various concentrations of the anions (2×10^{-5} - 3.2×10^{-3} M) in the same solvent. Based on the absorption spectral

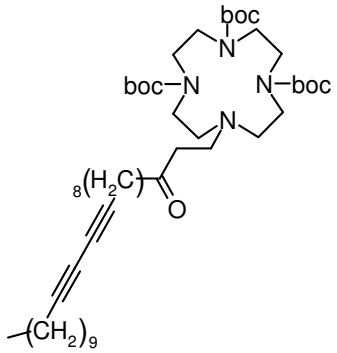
changes the colorimetric response (CR) was derived by using equation 1 as stated in the article.

Emission titration. The standard solutions mentioned above were used for emission titration studies. For all measurements the liposome solutions were excited at 510 nm, with an excitation and emission slit width of 10 nm. All titration experiments were performed using 1 x 10^{-4} M solutions of **LP-4-Zn**, 8.2 x 10^{-5} M solution of **LP-8-Zn** and 6.0 x 10^{-5} M solution of **LP-11-Cu** in water (HEPES 10 mmol, pH 7.2), with various anions like ATP, ADP, AMP, PPi, $H_2PO_4^-$, F^-, CN^-, Br^-, Cl^- and I^-.

5.4.3 Synthesis and Characterization of compounds

General. Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Detection via UV light at 254 nm / 366 nm or through discolouration with ninhydrin in EtOH. Column chromatography was performed on silica gel (70–230 mesh) from Merck. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Commercially available solvents of standard quality were used. Dry THF, which was prepared by distillation from potassium. If otherwise stated, purification and drying was done according to accepted general procedures.[24] Elemental analyses were carried out by the Center for Chemical Analysis of the Faculty of Natural Sciences of the University Regensburg.

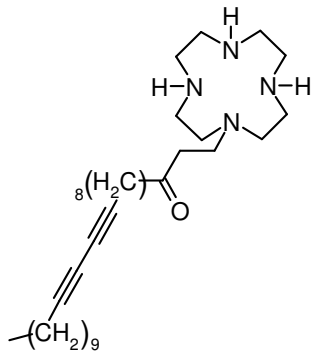
Synthesis. Compounds **1**, **5** and **9** were synthesised according to literature known procedures.[25]



Synthesis of compound **3**

Compound **2** (100 mg, 0.22 mmol) was dissolved in dry dichloromethane under N_2 atmosphere and compound **1** (118 mg, 0.22 mmol) and 0.25 mL of Et_3N were added. The reaction mixture was stirred at room temperature for 10 h then the reaction mixture was evaporated to dryness. The crude product was purified by silica column chromatography using ethyl acetate and petroleum ether as the eluent. The desired product was collected as the first fraction in the form of colourless sticky solid (ethyl acetate / petrol ether 7:3; R_f = 0.28). Yield: 170 mg (83 %).

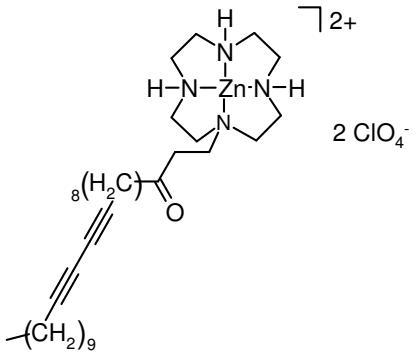
IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2940, 2928, 2857, 1687, 1537, 1465, 1408, 1364, 1246, 1158, 1158, 1105, 776. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.81 (t, ^{3}J = 7.1 Hz, 3 H, CH_3), 1.21 (m, 27 H, CH_3), 1.40 (s, 26 H, CH_2), 1.51 (t, ^{3}J = 7.5 Hz, 4 H, CH_2), 2.21 (t, ^{3}J = 7.1 Hz, H), 2.58 (bs, 4 H, CH_2), 3.27-3.47 (m, 16 H, CH_2 cyclen). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.4 (C_q, amide), 156.4 (C_q, amide), 79.5 (C_q, boc), 79.7 (C_q, boc), 77.5 (C_q), 77.0 (C_q), 76.6 (C_q), 65.3 (C_q), 65.2 (C_q), 52.6 (–, CH_2), 50.0 (–, CH_2), 48.1 (–, CH_2), 36.5 (–, CH_2), 36.4 (–, CH_2), 31.9 (–, CH_2), 29.5 (–, CH_2), 29.4 (–, CH_2), 29.3 (–, CH_2), 29.2 (–, CH_2), 29.2 (–, CH_2), 29.0 (–, CH_2), 28.9 (–, CH_2), 28.8 (–, CH_2), 28.76 (+, CH_3), 28.6 (–, CH_2), 28.5 (–, CH_2), 28.3 (–, CH_2), 28.28 (–, CH_2), 25.7 (–, CH_2), 22.7 (–, CH_2), 21.0 (–, CH_2), 19.1 (–, CH_2), 19.09 (–, CH_2), 14.1 (+, CH_3). – **MS** (ES-MS, DCM/MeOH + 10 mM NH_4OAc): m/z(%) = 844.6 (100) [MH^+].



Synthesis of compound **4**

Compound **3** (100 mg, 0.12 mmol) was dissolved in 25 mL of dichloromethane. To this solution 1 mL of trifluoroacetic acid was added under ice cooling. The reaction mixture was stirred at room temperature for 24 h and was evaporated. A colourless solid was obtained. The TFA-salt was redissolved in a water/methanol mixture (40/60 v/v) and passed over a strongly basic ion-exchanger column. The obtained solution was lyophilized to afford compound **4** as a wax, Yield: 78 mg (90 %).

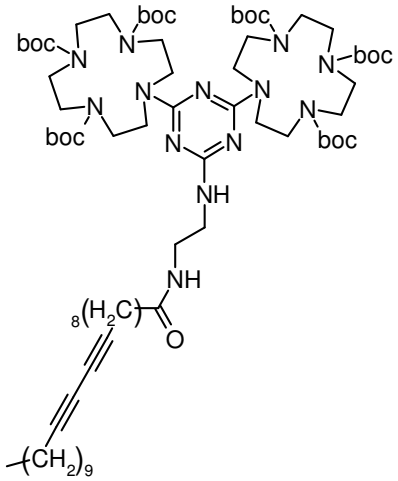
IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3290, 2970, 2928, 2857, 1687, 1537, 1501, 1465, 1408, 1364, 1246, 1158, 1246, 1158, 1105, 776. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.83 (t, ^{3}J = 7.1 Hz, 3H, CH_3), 1.18 (bs, 24 H, CH_2), 1.45 (m, 4 H, CH_2), 1.54 (t, ^{3}J = 7.8 Hz, 2 H, CH_2), 2.08-2.19 (m, 6 H, CH_2), 2.51-2.76 (m, 16 H, CH_2 cyclen), 3.30 (t, ^{3}J = 7.8 Hz, 2 H, CH_2). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.2 (C_q, amide), 65.2 (C_q), 53.6 (C_q), 51.5 (C_q), 47.1 (C_q), 46.4 (–, CH_2), 45.3 (–, CH_2), 37.4 (–, CH_2), 36.7 (–, CH_2), 31.9 (–, CH_2), 29.5 (–, CH_2), 29.4 (–, CH_2), 29.38 (–, CH_2), 29.2 (–, CH_2), 29.1 (–, CH_2), 28.9 (–, CH_2), 28.8 (–, CH_2), 28.78 (–, CH_2), 28.34 (–, CH_2), 28.30 (–, CH_2), 25.7 (–, CH_2), 22.7 (–, CH_2), 19.20 (–, CH_2), 19.17 (–, CH_2), 14.1 (+, CH_3). – **MS** (ESI, MeOH): m/z (%) = 544.4 (50) [MH^+], 583.4 (50) [K + MH^+], 622.4 (100) [2 K + MH^+].



Synthesis of compound **4-Zn**

Compound **4** (50 mg, 0.09 mmol) was dissolved in 20 mL of methanol. To this mixture 45 mg (0.12 mmol) of $Zn(ClO_4)_2$ dissolved in 5 mL of methanol was added. The reaction mixture was stirred at room temperature for 12 h and refluxed for another 5 h. The solvent was removed in vacuum, the crude product was dissolved in THF and precipitated using petroleum ether yielding **4-Zn** (55 mg, 67%). as a highly hygroscopic colourless solid.

MP: 137-140 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3293, 2925, 2854, 1660, 1646, 1461, 1371, 1264, 1086, 974, 857, 730. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.83 (t, ^{3}J = 7.2 Hz , 3 H, CH_3), 1.24 (bs, 24 H, CH_2), 1.51 (m, 4 H, CH_2), 2.22 (t, ^{3}J = 7.6 Hz, 4 H, CH_2), 2.40 (t, ^{3}J = 7.5 Hz , 2 H, CH_2), 2.65-3.16 (m, 16 H, CH_2 cyclen), 3.44 (m, 2 H, CH_2), 4.25 (m, 2 H, CH_2). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.2 (C_q, amide), 78.1 (C_q, acetylene), 78.05 (C_q, acetylene), 66.4 (C_q, acetylene), 65.0 (C_q, acetylene), 55.5 (–, CH_2), 52.8 (–, CH_2), 45.8 (–, CH_2), 45.2 (–, CH_2), 43.7 (–, CH_2), 37.1 (–, CH_2), 30.7 (–, CH_2), 30.6 (–, CH_2), 30.4 (–, CH_2), 30.24 (–, CH_2), 30.22 (–, CH_2), 30.20 (–, CH_2), 30.0 (–, CH_2), 28.9 (–, CH_2), 29.9 (–, CH_2), 29.6 (–, CH_2), 26.6 (–, CH_2), 23.8 (–, CH_2), 19.7 (–, CH_2), 14.1 (+, CH_3). – **MS** (ES-MS, H_2O/MeOH + 10 mM NH_4OAc): m/z (%) = 606.3 (100) $[M^{2+} - H^+]^+$, 666.3 (10) $[M^{2+} + CH_3COO^-]^+$.

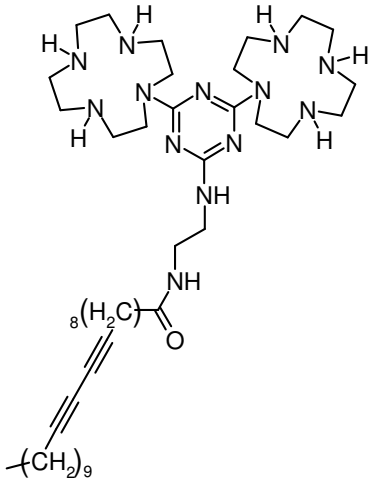


Synthesis of compound **7**

10, 12-Tricosadiynoic acid **6 (**71 mg, 0.20 mmol), DIPEA (128 µL, 0.74 mmol), TBTU (71 mg, 0.22 mmol), and HOBt (34 mg, 0.22 mmol) were dissolved under nitrogen atmosphere in dry DMF (4 mL) under ice cooling and stirred for 1 h. Compound **5** (200 mg, 0.19 mmol) was added. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h at RT. The reaction progress was monitored by TLC (ethyl acetate). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate/petrol ether 8:2; R_f = 0.34) yielding compound **7** (148 mg, 58 %) as colourless oil.

IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2970, 2928, 2857, 1687, 1537, 1501, 1465, 1408, 1364, 1246, 1158, 1246, 1158, 1105, 776. – **^{1}H-NMR** (400 MHz, $CDCl_3$): δ (ppm) = 0.82 (t, ^{3}J = 7.1 Hz, 3 H, COSY: CH_3), 1.17-1.28 (m, 14 H, COSY: CH_2, HSQC: *alkyl*-CH_2), 1.28-1.35 (m, 8 H, COSY: CH_2, HSQC: *alkyl*-CH_2), 1.36-1.52 (m, 58 H, COSY: CH_2, HSQC: *alkyl*-CH_2, HSQC: CH_3), 1.53-1.63 (m, 2 H, COSY, HMBC: CH_2), 2.13 (t, ^{3}J = 7.6 Hz, 2 H, COSY, HMBC: CH_2), 2.16-2.23 (m, 4 H, HMBC: CH_2), 2.83-4.07 (m, 36 H, COSY, HSQC: CH_2 cyclen, COSY, HSQC: CH_2), 5.06 (bs, 1 H, HMBC, HSQC: NH), 7.24 (bs, 1 H, HMBC, HSQC: NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 1 C, HSQC, COSY: CH_3), 19.07, 19.09 (–, 2 C, HMBC, HSQC: CH_2), 22.6, 29.13, 29.18, 29.21, 29.35, 29.44, 31.8 (–, 7 C, HSQC, COSY: CH_2, *alkyl*-CH_2), 25.7 (–, 1 C, HMBC, COSY: CH_2), 28.22, 28.24 (–, 2 C, HSQC, COSY: CH_2, *alkyl*-CH_2), 28.4 (+, 18 C, HSQC: CH_3), 28.69, 28.73, 28.85, 28.97 (–, 4 C, HSQC, COSY: CH_2, *alkyl*-CH_2), 36.5 (–, 1 C, HMBC, COSY: CH_2), 38.7 (–, 1 C, HMBC, COSY: CH_2), 40.6 (–, 1 C, HMBC, COSY: CH_2), 50.1 (–, 16 C, HMBC, HSQC: CH_2), 65.17, 65.22, 77.3, 77.4 (C_q, 4 C, HMBC) 79.6, 80.1 (C_q, 6 C, HMBC),

156.3, 156.9 (C_q, 6 C, HMBC), 165.9 (C_q, 3 C, HMBC) , 173.6 (C_q, 1 C, HMBC). – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1409.3 (100) [MH^+].

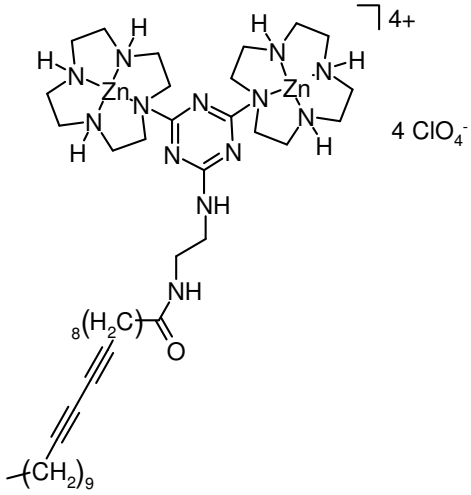


Synthesis of compound **8**

Compound **7** (108 mg, 77 µmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently 4.5 mL sat. HCl / ether were added, the solution was stirred 20 min at 0 °C and additional 20 h at room temperature. The solvent was removed in vaccuo yielding the protonated hydrochloride of compound **8** as a colourless solid (79 mg, 93 %). To obtain the free base of compound **8** a strong basic ion exchange resin was swollen for 15 min in water/MeOH (8:2) and washed neutral with water. A column was charged with resin (3.5 ml, 40.0 mmol hydroxy equivalents at a given capacity of 0.9 mmol/mL). The hydrochloride salt was dissolved in a mixture of $CHCl_3$/MeOH/water, put onto the column and eluated with the same solvent mixture. The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilized to yield 57 mg (quant.) of free base **8**, as a colourless solid.

MP: 62 - 65 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3285, 2923, 2852, 1645, 1532, 1486, 1415, 1352, 1284, 1119, 1050, 810, 721. – **^{1}H-NMR** (400 MHz, $CDCl_3$): δ (ppm) = 0.82 (bs, 3 H, COSY: CH_3), 1.21 (bs, 16 H, HSQC, COSY: CH_2, *alkyl*-CH_2), 1.31 (bs, 6 H, HSQC: *alkyl*-CH_2), 1.29-1.37 (m, 4 H, HMBC, HSQC: CH_2), 1.39-1.49 (m, 2 H, COSY, CH_2), 1.51-1.62 (m, 2 H, COSY: CH_2), 1.94-2.13 (m, 4 H, HMBC: CH_2), 2.63 (bs, 8 H, HMBC, HSQC, COSY: CH_2 cyclen), 2.74 (bs, 8 H, HMBC, HSQC, COSY: CH_2 cyclen), 2.88 (bs, 8 H, HMBC, HSQC, COSY: CH_2 cyclen), 3.33 (bs, 2 H, HMBC: CH_2), 3.39 (bs, 2 H, HMBC: CH_2), 3.72 (bs, 8 H, HMBC, HSQC, COSY: CH_2

cyclen), 5.21 (bs, 1 H, HMBC, HSQC, COSY: NH), 6.92 (bs, 1 H, HMBC, HSQC, COSY: NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 1 C, HSQC, COSY: CH_3), 19.1 (–, 2 C, HMBC, HSQC: CH_2), 22.6, 29.0, 29.13, 29.18, 29.21, 29.35, 29.45, 31.8 (–, 8 C, HSQC, COSY: CH_2, *alkyl*-CH_2), 25.7 (–, 1 C, HSQC, COSY: CH_2), 36.5 (–, 1 C, HSQC, COSY: CH_2), 28.22, 28.26 (–, 2 C, HMBC, HSQC, COSY: CH_2), 28.69, 28.76, 28.83 (–, 3 C, HSQC, COSY: *alkyl*-CH_2), 39.9 (–, 1 C, HMBC, HSQC: CH_2), 40.4 (–, 1 C, HMBC, HSQC: CH_2), 46.3 (–, 4 C, HMBC, HSQC, COSY: CH_2 cyclen), 48.0 (–, 4 C, HMBC, HSQC, COSY: CH_2 cyclen), 48.7 (–, 4 C, HMBC, HSQC, COSY: CH_2 cyclen), 49.0 (–, 4 C, HMBC, HSQC, COSY: CH_2 cyclen), 65.17, 65.22 (C_q, 2 C, HMBC, HSQC), 77.3, 77.4 (C_q, 2 C, HMBC, HSQC: solvent peak), 165.9 (C_q, 1 C, HMBC, HSQC), 166.5 (C_q, 2 C, HMBC, HSQC), 173.4 (C_q, 1 C, HMBC, HSQC). – **MS** (ESI(+), DCM/MeCN/TFA): m/z (%) = 404.8 (100) $[M + 2 H^+]^{2+}$, 270.1 (18) $[M + 3 H^+]^{3+}$, 808.8 (10) $[MH^+]$. **HRMS**: *m/z*: calcd for $C_{44}H_{81}N_{13}O$: 808.6765; found: 808.6750.

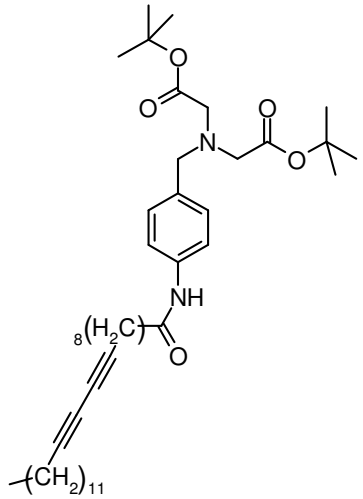


*Synthesis of compound **8-Zn***

Compound **8** (43 mg, 53 µmol) was dissolved in 1 mL of MeOH and heated to 65 °C to give a clear solution. $Zn(ClO_4)_2$ (44 mg, 117 µmol) dissolved in 1 ml of MeOH was added slowly to the stirred reaction mixture. The reaction mixture was stirred for additional 18 h at 65 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized to yield 70 mg (quant.) of **8**-**Zn** as a lightly brownish hygroscopic solid.

MP: 150 - 152 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2930, 2856, 1683, 1549, 1456, 1346, 1287, 1075, 974, 815. – **^{1}H-NMR** (600 MHz; $CDCl_3$ / CD_3OD 1:1): δ (ppm) = 0.84 (t,

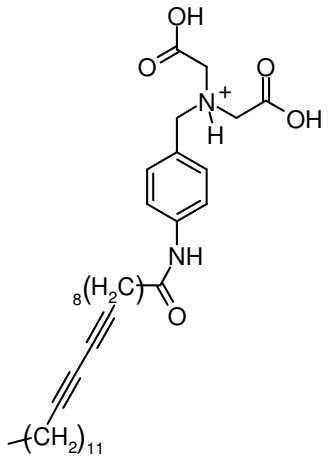
^{3}J = 7.0 Hz, 3 H, HSQC, COSY: CH_3), 1.17-1.27 (m, 18 H, HSQC, COSY: *alkyl*-CH_2), 1.32-1.40 (m, 4 H, HSQC, COSY: *alkyl*-CH_2), 1.42-1.51 (m, 4 H, HSQC, COSY: *alkyl*-CH_2), 1.52-1.62 (m, 2 H, HSQC, COSY: *alkyl*-CH_2), 2.12-2.25 (m, 6 H, HSQC, HMBC: *alkyl*-CH_2), 2.70-3.56 (m, 36 H, HSQC: CH_2 cyclen, *ED*-CH_2), 4.12-4.42 (m, 2 H, HSQC, NH). – **^{13}C-NMR** (150 MHz; $CDCl_3$/CD_3OD 1:1): δ (ppm) = 14.3 (+, 1 C, HSQC, COSY: CH_3), 19.5 (–, 2 C, HSQC: *alkyl*-CH_2), 23.1, 29.45, 29.56, 29.70, 29.72, 29.98, 30.04, 30.14, 32.41 (–, 9 C, HMBC, HSQC, COSY: *alkyl*-CH_2), 26.3 (–, 1 C, HSQC: *alkyl*-CH_2), 28.90 (–, 2 C, HSQC: *alkyl*-CH_2), 29.28 (–, 1 C, HSQC: *alkyl*-CH_2), 29.32 (–, 1 C, HSQC: *alkyl*-CH_2), 36.8 (–, 1 C, HSQC: *alkyl*-CH_2), 39.3, 40.9, 42.5, 44.2, 45.5, 45.69, 45.72, 45.8 , 46.8 (–, 18 C, HSQC: CH_2 cyclen, CH_2), 65.84 (C_q, 1 C, acetylene), 65.87 (C_q, 1 C, acetylene), 77.7 (C_q, 1 C, acetylene), 78.4 (C_q, 1 C, acetylene), 166.6 (C_q, 1 C, triazine), 171.2 (C_q, 2 C, triazine), 176.2 (C_q, 1 C, amide). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 526.8 (100) $[M^{4+}$ + 2 $CH_3COO^-]^{2+}$, 496.8 (50) $[M^{4+}–H^+$+ $CH_3COO^-]^{2+}$.



*Synthesis of compound **10***

In a dry 100 mL flask 224 mg (0.64 mmol) of compound **9** was dissolved in 50 mL of dry THF in ice cooled condition, to this 250 mg (0.64 mmol) of 10, 12-pentacosadiyonic acid chloride and few drops of Et_3N was added. This light yellow colour solution was stirred at RT for 12 h and heated to reflux for 2 h. A colourless precipitate was obtained, it was filtered off and the clear solution was evaporated in vacuum. The crude product was purified by silica column using dichloromethane and methanol as eluent. (R_f = 0.45 (DCM/MeOH, 90/10).

IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3310, 2921, 2849, 1743, 1664, 1601, 1531, 1465, 1410, 1367, 1307, 1250, 1216, 1144, 994, 844, 721. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.84 (t, 3 H, CH_3), 1.19-1.28 (bs, 26 H, CH_2), 1.42 (s, 18 H, CH_3), 1.67 (t, 2 H, CH_2), 2.17-2.13 (m, 8 H, CH_2), 2.46 (t, 2 H, CH_2), 3.35 (s, 4 H, CH_2), 3.79 (s, 2 H, CH_2), 7.26 (d, 2 H, J = 8.7 Hz, Ar-H), 7.44 (d, 2 H, J = 8.7 Hz, Ar-H). – **^{13}C-NMR** (75 MHz, $CDCl_3$): 171.7 (C_q, amide), 162.6 (C_q, ester), 137.4 (C_q, aromatic), 134.1 (C_q, aromatic), 129.7 (+, CH), 119.7 (+, CH), 81.0 (C_q, acetylene), 68.6 (C_q, acetylene), 68.2 (C_q, acetylene), 65.3 (–, CH_2), 65.2 (–, CH_2), 57.0 (C_q), 55.0 (C_q), 37.9 (–, CH_2), 37.6 (–, CH_2), 36.5 (–, CH_2), 33.8 (–, CH_2), 31.9 (–, CH_2), 31.5 (–, CH_2), 29.62 (–, CH_2), 29.60 (+, CH_3), 29.58 (–, CH_2), 29.45 (–, CH_2), 29.32 (–, CH_2), 29.18 (–,CH_2), 29.07 (–, CH_2), 28.88 (–,CH_2), 28.83 (–,CH_2), 28.74 (–, CH_2), 28.33 (– CH_2), 28.74 (–, CH_2), 28.33 (–, CH_2), 28.26 (–, CH_2), 28.15 (–, CH_2), 28.04 (–, CH_2), 28.01 (–, CH_2), 27.80 (–, CH_2), 25.6 (–, CH_2), 23.41 (–, CH_2), 22.70 (–, CH_2), 22.16 (–, CH_2), 19.17 (–, CH_2), 19.14 (–, CH_2), 14.16 (+, CH_3). – **MS** (ES-MS, DCM/MeOH + 10 mM NH_4OAc): m/z (%): 707.6 (100) [M^+], 708.6 (50) [MH^+].

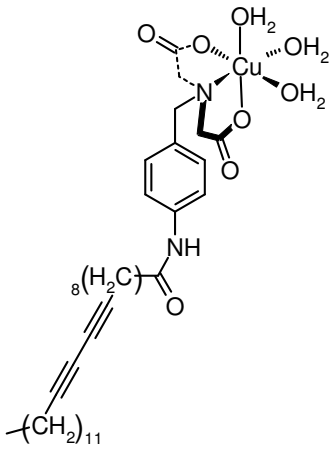


*Synthesis of compound **11***

200 mg (0.28 mmol) of Compound **10** was dissolved in TFA (5 ml) and stirred at RT for 2 h. After evaporating the TFA, the slurry was suspended in diethylether and decanted. Compound **11** (120 mg, 65%) obtained as a pale yellow hygroscopic substance.

IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2924, 2854, 1737, 1668, 1600, 1531, 1465, 1413, 1311, 1255, 1188, 1131, 903, 835, 722, 648. – **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.82 (t, 3 H, CH_3), 1.42 (bs, 26 H, CH_2), 1.53-1.61(m, 4 H, CH_2), 2.17-2.13 (m, 6 H, CH_2), 2.46 (t, 2 H, CH_2), 3.31 (s, 4 H, CH_2,), 3.79 (s, 2 H, CH_2,), 7.26 (d, J = 8.7 Hz, 2 H, Ar-H),

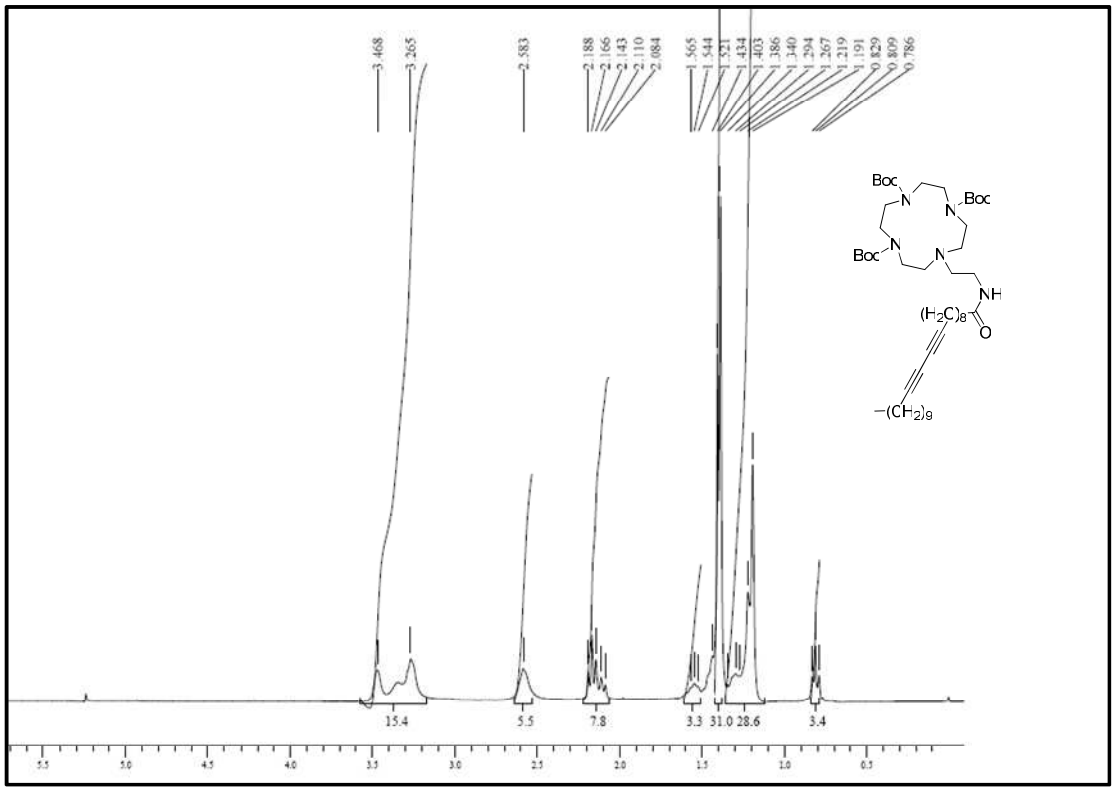
7.44 (d, J = 8.7 Hz, 2 H, Ar-H). – **^{13}C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.3 (C_q, amide), 168.7 (C_q, ester), 140.2 (C_q, aromatic), 131.7 (C_q, aromatic), 124.1 (+, CH), 120.36 (+, CH), 68.6 (C_q, acetylene), 68.2 (C_q, acetylene), 65.30 (–, CH_2), 65.24 (–, CH_2), 57.1 (C_q, acetylene), 55.0 (C_q, acetylene), 31.9 (–, CH_2), 29.57 (–, CH_2), 29.43 (–, CH_2), 29.29 (–, CH_2), 29.04 (–, CH_2), 28.98 (–, CH_2), 28.90 (–, CH_2), 28.82 (–, CH_2), 28.32 (–, CH_2), 22.63 (–, CH_2), 19.16 (–, CH_2), 14.16 (+, CH_3). – **MS** (ESI, DCM/MeOH): m/z (%) = 609.3 (100) [MH^+], 1218.1 (20) [2 MH^+].

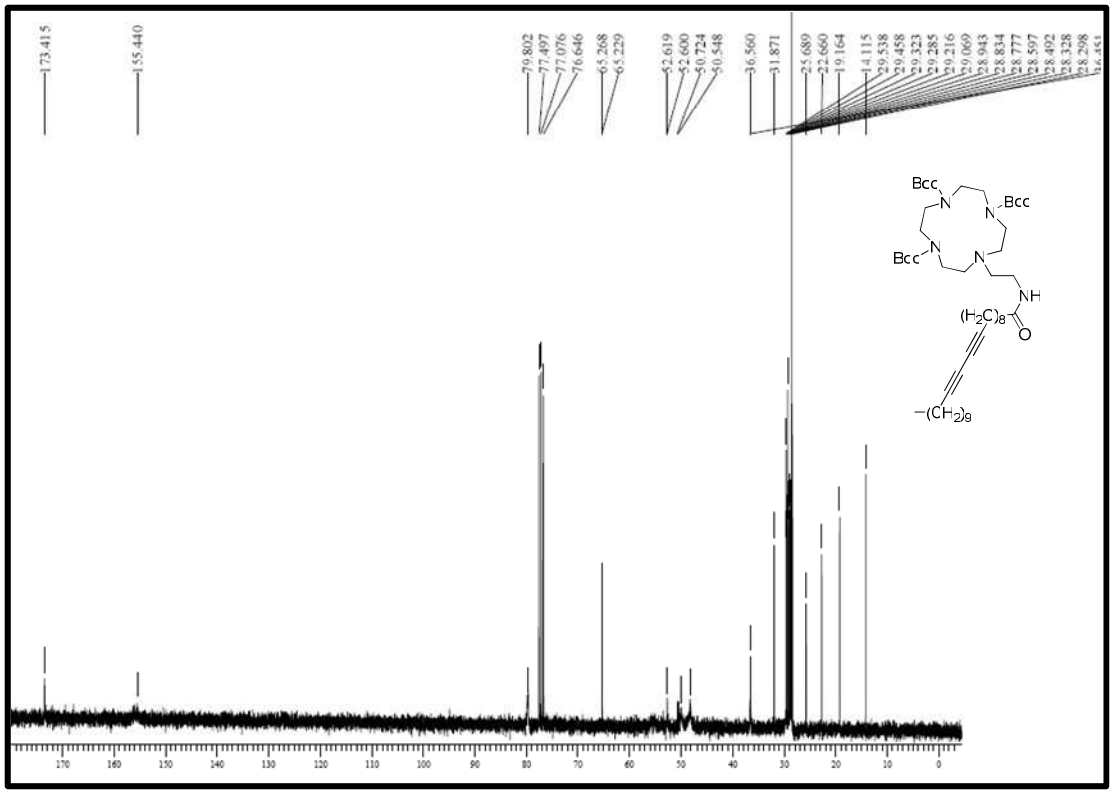


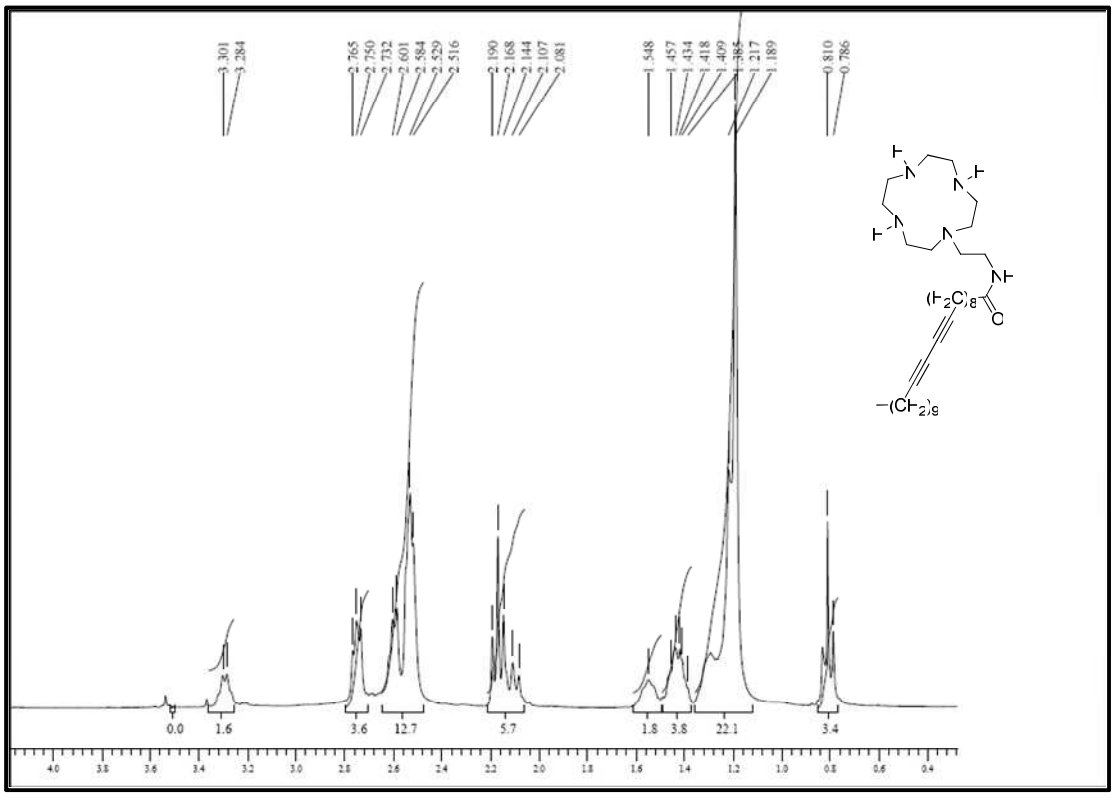
Synthesis of compound **11-Cu**

To a solution of **11** (100 mg, 0.12 mmol) in 10 ml of water $CuCl_2$ (23 mg, 0.12 mmol) dissolved in MeOH (10 ml) was added. Stirring was continued at room temperature for 10 h. The solvent was evaporated and the residue dissolved in a small amount of water. Methanol was added until a precipitate was formed and the solid was removed by filtration. Methanol was evaporated and the crude product was recrystallised from methanol yielding compound **11-Cu** (103 mg, 86 %) as a greenish blue solid. NMR spectrum of this complex was not recorded due to paramagnetic nature of this complex. **MP**: 217-221 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3308, 2920, 2851, 1662, 1612, 1557, 1466, 1406, 1363, 1302, 1250, 1184, 1079, 1008, 956, 924, 897, 831, 741. – **MS (**ES-MS, DCM/MeOH + 10 mM NH_4Ac): m/z (%) = 714.4 (100) [$M^+ + CH_3COO^- - 3\ H_2O$].

5.4.4 ^{1}H NMR and ^{13}C spectra of synthesized compounds



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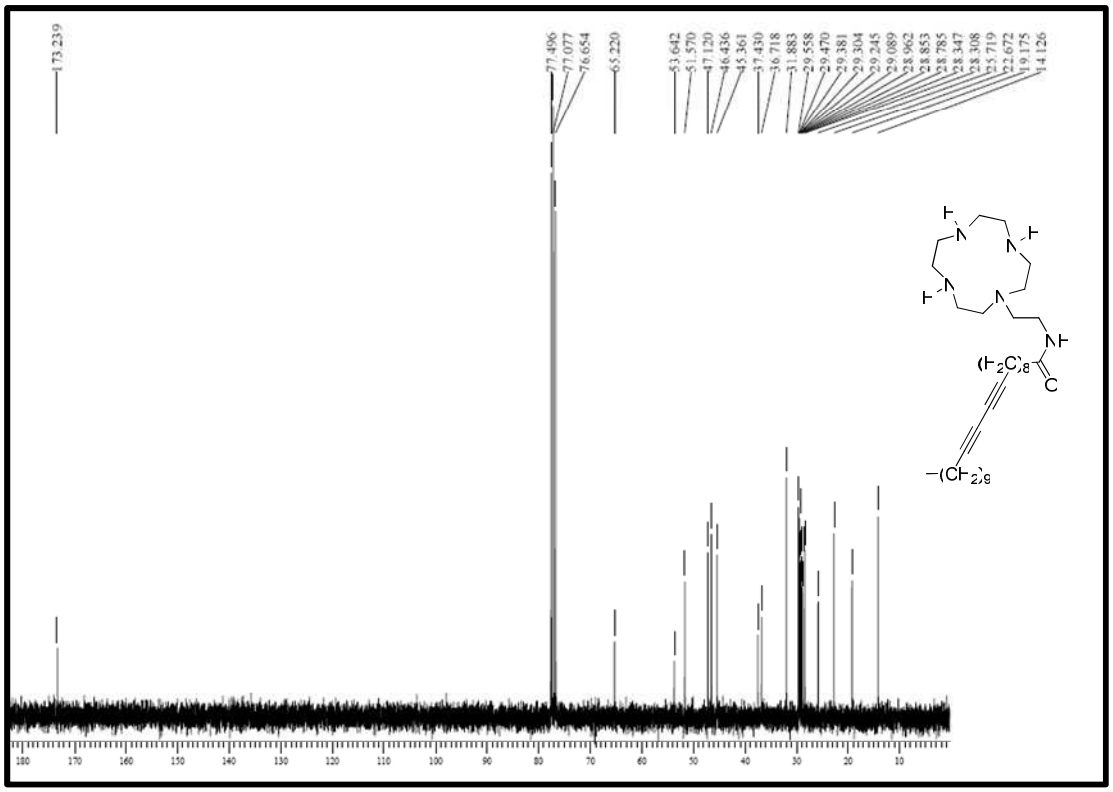
2.765
2.750
2.732
2.601
2.584
2.529
2.516
2.190
2.168
2.144
2.107
2.081
1.548
1.457
1.434
1.418
1.409
1.385
1.217
1.189
0.810
0.786
0.0
1.6
3.6
12.7
5.7
1.8
3.8
22.1
3.4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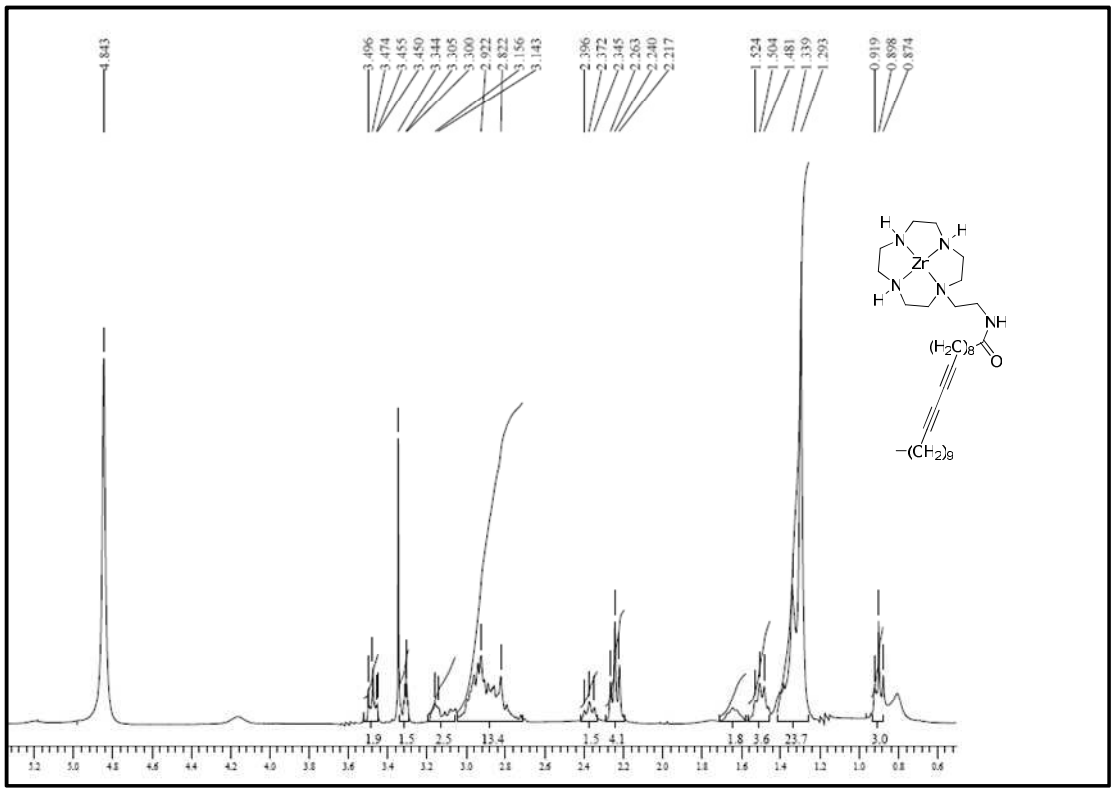
77.496
77.077
76.654
65.220
53.642
51.570
47.120
46.436
45.361
37.430
36.718
31.883
29.558
29.470
29.381
29.304
29.245
29.089
28.962
28.853
28.785
28.347
28.308
25.719
22.672
19.175
14.126


4.843
3.496
3.474
3.455
3.450
3.344
3.305
3.300
2.922
2.822
3.156
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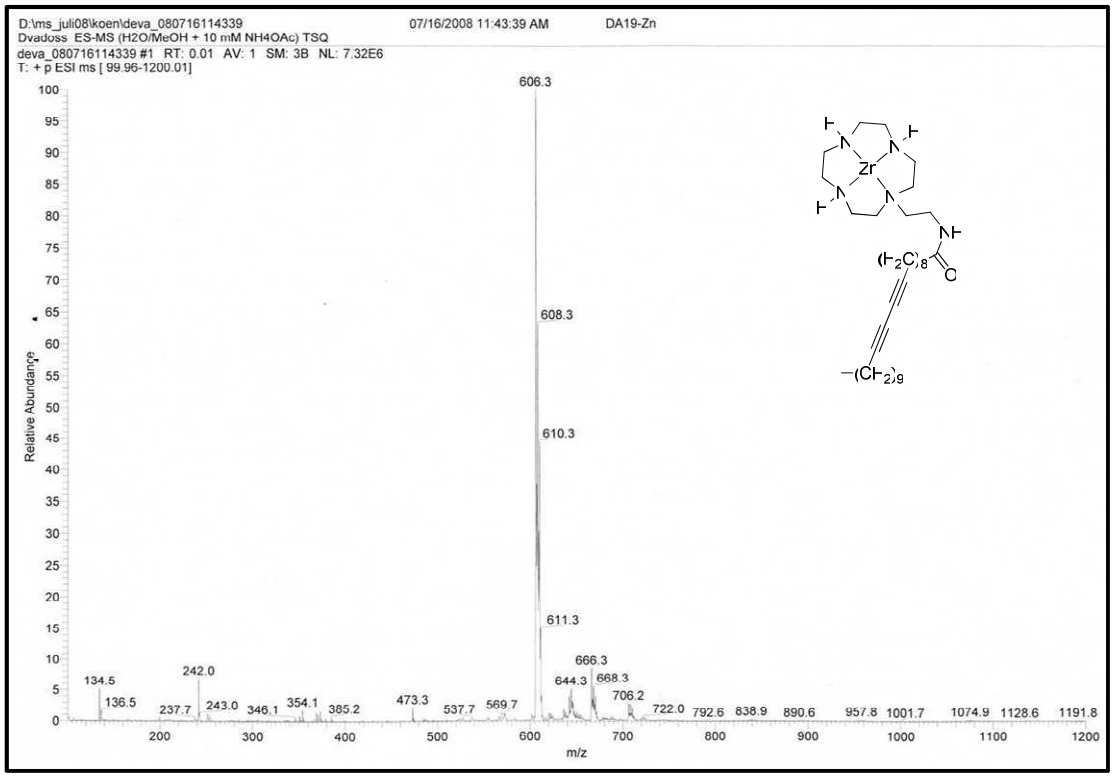
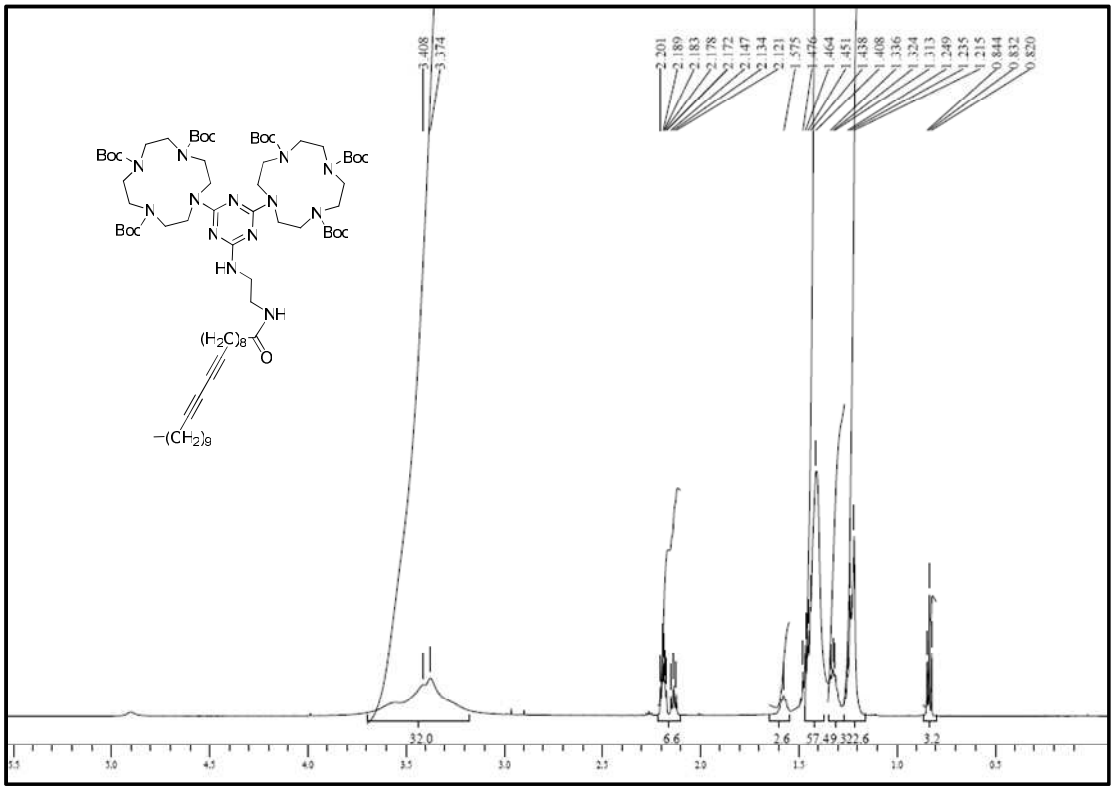
2.396
2.372
2.345
2.263
2.240
2.217
1.524
1.504
1.481
1.339
1.293
0.919
0.898
0.874
1.9
1.5
2.5
13.4
1.5
4.1
1.8
3.6
23.7
3.0
Zr
NH
(H2C)8
O
(CH2)9


D:\ms_juli08\koen\deva_080716114339
07/16/2008 11:43:39 AM
DA19-Zn
Dvadoss ES-MS (H2O/MeOH + 10 mM NH4OAc) TSQ
deva_080716114339 #1 RT: 0.01 AV: 1 SM: 3B NL: 7.32E6
T: + p ESI ms [99.96-1200.01]
606.3
608.3
610.3
611.3
666.3
668.3
644.3
706.2
242.0
134.5
136.5
237.7
243.0
346.1
354.1
385.2
473.3
537.7
569.7
722.0
792.6
838.9
890.6
957.8
1001.7
1074.9
1128.6
1191.8
Relative Abundance
m/z
Zr
NH
(H2C)8
O
(CH2)9


Boc
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NH
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O
−(CH2)9
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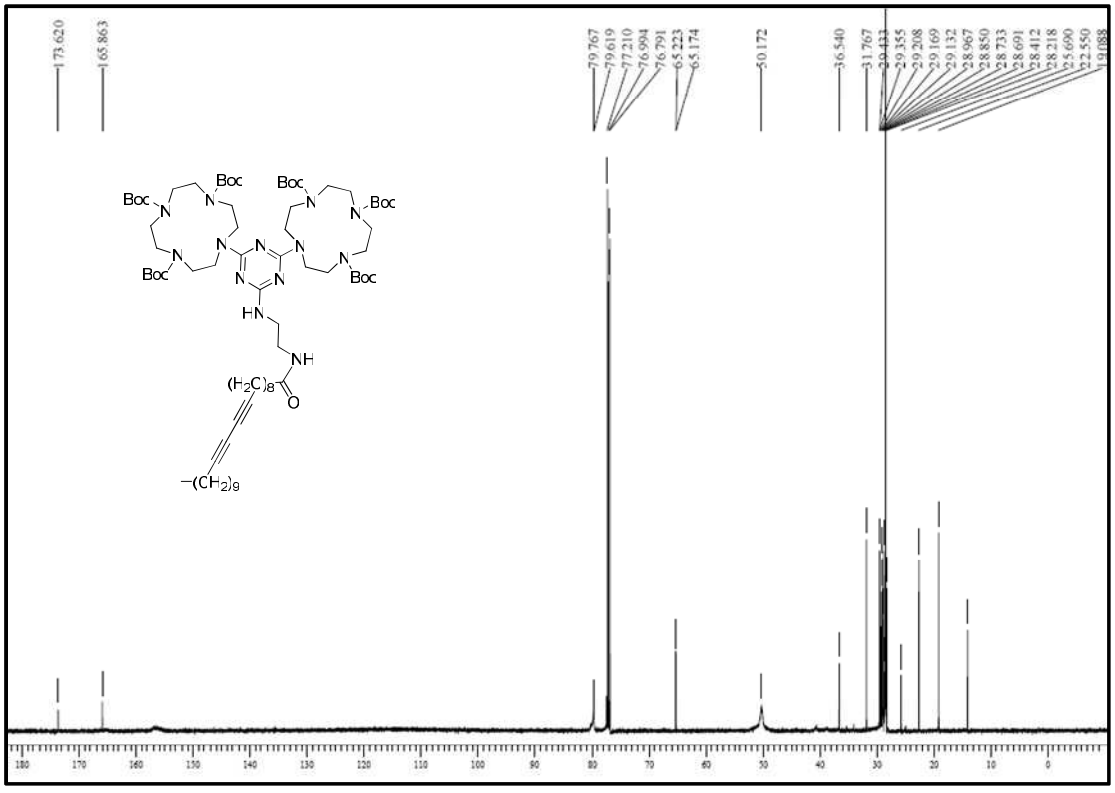
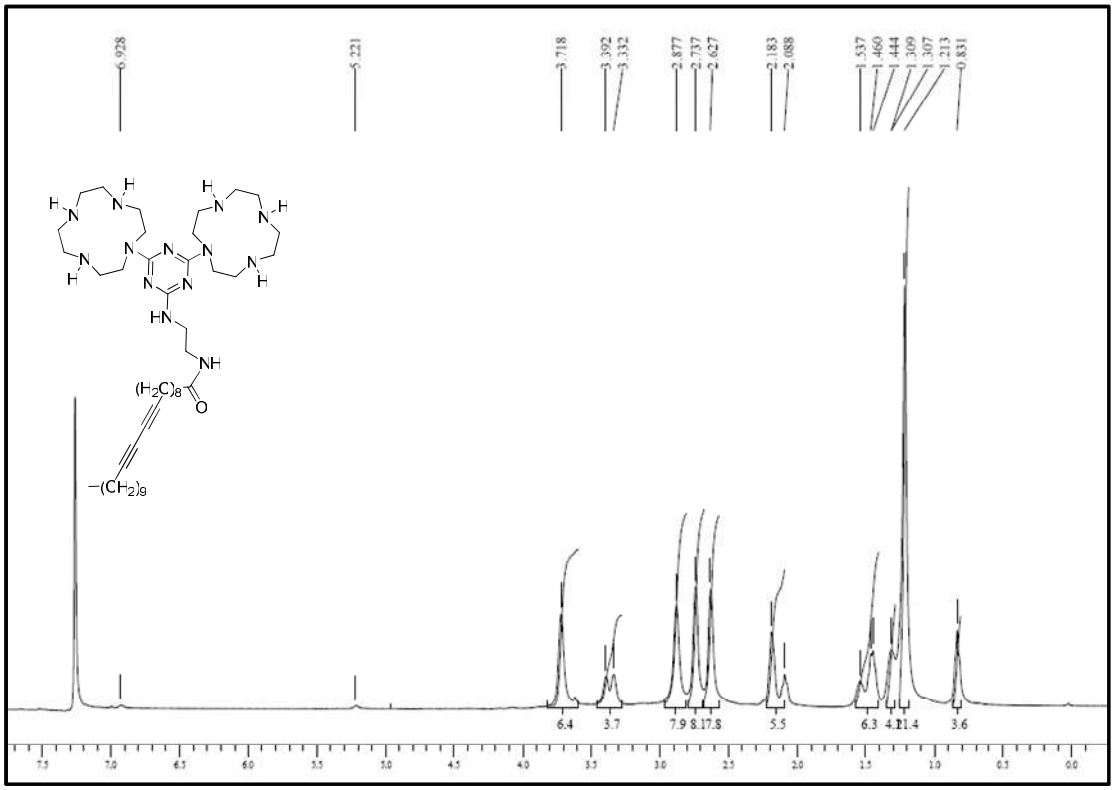
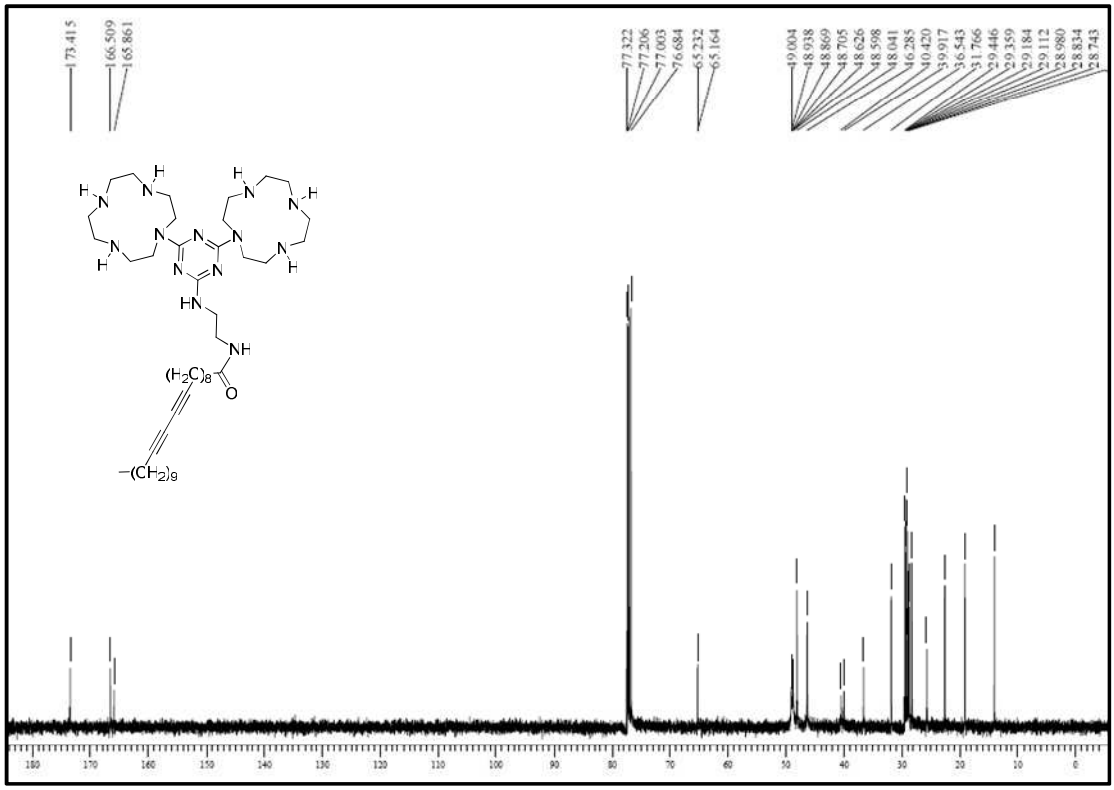
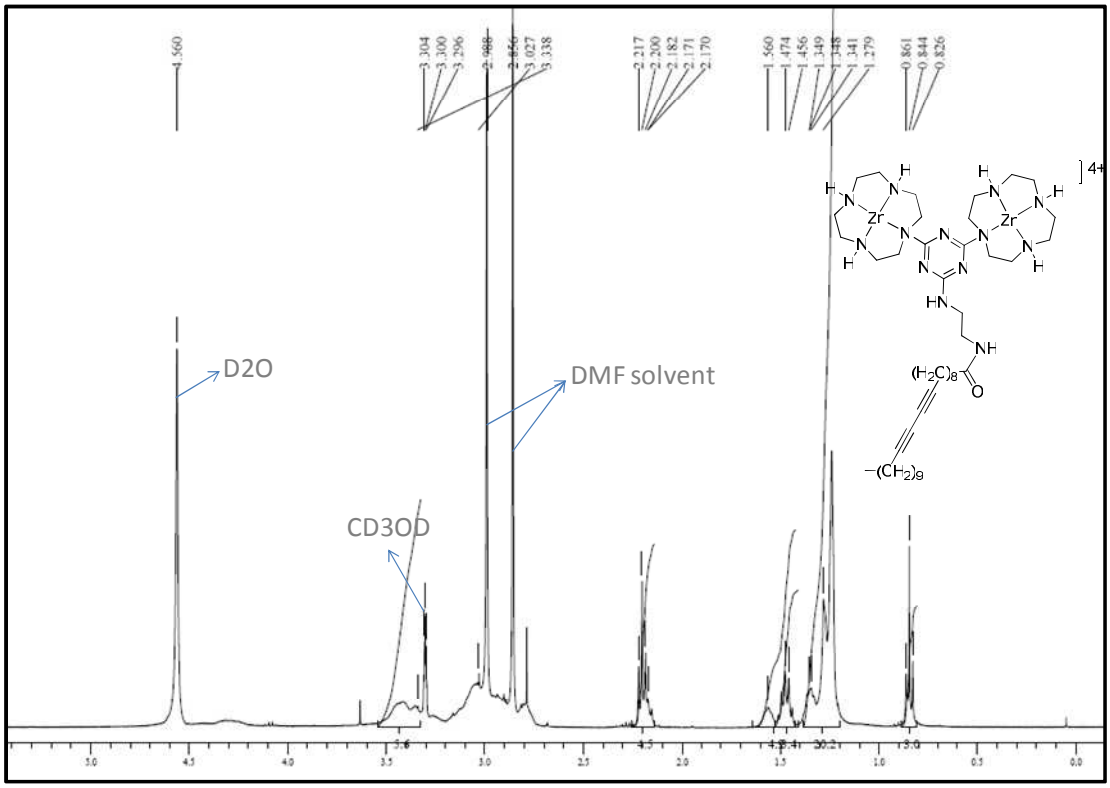
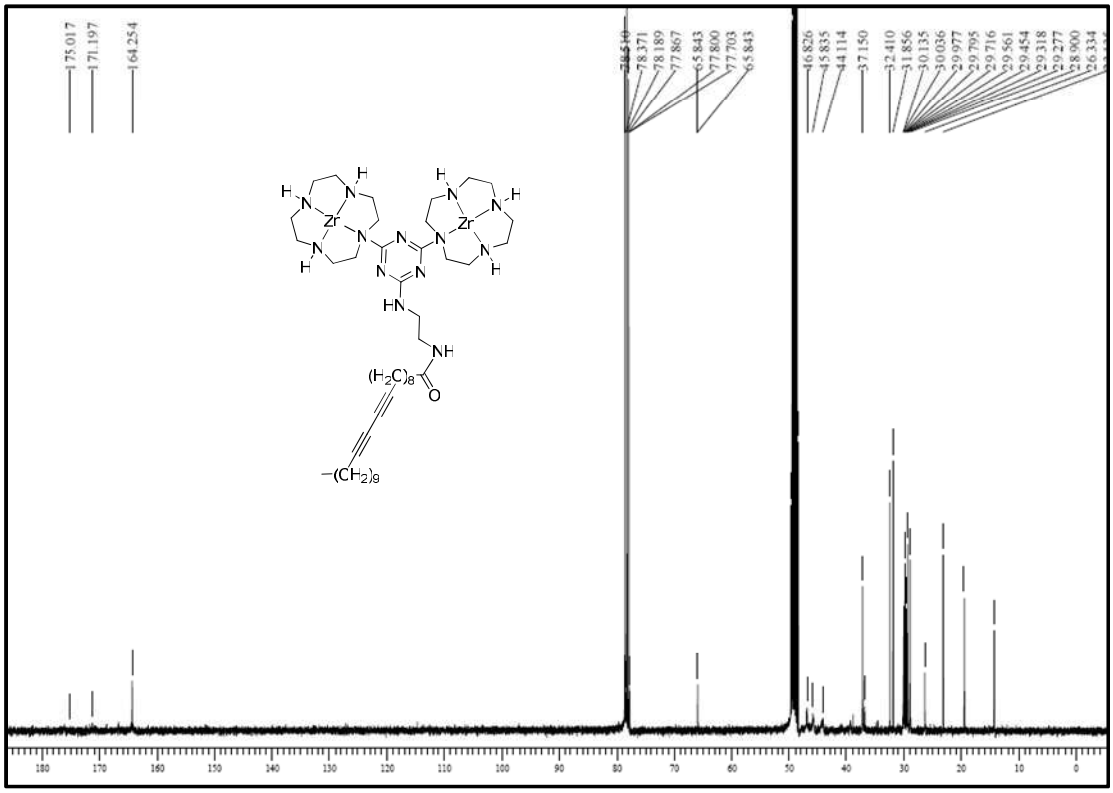
6.6
2.6
57.4
9.3
22.6
3.2

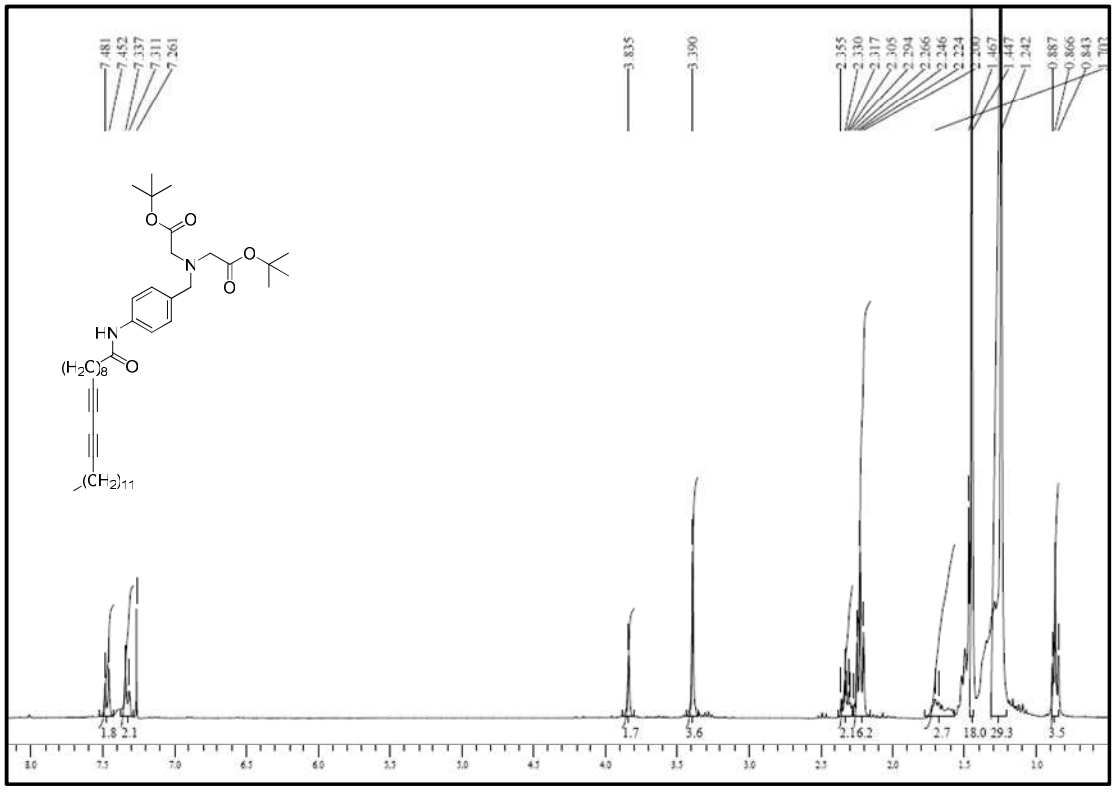

Boc
HN
NH
(H2C)8
O
−(CH2)9

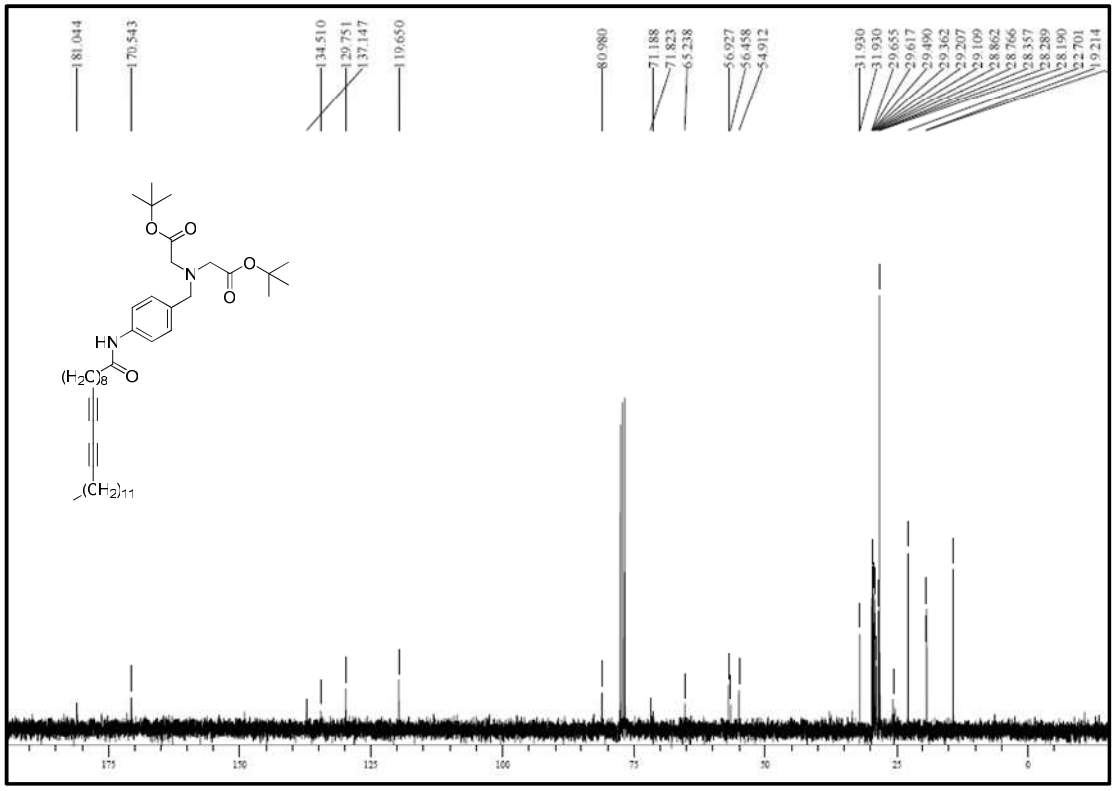

6.928
5.221
3.718
3.392
3.332
2.877
2.737
2.627
2.183
2.088
1.537
1.460
1.444
1.309
1.307
1.213
0.831
6.4
3.7
7.9
8.1
7.8
5.5
6.3
4.2
11.4
3.6


173.415
166.509
165.861
77.322
77.206
77.003
76.684
65.232
65.164
49.004
48.938
48.869
48.705
48.626
48.598
48.041
46.285
40.420
39.917
36.543
31.766
29.446
29.359
29.184
29.112
28.980
28.834
28.743


D2O
DMF solvent
CD3OD
4+




7.481
7.452
7.337
7.311
7.261
3.835
3.390
2.355
2.330
2.317
2.305
2.294
2.266
2.246
2.224
1.467
1.447
1.242
0.887
0.866
0.843
HN
(H2C)8
O
(CH2)11
1.8 2.1
1.7
3.6
2.16.2
2.7
18.0 29.3
3.5


181.044
170.543
134.510
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127.147
119.650
80.980
71.188
71.823
65.238
56.927
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54.912
31.930
31.930
29.655
29.617
29.490
29.362
29.207
29.109
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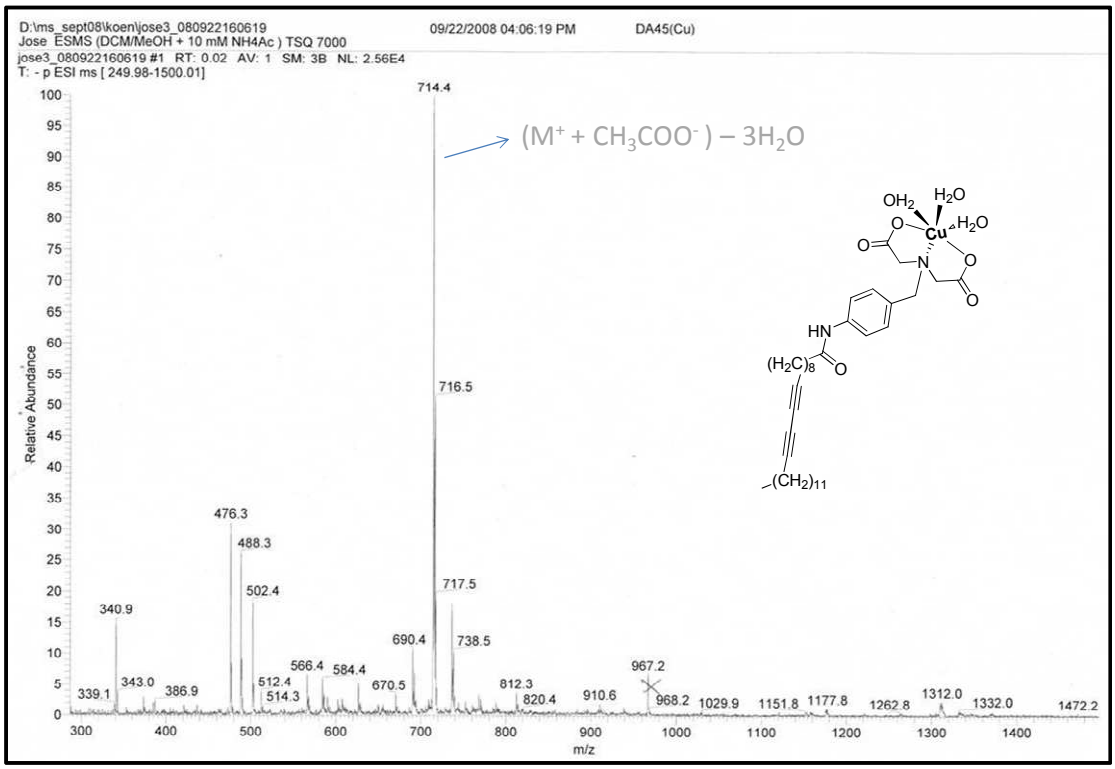
28.357
28.289
28.190
22.701
19.214
HN
(H2C)8
O
(CH2)11


D:\ms_sept08\koen\jose3_080922160619
09/22/2008 04:06:19 PM
DA45(Cu)
Jose ESMS (DCM/MeOH + 10 mM NH4Ac) TSQ 7000
jose3_080922160619 #1 RT: 0.02 AV: 1 SM: 3B NL: 2.56E4
T: - p ESI ms [249.98-1500.01]
(M+ + CH3COO-) – 3H2O
Relative Abundance
m/z
714.4
716.5
717.5
476.3
488.3
502.4
340.9
690.4
738.5
566.4
584.4
512.4
514.3
670.5
339.1
343.0
386.9
812.3
820.4
910.6
967.2
968.2
1029.9
1151.8
1177.8
1262.8
1312.0
1332.0
1472.2
OH2
H2O
H2O
Cu
O
N
HN
(H2C)8
(CH2)11

5.5 References

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6. Vesicular Receptors with co-embedded Amphiphilic Zinc Cyclen Complexes and Fluorophors for Phosphate Anion Sensing in Water[i]

Phosphate anion probes typically consist of a binding site and a luminescent reporter group. The luminescent moiety is either part of the chemosensor in close proximity of the analyte binding site or in indicator displacement assays non-covalently bound to the binding site and displaced by the analyte. We report here the preparation and binding properties of 80 nm vesicular synthetic receptors, which contain amphiphilic 1,4,7,10-tetraazacyclododecane (cyclen) Zn(II) complexes as phosphate anion binding sites and amphiphilic coumarin derivatives as fluorescent reporter groups.[ii] By colocalization of binding sites and reporter groups in the vesicle they respond to the presence of phosphate anions in aqueous solution at micromolar concentrations by a strong emission decrease.[iii] The technique avoids the covalent synthesis of labelled analyte binding sites and allows the rapid and versatile preparation of luminescent nanometer size synthetic receptors.

[i] S. Stadlbauer, B. Gruber. K. Woinaroschy, B. König *Inorg. Chem.* **2009**, *in preparation*.

[ii] All compounds shown in this chapter were synthesized by S. Stadlbauer, except compound **3** was prepared by K. Woinaroschy.

[iii] All vesicular receptors and the analytical measurements on their binding properties were done by B. Gruber. IDA assay for **1** was done by K. Woinaroschy.

6.1 Introduction

Molecular recognition of phosphate esters under physiological conditions is of current interest as they are ubiquitously present in nature[1] in RNA and DNA, in phosphorylated saccharides and phosphorylated proteins.[2] The nucleotide adenosine triphosphate (ATP) is the molecular currency for intracellular energy transfer,[3] and pyrophosphate ($P_2O_7^{4-}$, PP_i), the product of ATP hydrolysis, plays an important role in intracellular signalling.[4] Therefore the development of artificial phosphate anion receptors for use under physiological conditions is of continious interest. Such sensors are useful tools for the detection of biologically important phosphates,[5] with applications in molecular biology, life and environmental sciences.

There are two different ways to signal the binding of an analyte to a synthetic receptor: A luminescent group is located closely to the binding site and responds to the binding event by a change in its emission properties.[6] Alternatively, an indicator-displacement assay (IDA) based on the competitive binding of a pH indicator and the analyte to the non-labelled binding site is used to signal the interaction of the analyte and the receptor.[7]

Recent reports reveal that transition metal complexes with vacant coordination sites are well suited to serve as phosphate ion binding sites.[8] A widely used binding unit in phosphate chemosensors is the zinc(II)-dipicolylamine (Dpa) complex as demonstrated by Hamachi[9], Hong[10] and Smith[11]. Macrocyclic 1,4,7,10-tetraazacyclo-dodecane (cyclen) transition metal complexes were reported as phosphate binding sites by Kikuchi[12] and Kimura.[13] We have recently reported the use of zinc(II)-cyclen as promoters in ester hydrolysis,[14] detection of phosphorylated peptides[15] and proteins[16] and for a sterically guided molecular recognition of nucleotides, nucleobases and phosphates in supramolecular self-assembled systems (SAM,[17] unilamellar vesicles[18]).

However, phosphate detection principles using luminescent labels or indicator displacement have drawbacks: The synthesis of chemosensors can be laboursome and their analyte response is hard to predict, while IDA is an indirect method.

We have therefore combined parts of both techniques into a new approach using surface modified vesicles, which contain amphiphilic Zn(II)-cyclen complexes for phosphate anion binding and co-embedded amphiphilic fluorophors to signal the interaction. Such self-assembled supramolecular vesicular receptors signal the presence

of biological relevant phosphates in micro molar concentrations by an increased emission.

6.2 Results and Discussion

6.2.1 Syntheses of amphiphilic Zn(II)-cyclen complexes

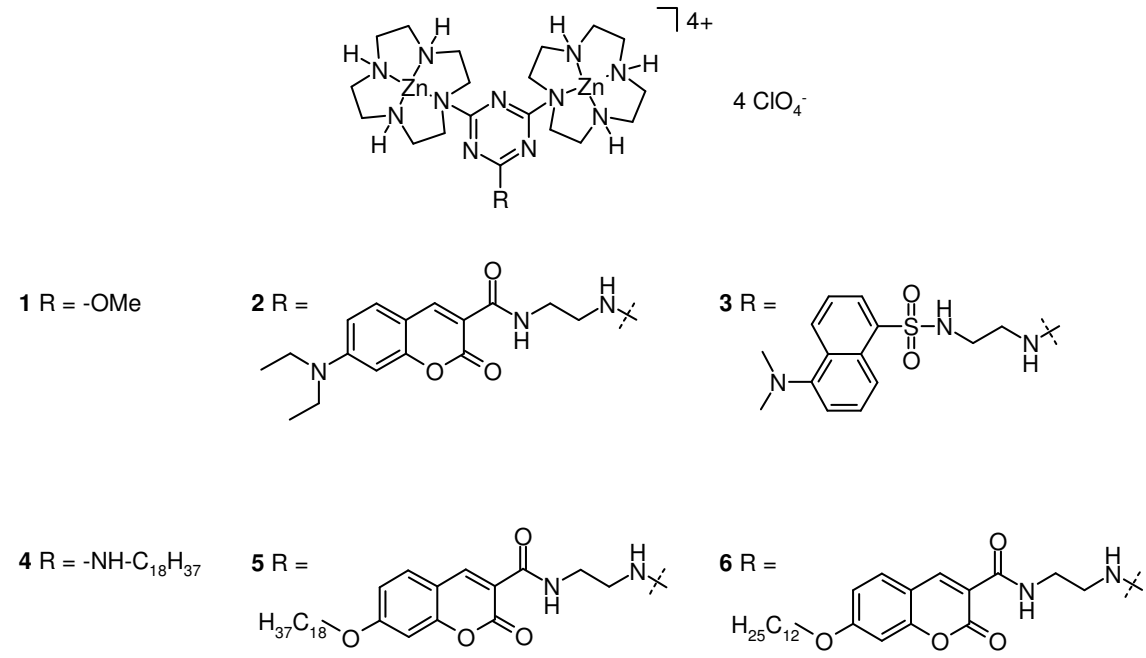
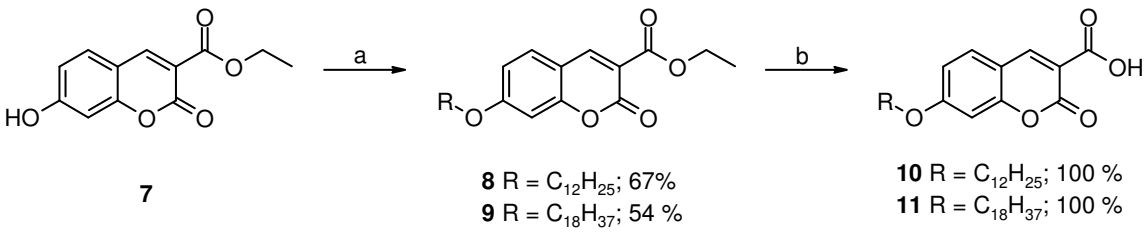


Figure 1. Fluorescent/non-fluorescent and amphiphilic/non-amphiphilic binuclear Zn(II)-cyclen complexes for phosphate binding in aqueous media.

The previously reported triazen-bis-zinc cyclen complex **1** was modified with fluorescent groups (**2, 3**), alkyl chain (**4**) or both (**5, 6**). Figure 1 summarizes all structures. Complexes **1**[14b], **2**[16] and **4**[17] were synthesized as previously reported. The synthesis of compound **3** is given in the Experimental Part. The synthesis of amphiphilic coumarin derivatives is shown in Scheme 1. Coumarin was selected as it is known for its high quantum yields and ease of preparation.[19] Williamson ether synthesis with alkyl bromide on coumarin **7**[19] and saponification gave the amphiphilic fluorescent labels **10** and **11**, respectively.



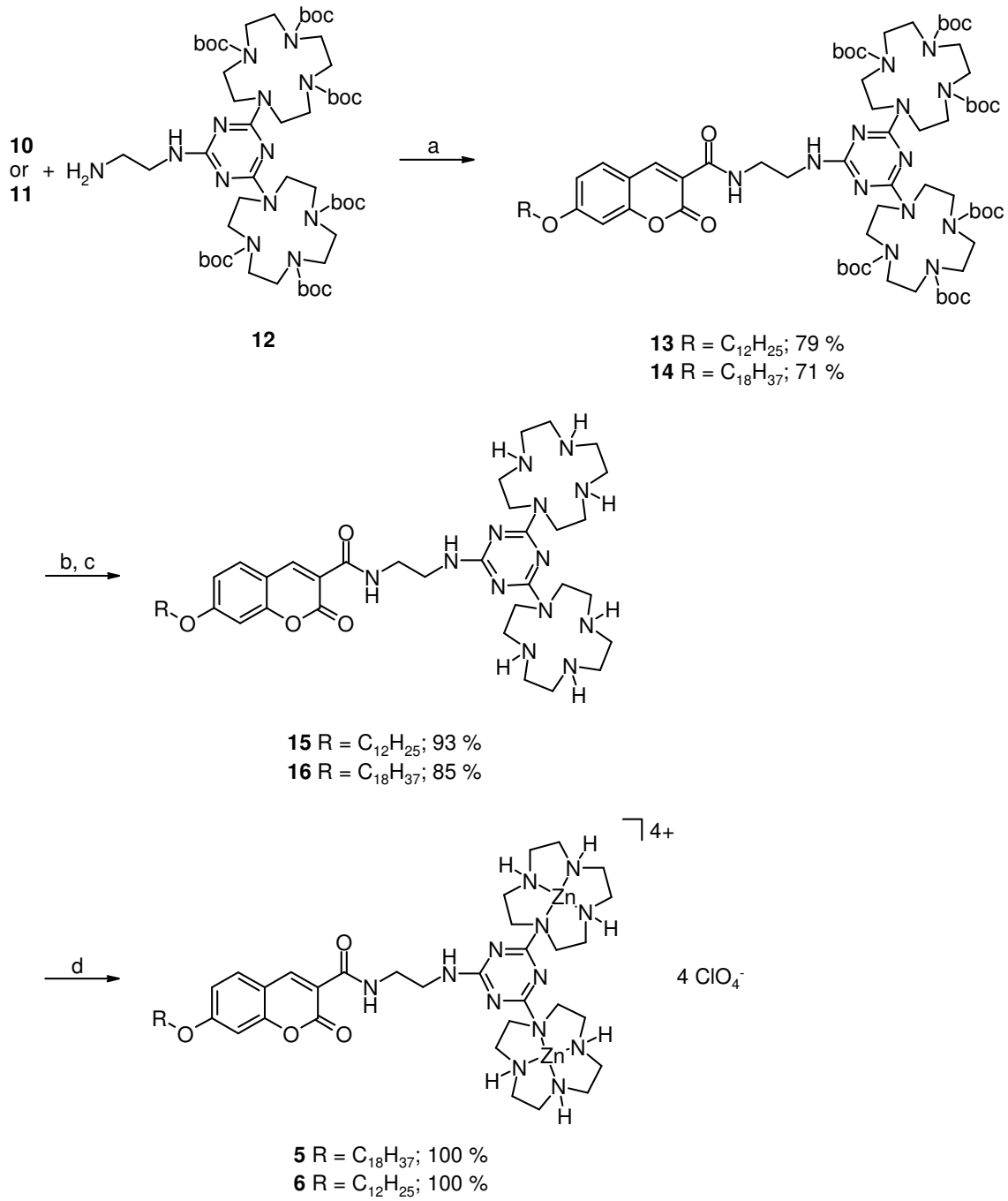
7

8 R = $C_{12}H_{25}$; 67%
9 R = $C_{18}H_{37}$; 54 %

10 R = $C_{12}H_{25}$; 100 %
11 R = $C_{18}H_{37}$; 100 %

Scheme 1. Synthesis of an amphiphilic coumarin derivative with various hydrophobic chains. (a) Br-$(CH_2)_n$-CH_3 (n = 11 or 17), K_2CO_3, DMF, 80 °C, 20 h; (b) NaOH, THF, reflux, 5 h.

Binuclear Zn(II)-cyclen complexes **5** and **6** were prepared by amide formation using standard peptide coupling conditions in solution as shown in Scheme 2. Removing of the Boc protecting groups and subsequent basic ion exchanger resin gave the free amine ligands which finally were treated with two equivalents of a methanolic solution of $Zn(ClO)_4$. Detailed experimental procedures and analytical data of the prepared compounds are provided in the Experimental Part.



Scheme 2. Synthesis of fluorescent amphiphilic binuclear Zn(II)-cyclen complexes. (a) TBTU, HOBt, DIPEA, DMF, 40 °C, 2.5 h; (b) HCl/ether, RT, o/n; (c) basic ion exchanger resin H_2O, MeOH; (d) $Zn(ClO_4)_2$, MeOH, 65 °C, 20 – 24 h.

6.2.2 Preparation of Zn(II)-cyclen modified vesicles (vesicular receptors)

Surface modified vesicles (vesicular receptors) were prepared from a mixture of commercially available synthetic phospholipid 1,2-distearoyl-sn-glycero-3-phospho-

choline (DSPC) and amphiphilic Zn(II)-cyclen complexes **5**, **6** (10 mol% in respect to used DSPC) by the well-established film-hydration-method.[20] For the non-fluorescent complex **4** amphiphilic dyes **10** or **11** were additionally incorporated into the liposomal membrane to equip the vesicular receptors with signalling units. The resulting multilamellar vesicles (MLVs) were homogenized by extrusion to yield small unilamellar vesicles (SUVs) of a defined size of 80 nm.

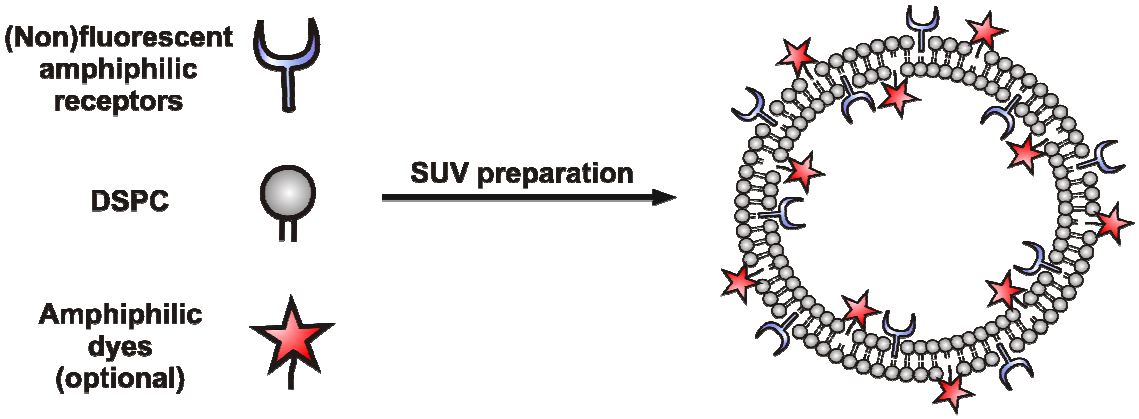


Figure 2. Schematic of functionalized vesicles with surface exposed receptors.

The individual receptor units of the obtained vesicles are assumed to be equally distributed in both layers of the liposomal membrane. Thus, we established a correction factor *f* describing the outer surface exposed receptors as a fraction of its entire quantity of matter. This factor enables the determination of the effective concentration of available binding sites on the outer layer of the vesicle (for details see Experimental Part).

6.2.3 Characterization, purification and stability of vesicle dispersion

The particle size, particle number and sample dispersity of the prepared vesicle dispersions were determined by dynamic light scattering (DLS).[21] The average hydrodynamic diameter of SUV dispersions of simple DSPC vesicles was found to be 110 (± 5) nm, while the average hydrodynamic particle diameter of dispersions of vesicular receptors was determined to be slightly smaller with 80 (± 5) nm. The obtained results are in good agreement with comparable liposome preparations that have been reported in literature.[22]

Generally, homogenized SUV dispersions are assumed to be free of impurities and thus no further purification is required. Nevertheless vesicles can be passed through

size exclusion chromatography (SEC) columns to ensure complete exclusion of unimolecular amphiphiles or lower molecular aggregates[23].

All prepared vesicular receptor dispersions and vesicle dispersions were stored as buffered aqueous solutions at 6 °C in the refrigerator and used within 2 weeks.

6.2.4 Phosphate anion binding studies

Initially, the binding properties of Zn(II)-cyclen **1** to various phosphate species were investigated by an indicator displacement assay utilizing pyrocatechol violet (PV) in HEPES buffered solution (10 mM, pH 7.4) by UV-VIS spectroscopy. Upon coordination to zinc ions PV shows a colour change resulting from a decreasing absorption at λ_{max} = 443 nm and an increasing absorption at λ_{max} = 636 nm (Figure **3**a).[24] By addition of aliquots of aqueous solutions (HEPES buffer 10 mM, pH 7.4) of the sodium and/or potassium salts of ATP, ADP, cAMP, GTP, PPi, hydrogen phosphate and phenyl phosphate to a 1:1 mixture of **1** and PV (35 µM each) the indicator is partially or fully displaced (Figure 3b). The binding constants (lg K) of **1** to the different phosphate anions (Table 1) were derived from the concentrations of PV and the respective phosphate anion at 50% release of the indicator. The addition of other anions, such as SO_4^{2-}, NO_3^{2-}, N_3^-, CO_3^{2-}, Br^-, Cl^-, ClO_4^-, tatrate, ascorbate or acetate, did not displace the PV indicator from the metal complex: The absorption at λ_{max} = 443 nm remains unchanged and only a slight decrease at λ_{max} = 636 nm is observed.[25]

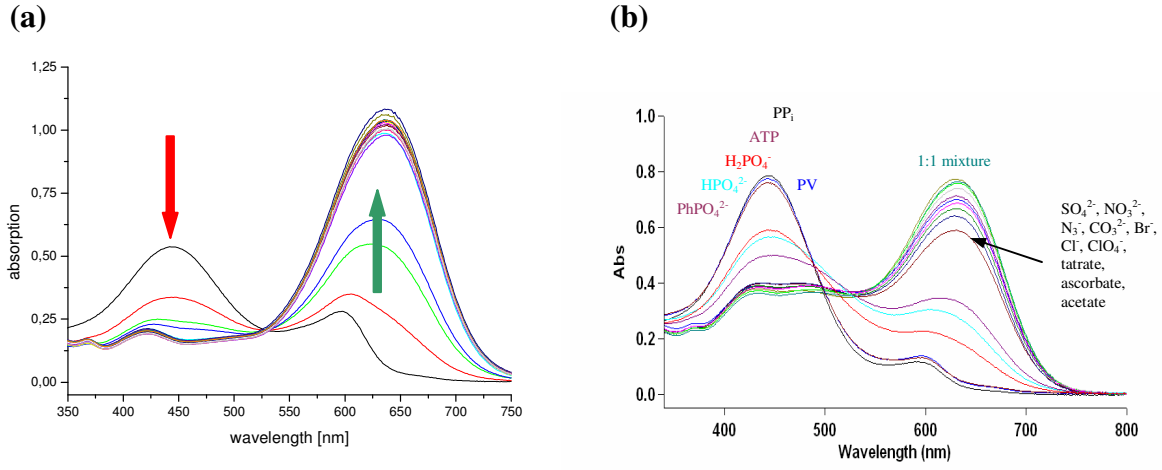


Figure 3. (a) Addition of **1** (0-105 µM) to a constant concentration of **PV** (35 µM). Titrations were performed at 25 °C in 10 mM HEPES buffer, pH 7.4. (b) UV/Vis spectra of a 1:1 mixture **1** and **PV** (50 µM, λ_{max} = 636 nm) in the presence of various anions (250 µM). Only phosphate anions are able to displace the indicator with λ_{max} = 443 nm. The displacement, and therefore the binding ability of **1**, is proportional to the number of negative charges on the phosphate.

The binding affinity is clearly influenced by the number of negative charges on the phosphate, as was previously reported for other phosphate anion receptors.[12a, 26] ATP, GTP and PP_i have the highest negative charge and thus show the highest binding constants (lg K ~ 6), whereas for cAMP (lg K = 3.8) with one negative charge partial displacement of the indicator was observed only upon addition of an excess of analyte (> 6 eq). As the IDA method represents an indirect method for the determination of the binding event, we used the Zn(II)-cyclen complexes **2** and **3** functionalized with a fluorescence label and investigated their response to phosphate anions (e.g. PP_i, ATP, GTP, ADP, Na_2HPO_4, GDP and other nucleotides). However, none of the added anions induced a significant change in the absorption or emission properties of **2** or **3** (data not shown). The coordination of a phosphate anion obviously does not influence the photophysical properties of the attached fluorophores.

Having acquired these informations on phosphate anion solution binding of complexes **1**, **2** and **3** we turned our attention to self-assembled surface modified vesicles for anion sensing[18b] and molecular recognition.[27] Thus a set of vesicular receptors modified by phosphate binding moieties **4** – **6** were prepared.

A vesicular receptor (**VR-4**) with the hydrophobic binuclear Zn(II)-cyclen complex **4** was synthesized and its binding affinity to various phosphate anions was investigated by IDA methods employing coumarin methyl sulfonate (CMS) as an indicator dye (Figure 4a). Pyrophosphate, due to its small size and high charge density, exhibited one of the highest affinities of the tested compounds with a binding constant lg *K* of 7.1. The highest binding constant was found for UTP (7.2), which exceeded the affinities of the other tested nucleotides ATP and GTP (both 6.5) by five times. This may be explained by binding of both phosphate and imide moieties of UTP to the Zn(II)-macrocycles.[17] However, no difference in binding affinities of the nucleoside diphosphates UDP and GDP was observed. GDP and UDP, both with a binding constant of 5.2, exhibited an affinity that is one to two orders of magnitude lower compared to their respective triphosphates. This is reasonable because of the weaker coordination capabilities of the diphosphates. However, the stronger decrease of lg *K* from UTP (7.2) to UDP (5.2) compared to GTP/GDP (6.5/5.2) cannot be explained on the basis of the present data. Fructose-1,6-bisphosphate also exhibited a rather high affinity (lg *K* = 6.4), which might be explained by the favourable interaction with two bis-Zn(II)-cyclen moieties revealing additive or cooperative action: Following the binding of the first phosphate group, the binding of the second phosphate group is facilitated by the

preformed substrate-receptor complex. Inorganic phosphate showed the same binding constant as the diphosphates GDP and UDP. At the given pH value inorganic phosphate exists predominantly as a dianion, having one negative charge less than GDP and UDP. The similar binding affinities might be explained by the lack of steric hindrance of inorganic phosphate as well as by an increase of its acidity upon complexation by the bis-Zn(II)-cyclen moieties resulting in an additional negative charge. The phosphate monoesters phenylphosphate and phosphoserine in contrast exhibited an affinity that is one order of magnitude lower than inorganic phosphate, obviously due to their larger steric demands and only two acidic protons present at the phosphate moiety. For all tested and compared compounds the respective binding constants to the vesicular receptor were found to be higher than those to complex **1**. Only a minor increase was found for the monophosphates phenylphosphate and inorganic phosphate, whereas the difference in the binding constants for pyrophosphate amounted to an entire order of magnitude. The ion selectivity of vesicular receptor **VR-4** was investigated by the addition of other anionic compounds like sulfate, azide and acetate (Figure 4b). Furthermore imidazole was tested as a potential ligand as the bis-Zn(II)-cyclen moieties are known to exhibit a weak affinity for histidine residues (Figure 4b).[28] None of these compounds exhibited a considerable affinity towards vesicular receptor **VR-4**. Thus, binding constants for these compounds could not be determined but were estimated to be smaller than lg $K = 2$.

(a) **(b)**

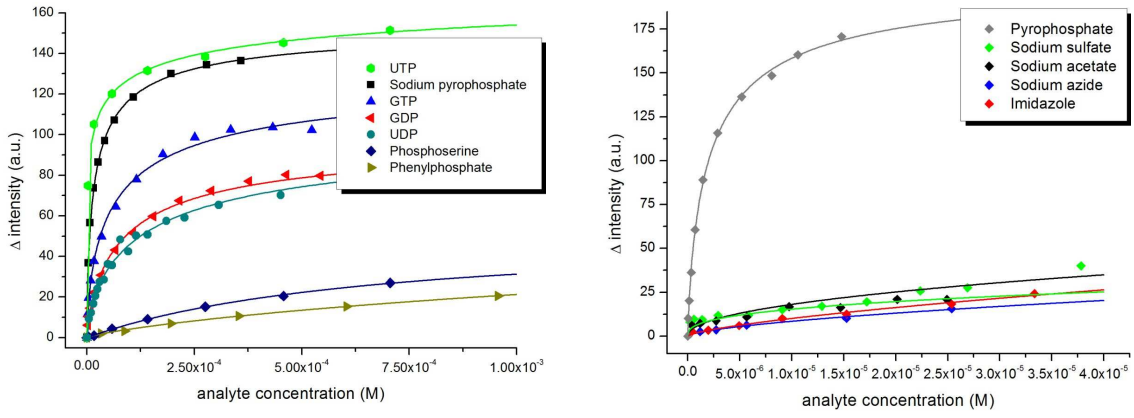


Figure 4. (a) Binding isotherms obtained by indicator displacement assay (IDA) for vesicular receptor **VR-4** and various phosphate species. (b) Phosphate ion selectivity of vesicular receptor **VR-4**.

		lg *K*			
Entry	**Phosphate species**	**1**	**VR-4**	**VR-5**	**VR-4/11**
1	Pyrophosphate	5.9[a]	7.1[c]	6.6	5.6
2	UTP	– [b]	7.2[c]	–	–
3	ATP	5.9[a]	6.5[c]	6.6	5.3
4	GTP	5.8[a]	6.5[c]	–	–
5	GDP	– [b]	5.2[c]	–	–
6	UDP	– [b]	5.2[c]	–	–
7	Fructose-1,6-bisphosphate	– [b]	6.4[c]	6.1	–
8	Inorganic phosphate	4.9[a]	5.2[c]	5.5	–
9	p-Ser	– [b]	4.3[c]	–	–
10	Ph-O-PO_3	4.0[a]	4.2[c]	–	–

Table 1. Summary of binding constants to various phosphate species obtained by indicator displacement assays in solution for the binuclear Zn(II)-cyclen motif. Apparent error limits for detected binding constants are ± 0.2.
[a] Binding affinities were obtained by IDA methods (indicator dye: PV) with UV-VIS absorption titration. [b] Binding experiments were not done. [c] Binding affinities were obtained by IDA methods (indicator dye: CMS) by emission titration (λ_{ex} = 396 nm, λ_{em} = 480 nm).

Using the amphiphilic fluorescent binuclear Zn(II)-cyclen **5** and its corresponding vesicular receptor **VR-5** a direct signalling of the phosphate binding event was possible (Figure 6): Upon addition of phosphate anions, such as PP_i, ATP, fructose-1,6-bisphosphate and inorganic phosphate, the emission intensity at 405 nm of the coumarin label decreased. The determined binding affinities exceed the micro molar range and are consistent with the corresponding values obtained by indicator displacement assay for the vesicular receptor **VR-4** and compound **1**. Modifying the tethered hydrophobic alkyl chain of the binuclear Zn(II)-cyclen complex from C_{18} to C_{12} (compound **6**) did not affect the binding affinity of the vesicular receptor to PP_i (data not shown).

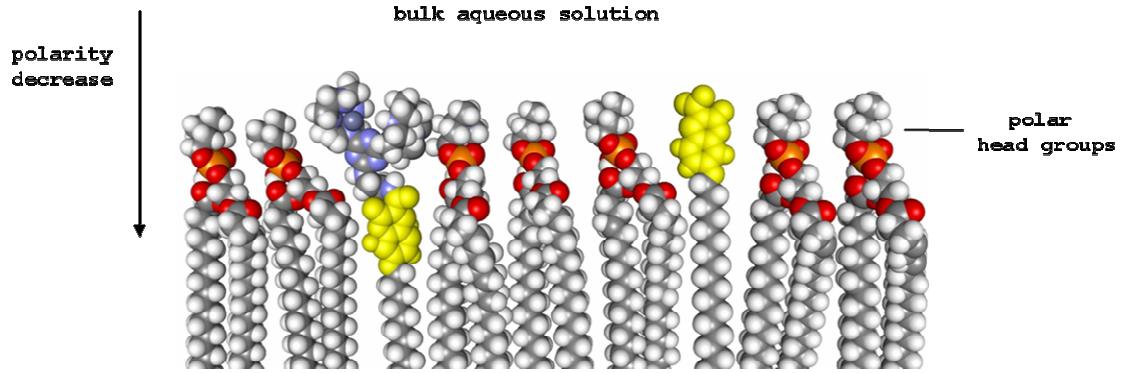


Figure 5. Supposed structural arrangement of embedded **5** and **11** in the vesicle bilayer formed by DSPC lipid. The coumarin fluorophore (yellow) is located at different depth in the membrane.

Monitoring of phosphate binding to compounds **2** and **3** by changes of their luminescence was not possible in aqueous solution, most likely due to an insufficient crosstalk of their binding and signalling sites. Compounds **5** and **6** are expected to be embedded with their alkyl chain and the coumarin dye into the vesicle bilayer, as reported for similar coumarin derivatives (Figure 5).[29] This should significantly restrict their movement in the highly ordered vesicle bilayer, which might be beneficial for the sensing properties.[30] In addition, the local polarity change at the fluorophore, which is a crucial factor for the response to phosphate binding, is expected to be larger at the vesicle membrane – water interface compared to bulk water.[29] Coumarin dye derivatives are known for their solvatochromism in various solvents of different polarity.[31]

Noteworthy, a vesicular receptor (**VR-4/11**) containing the amphiphilic non-fluorescent metal complex **4** and the amphiphilic coumarin dye **11** showed a fluorescence response upon addition of PP_i to the liposomal dispersion. Clearly a change in the emission at 405 nm was observable and allowed the calculation of the binding constant by non linear fitting methods (Figure 6b). The derived lg *K* values are within the error limits of the method in good agreement with the values given in table 1.[32]

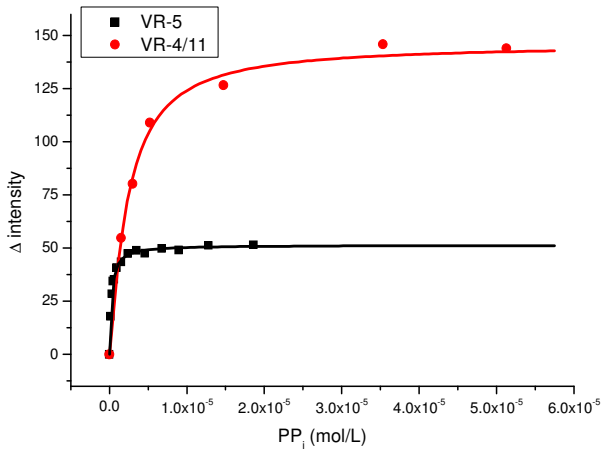


Figure 6. Binding isotherm obtained by direct emission signalling of PP_i binding to vesicular receptors **VR-5** and **VR-4/11**.

6.3 Conclusion

The modification of a vesicle surface with an amphiphilic binuclear Zn(II)-cyclen complex yields a molecular vesicle receptor with sensitivity and selectivity to phosphate anions under physiological conditions exceeding micro molar affinities. While phosphate sensing in solution utilizing the binuclear Zn(II)-cyclen complex **1** requires IDA methods, an embedding in a vesicle enables the resulting vesicular receptor for a direct signalling of the phosphate binding event. Even more, a statistical mixture of the phosphate binding moiety and an amphiphilic dye leads to vesicles showing emission changes in the presence of phosphate anions. The results clearly demonstrate the use of modified vesicles with embedded metal complexes in the field of molecular recognition. Especially, the ease of preparation of more complex vesicular receptors by simple addition of binding and signalling sites may allow the practical design of selective chemosensors using the existing knowledge on artificial binding sites from the field of supramolecular chemistry.

6.4 Experimental Part

6.4.1 General methods and material

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck or Baker).

Dynamic light scattering. PCS measurements were performed on a Malvern Zetasizer 3000 at 25 °C using 1 cm disposable polystyrene fluorescence cuvettes (VWR). Three subsequent measurements of 60 seconds each were performed for each sample. Data analysis was performed using the Malvern PCS software.

NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in 13C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

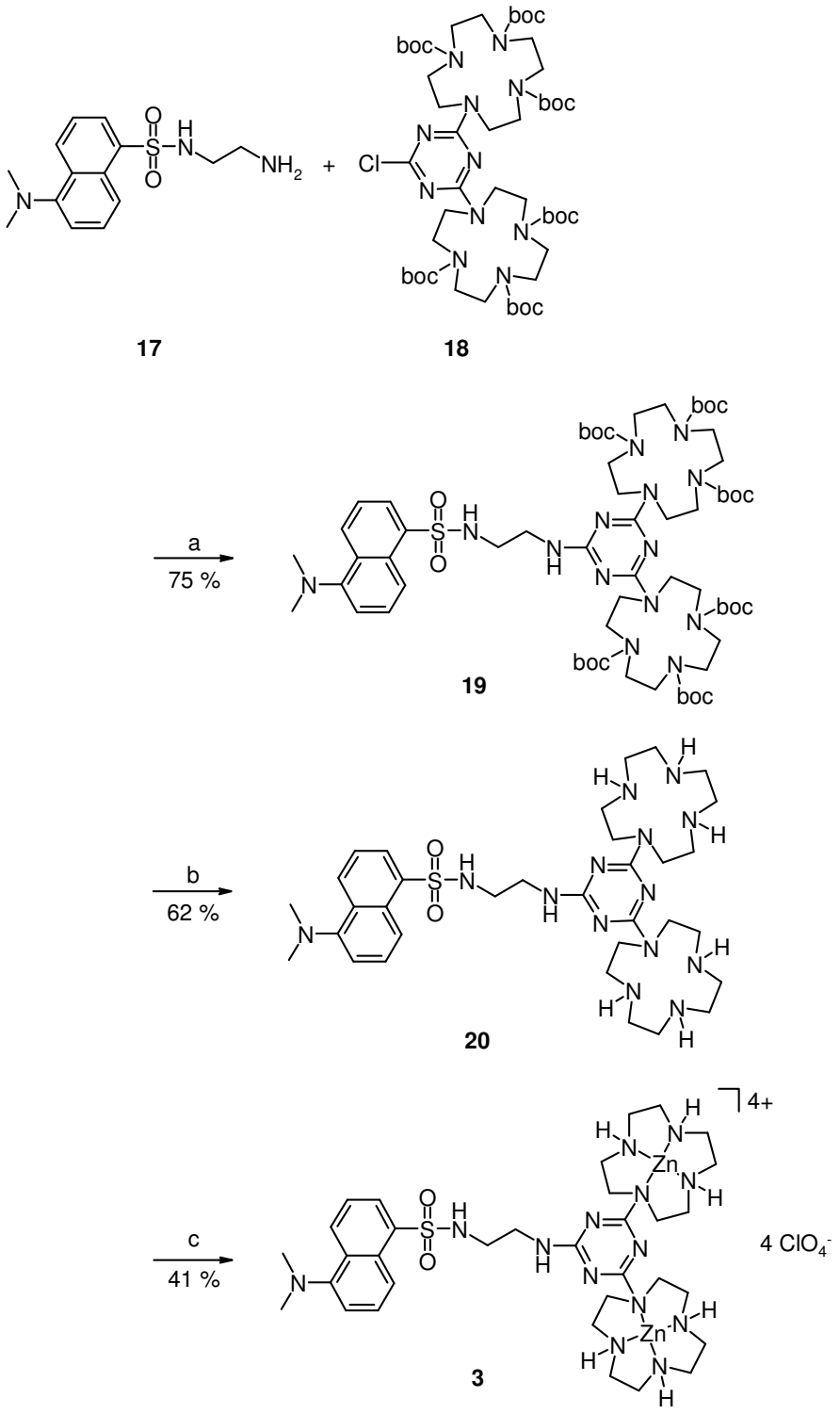
Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

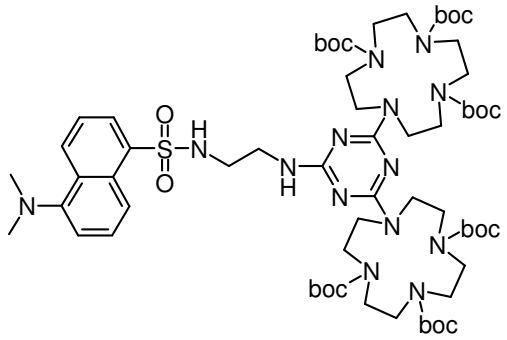
Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

General. Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Detection via UV light at 254 nm / 366 nm or through staining with ninhydrin in EtOH. Column chromatography was performed on silica gel (70–230 mesh) from Merck. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Commercially available solvents of standard quality were used. Dry THF, which was prepared by distillation from potassium. If otherwise stated, purification and drying was done according to accepted general procedures.[33] Elemental analyses were carried out by the Center for Chemical Analysis of the Faculty of Natural Sciences of the University Regensburg.

6.4.2 Synthesis



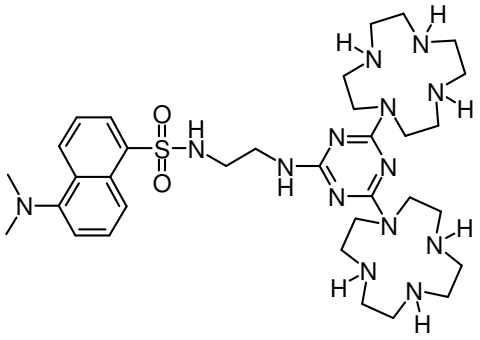
Scheme 3. Syntheses of fluorescent binuclear Zn(II) cyclen derivative **3**. (a) 5-Dimethylamino-naphthalene-1-sulfonic acid (2-amino-ethyl)-amide **17**, K_2CO_3, dioxane, reflux, 72 h; (b) TFA, DCM RT 12 h, basic ion exchanger resin; (c) $Zn(ClO_4)_2$, H_2O, 65 °C.



***5-Dimethylamino-naphthalene-1-sulfonic acid {2-[4,6-bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1,4,7-tricarboxylic acid tri-tert-butyl ester* (19)**

A solution of **18**[14b] (0.886 g, 0.84 mmol) in 25 mL of dioxane was stirred under nitrogen for 5 minutes, then a solution of 5-dimethylamino-naphthalene-1-sulfonic acid (2-amino-ethyl)-amide **17**[34] (0.615 g, 2.10 mmol) in dioxane (75 mL) was added dropwise. Then K_2CO_3 (0.580 g, 4.19 mmol, 5 eq.) was added. The mixture was refluxed for 72 h at 140 ºC under inert atmosphere. After completion, the reaction mixture was filtered in order to remove all inorganic salts and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on neutral alumina (EE/PE 3:7 to 2:3). The product was obtained as a yellow solid (0.820 g, 0.62 mmol, 75 %).

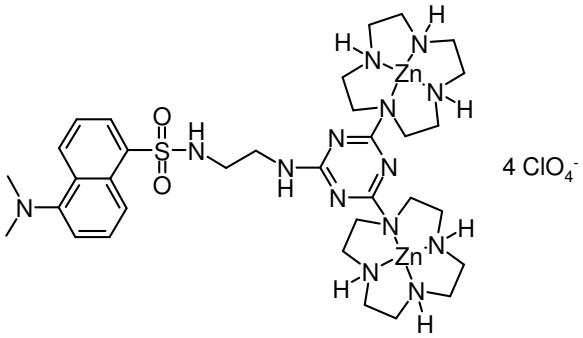
MP: 113 °C (sublimation). – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 1.41 (bs, 54 H, CH_3-Boc), 2.86 (s, 6 H, CH_3N), 3.10-3.68 (m, 36 H, CH_2 chain and CH_2 cyclen), 4.77 (bs, 1 H, $NHSO_2$), 6.95 (bs, 1 H, NH-triazine), 7.13 (d, 1 H, ^3J = 7.6 Hz, CH), 7.47 (dd, 1 H, ^3J = 7.6, 8.7 Hz, CH), 7.48 (dd, 1 H, ^3J = 7.3, 8.5 Hz, CH), 8.21 (d, 1 H, ^3J = 7.3 Hz, CH), 8.36 (d, 1 H, ^2J = 8.7 Hz, CH), 8.5 (d, 1 H, ^2J = 8.5 Hz, CH). – **^{13}C-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 28.5 (+, CH_3-Boc), 41.8 (–, CH_2NHSO_2), 43.6 (–, CH_2NH-triazine), 45.4 (+, $(CH_3)_2N$), 50.09, 50.16, 50.25, 50.29 (–, CH_2 cyclen), 79.78, 80.1, 80.3 (C_q, Boc), 115.0, 123.1, 127.78, 127.82, 129.8, 129.8, 129.9, 129.9 (+, CH); 135.08 (C_q, Cq-SO_2), 151.7 (C_q, Cq-N-$(CH_3)_2$), 156.2 (C_q, Boc), 165.8 (C_q, triazine). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3267, 2975, 2933, 2361, 2200, 1686, 1541, 1499, 1474, 1410, 1366, 1321, 1249, 1163, 1106, 1050, 970, 946, 858, 777. – **UV** (CH_2Cl_3): λ_{max} (lg ε) = 340 nm (3.655). – **MS** (ESI, DCM/MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 1313.9 (100) $[MH]^+$. – **Elemental analysis** calcd (%) for $C_{63}H_{104}N_{14}O_{14}S$: C 57.59, H 7.98, N 14.93; found: C 57.22, H 8.11, N 14.66. – **MF**: $C_{63}H_{104}N_{14}O_{14}S$ – **FW**: 1313.66 g/mol



***5-Dimethylamino-naphthalene-1-sulfonic acid {2-[4,6-bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-amide* (20)**

A solution of **19** (0.770 g, 0.59 mmol) in CH_2Cl_2 was treated with TFA (3.6 mL, 47 mmol) and the reaction mixture was stirred for 24 h. After completion of reaction the solvent was removed under reduced pressure. The obtained pale yellow solid (quantitative yield) was solved in water and passed through a column of pre-swelled (pH = 7) basic ion exchanger resin. The fractions having a basic pH were collected and the resulting aqueous solution was lyophilised. The product was obtained as a yellow solid (0.258 g, 0.36 mmol, 62 %).

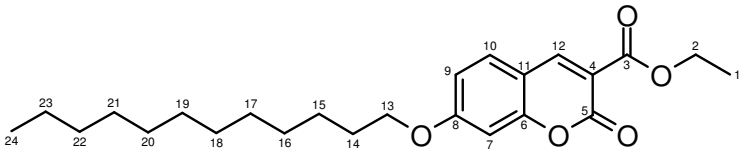
MP: 92 °C. – **^{1}H NMR** (300 MHz; MeOD): δ (ppm) = 2.62 (bs, 8 H, CH_2 cyclen), 2.70 (bs, 8 H, CH_2 cyclen), 2.87 (s, 14 H, $(CH_3)_2N$ and CH_2 cyclen), 3.00 (t, 2 H, ^{3}J = 6.0 Hz, CH_2NH-triazine), 3.32 (t, 2 H, ^{3}J = 6.0 Hz, CH_2NHSO_2), 3.7 (bs, 8 H, CH_2 cyclen), 7.23 (d, 1 H, ^{3}J = 7.6 Hz, CH), 7.50 (dd, 1 H, ^{3}J = 7.6, 8.6 Hz, CH), 7.55 (dd, 1 H, ^{3}J= 7.3, 8.5 Hz, CH), 8.17 (d, 1 H, ^{3}J = 7.3 Hz, CH), 8.26 (d, 1 H, ^{3}J = 8.6 Hz, CH), 8.53 (d, 1 H, ^{3}J = 8.5 Hz, CH). – **^{13}C NMR** (300 MHz; MeOD): δ (ppm) = 41.2 (–, CH_2NHSO_2), 44.5 (–, CH_2NH-triazine), 45.8 (–, $(CH_3)_2N$), 46.9, 48.6, 48.7, 48.9 (–, CH_2 cyclen), 116.28 (+, CH), 120.4 (+, CH), 124.2 (+, CH), 129.0 (+, CH), 130.1 (+, CH), 131.0 (C_q), 131.1 (+, CH), 131.2 (C_q), 136.9 (C_q, Cq-SO_2), 153.2 (C_q, Cq-N-$(CH_3)_2$), 167.2 (C_q, Cq-triazine cyclen), 168.0 (C_q, Cq-triazine NH). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3397, 2938, 2840, 2361, 2200, 1542, 1497, 1416, 1362, 1294, 1142, 1063, 940, 792, 625, 572. – **UV** (CH_2Cl_3): λ_{max} (lg ε) = 336 nm (3.766). – **MS** (ESI, TFA/AcN/H_2O): *m/z* (%): 357.4 (100) $[M + 2\ H^+]^{2+}$, 713.6 (20) $[MH]^+$. – **HRMS** Calcd for $C_{33}H_{56}N_{14}O_2S$: 712.4331; found: 712.4421. – **MF**: $C_{33}H_{56}N_{14}O_2S$ – **FW**: 712.96 g/mol



***Bis-Zn(II)-cyclen dansyl* (3)**

Compound **20** (120 mg, 0.17 mmol) was dissolved in 1 mL of water and heated to 65 °C to give a clear yellow solution. Subsequently zinc(II)-perchlorate (64 mg, 172 µmol) dissolved in 1 ml of water was added slowly. The pH was adjusted by addition of 1 M NaOH (approx. 2 mL) to pH 7. The reaction mixture was stirred for additional 23 h at 70 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized. The crude product (200 mg) was recrystallized from an EtOH / H_2O (4:1) mixture as a yellow solid (89 mg, 0.07 mmol, 41 %).

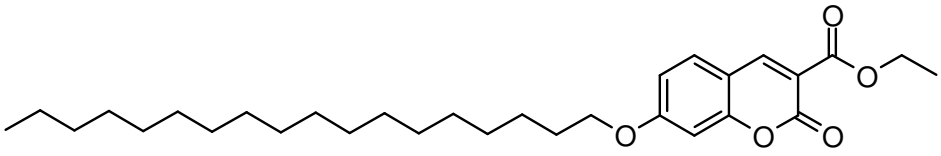
MP: 180-182°C. – **^{1}H NMR** (300 MHz; CD_3CN): δ (ppm) = 2.65-2.90 (m, 18 H, CH_2 cyclen,$(CH_3)_2N$), 2.94-3.15 (m, 14 H, CH_2 cyclen, CH_2NHSO_2), 3.23-3.46 (m, 6 H, CH_2 cyclen, CH_2NH-triazine), 4.24-4.44 (m, 4 H, CH_2 cyclen), 6.11 (m, 1 H, NH-triazine), 7.23 (d, 1 H, ^{3}J = 7.4 Hz, CH), 7.53 (dd, 1 H, ^{3}J = 7.4, 8.8 Hz, CH), 7.57 (dd, 1 H, ^{3}J = 7.4, 8.2 Hz, CH), 8.15 (d, 1 H, ^{3}J = 7.4 Hz, CH), 8.18 (d, 1 H, ^{3}J = 8.8 Hz, CH), 8.51 (d, 1 H, ^{3}J = 8.2 Hz, CH). – **^{13}C NMR** (300 MHz; CD_3CN): δ (ppm) = 41.9 (–, CH_2NH-triazine), 43.0 (–, CH_2NHSO_2), 44.4 (+, $(CH_3)_2N$), 45.3, 45.8, 46.1, 46.4 (–, CH_2 cyclen), 114.8, 118.3, 123.1, 127.9, 128.6, 128.9, 129.3, 129.9 (+, CH), 135.0 (C_q, Cq-SO_2), 151.7 (C_q, Cq-N-$(CH_3)_2$), 165.5 (C_q, Cq-triazine). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3427, 3283, 2931, 2361, 2200, 1636, 1560, 1419, 1346, 1312, 1143, 1110, 1090, 979, 795, 627, 575. – **UV** (HEPES pH 7.4, 25 mM): λ_{ex} (lg ε) = 330 nm (3.575), 227 (4.637). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 479.1 (100) $[M^{4+} + 2\ CH_3COO^-]^{2+}$, 449.1 (82) $[M^{4+} – H^+ + CH_3COO^-]^{2+}$, 420.1 (20) $[M^{4+} – 2\ H^+]^{2+}$. – **Elemental analysis** Calcd (%) for $C_{33}H_{56}N_{14}O_{18}SCl_4Zn_2 \cdot$ EtOH: C 32.65, H 4.85, N 15.23; found: C 32.52, H 4.87, N 15.04. – **MF**: $C_{33}H_{56}N_{14}O_2SZn_2(ClO_4)_4$ – **FW**: 1241.52 g/mol



***7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid ethyl ester* (8)**

Under nitrogen atmosphere hydroxy-coumarin ethylester **7** (779 mg, 3.3 mmol) was dissolved in dry DMF (12 mL) and K_2CO_3 (2.68 g, 11.6 mmol) was added. Subsequently 1-bromododecane (1.2 mL, 5.0 mmol) was given dropwise to the stirred to suspension. The reaction mixture was stirred over night (20 h) at 80 °C. The reaction progress was monitored by TLC (chloroform). K_2CO_3 was filtered off and the filtrate was concentrated. The crude product was purified by flash column chromatography on flash silica gel (chloroform; $R_f = 0.48$) yielding compound **8** (537 mg, 1.33 mmol, 40 %) as yellow solid.

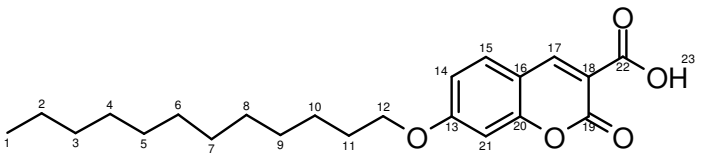
MP: 71 °C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 0.87 (t, ^3J = 7.1 Hz, 3 H, COSY: $C^{24}H_3$), 1.17-1.36 (m, 16 H, COSY: $C^{16}H_2$ – $C^{23}H_2$), 1.38 (t, ^3J = 7.1 Hz, 3 H, COSY: C^1H_3), 1.43-1.51 (m, 2 H, COSY: $C^{15}H_2$), 1.81 (quin, 2 H, COSY: $C^{14}H_2$), 4.03 (t, ^3J = 6.1 Hz, 2 H, COSY: $C^{13}H_2$), 4.39 (q, ^3J = 7.1 Hz, 2 H, COSY: C^2H_2), 6.78 (d, ^3J = 1.9 Hz, 1 H, HMBC: C^7H), 6.87 (dd, ^3J = 1.9 Hz, 8.5 Hz, 1 H, COSY: $C^{10}H$), 7.48 (d, ^3J = 8.5 Hz, 1 H, COSY: C^9H), 8.49 (s, 1 H, HMBC: $C^{12}H$). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, HSQC, COSY: $C^{24}H_3$), 14.3 (+, 1 C, HSQC, COSY: C^1H_3), 22.6, 29.26, 29.29, 29.47, 29.52, 29.59, 31.9 (–, 8 C, HSQC, COSY: $C^{16}H_2$ – $C^{23}H_2$), 25.9 (–, 1 C, HSQC, COSY: $C^{15}H_2$), 28.8 (–, 1 C, HSQC, COSY: $C^{14}H_2$), 61.3 (–, 1 C, HSQC, COSY: C^2H_2), 70.0 (–, 1 C, HSQC, COSY: $C^{13}H_2$), 100.8 (+, 1 C, HSQC, HMBC: C^7H), 114.0 (+, 1 C, HSQC, COSY: $C^{10}H$), 130.6 (+, 1 C, HSQC, COSY: C^9H), 149.0 (+, 1 C, HSQC, HMBC: $C^{10}H$), 111.4 (C_q, 1 C, HMBC: C^{11}), 113.9 (C_q, 1 C, HMBC: C^6), 157.2 (C_q, 1 C, HMBC: C^5), 157.6 (C_q, 1 C, HMBC: C^4), 163.5 (C_q, 1 C, HMBC: C^3), 164.7 (C_q, 1 C, HMBC: C^8). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2918, 2847, 1748, 1693, 1598, 1553, 1469, 1434, 1378, 1301, 1213, 1110, 1027, 793, 722. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 352 nm (4.423). – **MS** (CI (NH_3): m/z (%) = 403.2 (100) [MH^+], 420.2 (38) [$M + NH_4^+$]. – **HRMS** Calcd for $C_{24}H_{34}O_5$: 402.2406; Found: 402.2398. – **MF**: $C_{24}H_{34}O_5$ – **FW**: 402.54 g/mol



***7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid* (9)**

Under nitrogen atmosphere hydroxy-coumarin ethylester **7** (1.3 g, 5.6 mmol) were dissolved in dry DMF and K_2CO_3 (2.7 g, 19.5 mmol) was added. Subsequently 1-ocatedecylamine (2.8 g, 8.4 mmol) was given dropwise to the stirred to suspension. The reaction mixture was stirred over night (20 h) at 60 °C. The reaction progress was monitored by TLC (chloroform). K_2CO_3 was filtered off and the filtrate was concentrated. The crude product was purified by flash column chromatography on flash silica gel (chloroform; R_f = 0.34) yielding compound **9** (1.4 g, 2.8 mmol, 50 %) as yellow solid.

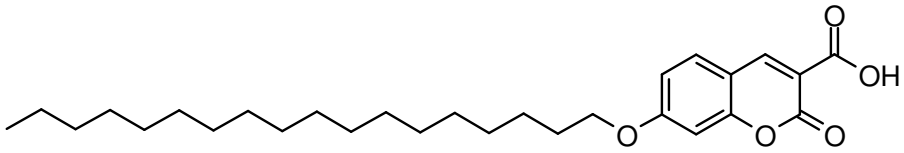
MP: 84 °C. –**^{1}H-NMR** (300 MHz; $CDCl_3$): δ (ppm) = 0.87 (t, ^{3}J = 6.7 Hz, 3 H, CH_3), 1.12-1.35 (m, 28 H, CH_2), 1.39 (t, ^{3}J = 7.1 Hz, 3 H, CH_3), 1.43-1.54 (m, 2 H, CH_2), 1.81 (quin, 2 H, CH_2), 3.63 (t, ^{3}J = 6.6 Hz, 1.7 H, CH_2), 4.02 (t, ^{3}J = 6.6 Hz, 0.3 H, CH_2), 4.39 (t, ^{3}J = 7.1 Hz, 2 H, CH_2), 6.79 (d, ^{3}J = 2.2 Hz, 1 H, CH), 6.87 (dd, ^{3}J = 2.3 Hz, 8.6 Hz, 1 H, CH), 7.48 (d, ^{3}J = 8.8 Hz, 1 H, CH), 8.49 (s, 1 H, CH). – **^{13}C-NMR** (75 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, CH_3), 14.3 (+, 1 C, CH_3), 22.7 (–, 1 C, CH_2), 25.8 (–, 0.2 C, CH_2), 25.9 (–, 0.8 C, CH_2), 28.9 (–, 1 C, CH_2), 29.32 (–, 1 C, CH_2), 29.38 (–, 1 C, CH_2), 29.46 (–, 0.2 C, CH_2), 29.54 (–, 0.8 C, CH_2), 29.59 (–, 1 C, CH_2), 29.67, 29.71 (–, 8 C, CH_2), 31.9 (–, 0.9 C, CH_2), 32.8 (–, 0.1 C, CH_2), 61.7 (–, 0.8 C, CH_2), 63.1 (–, 0.2 C, CH_2), 69.0 (–, 1 C, CH_2), 100.8 (+, 1 C, CH), 111.5 (C_q, 1 C), 113.8 (C_q, 1 C), 114.0 (+, 1 C, CH), 130.7 (+, 1 C, CH), 149.1 (+, 1 C, CH), 157.3 (C_q, 1 C), 157.7 (C_q, 1 C), 163.5 (C_q, 1 C), 164.8 (C_q, 1 C). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2916, 2849, 1746, 1702, 1624, 1510, 1472, 1376, 1228, 1180,1040, 847, 793, 718. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 352 nm (4.246). – **MS** (EI): m/z (%) = 486.3 (20) [$M^{+\cdot}$], 440.4 (12) [$M^{+\cdot}$ - EtOH], 247.0 (50) [$M^{+\cdot}$ - $C_{17}H_{35}^{\cdot}$], 234.0 (100) [$M^{+\cdot}$ - $C_{18}H_{36}$], 189.0 (90) [$M^{+\cdot}$ - $C_{18}H_{36}$ - $EtO^{\cdot}$]. – **HRMS** Calcd for $C_{30}H_{46}O_5$ 486.3345; Found: 486.3347. – **MF**: $C_{30}H_{46}O_5$ – **FW**: 486,70 g/mol



7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid **(10)**

Ethyl ester of compound **8** (386 mg, 0.96 mmol) was dissolved in THF (6.0 mL) and heated to reflux. Subsequently 2 M NaOH (15.4 mL) were added and the solution was refluxed for 5 h. Reaction control was performed by TLC (chloroform). The reaction mixture was cooled to room temperature and further to 0 °C by an ice bath. The yellow solution was acidified with 1 M HCl until a white precipitate was formed which was isolated by filtration and washed with cold water. Compound **10** was obtained as a white solid (360 mg, 0.96 mmol, 100 %).

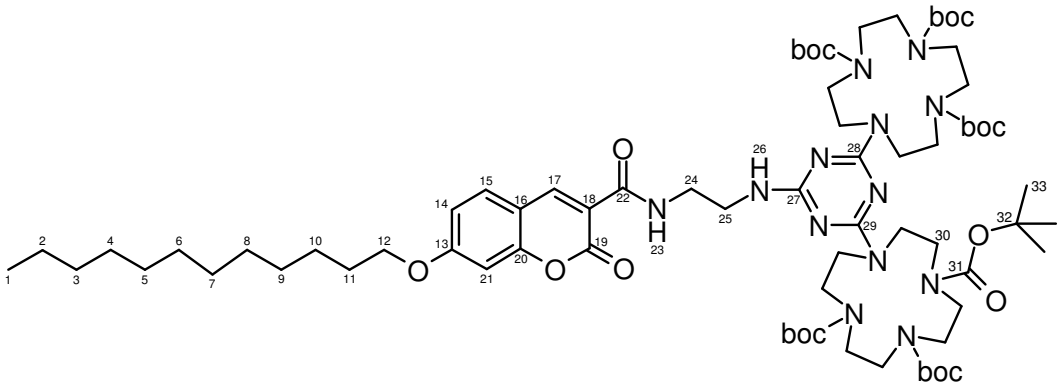
MP: 126 °C. – **^{1}H-NMR** (600 MHz; $CDCl_3$): δ (ppm) = 0.87 (t, ^{3}J = 7.0 Hz, 3 H, HSQC, COSY: $C^{1}H_3$), 1.16-1.33 (m, 14 H, HSQC, COSY: $C^{2}H_2$ – $C^{8}H_2$), 1.33-1.42 (m, 2 H, HSQC, COSY: $C^{9}H_2$), 1.47 (quin, ^{3}J = 7.4 Hz, 2 H, HSQC, COSY: $C^{10}H_2$), 1.84 (quin, ^{3}J = 7.6 Hz, 2 H, HSQC, COSY: $C^{11}H_2$), 4.08 (t, ^{3}J = 6.5 Hz, 2 H, HSQC, COSY: $C^{12}H_2$), 6.89 (d, ^{4}J = 2.0 Hz, 1 H, HSQC, COSY: $C^{14}H_2$), 6.99 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.2 Hz, 1 H, HSQC, COSY: $C^{15}H_2$), 7.62 (d, ^{3}J = 8.7 Hz, 1 H, HMBC, HSQC: $C^{21}H_2$), 8.84 (s, 1 H, HMBC, HSQC: $C^{17}H_2$), 12.16 (bs, 1 H, HSQC: $COOH^{23}$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, HSQC, COSY: $C^{1}H_3$), 22.6, 29.29, 29.46, 29.51, 29.58, 29.59, 31.9 (–, 7 C, HMBC, HSQC: $C^{2}H_2$ – $C^{8}H_2$), 25.8 (–, 1 C, HSQC, COSY: $C^{10}H_2$), 28.8 (–, 1 C, HSQC, COSY: $C^{11}H_2$), 29.23 (–, 1 C, HSQC, COSY: $C^{9}H_2$), 69.4 (–, 1 C, HSQC, COSY: $C^{12}H_2$), 101.2 (+, 1 C, HSQC, COSY: $C^{14}H$), 110.6 (C_q, 1 C, HMBC, HSQC: C^{18}), 112.1 (C_q, 1 C, HMBC, HSQC: C^{16}), 115.4 (+, 1 C, HSQC, COSY: $C^{15}H$), 131.6 (+, 1 C, HMBC, HSQC: $C^{21}H$), 151.2 (+, 1 C, HMBC, HSQC: $C^{17}H$), 157.1 (C_q, 1 C, HMBC, HSQC: C^{20}), 163.1 (C_q, 1 C, HMBC, HSQC: C^{19}), 164.6 (C_q, 1 C, HMBC, HSQC: C^{22}), 165.9 (C_q, 1 C, HMBC, HSQC: C^{13}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2914, 2846, 1734, 1686, 1619, 1560, 1504, 1466, 1429, 1384, 1258, 1217, 1121, 922, 807, 720. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 277 (4.305), 358 nm (4.418). – **MS** (ESI(–), EE/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 373.1 (100) $[M - H^+]^-$, 329.1 (13) $[M - CO_2]^-$, 747.4 (8) $[2\ M - H^+]^-$. – **HRMS** Calcd for $C_{22}H_{30}O_5$: 374.2093; Found: 374.2088. – **MF**: $C_{22}H_{30}O_5$ – **FW**: 374.48 g/mol



***7-Octadecyloxy-2-oxo-2H-chromene-3-carboxylic acid* (11)**

Ethyl ester of compound **9** (297 mg, 0.61 mmol) was dissolved in THF (5.0 mL) and heated to reflux. Subsequently 2 M NaOH (10.3 mL) were added and the solution was refluxed for 4 h. Reaction control was performed by TLC (chloroform). The reaction mixture was cooled to room temperature and further to 0 °C by an ice bath. The yellow solution was acidified with 1 M HCl until a white precipitate was formed which was isolated by filtration and washed with cold water. After drying in vaccuo compound **11** was obtained as a white solid (278 mg, 0.61 mmol, 100 %).

MP: 125 °C. – **^{1}H-NMR** (300 MHz; $CDCl_3$): δ (ppm) = 0.86 (t, ^{3}J = 6.7 Hz, 3 H, CH_3), 1.03-1.38 (m, 28 H, CH_2), 1.41-1.58 (m, 2 H, CH_2), 1.68-1.97 (m, 2 H, CH_2), 3.64 (t, ^{3}J = 6.7 Hz, 0.2 H, CH_2), 4.08 (t, ^{3}J = 6.4 Hz, 1.8 H, CH_2), 6.89 (d, ^{4}J = 2.5 Hz, 1 H, CH), 6.99 (dd, ^{3}J = 8.8 Hz, ^{4}J = 2.5 Hz, 1H, CH), 7.62 (d, ^{3}J = 8.8 Hz, 1 H CH), 8.84 (s, 1 H, CH). – **^{13}C-NMR** (75 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, CH_3), 22.7 (–, 1 C, CH_2), 25.9 (–, 1 C, CH_2), 28.8 (–, 1 C, CH_2), 29.3 (–, 1 C, CH_2), 29.4, (–, 1 C, CH_2), 29.53 (–, 1 C, CH_2), 29.58 (–, 1 C, CH_2), 29.67 (–, 2 C, CH_2), 29.71 (–, 6 C, CH_2), 31.9 (–, 1 C, CH_2), 69.4 (–, 1 C, CH_2), 101.2 (+, 1 C, CH), 110.6 (C_q, 1 C), 112.2 (C_q, 1 C), 115.5 (+, 1 C, CH), 131.7 (+, 1 C, CH), 151.3 (+, 1 C, CH), 157.1 (C_q, 1 C), 163.2 (C_q, 1 C), 164.6 (C_q, 1 C), 166.0 (C_q, 1 C). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2915, 2850, 1733, 1686, 1622, 1560, 1505, 1471, 1383, 1256, 1221, 1122, 1005, 820, 798. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 358 nm (4.009). – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 459.3 (100) [MH^+], 476.3 (25) [$M + NH_4^+$]. – **HRMS** Calcd for $C_{28}H_{42}O_5$ 458.3032; Found: 458.3026. –**MF**: $C_{28}H_{42}O_5$ – **FW**: 458.64 g/mol

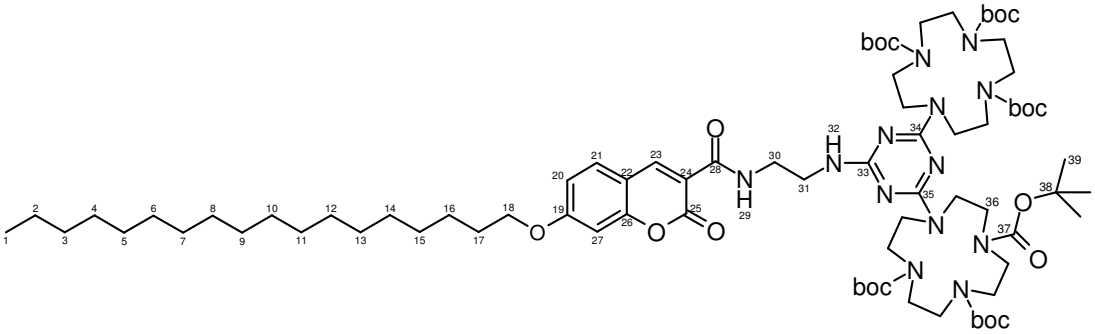


***7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid {2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-ethyl}-1,4,7-tricarboxylic acid tri-tert-butyl ester* (13):**

7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid **10** (200 mg, 0.53 mmol), DIPEA (368 µL, 2.14 mmol), TBTU (189 mg, 0.59 mmol), and HOBt (90 mg, 0.59 mmol) were dissolved under nitrogen atmosphere in dry DMF/THF (2 mL/4 mL) under ice cooling and stirred for 1 h. Subsequently **12** (635 mg, 0.59 mmol) dissolved in DMF (2 mL) was added dropwise. The reaction was allowed to warm to room temperature and was stirred 30 min at rt and 2.5 h at 40 °C. The reaction progress was monitored by TLC (ethyl acetate/petrol ether). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate/petrol ether 1:1; R_f = 0.25) yielding compound **13** (608 mg, 0.42 mmol, 79 %) as a lightly yellow solid.

MP: 113 °C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 0.85 (t, ^{3}J = 6.9 Hz, 3 H, HSQC, COSY: C^1H_3), 1.20-1.33 (m, 18 H, HSQC, COSY: C^2H_2 – $C^{10}H_2$), 1.41 (s, 18 H, HSQC, COSY: $C^{33}H_3$), 1.42 (s, 36 H, HSQC, COSY: $C^{33}H_3$), 1.80 (quin, ^{3}J = 7.3 Hz, 2 H, HSQC, COSY: $C^{11}H_2$), 3.02-3.89 (m, 36 H, HSQC, COSY: $C^{24}H_2$, $C^{25}H_2$, $C^{30}H_2$), 4.02 (t, ^{3}J = 6.5 Hz, 2 H, HSQC, COSY: $C^{12}H_2$), 4.99 (bs, 1 H, HMBC, HSQC: NH^{23}), 6.81 (d, ^{4}J = 2.2 Hz, 1 H, HMBC, HSQC, COSY: $C^{21}H_2$), 6.90 (dd, ^{4}J = 2.3 Hz, ^{3}J = 8.8 Hz, 1 H, HMBC, HSQC, COSY: $C^{14}H$), 7.54 (d, ^{3}J = 8.8 Hz, 1 H, HMBC, HSQC, COSY: $C^{15}H$), 8.79 (s, 1 H, HMBC, HSQC: $C^{17}H$), 8.87 (m, 1 H, HMBC, HSQC: NH^{26}). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 1 C, HSQC, COSY: C^1H_3), 22.6, 25.9, 29.23, 29.26, 29.46, 29.49, 29.55, 29.57, 31.8 (–, 9 C, HSQC, COSY: C^2H_2 – $C^{10}H_2$), 28.43, 28.47 (+, 18 C, HSQC, COSY: $C^{33}H_3$), 28.8 (–, 1 C, HSQC, COSY: $C^{11}H_2$), 39.8, 40.6 (–, 2 C, HSQC, COSY: $C^{24}H_2$, $C^{25}H_2$), 50.2 (–, 16 C, HSQC, COSY: $C^{30}H_2$), 69.0 (–, 1 C, HMBC, HSQC: $C^{12}H_2$), 79.7 (C_q, 6 C, HMBC, HSQC: C^{32}), 100.7 (+, 1 C, HMBC, HSQC, COSY: $C^{21}H$), 112.1 (C_q, 1 C, HMBC,

HSQC: C^{16}), 114.35 (+, 1 C, HMBC, HSQC, COSY: $C^{14}H$), 114.41 (C_q, 1 C, HMBC, HSQC: C^{19}), 130.8 (+, 1 C, HMBC, HSQC, COSY: $C^{15}H$), 148.3 (+, 1 C, HMBC, HSQC, COSY: $C^{17}H$), 156.3 (C_q, 6 C, HMBC, HSQC: C^{31}), 156.7 (C_q, 1 C, HMBC, HSQC: C^{20}), 161.7 (C_q, 1 C, HMBC, HSQC: C^{18}), 162.7 (C_q, 1 C, HMBC, HSQC: C^{22}), 164.5 (C_q, 1 C, HMBC, HSQC: C^{13}), 165.9 (C_q, 3 C, HMBC, HSQC: C^{27}, C^{28}, C^{29}). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2973, 2929, 2878, 1686, 1603, 1535, 1501, 1466, 1408, 1247, 1160, 1026, 971, 858, 776. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 351 nm (4.336). – **MS** (ESI(+), EE/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1437.3 (100) [MH^+], 569.0 (12) [M + 2 H^+ – 3 Boc]$^{2+}$, 518.9 (20) [M + 2 H^+ – 4 Boc]$^{2+}$, 468.8 (17) [M + 2 H^+ – 5 Boc]$^{2+}$, 418.8 (13) [M + 2 H^+ – 6 Boc]$^{2+}$. – **MF**: $C_{73}H_{121}N_{13}O_{16}$ – **FW**: 1436.84 g/mol

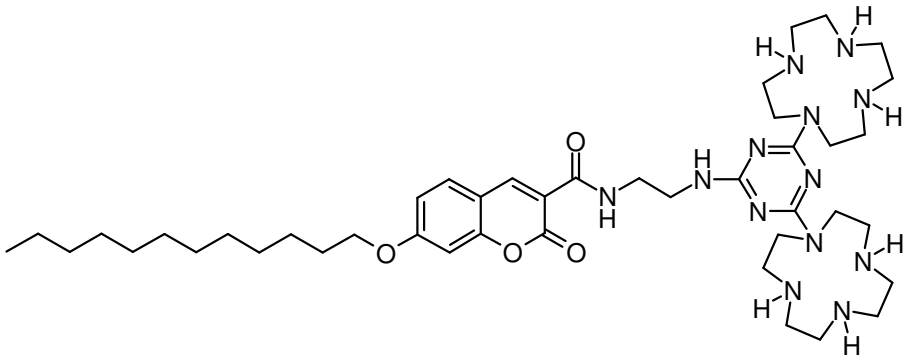


7-Octadecyloxy-2-oxo-2H-chromene-3-carboxylic acid {2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1,4,7-tricarboxylic acid tri-tert-butyl ester **(14)**

7-Octadecyloxy-2-oxo-2H-chromene-3-carboxylic acid **11**(160 mg, 0.35 mmol), DIPEA (360 µL, 2.09 mmol), TBTU (123 mg, 0.38 mmol), and HOBt (59 mg, 0.38 mmol) were dissolved under nitrogen atmosphere in dry DMF/THF (2 mL / 4 mL) under ice cooling and stirred for 1 h. Subsequently **12** (415 mg, 0.38 mmol) dissolved in DMF (2 mL) was added dropwise. The reaction was allowed to warm to room temperature and was stirred 30 min at rt and 4.5 h at 40 °C. The reaction progress was monitored by TLC (ethyl acetate/petrol ether). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate/petrol ether 1:1; R_f = 0.25) yielding compound **14** (375 mg, 0.25 mmol, 71 %) as a colourless solid.

^{1}H-NMR (600 MHz; $CDCl_3$): δ (ppm) = 0.86 (t, ^{3}J = 7.2 Hz, 3 H, HSQC, HMBC: C^1H_3), 1.24 (m, 28 H, HSQC, HMBC: C^2H_2 – $C^{15}H_2$), 1.31-1.64 (m, 56 H, HSQC, HMBC: boc-CH_3, $C^{16}H_2$), 1.78-1.83 (m, 2 H, HSQC, HMBC: $C^{17}H_2$), 3.00-3.92 (m,

36 H, HSQC, HMBC: cyclen-$C^{36}H_2$, $C^{30}H_2$, $C^{31}H_2$), 4.02 (t, ^3J = 6.5 Hz, 2 H, HSQC, HMBC: $C^{18}H_2$), 5.04 (bs, 1 H, HSQC, HMBC: $N^{32}H$), 6.82 (d, ^4J = 2.3 Hz, 1 H, HSQC, HMBC: $C^{27}H$), 6.90 (dd, ^3J = 8,7 Hz, ^4J = 2.3 Hz, 1 H, HSQC, HMBC: $C^{20}H$), 7.55 (d, ^3J = 8,7 Hz, 1 H, HSQC, HMBC: $C^{21}H$), 8.79 (s, 1 H, HSQC, HMBC: C^{23}), 8.88 (m, 1 H, HSQC, HMBC: $N^{29}H$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, HSQC, HMBC: C^1H_3), 22.6 (–, 1 C), 25.9 (–, 1 C), 29.26 (–, 1 C), 29.30 (–, 1 C), 29.52 (–, 1 C), 29.59 (–, 2 C), 29.61 (–, 1 C), 29.63 (–, 5 C), 31.9 (–, 1 C) HSQC, HMBC: C^2H_2 – $C^{16}H_2$), 28.4, 28.5 (+, 18 C, HSQC, HMBC: $C^{39}H_3$), 28.8 (–, 1 C, HSQC, HMBC: $C^{17}H_2$), 39.7 (–, 1 C, HSQC, HMBC: $C^{30}H_2$), 40.6 (–, 1 C, HSQC, HMBC: $C^{31}H_2$), 50.3 (–, 16 C, HSQC, HMBC: $C^{36}H_2$), 69.0 (–, 1 C, HSQC, HMBC: $C^{18}H_2$), 79.7 (C_q, 6 C, HSQC: C^{37}), 100.7 (+, 1 C, HSQC, HMBC: $C^{27}H$), 112.4 (C_q, 1 C, HSQC, HMBC: C^{22}), 114.37 (+, 1 C, HSQC, HMBC: $C^{20}H$), 114.39 (C_q, 1 C, HSQC, HMBC: C^{24}), 130.9 (+, 1 C, HSQC, HMBC: $C^{21}H$), 148.3 (+, 1 C, HSQC, HMBC: $C^{23}H$), 156.7 (C_q, 7 C, HSQC, HMBC: C^{26}, C^{38}), 161.7 (C_q, 1 C, HSQC, HMBC: C^{25}), 162.7 (C_q, 1 C, HSQC, HMBC: C^{28}), 164.5 (C_q, 1 C, HSQC, HMBC: C^{19}), 165.9 (C_q, 3 C, HSQC, HMBC: C^{33}, C^{34}, C^{35}). – **UV** ($CHCl_3$): λ_{max} (lg ε) = 350 nm (4.333). – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1521.4 (100) $[MH^+]$, 769.7 (46) $[MH^+ + NH_4^+]^{2+}$, 761.2 $[M + 2\ H^+]^{2+}$. – **MF**: $C_{79}H_{133}N_{13}O_{16}$ – **FW**: 1521.09 g/mol

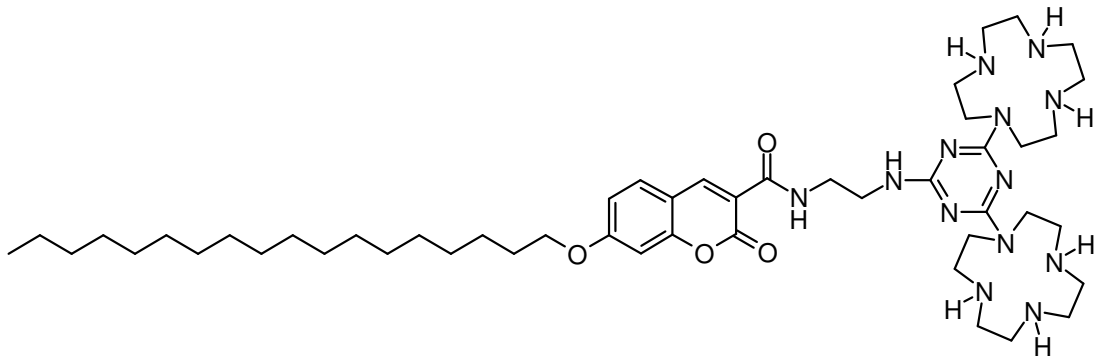


***7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid {2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-amide* (15)**

Compound **13** (200 mg, 0.14 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently TFA (901 µL, 11.7 mmol) was added. The solution was stirred 15 min at 0 °C and additionally 20 h at room temperature. The solvent was removed in vacuo, yielding quantitatively the protonated TFA salt of compound **15** as a pale yellow solid.

To obtain the free base of compound **15** a weak basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (1114 mg, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The hydrochloride salt was dissolved in water / MeOH (8:2), put onto the column and eluated with water / MeOH (8:2). The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 110 mg (0.13 mmol, 93 %) of free base **15**, as pale yellow solid.

MP: 174°C. – **^{1}H-NMR** (400 MHz; $CDCl_3$): δ (ppm) = 0.85 (t, ^{3}J = 6.9 Hz, 3 H, CH_3), 1.19-1.37 (m, 16 H, CH_2), 1.39-1.49 (m, 2 H, CH_2), 1.80 (quin, ^{3}J = 7.2 Hz, 2 H, CH_2), 2.82-3.84 (m, 36 H, CH_2), 4.02 (t, ^{3}J = 6.2 Hz, 2 H, CH_2), 6.80 (d, ^{4}J = 1.8 Hz, 1 H, CH), 6.93 (dd, ^{3}J = 8.7 Hz, ^{4}J = 1.7 Hz, 1 H, CH), 7.02 (bs, 1H, NH), 7.66 (d, ^{3}J = 8.8 Hz, 1 H, CH), 8.73 (s, 1 H, CH), 9.18 (bs, 1 H, NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 1 C, CH_3), 22.6 (–, 1 C, CH_2), 25.9 (–, 1 C, CH_2), 28.8 (–, 1 C, CH_2), 29.27 (–, 2 C, CH_2), 29.46 (–, 1 C, CH_2), 29.51 (–, 1 C, CH_2), 29.56 (–, 2 C, CH_2), 31.8 (–, 1 C, CH_2), 39.2 (–, 1 C, CH_2), 39.3 (–, 1 C, CH_2), 42.5 (–, 1 C, CH_2), 43.3 (–, 2 C, CH_2), 43.7 (–, 1 C, CH_2), 44.8 (–, 4 C, CH_2), 45.8 (–, 4 C, CH_2), 46.9 (–, 4 C, CH_2), 69.1 (–, 1 C, CH_2), 100.8 (+, 1 C, CH), 111.9 (C_q, 1 C), 113.4 (C_q, 1 C), 114.5 (+, 1 C, CH), 131.3 (+, 1 C, CH), 148.5 (+, 1 C, CH), 156.8 (C_q, 1 C), 161.6 (C_q, 1 C), 163.7 (C_q, 1 C), 164.9 (C_q, 1 C), 165.9 (C_q, 1 C), 167.2 (C_q, 1 C), 168.0 (C_q, 1 C). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2926, 2855, 1676, 1597, 1536, 1496, 1418, 1366, 1297, 1198, 1174, 1120, 1017, 795, 719. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 352 nm (4.197). – **LC-MS** (+ c ESI Q1MS): m/z (%) = 418.7.0 (100) $[M + 2\ H^+]^{2+}$, 836.6 (10) $[MH^+]$. – **HRMS** Calcd for $C_{43}H_{74}N_{13}O_4$ 836.5987; Found: 836.5960. – **MF**: $C_{43}H_{73}N_{13}O_4$ – **FW**: 836.14 g/mol

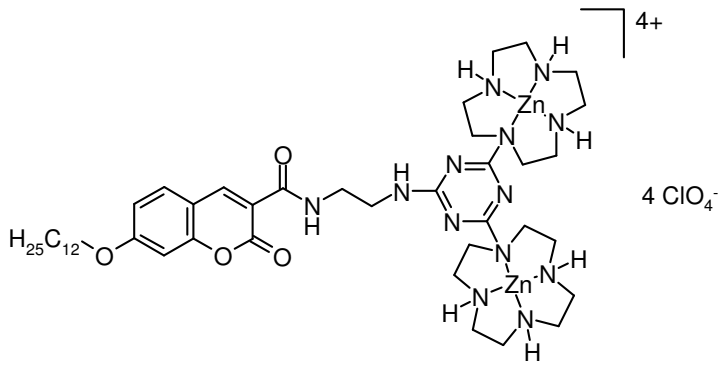


***7-Octadecyloxy-2-oxo-2H-chromene-3-carboxylic acid {2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-amide* (16)**

Compound **14** (375 mg, 0.26 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently HCl/ether (14 mL) was added. The solution was stirred 15 min at 0 °C and additionally 24 h at room temperature. The solvent was removed in vacuo, yielding quantitatively the protonated HCl salt of compound **16** as a pale yellow solid. To obtain the free base of compound **16** a weak basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (1920 mg, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The hydrochloride salt was dissolved in water / MeCN (5:1), put onto the column and eluated with water / MeCN (5:1). The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 200 mg (0.22 mmol, 85 %) of free base **16**, as colourless solid.

Note: NMR investigations on compound **16** were not feasible as the resolution on the NMR was not sufficient.

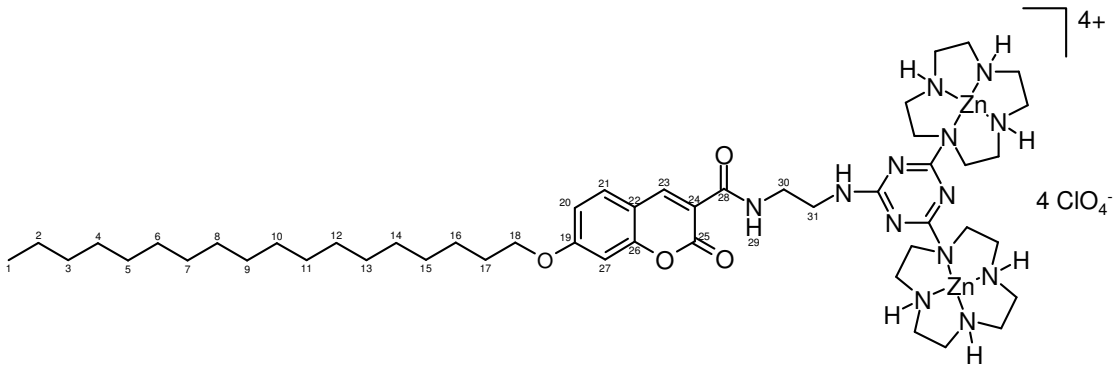
MP: 179 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3347, 2921, 2850, 1708, 1594, 1538, 1496, 1418, 1362, 1274, 1222, 1142, 1017, 809, 739. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 354 nm (4.360). – **LC-MS** (+ c ESI Q1MS): m/z (%) = 460.8 (100) $[M + 2\ H^+]^{2+}$, 920.7 (5) $[MH^+]$. – **HRMS** Calcd for $C_{49}H_{86}N_{13}O_4$ 920.6926; Found: 920.6901. – **MF**: $C_{49}H_{85}N_{13}O_4$ – **FW**: 920.30 g/mol



***Binuclear Zn(II)-cyclen-coumarin C12* (6)**

A solution of compound **15** (50.0 mg, 60 µmol) in MeOH (1 mL) was heated to 65 °C and subsequently a methanolic solution of $Zn(ClO_4)_2$ (100 mM, 1196 µL, 120 µmol) was added dropwise. After stirring the reaction mixture for 20 h at 65 °C, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **5** as a colourless solid in quantitative yield (82 mg, 60 µmol).

MP: 232 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3537, 3303, 2929, 2854, 1702, 1599, 1546, 1424, 1347, 1283, 1224, 1058, 964, 848. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 353 nm (3.740). – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 540.9 (100) $[M^{4+} + 2\,CH_3COO^-]^{2+}$, 561.9 (26) $[M^{4+} + ClO_4^- + CH_3COO^-]^{2+}$, 510.9 (20) $[M^{4+} - H^+ + CH_3COO^-]^{2+}$. – **MF**: $C_{43}H_{73}N_{13}O_4Zn_2(ClO_4)_2$ – **FW**: 1364.70g/mol



***Binuclear Zn(II)-cyclen-coumarin C18* (5)**

Compound **16** (100 mg, 0.11 mmol) was dissolved in 5 mL water and heated to 65 °C to get a clear yellow solution. Subsequently zinc(II)-perchlorate (81 mg, 0.22 mmol) dissolved in 5 ml water was added slowly to the stirred reaction mixture. The reaction mixture was stirred for additional 24 h at 65 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized to yield 158 mg (0.11 mmol, 100 %) of **6** as a lightly yellow solid.

MP: 209 °C. – **^{1}H-NMR** (600 MHz; $CDCl_3$ / CD_3CN 1:1): δ (ppm) = 0.86 (t, ^{3}J = 7.1 Hz, 3 H, HSQC, HMBC: $C^{1}H_3$), 1.20-1.31 (m, 26 H, HSQC, HMBC: $C^{2}H_2$ – $C^{14}H_2$), 1.31-1.37 (m, 2 H, HSQC, HMBC, ROESY: $C^{15}H_2$), 1.41-1.46 (2 H, HSQC, HMBC, ROESY: $C^{16}H_2$), 1.73-1.80 (m, 2 H, HSQC, HMBC, ROESY: $C^{17}H_2$), 2.51-3.57 (m, 36 H, HSQC, HMBC: cyclen-CH_2, $C^{30}H_2$, $C^{31}H_2$), 4.06 (t, ^{3}J = 6.5 Hz, 2 H, HSQC, HMBC: $C^{18}H_2$), 6.11 (s, 1 H, HSQC, ROESY: cyclen-NH), 6.20 (s, 1 H, HSQC, ROESY: cyclen-NH), 6.29 (s, 1 H, HSQC, ROESY: cyclen-NH), 6.44 (s, 1 H, HSQC, ROESY: cyclen-NH), 6.87 (d, ^{4}J = 2.2 Hz, 1 H, HSQC, HMBC, ROESY: $C^{27}H$), 6.95 (dd, ^{3}J = 8.7 Hz, ^{4}J = 2.3 Hz, 1 H, HSQC, HMBC, ROESY: $C^{20}H$), 7.74 (d, ^{3}J = 8.7 Hz, 1 H, HSQC, HMBC, ROESY: $C^{21}H$), 8.85 (s, 1 H, HSQC, HMBC, ROESY: $C^{23}H$), 9.22 (m, 1 H, HSQC, ROESY: NH^{29}). – **^{13}C-NMR** (150 MHz; $CDCl_3$ / CD_3CN 1:1): δ (ppm) = 14.3 (+, 1 C, HSQC, HMBC: $C^{1}H_3$), 26.3 (–, 1 C, HSQC, HMBC, ROESY: $C^{16}H_2$), 23.1, 29.7, 29.94, 29.97, 30.1, 32.3 (–, 13 C, HSQC, HMBC: $C^{2}H_2$ – $C^{14}H_2$), 29.3 (–, 1 C, HSQC, HMBC, ROESY: $C^{17}H_2$), 29.75 (–, 1 C, HSQC, HMBC: $C^{15}H_2$), 39.9, 42.6, 44.3, 45.8, 45.9, 46.3, 46.9 3 (–, 18 C, HSQC, HMBC, ROESY: cyclen-CH_2, $C^{30}H_2$, $C^{31}H_2$), 69.8 (–, 1 C, HSQC, HMBC, ROESY: $C^{18}H_2$), 101.4 (+, 1 C, HSQC, HMBC, ROESY: $C^{27}H$), 112.8 (C_q, 1 C, HSQC, HMBC: C^{22}), 113.8 (C_q, 1 C, HSQC, HMBC: C^{24}), 115.1 (+, 1 C, HSQC, HMBC, ROESY: $C^{20}H$), 132.3 (+, 1 C, HSQC, HMBC, ROESY: $C^{21}H$), 149.8 (+, 1 C, HSQC, HMBC, ROESY: $C^{23}H$), 157.5 (C_q, 1 C, HSQC, HMBC: C^{26}), 162.1 (C_q, 1 C, HSQC, HMBC: C^{25}), 164.8 (C_q, 1 C, HSQC, HMBC: C^{28}), 165.6 (C_q, 1 C, HSQC, HMBC: C^{19}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3355, 2919, 2851, 1705, 1655, 1612, 1534, 1426, 1276, 1225, 1051, 926. – **UV** ($CHCl_3$): λ_{max} (lg ε) = 355 nm (3.523). –**MS** (ESI(+), EE/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 583.0 (100) $[M^{4+} + 2\ CH_3COO^-]^{2+}$. – **MF**: $C_{49}H_{85}N_{13}O_4Zn_2(ClO_4)_4$ – **FW**: 1448.86 g/mol

6.4.3 Preparation of vesicles and vesicular receptors

6.4.3.1 Vesicle preparation

In a small round-bottom flask 2 - 12 mg of DSPC were dissolved in 5 - 10 mL of chloroform and optionally 10 mol% of amphiphilic receptors and/or dyes were added. After warming to 75 °C under vigorous shaking, the solvent was slowly removed under reduced pressure to yield a thin lipid film. Traces of solvent were removed by high vacuum. Treatment with an appropriate amount of buffer (HEPES 25 mM, pH 7.4) to obtain lipid concentrations of 1.5 – 2.5 mM and heating to 75 °C for 15 – 30 min yielded a turbid MLV-suspension. SUV-dispersions were obtained by extrusion through 100 nm-pore size polycarbonate membranes with a LiposoFast liposome extruder from Avestin.[20, 35]

6.4.3.2 Size exclusion chromatography

Vesicle dispersions were separated from low molecular weight solutes on minicolumns of Sephadex LH-20 gel filtration media as described in literature.[23]

6.4.3.3 Dynamic Light Scattering

PCS measurements were performed on a Malvern Zetasizer 3000 at 25 °C using 1 cm disposable polystyrene fluorescence cuvettes (VWR). Three subsequent measurements of 60 seconds each were performed for each sample. Data analysis was performed using the Malvern PCS software.

6.4.3.4 Preparation of vesicular receptors VR-4, VR-5 and VR-4/11

VR-4, VR-5:

Compounds **4** and compound **5** were incorporated into DSPC–SUVs by following the general preparation procedure described above. The mixed lipid films were hydrated with 25 mM HEPES buffer (pH 7.4) to obtain final concentrations of 2.4 mM DSPC and 0.24 mM of compound **4** or **5**.

VR-4/11:

Compound **4** and compound **11** were incorporated into DSPC–SUVs by following the general preparation procedure described above. The mixed lipid films were hydrated with 25 mM HEPES buffer (pH 7.4) to obtain final concentrations of 2.4 mM DSPC and 0.24 mM of compound **4** and **11**.

6.4.3.5 Characterization of vesicular receptors VR-4, VR-5 and VR-4/11

	particle size	λ_{ex}	λ_{em}
VR-4	80 ± 5 nm	–	–
VR-5	95 ± 5 nm	349 nm	406 nm
VR-4/11	85 ± 5 nm	349 nm	406 nm

6.4.4 Binding studies

All titrations were carried out at 25 °C in HEPES buffer (25 mM, pH 7.4) and corrected for dilution. Data analysis was performed with Origin 8 software.

6.4.4.1 Vesicular receptor concentration

For all binding studies the concentration of vesicular receptors refers to the outer surface exposed binding units. The following equation describes the correction factor *f* for surface exposed receptor molecules as a fraction its entire quantity of matter:

$$f = \frac{\sigma_o}{\sigma_o + \sigma_i}$$

The ratio of outer surface (σ_o) and inner (σ_i) surface of the respective vesicles was calculated using the hydrodynamic diameters obtained from dynamic light scattering (see 6.4.3.5) and the assumption that the bilayer thickness for the prepared vesicles generally amounts to 5 nm.[36]

6.4.4.2. Direct emission signalling of vesicular receptor binding

Evaluation of phosphate binding towards vesicular receptors **VR-5** and **VR-4/11** was performed by plotting Δ emission values against analyte concentration and employing non-linear curve fitting using the Hill equation. The initial concentration of vesicular

receptors for all titrations was 5.0 x 10^{-7} M, after each analyte addition, the cuvette was shaken for 1 minute before the fluorescence spectrum (λ_{ex} = 348 nm, λ_{em} = 406 nm) was recorded.

6.4.4.3 Indicator displacement assays (IDA)

Evaluation of the indicator binding towards receptors **1** and **VR-4** was performed by utilizing Hill plots, whereas for the subsequent displacement titrations a competitive binding model was used.[37] For all titrations the initial indicator concentration was 3.5 x 10^{-5} M for PV and 5.0 x 10^{-7} M for CMS. After each addition, the cuvette was shaken for 1 minute before the absorption (PV: λ_{max} = 636 nm) or fluorescence spectrum (CMS: λ_{ex} = 396 nm, λ_{em} = 480 nm) was recorded.

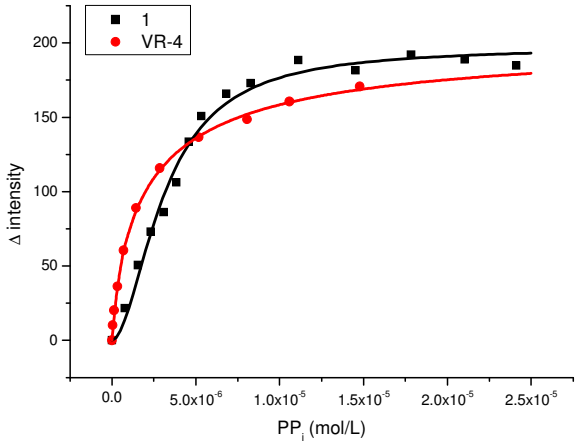
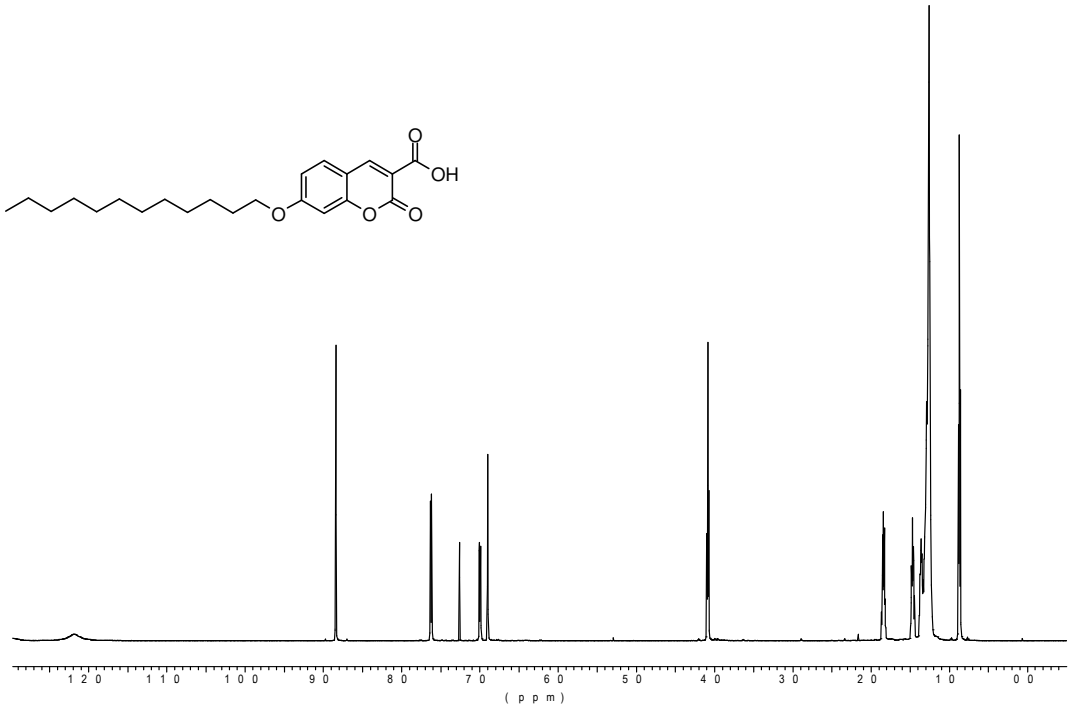


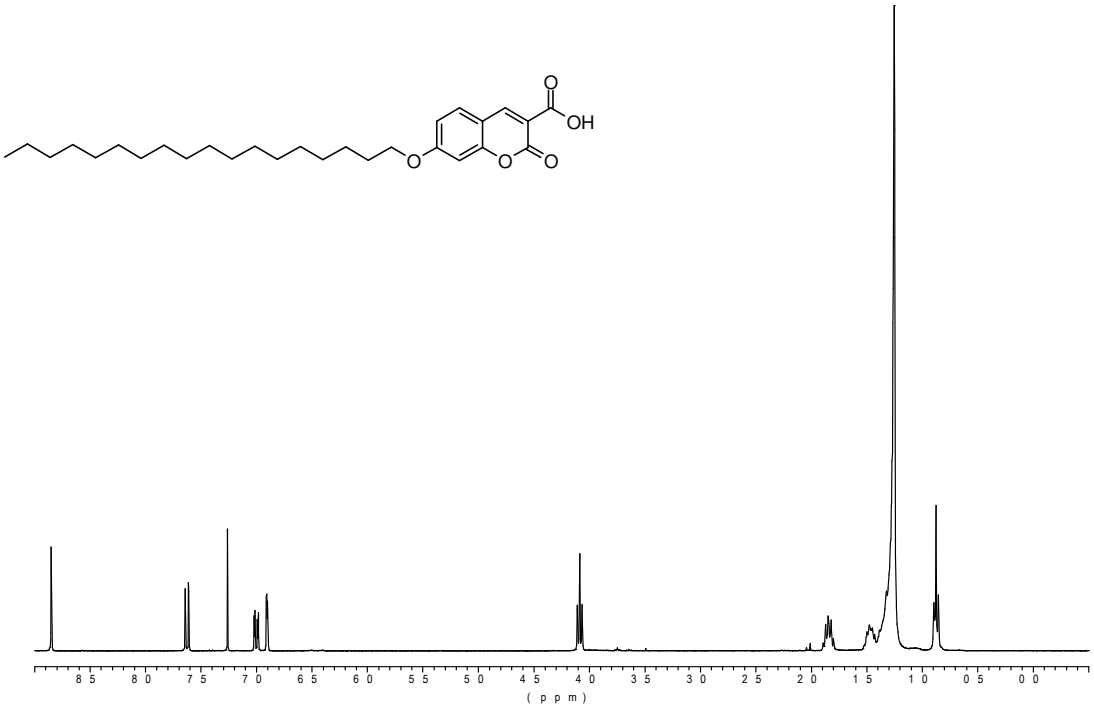
Figure 7. Comparison of binding isotherms obtained by indirect IDA methods of **1** and **VR-4** to PP_i.

6.4.5 ^{1}H and ^{13}C spectra of synthesized compounds

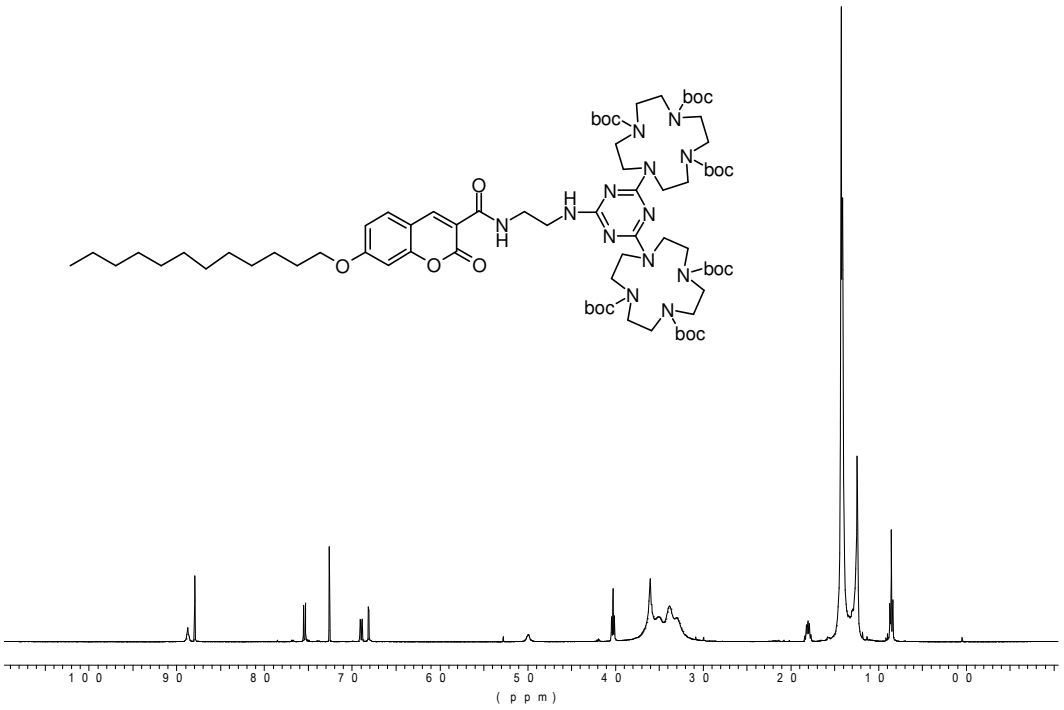
Compound **10**



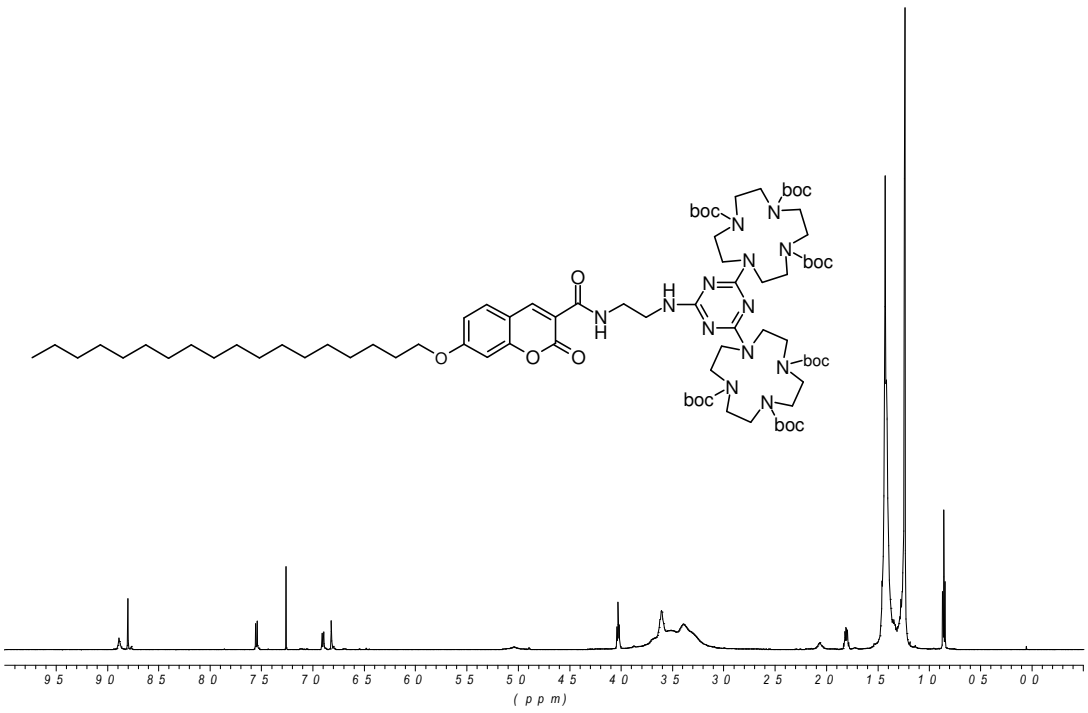
Compound **11**



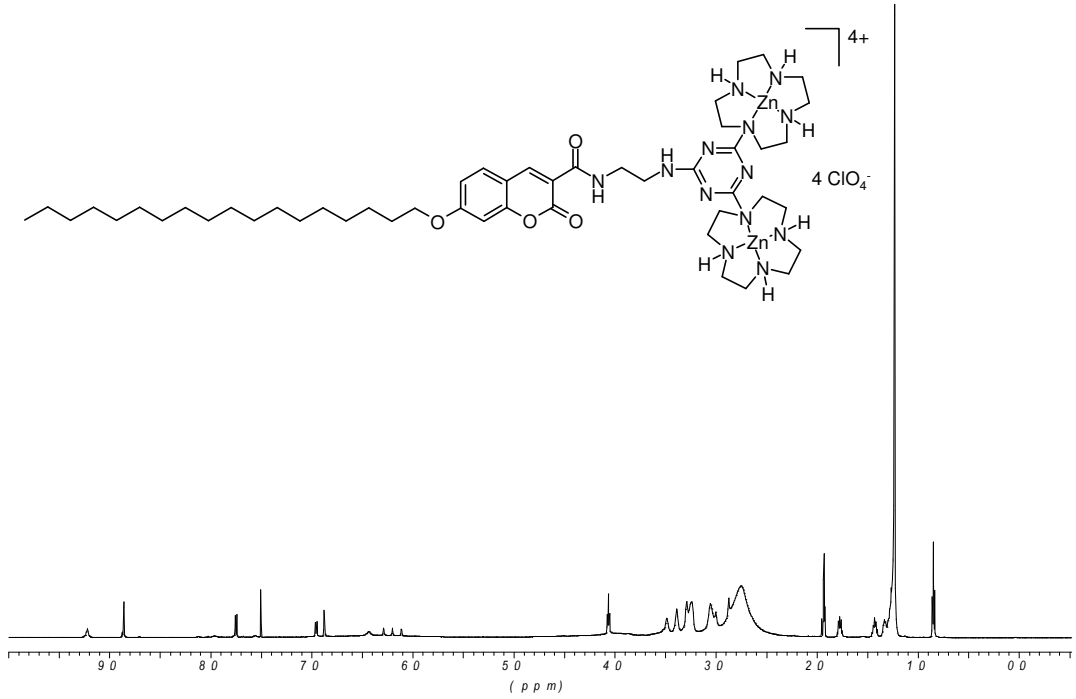
Compound **13**



Compound **14**



Compound **5**



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solvatisation leading to a stronger fluorescence response upon phosphate binding of **VR-4/11** compared to **VR-5**.

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7. Synthesis of Non-Fluorescent and Fluorescent Amphiphilic Metal Chelating Artificial Lipids for Surface Modification of Biomembrane Mimics

This chapter reports the synthesis of new non-fluorescent and fluorescent amphiphilic Lewis acidic metal complexes (metal chelating artificial lipids) based on 1,4,7,10-tetraazacyclododecane Zn(II) complexes, dipicolylamine (Dpa) complexes and a nitrilotriacetato acid (NTA) complex. The prepared metal chelating artificial lipids will be used for fabrication of complex self-assembled supramolecular surfaces by one or more different chemosensors for molecular recognition at interfaces.

All compounds were prepared at the University of Regensburg by Stefan Stadlbauer.

7.1 Introduction

Liposomes, also called vesicles, assembled from phospholipids, surfactants or block copolymers are considered as most related to biological cells as they consist of a single lipid bilayer.[1] Attracting the interest of chemists, biologist and physicists they are employed in a broad field of applications in various areas of technology and industry.[2] Utilized as targeting drug delivery systems due to their properties, liposomes are able to encapsulate a variety of small molecules[3] and they are considered as biomembrane mimics.[2, 4]

The process of molecular recognition and interaction between liposomes is regarded as a model system for the recognition between cells, as the process itself shows similarities. [1, 5] Several metal chelating lipids[6] based on iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA) have been developed by Arnold[7], Tampe[8] and Mallik,[9] and were applied in modification of mono- and bilayer assemblies or liposomes for protein targeting, 2D protein crystallisation and biological sensing. Furthermore Mallik *et al* impressively demonstrated the use of liposomes for the design of a "multi-prong" enzyme inhibitor for human carbonic anhydrase II (hCA II) incorporating a mixture of two weak inhibitors in a liposomal surface.[10] Recently, we used metal chelating 1,4,7,10-tetraazacyclododecane (cyclen) lipids as synthetic surface receptors incorporated in a self assembled monolayer (SAM)[11] or unilamellar vesicles[12] for sterically guided molecular recognition of nucleotides, nucleobases and phosphates. Further we employed Zn(II)-cyclen complexes,[13a] a zinc(II)- and manganese(II)-Dpa complex[13b] for staining of phosphoproteins on SDS-page.

Herein, we report the synthesis of new fluorescent/non-fluorescent metal chelating artificial lipids based on known Lewis acidic metal complexes:[6b] 1,4,7,10-tetraazacyclododecane (cyclen) complexes[14], dipicolylamine (Dpa) complexes[15] and nitrilotriacetato acid (NTA) complexes[6b, 16].

7.2 Results and Discussion

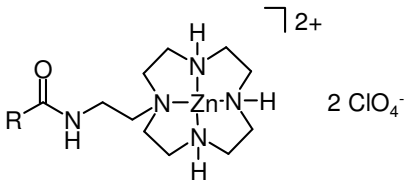
Synthesis – an Overview

Cyclen metal complexes, Dpa metal complexes and NTA metal complexes are widely used in molecular recognition and were therefore selected as polar head groups in the synthesis metal chelating lipids. The prepared compounds enable a rapid preparation of complex self-assembled supramolecular surfaces by embedding one or more different metal chelating lipids into the interfaces (SAM or vesicle).

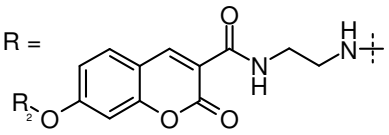
Recently we demonstrated the use of surface modified vesicles (vesicular receptors) by amphiphilic fluorescent Zn(II)-cyclen compounds **6** and **7** for direct sensing and of compound **4** utilizing the indirect indicator displacement assay (IDA) of various phosphorous species in aqueous media, respectively.[12a] An alternative phosphate binding site based on Dpa-tyrosine was employed for the preparation of non-fluorescent (**10** – **13**) and fluorescent (**14**, **15**) vesicular receptors. Additionally, mononuclear amphiphilic cyclen (**1** – **3**) and dpa (**9**) metal complexes were prepared for the investigations of compound mobility in the vesicle bilayer at different temperatures. A NTA based amphiphilic copper complex was synthesized, allowing the preparation of mixed vesicular receptors, which may show specificity to analytes containing phosphate and histidine. As changes in the length of the hydrophobic alkyl chain did not affect the binding affinity of **6** and **7** to ATP,[12a] C_{10} was introduced to mononuclear Dpa and NTA complex for an easier practical synthesis procedure.

All synthesized metal chelating lipids are sumerized in Figure 1 and 2: Figure 1 shows fluorescent/non-fluorescent mono-, bi- and trinuclear zinc(II)–1,4,7,10-tetraazacyclododecane (cyclen) lipids, Figure 2 shows fluorescent/non-fluorescent mono- and binuclear Dpa based metal chelating lipids and Figure 1 shows a NTA based chelating lipid.

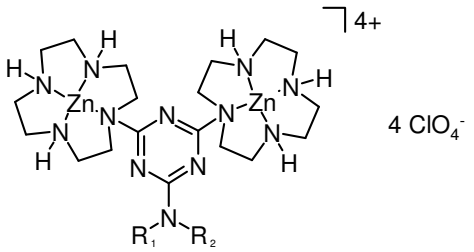
1,4,7,10-Tetraazacyclododecane (cyclen)



1 R = $-C_{17}H_{35}$

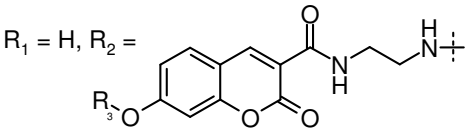


2 $R_2 = C_{18}H_{37}$
3 $R_2 = C_{12}H_{25}$

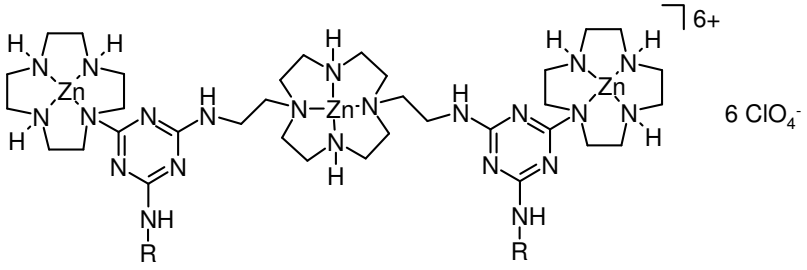


4: $R_1 = H$
$R_2 = C_{18}H_{37}$

5: $R_1 = C_{18}H_{37}$
$R_2 = C_{18}H_{37}$



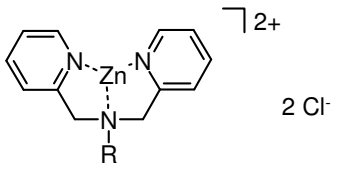
6 $R_3 = C_{18}H_{37}$
7 $R_3 = C_{12}H_{25}$



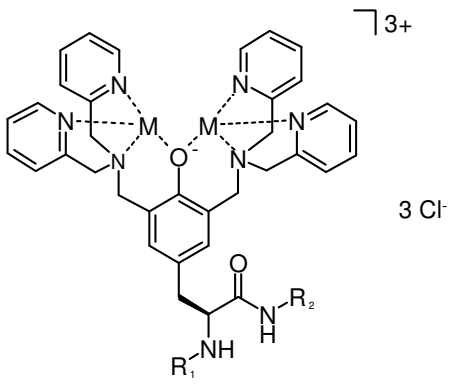
8: R = $C_{18}H_{37}$

Figure 1. Synthesized metal chelating artificial lipids based on Lewis acidic zinc(II)–1,4,7,10-tetraazacyclododecane (cyclen).

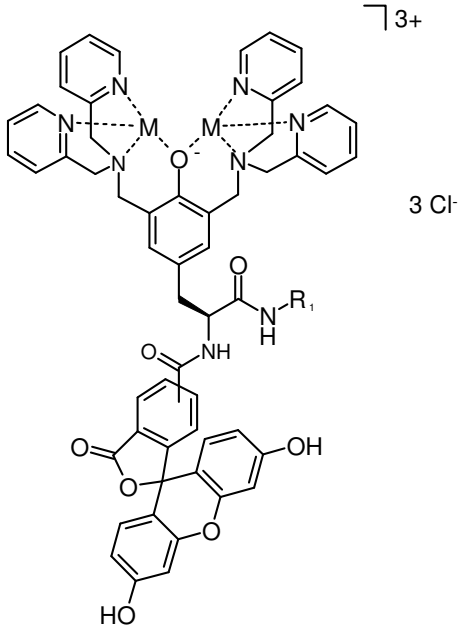
Dipicolylamine (Dpa)



9: R = $C_{10}H_{21}$



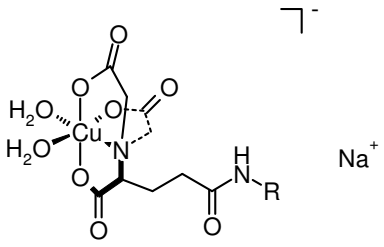
10 M = Zn^{2+}, R_1 = boc, R_2 = $C_{18}H_{37}$
11 M = Mn^{2+}, R_1 = boc, R_2 = $C_{18}H_{37}$
12 M = Zn^{2+}, R_1 = H, R_2 = $C_{18}H_{37}$
13 M = Mn^{2+}, R_1 = H, R_2 = $C_{18}H_{37}$



14 M = Zn^{2+}, R_1 = $C_{18}H_{37}$
15 M = Mn^{2+}, R_1 = $C_{18}H_{37}$

Figure 2. Synthesized metal chelating artificial lipids based on Lewis acidic zinc(II)–dipicolylamine (Dpa).

Nitrilotriacetato acid (NTA)

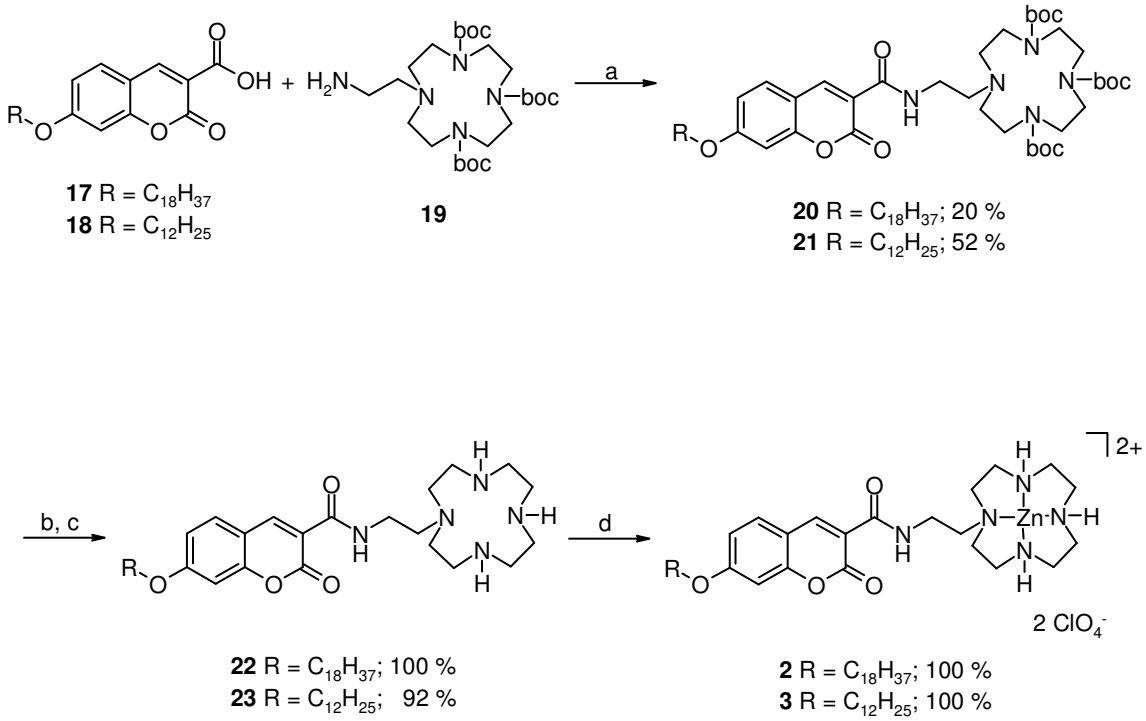


16: R = $C_{10}H_{21}$

Figure 3. Synthesized metal chelating artificial lipid based on Lewis acidic copper(II)–nitrilotriacetoato acid (NTA).

7.2.1 Synthesis of cyclen based metal chelating artificial lipids

Metal chelating cyclen based lipids **1**[17] and **4** – **8**[18] were synthesized as previously reported. Mononuclear chelating cyclen lipids with different hydrophobic chain length (C_{12} or C_{18}) were prepared by amide formation of literature known compounds **17**[12a] and **18**,[12a] respectively, and **19**.[19] Removal of the Boc groups, passing through a basic ion exchanger resin and subsequent zinc complex formation by treating the free amine ligand **22** and **23**, with one equivalent of $ZnCl_2$ each yielded the fluorescent amphiphilic Zn(II)-cyclen metal complexes **2** and **3**.

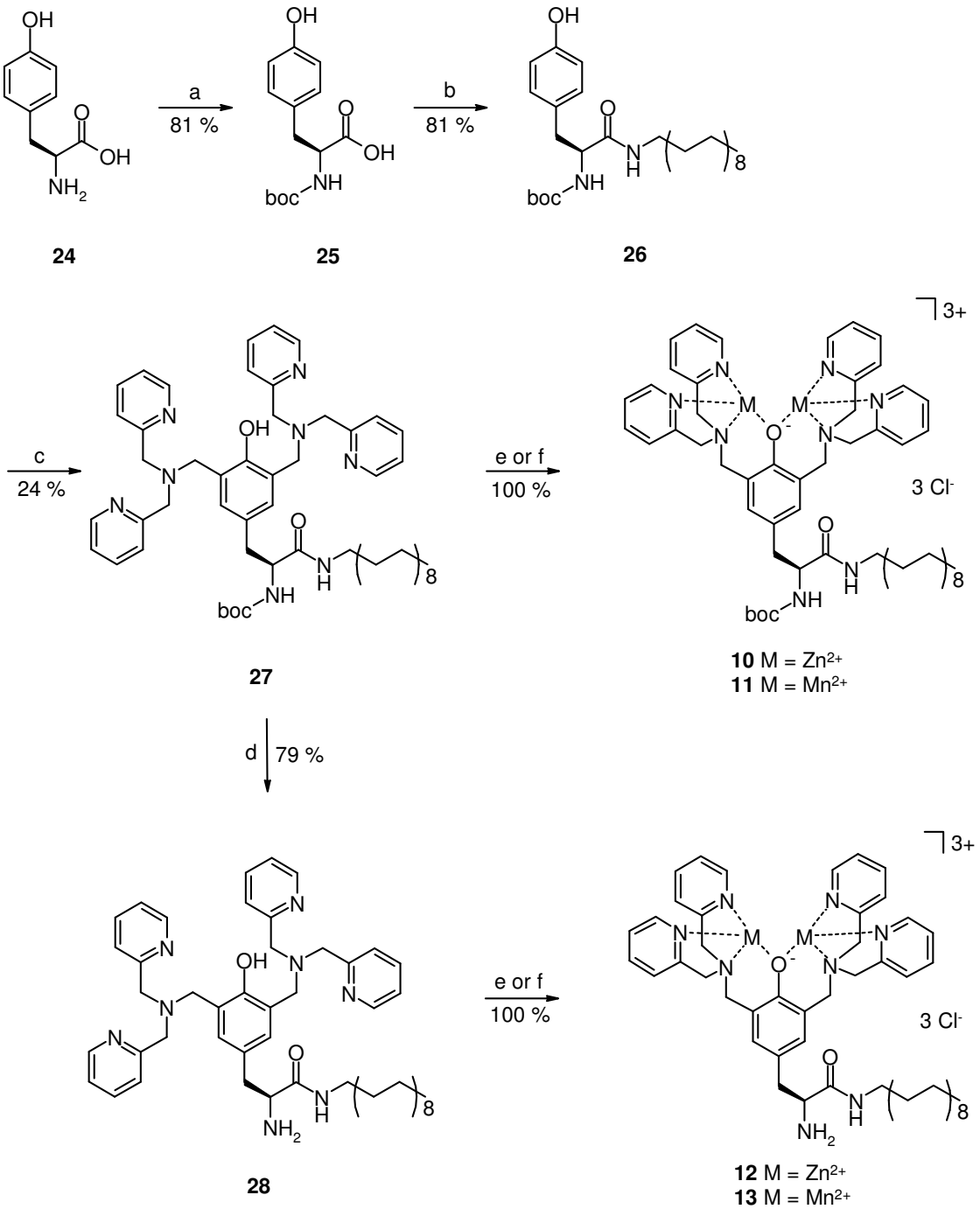


Scheme 1. Synthesis of fluorescent amphiphilic mononuclear Zn(II)-cyclen complexes. (a) TBTU, HOBt, DIPEA, DMF, 40 °C, 2-3 h; (b) HCl/ether, DCM, RT, o/n; (c) basic ion exchanger resin H_2O, MeOH; (d) $Zn(ClO_4)_2$, MeOH, 65 °C, 22 - 23 h.

7.2.2 Synthesis of Dpa based metal chelating artificial lipids

We focused on the preparation of a non-fluorescent (Scheme 2) and a fluorescent labelled (Scheme 3) tyrosine based bis-Dpa metal chelating lipids. The metal chelating binuclear Dpa lipid was synthesized by a modified procedure for the binuclear tyrosine

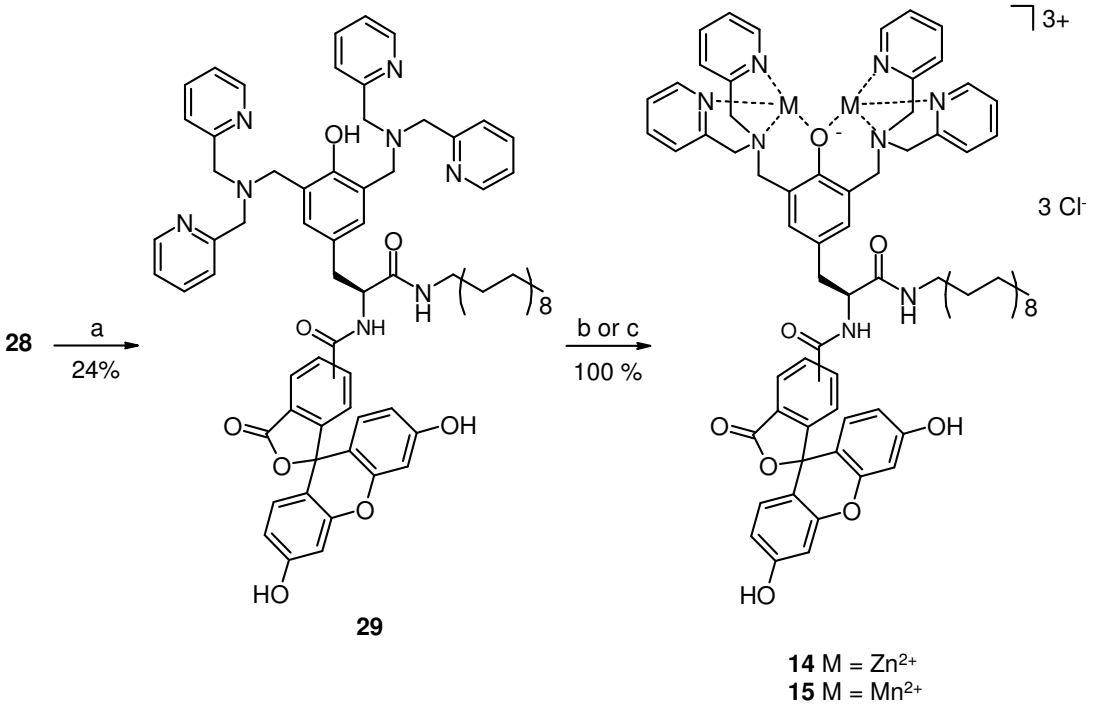
based bis-Dpa (Boc-dpa-Tyr-OMe) reported by Hamachi *et al.*[15c] Derivatization of the tyrosine scaffold on large scale was possible by standard peptide solution chemistry.



Scheme 2. Synthesis of amphiphilic tyrosine based bis-dpa zinc / manganese complex. (a) Boc_2O, DCM, RT, 20 h; (b) EDC, HOBt, DIPEA, DMF, Toluol, 60 °C, 22h; (c) 2,2`-dipicoloylamine, paraformaldehyde, H_2O, ^{i}PrOH, 80 °C, 30 min, reflux, 17 h; (d) HCl / ether, DCM, RT, 16 h; (e) $ZnCl_2$, MeOH, RT, 2 - 3 h; (f) $MnCl_2$, MeOH, RT, 2 - 3 h.

Amide formation of **25**[20] with octadecylamine gave amphiphilic tyrosine derivative **26** in good yields. *Mannich* type reaction using 2,2`-dipycolylamine and paraformaldehyde

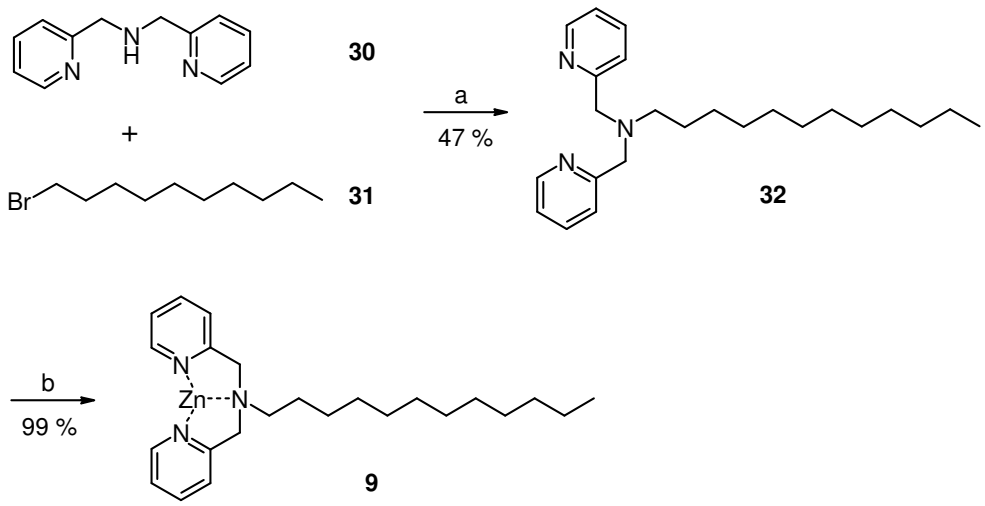
and according to literature known procedure gave the protected metal chelating lipid **27**.[15c] Removal of the Boc group in acidic conditions (HCl/ether) yielded compound **28**. Finally metal chelating lipids **27** and **28** were treated with two equivalents of a $ZnCl_2$ and $MnCl_2$, respectively.



Scheme 3. Synthesis of fluorescent amphiphilic tyrosine based bis-Dpa zinc / manganese complex. (a) 5/6-carboxy fluorescein, DIPEA, TBTU, HOBt, DMF, 40 °C, 22 h; (b) $ZnCl_2$, MeOH, RT, 2 - 3 h; (c) $MnCl_2$, MeOH, RT, 2 - 3 h.

As a fluorescence signalling unit we introduced a fluorescein dye. Therefore the amide of **28** and an isomeric mixture of 5/6-carboxy fluorescein was prepared and subsequent treatment with $ZnCl_2$ or $MnCl_2$ gave the fluorescent amphiphilic binuclear tyrosine based metal complexes **14** and **15**.

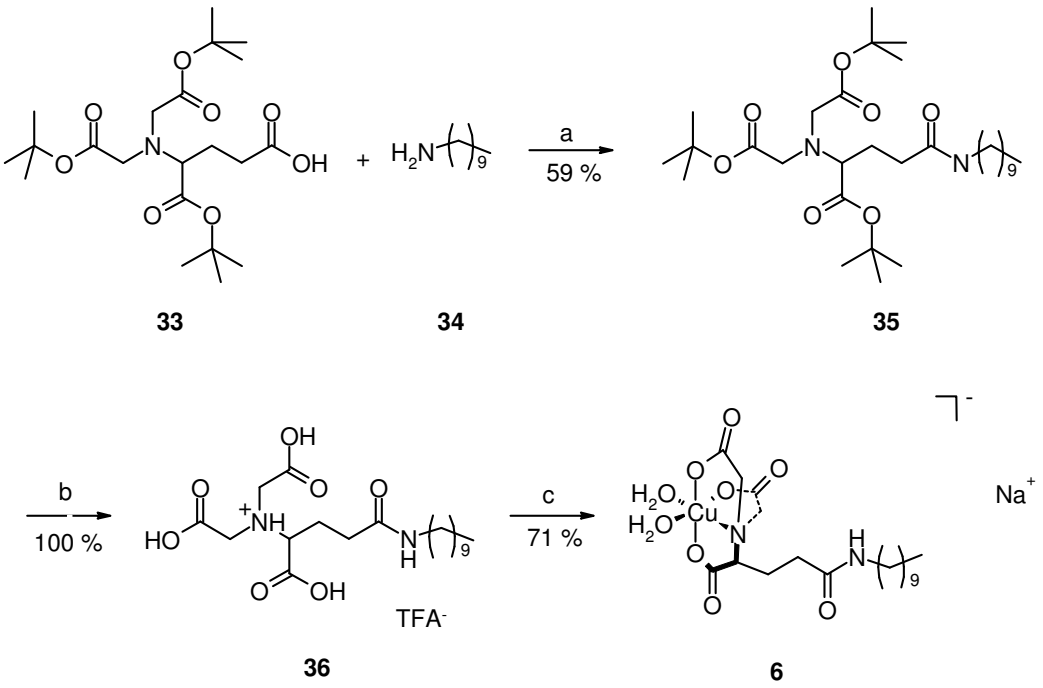
Further, an amphiphilic mononuclear zinc(II)-Dpa was prepared: 2,2`Dipycolylamine **30**[21] was allowed to react with 1-bromododecane **31** giving Dpa-dodecane **32**, which was converted to the Zn(II)-Dpa-dodecane **9** by $ZnCl_2$ (Scheme 4).



Scheme 4. Synthesis of amphiphilic zinc dpa complex. (a) NEt_3, toluol, 90 °C, 3d; (b) $ZnCl_2$, MeOH, RT, 16 h.

7.2.3 Synthesis of NTA based metal chelating artificial lipids

Glutamic acid based NTA moiety **33**[22] was reacted with decylamine **34** and subsequently treated with trifluoroacetic acid giving the NTA compound **36**. Finally, copper (II) complex formation was done with $Cu_2(OH)_2CO_3$ (Scheme 5).



Scheme 5. Synthesis of amphiphilic copper NTA complex. (a) EDC, HOBt, DIPEA, DMF, 40 °C; (b) TFA, RT; (c) $Cu_2(OH)_2CO_3$, MeOH.

7.3 Conclusion and Outlook

In summary, we prepared amphiphilic metal chelating artificial lipids based on cyclen, Dpa and NTA metal complexes as binding sites at interfaces of SAMs, LB films or vesicles. Detailed investigations of the binding properties of modified surfaces incorporating the new amphiphilic complexes are in progress.

7.4 Experimental Part

7.4.1 General methods and material

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck or Baker).

NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in 13C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for quaternary C_q. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

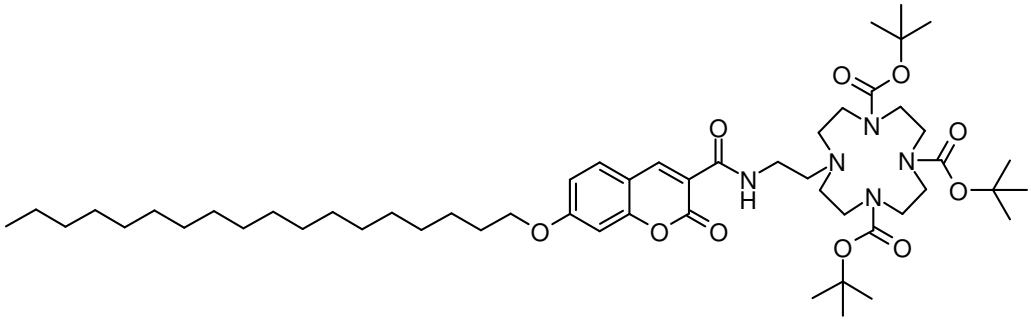
Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

General. Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Detection via UV light at 254 nm / 366 nm or through staining with ninhydrin in EtOH. Column chromatography was performed on silica gel (70–230 mesh) from Merck. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Commercially available solvents of standard quality were used. Dry THF, which was prepared by distillation from potassium. If otherwise stated, purification and drying was done according to accepted general procedures.[23] Elemental analyses were carried out by the center for Chemical Analysis of the Faculty of Natural Sciences of the University Regensburg.

7.4.2 Synthesis

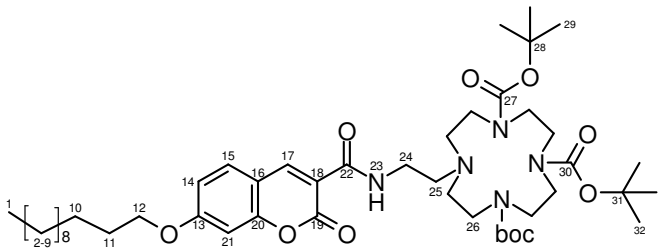


10-{2-[(7-Octadecyloxy-2-oxo-2H-chromene-3-carbonyl)-amino]-ethyl}-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester **(20)**

7-Octadecyloxy-2-oxo-2H-chromene-3-carboxylic acid **17** (250 mg, 0.55 mmol), DIPEA (376 µL, 2.18 mmol), TBTU (203 mg, 0.60 mmol), and HOBt (97 mg, 0.60 mmol) were dissolved under nitrogen atmosphere in 9 mL of a mixture of dry DMF / THF (1:2) under ice cooling and stirred for 1 h at rt. Subsequently amine **19** (281 mg, 0.55 mmol) dissolved in dry DMF (2 mL) was added dropwise. The reaction was allowed to warm to room temperature and was stirred 3.5 h at 40 °C. The reaction progress was monitored by TLC (ethyl acetate / petrol ether 1:1). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate / petrol ether 1:1, R_f = 0.30) yielding compound **20** (104 mg, 0.11 mmol, 20 %) as a colourless oil.

^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 0.83 (t, ^{3}J = 6.8 Hz, 3 H, CH_3), 1.05-1.35 (m, 28 H, CH_2), 1.41 (s, 29 H, boc-CH_3, CH_2), 1.79 (quin, ^{3}J = 7.0 Hz, 2 H, CH_2), 2.61-2.89

(m, 6 H, CH_2), 3.15-3.65 (m, 14 H, CH_2), 4.01 (t, ^3J = 6.2 Hz, 2 H, CH_2), 6.80 (s, 1 H, CH), 6.88 (dd, ^3J = 8.7 Hz, ^4J = 1.8 Hz, 1 H, CH), 7.52 (d, ^3J = 8.5 Hz, 1 H, CH), 8.74-8.81 (m, 2 H, CH, NH). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 1 C, CH_3), 22.6 (–, 1 C, CH_2), 25.8 (–, 1 C, CH_2), 28.4 (+, 9 C, CH_3), 28.6 (–, 2 C, CH_2), 28.8 (–, 1 C, CH_2), 29.20 (–, 1 C, CH_2), 29.24 (–, 1 C, CH_2), 29.42 (–, 1 C, CH_2), 29.46 (–, 1 C, CH_2), 29.57 (–, 6 C, CH_2), 31.8 (–, 1 C, CH_2), 35.4 (–, 1 C, CH_2), 47.6, 47.9 (–, 4 C, CH_2), 49.9 (–, 2 C, CH_2), 51.3 (–, 1 C, CH_2), 53.8 (–, 1 C, CH_2), 55.2 (–, 1 C, CH_2), 69.0 (–, 1 C, CH_2), 79.2 (C_q, 1 C), 79.47 (C_q, 1 C), 79.52 (C_q, 1 C), 100.7 (+, 1 C, CH), 112.1 (C_q, 1 C), 114.2 (C_q, 1 C), 114.4 (+, 1 C, CH), 130.8 (+, 1 C, CH), 148.2 (+, 1 C, CH), 155.3 (C_q, 1 C), 155.6 (C_q, 1 C), 156.1 (C_q, 1 C), 156.6 (C_q, 1 C), 161.8 (C_q, 1 C), 162.3 (C_q, 1 C), 164.5 (C_q, 1 C). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2926, 2852, 1685, 1604, 1531, 1461, 1414, 1365, 1247, 1220, 1153, 963, 857, 753. – **UV** ($CHCl_3$): λ_{max} (log ε) = 352 nm (4.439). – **MS** (ESI(+), DCM/MeOH + 0.1 % TFA): m/z (%) = 956.8 (100) [MH^+]. – **HRMS** Calcd for $C_{53}H_{89}N_5O_{10}$ 956.6688; Found: 956.6697. – **MF**: $C_{53}H_{89}N_5O_{10}$ – **FW**: 956.32 g/mol

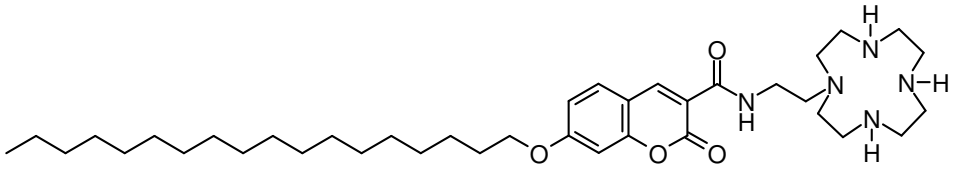


***7-tert-Butoxycarbonylmethyl-10-{2-[(6-dodecyloxy-naphthalene-2-carbonyl)-amino]-ethyl}-1,4,7,10-tetraaza-cyclododecane-1,4-dicarboxylic acid 4-tert-butyl ester 1-isopropyl ester* (21)**

7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid **18** (100 mg, 0.27 mmol), DIPEA (184 µL, 1.07 mmol), TBTU (94 mg, 0.29 mmol), and HOBt (45 mg, 0.29 mmol) were dissolved under nitrogen atmosphere in dry DMF / THF (2:1, 6 mL) under ice cooling and stirred for 1 h. Subsequently amine **19** (138 mg, 0.27 mmol) dissolved in DMF (2 mL) was added dropwise. The reaction was allowed to warm to room temperature and was stirred 30 min at rt and 4 h at 40 °C. The reaction progress was monitored by TLC (ethyl acetate/petroleum ether 1:1). After completion of the reaction the solvent was removed and the crude product was purified by flash column chromatography on

flash silica gel (ethyl acetate/petroleum ether 1:1; R_f = 0.4) yielding compound **21** (120 mg, 0.14 mmol, 52 %) as a white solid.

^{1}H-NMR (400 MHz; $CDCl_3$): δ (ppm) = 0.87 (t, ^{3}J = 6.8 Hz, 3 H, HSQC, COSY: C^1H_3), 1.14-1.38 (m, 16 H, HSQC, COSY: $C^2H_2 – C^9H_2$), 1.44 (s, 18 H, HSQC: $C^{29}H_3$), 1.45 (s, 11 H, HSQC: $C^{32}H_3$, HSQC, HMBC: $C^{10}H_2$), 1.82 (quin, 2 H, HSQC, ROESY: $C^{11}H_2$), 2.51-2.95 (m, 6 H, HSQC, HMBC, ROESY: $C^{25}H_2$, HSQC, HMBC: $C^{26}H_2$), 3.10-3.72 (m, 14 H, HSQC, HMBC, ROESY: $C^{24}H_2$, HSQC, HMBC: $C^{26}H_2$), 4.04 (t, ^{3}J = 6.5 Hz, 2 H, HMBC, ROESY: $C^{12}H_2$), 6.83 (d, ^{4}J = 2.3 Hz, 1 H, HSQC, ROESY: $C^{21}H$), 6.91 (dd, ^{4}J = 2.3 Hz, ^{3}J = 8.7 Hz, 1 H, HSQC, ROESY: $C^{14}H$), 7.55 (d, ^{3}J = 8.7 Hz, 1 H, HSQC, ROESY: $C^{15}H$), 8.80 (m, 2 H, HSQC, ROESY: $C^{17}H$, NH). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, HSQC: C^1H_3), 22.6, 29.27, 29.30, 29.48, 29.53, 29.59, 29.60, 31.9 (–, 8 C, HSQC: C^2H_2- C^9H_2), 25.9 (–, 1 C, HSQC, HMBC: $C^{10}H_2$), 28.5 (+, 6 C, HSQC, HMBC: $C^{29}H_3$), 28.7 (+, 3 C, HSQC, HMBC: $C^{32}H_3$), 28.9 (–, 1 C, HSQC, ROESY: $C^{11}H_2$), 35.5 (–, 1 C, HSQC, HMBC, ROESY: $C^{24}H_2$), 47.6 (–, 1 C, HSQC, HMBC: $C^{26}H_2$), 48.0 (–, 3 C, HSQC, HMBC: $C^{26}H_2$), 49.9 (–, 2 C, HSQC, HMBC: $C^{26}H_2$), 51.4 (–, 1 C, HSQC, ROESY: $C^{25}H_2$), 53.9 (–, 1 C, HSQC, HMBC: $C^{26}H_2$), 55.3 (–, 1 C, HSQC, HMBC: $C^{26}H_2$), 69.0 (–, 1 C, HSQC, HMBC, ROESY: $C^{12}H_2$), 100.8 (+, 1 C, HSQC, ROESY: $C^{21}H$), 114.4 (+, 1 C, HSQC, ROESY: $C^{14}H$), 130.9 (+, 1 C, HSQC, ROESY: $C^{15}H$), 148.3 (+, 1 C, HSQC, ROESY: $C^{17}H$), 79.2 (C_q, 1 C, HSQC: C^{31}), 79.5 (C_q, 2 C, HSQC: C^{28}), 112.2 (C_q, 1 C, HSQC, HMBC: C^{16}), 114.4 (C_q, 1 C, HSQC, HMBC: C^{18}), 155.4, 155.7, 156.1 (C_q, 3 C, HSQC, HMBC: C^{27}, C^{30}), 156.7 (C_q, 1 C, HSQC, HMBC: C^{20}), 161.9 (C_q, 1 C, HSQC, HMBC: C^{19}), 162.4 (C_q, 1 C, HSQC, HMBC: C^{22}), 164.6 (C_q, 1 C, HSQC, HMBC: C^{13}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2925, 2856, 1684, 1599, 1531, 1458, 1413, 1364, 1247, 1154, 969, 854, 773. – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 872.7 (100) [MH^+]. – **HRMS** Calcd for $C_{47}H_{77}N_5O_{10}$ 871.5670; Found: 871.5648. – **MF**: $C_{47}H_{77}N_5O_{10}$ – **FW**: 872.17

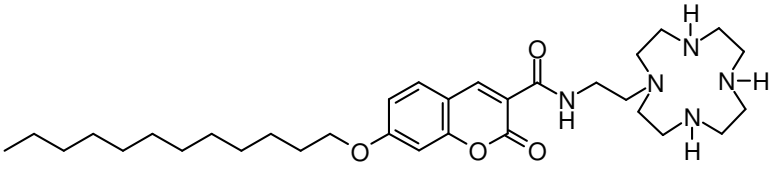


***7-Octadecyloxy-2-oxo-2H-chromene-3-carboxylic acid [2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethyl]-amide* (22)**

Compound **20** (104 mg, 0.11 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently 6 mL HCl saturated ether were added. The solution was stirred 15 min at 0 °C and additionally 20 h at room temperature. The solvent was removed in vacuo, yielding quantitatively the protonated hydrochloride of compound **22** as a pale yellow solid. To obtain the free base of compound **22** a weakly basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (435 mg, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The hydrochloride salt was dissolved in water / MeOH (9:1), put onto the column and eluated with water / MeOH (9:1). The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 72 mg (0.11 mmol, 100 %) of free base **22**, as colourless semi-solid.

Note: NMR investigations on compound **22** were not feasible as the resolution on the NMR was not sufficient.

MP: 159 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ =3349, 2918, 2851, 1706, 1616, 1537, 1468, 1374, 1259, 1224, 1144, 1022, 973, 795, 719. – **UV** ($CHCl_3$): λ_{max} (log ε) = 353 nm (4.116). – **LC-MS** (+ c ESI Q1MS): m/z (%) = 328.6 (100) $[M + 2 H^+]^{2+}$, 349.1 (85) $[M + 2 H^+ + MeCN]^{2+}$, 656.4 (55) $[MH^+]$. – **HRMS** Calcd for $C_{38}H_{66}N_5O_4$ 656.5115; Found: 656.5104. – **MF**: $C_{38}H_{65}N_5O_4$ –**FW**: 655.97



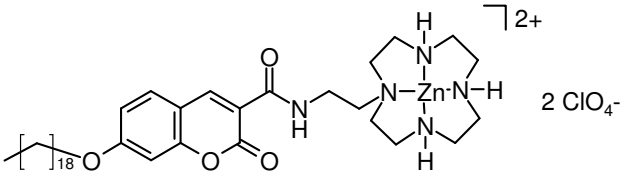
***7-Dodecyloxy-2-oxo-2H-chromene-3-carboxylic acid [2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethyl]-amide* (23)**

Compound **21** (108 mg, 0.12 mmol) was dissolved in DCM (4 mL) and cooled to 0 °C. Subsequently TFA (801 µL, 10.4 mmol) was added. The solution was stirred 15 min at

0 °C and additionally 20 h at room temperature. The solvent was removed in vacuo, yielding quantitatively the protonated TFA salt of compound **23** as a pale yellow solid. To obtain the free base of compound **23** a weak basic ion exchanger resin was swollen for 15 min in water and washed neutral with water. A column was charged with resin (495 mg, 40.0 mmol hydroxy equivalents at a given capacity of 5 mmol/g). The TFA salt was dissolved in water / MeOH (8:2), put onto the column and eluated with water / MeOH (8:2). The elution of the product was controlled by pH indicator paper (pH > 10) and was completed when pH again was neutral. The eluate was concentrated and lyophilised to yield 64 mg (0.11 mmol, 92 %) of free base **23**, as pale yellow solid.

Note: NMR investigations on compound **23** were not feasible as the resolution on the NMR was not sufficient.

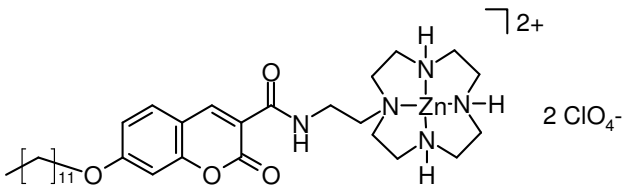
IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2924, 2851, 1673, 1617, 1540, 1456, 1374, 1273, 1199, 1175, 1121, 1026, 832, 797, 720. – **UV** ($CHCl_3$): λ_{max} (log ε) = 352 nm (4.108). – **LC-MS** (+ c ESI Q1MS): m/z (%) = 572.3 (100) [MH^+], 307.0 (95) [M + 2 H^+ + MeCN]$^{2+}$, 286.5 (40) [M + 2 H^+]$^{2+}$. – **MF**: $C_{32}H_{53}N_5O_4$ – **FW**: 571.81



***Mononuclear Zn(II)-cyclen coumarin C18* (2)**

A solution of compound **22** (30.1 mg, 46 µmol) in MeOH (2 mL) was heated to 65 °C and subsequently a methanolic solution of $Zn(ClO_4)_2$ (20 mM, 2086 µL, 46 µmol) was added dropwise. After stirring the reaction mixture for 22 h at 65 °C, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **2** as a colourless solid in quantitative yield (42 mg, 46 µmol).

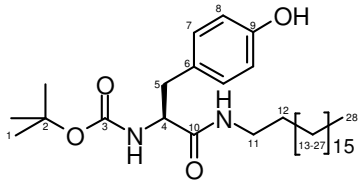
MP: 206 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3357, 2916, 2849, 1707, 1616, 1536, 1467, 1445, 1374, 1260, 1233, 1144, 1020, 795, 719. – **UV** ($CHCl_3$): λ_{max} (log ε)= 351 nm (3.785). – **MS** (ESI(+), MeOH + 10 mmol/L NH_4Ac): m/z (%) = 718.5 (100) [M^{2+} - H^+]$^+$. – **MF**: $C_{38}H_{65}N_5O_4Zn(ClO_4)_2$ – **FW**: 920.25 g/mol



***Mononuclear Zn(II)-cyclen coumarin C12* (3)**

To a solution of compound **23** (13.2 mg, 23 µmol) in MeOH (1 mL) was added dropwise a methanolic solution of $Zn(ClO_4)_2$ (22 mM, 1049 µL, 23 µmol). After stirring the reaction mixture for 23 h at 65 °C, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **3** as a colourless hygroscopic semi-solid in quantitative yield.

IR (ATR) [cm^{-1}]: $\tilde{\nu}$ = 3478, 2921, 2852, 1691, 1617, 1546, 1465, 1379, 1281, 1229, 1203, 1073, 795, 621. – **UV** ($CHCl_3$): λ_{max} (log ε) = 353 nm (3.803). **MS** (ESI(+), MeOH + 10 mmol/L NH_4Ac): m/z (%) = 634.4 (95) $[M^{2+} - H^+]^+$, 391.3 (100) $[softener]^+$. – **MF**: $C_{32}H_{53}N_5O_4Zn(ClO_4)_2$ – **FW**: 836.09 g/mol

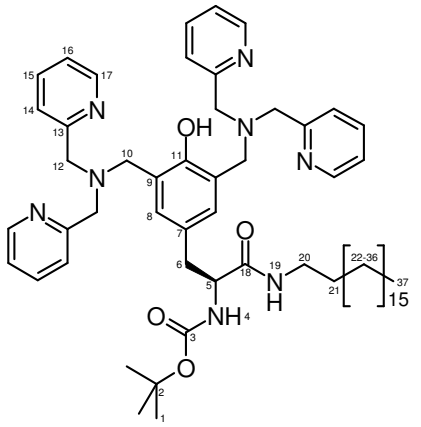


***[2-(4-Hydroxy-phenyl)-1-octadecylcarbamoyl-ethyl]-carbamic acid tert-butyl ester* (26)**

Boc-Tyr-OH **25** (2.50 g, 8.9 mmol), DIPEA (5.0 mL, 29.3 mmol), EDC (1.73 mL, 9.8 mmol), and HOBt (1.32 g, 9.8 mmol) were dissolved in DMF (4 mL) under ice cooling and stirred for 45 min. Subsequently a solution of octadecylamine (2.89 g, 9.8 mmol) in 25 mL DMF was added slowly. The reaction was allowed to warm to room temp. and was stirred over night (22 h) at 60 °C. The reaction progress was monitored by TLC ($CHCl_3$ / MeOH 9:1). After completion of the reaction the solvent was removed and the crude product was loaded on flash silica gel and purified by flash column chromatography ($CHCl_3$ / MeOH 95:5, R_f = 0.40) yielding **26** (3.82 g, 7.2 mmol, 81 %) as a colourless solid.

MP: 115 °C. – **^{1}H-NMR** (600 MHz; $CDCl_3$): δ (ppm) = 0.87 (t, ^{3}J = 7.0 Hz, 3 H, $C^{28}H_3$), 1.25 (s, 30 H, $C^{13}H_2$ – $C^{27}H_2$), 1.36 (bs, 2 H, COSY: $C^{12}H_2$), 1.42 (s, 9 H, COSY: C^1H_3), 2.83-3.02 (m, 2 H, COSY: C^5H_2), 3.07-3.24 (m, 2 H, COSY: $C^{11}H_2$), 4.21 (m, 1 H, HMBC, COSY: C^4H), 5.17 (bs, 1 H, COSY: NH^a), 5.91 (bs, 1 H, COSY:

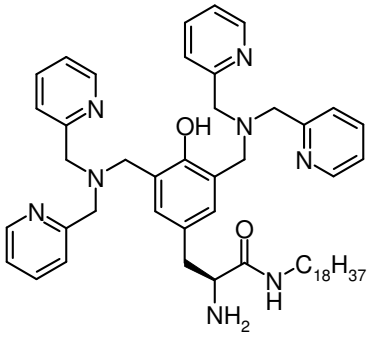
NH^b), 6.74 (d, ^2J = 8.5 Hz, 2 H, HMBC: C^8H), 7.02 (d, ^2J = 7.7 Hz, 2 H, HMBC: C^7H). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, $C^{28}H_3$), 22.7 (–, 1 C, alkyl-CH_2), 26.8 (–, 1 C, alkyl-CH_2), 28.3 (+, 3 C, C^1H_3), 29.2 (–, 1 C, alkyl-CH_2), 29.3 (–, 1 C, alkyl-CH_2), 29.5 (–, 1 C, $C^{12}H_2$), 29.60, 29.65, 29.69 (–, 10 C, alkyl-CH_2), 31.9 (–, 1 C, alkyl-CH_2), 37.9 (–, 1 C, C^5H_2), 39.6 (–, 1 C, $C^{11}H_2$), 56.2 (+, 1 C, HMBC: C^4H_3), 80.3 (C_q, 1 C, C^2), 115.6 (+, 2 C, HMBC: C^7H), 130.4 (+, 2 C, HMBC: C^8H), 128.1 (C_q, 1 C, HMBC: C^6), 155.2 (C_q, 1 C, HMBC: C^9), 155.6 (C_q, 1 C, HMBC: C^3), 171.3 (C_q, 1 C, HMBC: C^{10}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ [cm] = 3335, 3306, 2959, 2917, 1682, 1655, 1523, 1468, 1367, 1295, 1236, 1168, 1046, 896, 799. – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 533.6 (100) [MH^+], 477.4 (19) [MH^+ - C_4H_8]$^+$, 555.5 (36) [MNa^+]. – **Elemental analysis** calcd. (%) for $C_{32}H_{56}N_2O_4$: C 72.14, H 10.59, N 5.26; found C 72.13, H 10.63, N 5.00. – **MF**: $C_{32}H_{56}N_2O_4$ – **FW**: 532.81 g/mol



***(2-{3,5-Bis-[(bis-pyridin-2-ylmethyl-amino)-methyl]-4-hydroxy-phenyl}-1-octadecyl carbamoyl-ethyl)- carbamic acid tert-butyl ester* (27)**

2,2'-Dipicolylamine (2.35 g, 11.8 mmol) and paraformaldehyde (0.56 g, 18.9 mmol) were dissolved in 30 mL water / isopropanol (5:3) and the pH was adjusted to 8 by adding 1 M NaOH. After stirring at 80 °C for 35 min, compound **26** (2.51 g, 4.7 mmol) was added, and the reaction mixture was refluxed for 17 h. After cooling to room temperature the solvent was evaporated and the residue was dissolved in ethyl acetate. The solution subsequently was washed with saturated $NaHCO_3$ (3x) and brine (3x) followed by drying over $MgSO_4$. After removal of the solvent in vacuo the crude product was purified by flash column chromatography on flash silica gel (ethyl acetate / MeOH 2:1, R_f = 0.1) obtaining **27** (1.1 g, 1.15 mmol, 24 %) as a colourless oil.

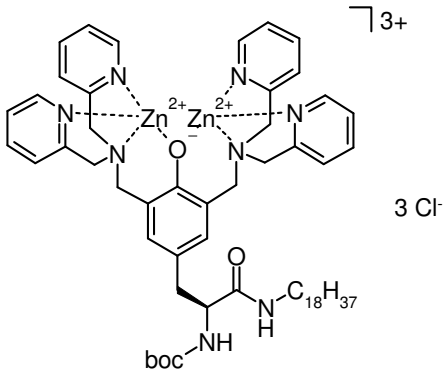
^{1}H-NMR (400 MHz; acetone-d6): δ (ppm) = 0.86 (t, ^{3}J = 6.9 Hz, 3 H, $C^{37}H_3$), 1.14-1.28 (m, 30 H, $C^{22}H_2$ – $C^{36}H_2$), 1.29 (s, 11 H, HSQC: $C^{1}H_3$, $C^{21}H_2$), 2.87 (dd, ^{3}J = 13.5 Hz, ^{2}J = 6.5 Hz, 1 H, HSQC, COSY: $C^{6}H_2$), 2.94 (dd, ^{3}J = 13.5 Hz, ^{2}J = 7.4 Hz, 1 H, HSQC, COSY: $C^{6}H_2$), 3.02 (dd, ^{3}J = 12.9 Hz, ^{2}J = 6.6 Hz, 2 H, HSQC: $C^{20}H_2$), 3.74 (d, ^{2}J = 13.7 Hz, 2 H, HMBC, COSY: $C^{10}H_2$), 3.80 (d, ^{2}J = 13.7 Hz, 2 H, HMBC, COSY: $C^{10}H_2$), 3.85 (s, 8 H, COSY: $C^{12}H_2$), 3.34 (dd, ^{3}J = 14.4 Hz, ^{2}J = 7.1Hz, 1 H, COSY: $C^{20}H_2$), 5.98 (d, ^{3}J = 8.2 Hz, 1 H, HSQC, COSY: $N^{4}H$), 7.10 (s, 2 H, HMBC, COSY: $C^{8}H$), 7.20 (ddd, ^{3}J = 7.4 Hz, 4.9 Hz, 1.1 Hz, 4 H, HMBC: $C^{16}H$), 7.29 (bs, 1 H, HSQC, COSY: $N^{19}H$), 7.55 (d, ^{3}J = 7.8 Hz, 4 H, HMBC: $C^{14}H$), 7.69 (dt, ^{3}J = 7.7 Hz, ^{2}J = 1.9 Hz, 4 H, HMBC: $C^{15}H$), 8.52 (m, 4 H, HMBC: $C^{17}H$), 10.96 (bs, 1 H, HSQC: OH). – **^{13}C-NMR** (100 MHz; acetone-d6): δ (ppm) = 14.3 (+, 1 C, $C^{37}H_3$), 23.3 (–, 1 C, alkyl-CH_2), 27.5 (–, 1 C, alkyl-CH_2), 28.5 (+, 3 C, $C^{1}H_3$), 29.9 (–, 1 C, alkyl-CH_2), 30.22 (–, 1 C, alkyl-CH_2), 30.27 (–, 1 C, alkyl-CH_2), 30.4 (–, 9 C, alkyl-CH_2, solvent peak), 32.6 (–, 1 C, alkyl-CH_2), 38.8 (–, 1 C, COSY: $C^{6}H_2$), 39.7 (–, 1 C, HSQC: $C^{20}H_2$), 55.3 (–, 2 C, HMBC, COSY: $C^{10}H_2$), 60.1 (–, 4 C, HMBC, COSY: $C^{12}H_2$), 56.9 (+, 1 C, HMBC: $C^{5}H_3$), 79.0 (C_q, 1 C, HMBC, HSQC, C^{2}), 122.8 (+, 4 C, HMBC: $C^{16}H$), 123.8 (+, 4 C, HMBC: $C^{14}H$), 124.6 (C_q, 2 C, HMBC: C^{9}), 127.7 (C_q, 1 C, HMBC: C^{7}), 131.3 (+, 2 C, HMBC, COSY: $C^{8}H$), 137.3 (+, 4 C, HMBC, COSY: $C^{15}H$), 149.7 (+, 4 C, HMBC, COSY: $C^{17}H$), 155.8 (C_q, 1 C, HMBC: C^{11}), 155.8 (C_q, 1 C, HMBC: C^{11}), 159.9 (C_q, 1 C, HMBC: C^{3}), 160.2 (C_q, 4 C, HMBC: C^{13}), 171.9 (C_q, 1 C, HMBC: C^{18}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ [cm] = 3383, 2922, 2852, 1706, 1656, 1590, 1569, 1433, 1364, 1288, 1245, 1167, 1048, 995, 791. – **MS** (ESI(+), DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 478.5 (100) [M + 2 H^+]$^{2+}$, 955.8 (85) [MH^+]. – **Elemental analysis** calcd. (%) for $C_{58}H_{82}N_8O_4$: C 72.92, H 8.65, N 11.73; found C 72.38, H 8.34, N 11.63 – **MF**: $C_{58}H_{82}N_8O_4$ – **FW**: 955.35 g/mol



***2-Amino-3-{3,5-bis-[(bis-pyridin-2-ylmethyl-amino)-methyl]-4-hydroxy-phenyl}-N-octadecyl-propionamide* (28)**

Boc protected **27** (700 mg, 0.73 mmol) was dissolved in DCM under ice cooling and mixed with HCl/ether (3.7 mL; 1 mL / 0.2 mmol Boc group). The reaction was allowed to warm to room temp. and was stirred for additional 16 hours. After completion the reaction mixture was evaporated to dryness, the residue was suspended in saturated $NaHCO_3$ and extracted with ethyl acetate (4x). Subsequently the organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo yielding **28**·(497 mg, 0.58 mmol, 79 %) as a pale yellow oil.

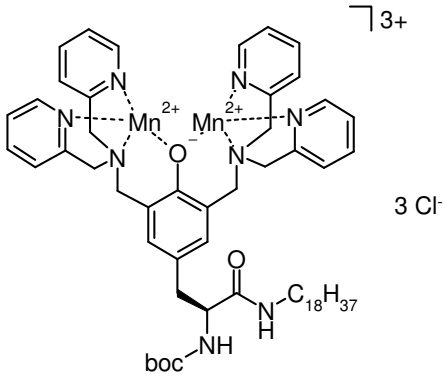
^{1}H-NMR (300 MHz; DMSO-d6): δ (ppm) = 0.82 (t, ^{3}J = 6.9 Hz, 3 H, CH_3), 1.04 (bs, 4 H, alkyl-CH_2), 1.19 (s, 28 H, alkyl-CH_2), 2.57 (dd, ^{3}J = 13.5 Hz, ^{2}J = 7.1 Hz, 1 H, Tyr-CH_2), 2.73 (dd, ^{3}J = 13.5 Hz, ^{2}J = 7.1 Hz, 1 H, Tyr-CH_2), 2.91 (m, 2 H, CH_2), 3.29 (t, ^{3}J = 6.4 Hz, 1 H, $C^{\alpha}H$), 3.65 (s, 4 H, Ar-CH_2-pyr), 3.74 (s, 8 H, N-CH_2-pyr), 6.98 (s, 2 H, Ar-CH), 7.21 (m, 4 H, Pyr-CH), 7.46 (d, ^{3}J = 8.0 Hz, 4 H, Pyr-CH), 7.70 (dt, ^{3}J = 7.6 Hz, 1.7 Hz, 4 H, pyr-CH), 8.47 (dd, ^{3}J = 4.4 Hz, 0.3 Hz, 4 H, pyr-CH). – **^{13}C-NMR** (75 MHz; DMSO-d6): δ (ppm) = 13.8 (+, 1 C, CH_3), 22.0 (–, 1 C, alkyl-CH_2), 26.1 (–, 1 C, alkyl-CH_2), 28.52 (–, 1 C, alkyl-CH_2), 28.57 (–, 1 C, alkyl-CH_2), 28.78, 28.81, 28.89 (–, 11 C, alkyl-CH_2), 31.4 (–, 1 C, alkyl-CH_2), 38.0 (–, 1 C, CH_2), 40.2 (–, 1 C, CH_2), 53.9 (–, 2 C, CH_2), 55.9 (+, 1 C, CH), 58.8 (–, 4 C, CH_2), 122.1 (+, 4 C, CH), 122.5 (+, 4 C, CH), 123.1 (C_q, 1 C), 127.4 (C_q, 1 C), 129.8 (+, 2 C, CH), 136.5 (+, 4 C, CH), 148.5 (+, 4 C, CH), 153.7 (C_q, 1 C), 158.5 (C_q, 4 C), 173.5 (C_q, 1 C). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ [cm] = 2921, 2852, 1658, 1590, 1522, 1474, 1433, 1372, 1233, 1149, 1048, 996, 754. – **MS** (ESI(+), DCM/MeCN/TFA): m/z (%) = 428.4 (100) $[M + 2\ H^+]^{2+}$, 299.6 (16) $[M + 3\ H^+ + MeCN]^{3+}$, 855.8 (14) $[MH^+]$, 286 (12) $[M + 3\ H^+]$. – **HRMS** Calcd for $C_{53}H_{75}N_8O_2$ 855.6013; Found: 855.6000. – **MF**: $C_{53}H_{74}N_8O_2$ – **FW**: 855.23 g/mol



Binuclear Zn(II)-Dpa Boc-tyrosine C18 complex **(10)**

To a solution of compound **27** (50 mg, 52 µmol) in MeOH (4 mL) was added dropwise a methanolic solution of $ZnCl_2$ (100 mM, 1052 µL, 104 µmol). After stirring the reaction mixture for 2.5 h at room temperature, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **10** as a colourless solid in quantitative yield.

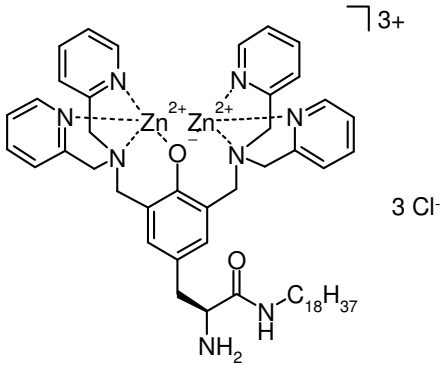
MP: 192 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2923, 2856, 2357, 1695, 1608, 1365, 1257, 1157, 1052, 1023, 761. – **UV** (MeOH): λ_{max} (log ε) = 262 nm (4.108), 296 (3.451). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1199.7 (100) $[M^{3+} + 2\ CH_3COO^-]^+$, 571.0 (15) $[M^{3+} + CH_3COO^-]^{2+}$. – **MF**: $C_{58}H_{82}N_8O_4Zn_2Cl_4$ – **FW**: 1227.91 g/mol



Binuclear Mn(II)-Dpa Boc-tyrosine C18 complex **(11)**

To a solution of compound **27** (50 mg, 52 µmol) in MeOH (4 mL) was added dropwise a methanolic solution of $MnCl_2$ (100 mM, 1047 µL, 104 µmol). After stirring the reaction mixture for 3 h at room temperature, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **11** as a colourless solid in quantitative yield.

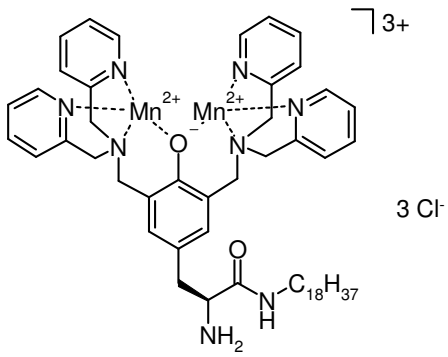
MP: 152 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2926, 2854, 2192, 1700, 1655, 1603, 1472, 1439, 1356, 1257, 1164, 1013, 759. – **UV** (MeOH): λ_{max} (log ε) = 255 nm (4.122), 305 (3.491). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1181.8 (100) $[M^{3+} + 2\ CH_3COO^-]^+$, 1081.7 (8) $[M^{3+} + 2\ CH_3COO^- - boc]^+$, 561.4 (7) $[M^{3+} + CH_3COO^-]^{2+}$, 511.4 (6) $[M^{3+} + CH_3COO^- - boc]^{2+}$. – **MF**: $C_{58}H_{82}N_8O_4Mn_2Cl_4$ – **FW**: 1207.03 g/mol



Binuclear Zn(II)-Dpa tyrosine C18 complex **(12)**

To a solution of compound **28** (36.5 mg, 43 µmol) in MeOH (2 mL) was added dropwise a methanolic solution of $ZnCl_2$ (100 mM, 858 µL, 86 µmol). After stirring the reaction mixture for 2 h at room temperature, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **12** as a colourless solid in quantitative yield.

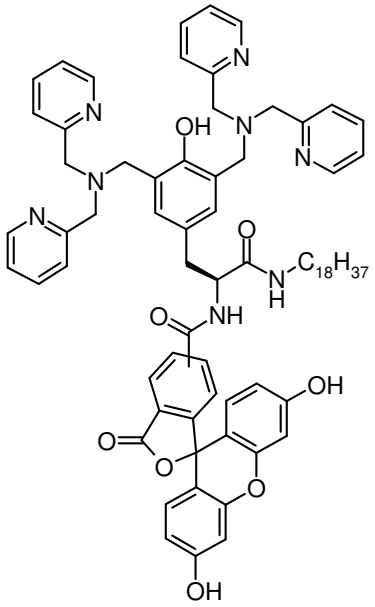
MP: 147 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2924, 2852, 1676, 1608,1573, 1477, 1440,1303, 1270, 1156, 1100, 1053, 1023, 763. – **UV** (MeOH): λ_{max} (log ε) = 261 nm (4.076), 296 (3.436). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1099.7 (100) $[M^{3+} + 2\ CH_3COO^-]^+$, 520.4 (69) $[M^{3+} + CH_3COO^-]^{2+}$. – **MF**: $C_{53}H_{74}N_8O_2Zn_2Cl_4$ – **FW**: 1127.79 g/mol



Binuclear Mn(II)-Dpa tyrosine C18 complex **(13)**

To a solution of compound **28** (32.9 mg, 38 µmol) in MeOH (2 mL) was added dropwise a methanolic solution of $MnCl_2$ (100 mM, 769 µL, 77 µmol). After stirring the reaction mixture for 2.5 h at room temperature, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **13** as a colourless solid in quantitative yield (42 mg, 38 µmol).

MP: 180 °C. – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 2921, 2849, 1675, 1603, 1468, 1439, 1294, 1266, 1153, 1097, 1052, 1014, 762. – **UV** (MeOH): λ_{max} (log ε) = 255 nm (4.016), 306 (3.420). – **MS** (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 1081.7 (100) $[M^{3+} + 2\,CH_3COO^-]^+$, 511.4 (56) $[M^{3+} + CH_3COO^-]^{2+}$. – **MF**: $C_{53}H_{74}N_8O_2Mn_2Cl_4$ – **FW**: 1106.91 g/mol



***Isomeric mixture of fluorescein labelled binuclear Dpa tyrosine C18* (29)**

A isomeric mixture of 5/6-carboxyfluorescein (246 mg, 0.65 mmol), DIPEA (323 µL, 1.87 mmol), TBTU (315 mg, 0.98 mmol), and HOBt (133 mg, 0.98 mmol) were dissolved under nitrogen atmosphere in dry DMF (4 mL) under ice cooling and stirred for 1 h. Subsequently **28** (400 mg, 0.47 mmol) in 4 mL DMF was added slowly. The reaction was allowed to warm to room temperature and was stirred 22 h at 40 °C. The reaction progress was monitored by TLC ($CHCl_3$ / MeOH 9:1). After completion of the reaction the solvent was removed and the crude product was purified by preparative HPLC yielding the isomers **29** as an orange-yellow solid.

Purification by preparative HPLC:

Column: LabID75 Phenomenex Luna 250 x 21.2 mm 10 um / Ser.Nr. 453159-3

Flow: 21 mL / min

Concentration 10 mg / mL

Injection volume: 500 µL

Detection wavelength: 220 nm

Gradient: 0 min – 5 % MeCN / H_2O (+ 0.0059 % TFA)

43 min – 72 % MeCN / H_2O (+ 0.0059 % TFA)

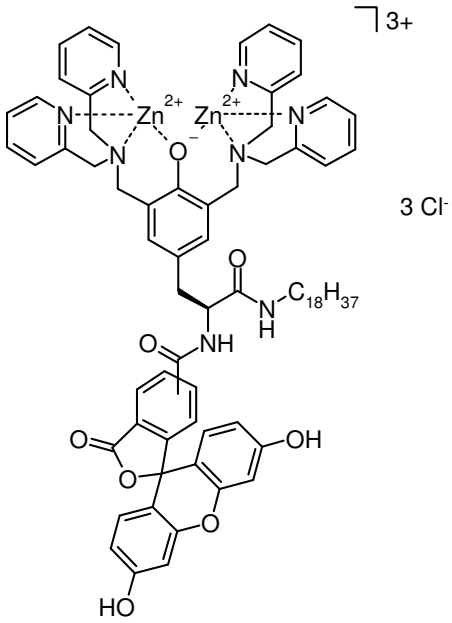
45 min – 98 % MeCN / H_2O (+ 0.0059 % TFA)

55 min – 98 % MeCN / H_2O (+ 0.0059 % TFA)

Retention time: ca. 42 min

MP: 97 – 99 °C. – **UV** (MeOH): λ_{max} (log ε) = 224 (4.629), 260 (4.158), 454 (3.500), 481 (3.481). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ [cm] = 2924, 2853, 1757, 1670, 1609, 1438, 1377, 1246, 1178, 1126, 955, 837, 798, 755, 719. – **MS** (ESI(+), DCM/MeCN/TFA):

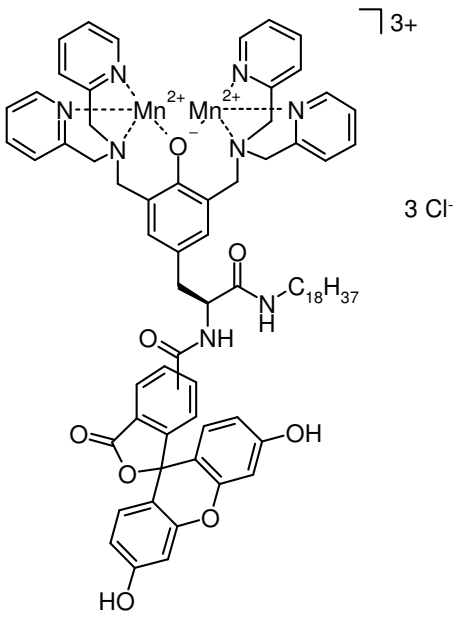
m/z (%) = 607.6 (100) $[M + 2\ H^+]^{2+}$, 405.3 (65) $[M + 3\ H^+]^{2+}$, 1213.9 (11) $[MH^+]$. – **LC-MS** (+ p ESI Q1MS ; RT 40 min): m/z (%) = 607.6 (100) $[M + 2\ H^+]^{2+}$, 1213.9 (65) $[MH^+]^+$, 405.3 (38) $[M + 3\ H^+]^{2+}$. – **MF**: $C_{74}H_{84}N_8O_8$ – **FW**: 1213.53 g/mol



Fluorescein binuclear Zn(II)-Dpa tyrosine C18 complex **(14)**

To a solution of compound **29** (10.5 mg, 8.7 µmol) in MeOH (2 mL) was added dropwise a methanolic solution of $MnCl_2$ (100 mM, 174 µL, 17.4 µmol). After stirring the reaction mixture for 2.5 h at room temperature, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **14** as an orange solid in quantitative yield (13 mg, 8.7 µmol).

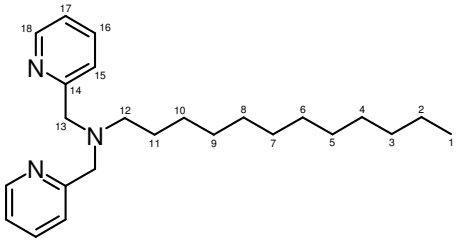
MS (ESI(+), H_2O/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 701.7 (100) $[M^{3+} + CH_3COO^-]^+$, 1398.0 (8) $[M^{3+} - H^+ + CH_3COO^-]^+$, 1458.0 (7) $[M^{3+} + 2\ CH_3COO^-]^+$. – **MF**: $C_{74}H_{84}N_8O_8Zn_2Cl_4$ – **FW**: 1486.10g/mol



Fluorescein binuclear Mn(II)-Dpa tyrosine C18 complex **(15)**

To a solution of compound **29** (8.6 mg, 7.1 µmol) in MeOH (2 mL) was added dropwise a methanolic solution of $MnCl_2$ (100 mM, 142 µL, 14.2 µmol). After stirring the reaction mixture for 3 h at room temperature, the methanol was removed in vacuo. The residue was dissolved in water and was lyophilized yielding complex **15** as an orange solid in quantitative yield (10 mg, 7.1 µmol).

MS (ESI(+), MeOH + 10 mmol/L NH_4Ac): m/z (%) = 690.5 (100) $[M^{3+} + CH_3COO^-]^+$, 1380.0 (5) $[M^{3+} - H^+ + CH_3COO^-]^+$, 1440.0 (10) $[M^{3+} + 2\ CH_3COO^-]^+$. – **MF**: $C_{74}H_{83}N_8O_8Mn_2Cl_4$ – **FW**: 1465.22 g/mol

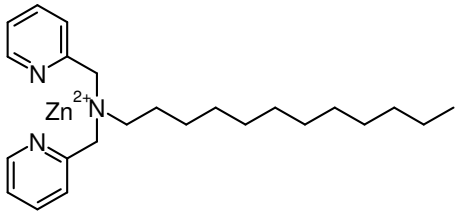


Dodecyl-bis-pyridin-2-ylmethyl-amine **(32)**

To a solution of 1-bromdodecane **31** (1.0 g, 5.02 mmol) and triethylamine (769 µL, 5.52 mmol) in 6 mL of toluene, 2,2`-dipicolylamine **30** (1.38 g, 5.52 mmol) dissolved in toluene (6 mL) were added. The reaction mixture was heated to 90 °C for 3 d under vigorous stirring, concentrated and taken up in chloroform. The organic phase was washed with brine (3x) and dried over $MgSO_4$. The crude product was purified by flash

column chromatography on flash silica gel ($CHCl_3$ / MeOH 95:5, R_f = 0.25) yielding **32** (0.86 g, 2.34 mmol, 46 %) as a dark brown oil.

^{1}H-NMR (600 MHz; $CDCl_3$): δ (ppm) = 0.84 (t, ^{3}J = 7.1 Hz, 3 H, C^1H_3), 1.13-1.30 (m, 18 H, $C^{2\text{-}10}H_2$), 1.52 (quin, ^{3}J = 7.3 Hz, 2 H, HMBC: $C^{11}H_2$), 2.53 (t, ^{3}J = 7.4 Hz, 2 H, HMBC: $C^{12}H_2$), 3.81 (s, 4 H, HSQC: $C^{13}H_2$), 7.10 (ddd, 2 H, ^{3}J = 7.4 Hz, ^{3}J = 4.9 Hz, ^{4}J = 1.3 Hz, HMBC: $C^{17}H$), 7.53 (ddd, 2 H, ^{3}J = 7.8 Hz, ^{4}J = 1.3 Hz, ^{5}J = 0.9 Hz, HMBC: $C^{15}H$), 7.61 (ddd, 2 H, ^{3}J = 7.8 Hz, ^{3}J = 7.4 Hz, ^{4}J = 1.8 Hz, HMBC: $C^{16}H$), 8.48 (ddd, 2 H, ^{3}J = 4.8 Hz, ^{4}J = 1.8 Hz, ^{5}J = 0.9 Hz, HMBC: $C^{18}H$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.0 (+, 1 C, C^1H_3), 22.6 (–, 1 C, HMBC: C^2H_2), 26.9 (–, 1 C, HMBC: $C^{11}H_2$), 27.2 (–, 1 C, HMBC: $C^{10}H_2$), 29.3 (–, 1 C, alkyl-CH_2), 29.4 (–, 1 C, alkyl-CH_2), 29.5 (–, 1 C, alkyl-CH_2), 29.52 (–, 1 C, alkyl-CH_2), 29.55 (–, 1 C, alkyl-CH_2), 29.57 (–, 1 C, alkyl-CH_2), 31.83 (–, 1 C, alkyl-CH_2), 54.4 (–, 1 C, HMBC: $C^{12}H_2$), 60.3 (–, 2 C, HMBC: $C^{13}H_2$), 122.8 (+, 2 C, $C^{17}H$), 122.9 (+, 2 C, $C^{15}H$), 136.3 (+, 2 C, C^{16}), 148.9 (C_q, 2 C, C^{18}), 159.7 (C_q, 2 C, C^{14}). – **IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ = 396, 3059, 3008, 2925, 2853, 1661, 1589, 1467, 1433, 1362, 1305, 1246, 1146, 1046, 892, 758. – **MS** (ESI(+), DCM/MeCN/TFA): m/z (%) = 368.4 [MH^+]. – **HRMS** Calcd for $C_{24}H_{37}N_3$: 367.2987; Found: 367.2988. – **Elemental analysis** calcd. (%) for $C_{24}H_{37}N_3$: C 78.42, H 10.15, N 11.43; found C 77.70, H 10.00, N 10.82 – **MF**: $C_{24}H_{37}N_3$ – **FW**: 367.58 g/mol

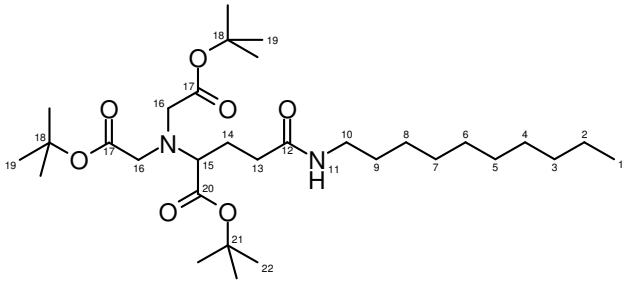


Zn-dpa-dodecane (9):

dpa-Dodecane **32** (762 mg, 2.07 mmol) was dissolved in 10 mL of MeOH and a solution of $ZnCl_2$ in MeOH (18 mL) was added. The reaction mixture was vigorously stirred at room temperature for 16 h. Subsequently MeOH was evaporated obtaining Zn-dpa-dodecane **9** (1035 mg, 2.05 mmol, 99.0 %) as a light brownish solid.

MP: 118 °C. – **^{1}H-NMR** (300 MHz; $CDCl_3$): δ (ppm) = 0.82 (t, ^{3}J = 7.0 Hz, 3 H, alkyl-CH_3), 0.92-1.44 (m, 20 H, alkyl-CH_2), 2.38-2.72 (m, 2 H, alkyl CH_2), 4.18 (s, 4 H, N-CH_2-Pyr), 7.34 (d, ^{3}J = 7.7 Hz, 2 H, pyr-CH), 7.39-7.52 (m, 2 H, pyr-CH), 7.86 (dt, ^{3}J = 1.6 Hz, 7.7 Hz, 2 H, pyr-CH), 9.14 (d, 2 H, ^{3}J = 4.8 Hz, pyr-CH). – **^{13}C-**

NMR (75 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, alkyl-CH_3), 22.7 (–, 1 C, alkyl-CH_2), 23.4 (–, 1 C, alkyl-CH_2), 27.2 (–, 1 C, alkyl-CH_2), 29.2 (–, 1 C, alkyl-CH_2), 29.3 (–, 1 C, alkyl-CH_2), 29.36 (–, 1 C, alkyl-CH_2), 29.4 (–, 1 C, alkyl-CH_2), 29.5 (–, 2 C, alkyl-CH_2), 31.9 (–, 1 C, alkyl-CH_2), 53.2 (–, 1 C, alkyl-CH_2), 57.1 (–, 2 C, N-CH_2-pyr), 123.3 (+, 2 C, pyr-CH), 124.5 (+, 2 C, pyr-CH), 139.9 (+, 2 C, pyr-CH), 149.5 (+, 2 C, pyr-CH), 153.9 (Cq, 2 C, pyr). –**IR** (ATR) [cm^{-1}]: $\tilde{\nu}$ [cm] = 2919, 2851, 1605, 1573, 1480, 1467, 1443, 1376, 1290, 1155, 1084, 1050, 962, 773, 759, 730. –**MS** (ESI(+),DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 490.2 (100) $[M^{2+} + CH_3COO^-]^{2+}$, 466.2 (12) $[M^{2+} + Cl^-]^{2+}$. – **HRMS** Calcd for $C_{24}H_{37}N_3ZnCl$ $[M^{2+} + Cl^-]^+$: 466.1967; Found: 466.1960. – **MF**: $C_{24}H_{37}N_3ZnCl_2$ – **FW**: 503.86 g/mol

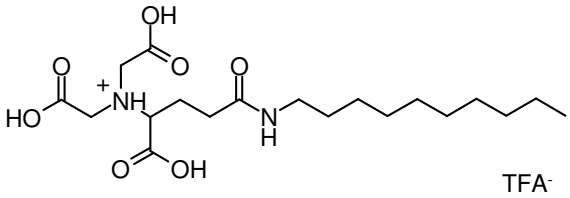


***2-(Bis-tert-butoxycarbonylmethyl-amino)-4-decylcarbamoyl-butyric acid tert-butyl ester* (35)**

Under N_2-atmosphere compound **33** (472 mg, 1.09 mmol), DIPEA (0.94 mL, 5.47 mmol), EDC (0.23 mL, 1.31 mmol), and HOBt (177 mg, 1.31 mmol) were dissolved in DMF (10 mL) under ice cooling. Decylamine **34** (0.26 mL, 1.31 mmol) dissolved in DMF was added slowly. The reaction was allowed to warm to r.t. and was stirred over night (20 h) at 40 °C. The reaction progress was monitored by TLC (EE). After completion the solution was mixed with water (25 mL) and extracted with citric acid (3x) and sat. NaCl (3x). The organic layer was dried over $MgSO_4$. The solvent was evaporated and the crude was purified using column chromatography on silica gel (EE/PE 1:1, R_f = 0.65) yielding **35** (365 mg, 0.64 mmol, 59 %) as a colourless solid after drying in vacuum.

^{1}H-NMR (600 MHz; $CDCl_3$): δ (ppm) = 0.80-0.90 (t, ^3J = 7.1 Hz, 3 H, HSQC: C^1H_3), 1.19-1.32 (m, 14 H, C^2H_2 – C^8H_2), 1.41-1.42 (s, 9 H, $C^{22}H_3$), 1.43-1.44 (s, 18 H, $C^{19}H_3$), 1.45-1.51 (m, 2 H, HMBC: C^9H_3), 1.73-1.83 (m, 1 H, HMBC: $C^{14}H_2$ diastereotop), 2.01-2.13 (m, 1 H, HMBC: $C^{14}H_2$ diastereotop), 2.32-2.40 (m, 1 H, HMBC: $C^{13}H_2$ diastereotop), 2.47-2.54 (m, 1 H, HMBC: $C^{13}H_2$ diastereotop), 3.09-3.17 (m, 1 H,

HMBC: $C^{10}H_2$ diastereotop), 3.18-3.26 (m, 2 H, HMBC: $C^{10}H_2$ diastereotop, $C^{15}H$), 3.32-3.47 (dd, ^4J = 17.1 Hz, ^4J = 41.9 Hz, 4 H, HMBC: $C^{16}H_2$), 6.75-6.83 (t, 1 H, ^3J = 5.3 Hz, HSQC: $N^{11}H$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ (ppm) = 14.1 (+, 1 C, HSQC: C^1), 22.6 (–, 1 C, HSQC: C^1), 26.6 (–, 1 C, HSQC: C^{14}), 27.0 (–, 1 C, HSQC: C^1), 28.07 (+, 6 C, HSQC: C^{19}), 28.15 (+, 3 C, HSQC: C^{22}), 29.2 (–, 1 C, HSQC: C^1), 29.3 (–, 1 C, HSQC: C^1), 29.52 (–, 1 C, HSQC: C^1), 29.55 (–, 1 C, HSQC: C^1), 29.6 (–, 1 C, HSQC: C^1), 31.8 (–, 1 C, HSQC: C^9), 32.7 (–, 1 C, HSQC: C^{13}), 39.5 (–, 1 C, HMBC: C^{10}), 54.5 (–, 2 C, HMBC: C^{16}), 64.8 (+, 1 C, HSQC: C^{15}), 80.9 (C_q, 2 C, HMBC: C^{18}), 81.4 (C_q, 1 C, HMBC: C^{21}), 170.8 (C_q, 2 C, HMBC: C^{17}), 171.8 (C_q, 1 C, HMBC: C^{20}), 173.0 (C_q, 2 C, HMBC: C^{17}). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3000, 2927, 2854, 1730, 1649, 1541, 1456, 1368, 1149. – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 571.6 (100) [MH^+], 515.5 (8) [MH^+ - C_4H_8], 593.6 (5) [MNa^+]. – **Elemental analysis** calcd. (%) for $C_{31}H_{58}N_2O_7$: C 65.23, H 10.24, N 4.91; found C 64.84, H 10.20, N 4.66. – **MF**: $C_{31}H_{58}N_2O_7$ –**FW**: 570.82 g/mol

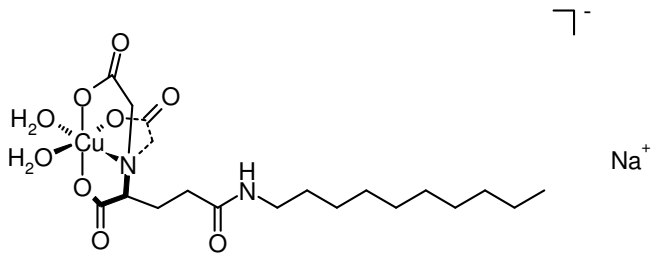


***2-(Bis-carboxymethyl-amino)-4-decylcarbamoyl-butyric acid triflate* (36)**

Compound **35** (107 mg, 0.19 mmol) was suspended in TFA (4 mL). The reaction mixture was stirred at room temperature for 20 h. Reaction progress was monitored by TLC (EE). TFA was evaporated in vacuo and the triflate salt of compound **36** was redissolved in water and was lyophilized giving product **36** (98 mg, 0.19 mmol, 100 %) as a white hygroscopic solid.

^{1}H-NMR (300 MHz; CD_3OD): δ (ppm) = 0.81-0.96 (t, ^3J = 6.7 Hz, 3 H, CH_3), 1.18-1.40 (m, 14 H, CH_2, 1.41-1.60 (m, 2 H, CH_2), 1.78-2.17 (m, 2 H, Glu-CH_2), 2.29-2.49 (t, ^3J = 7.0 Hz, 2 H, Glu-CH_2), 3.05-3.22 (t, ^3J = 7.0 Hz, 2 H, CH_2), 3.42-3.52 (m, 1 H, CH), 3.53-3.73 (m, 4 H, N-CH_2). – **^{13}C-NMR** (75 MHz; CD_3OD): δ (ppm) = 14.5 (+, 1 C, CH_3), 23.8 (–, 1 C, CH_2), 27.2 (–, 1 C, CH_2), 28.1 (–, 1 C, CH_2), 30.4 (–, 1 C, CH_2), 30.5 (–, 2 C, CH_2), 30.8 (–, 2 C, CH_2), 33.1 (–, 1 C, CH_2), 33.7 (–, 1 C, CH_2), 40.5 (–, 1 C, CH_2), 54.9 (–, 2 C, N–CH_2), 66.0 (+, 1 C, CH), 175.3 (C_q, 1 C, CONH), 175.5 (C_q, 1 C, CH*COOH*), 175.8 (C_q, 2 C, NCH_2*COOH*). – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 3340,

3028, 2921, 2852, 2547, 1725, 1643, 1538, 1427, 788, 715. – **MS** (ESI(+), H_2O/MeCN): m/z (%) = 401.2 (100) $[M - H^+]^-$, 457.2 (15) [starting material – 2 C_4H_8 + $H^+]^-$, 803 (6) $[2M-H^+]^-$. – **MF**: $C_{19}H_{34}N_2O_7 \cdot$ TFA – **FW**: 516.51 g/mol; without TFA: 402,49 g/mol



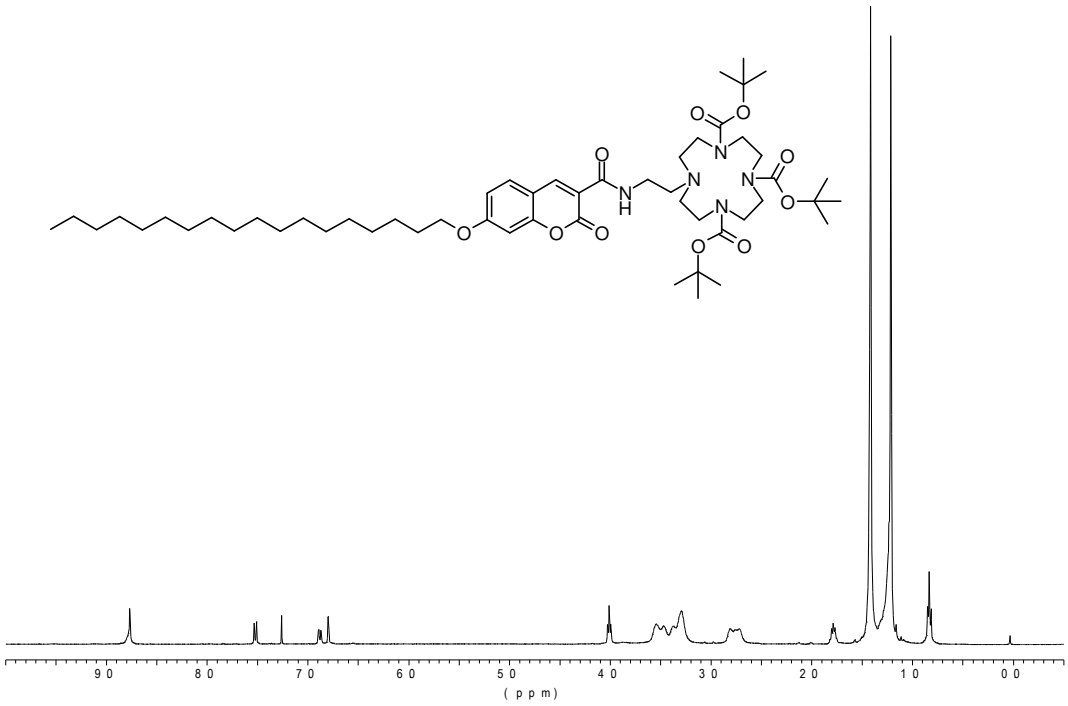
***Glu-NTA-2-(Bis-carboxymethyl-amino)-4-decylcarbamoyl-butyric acid* (6):**

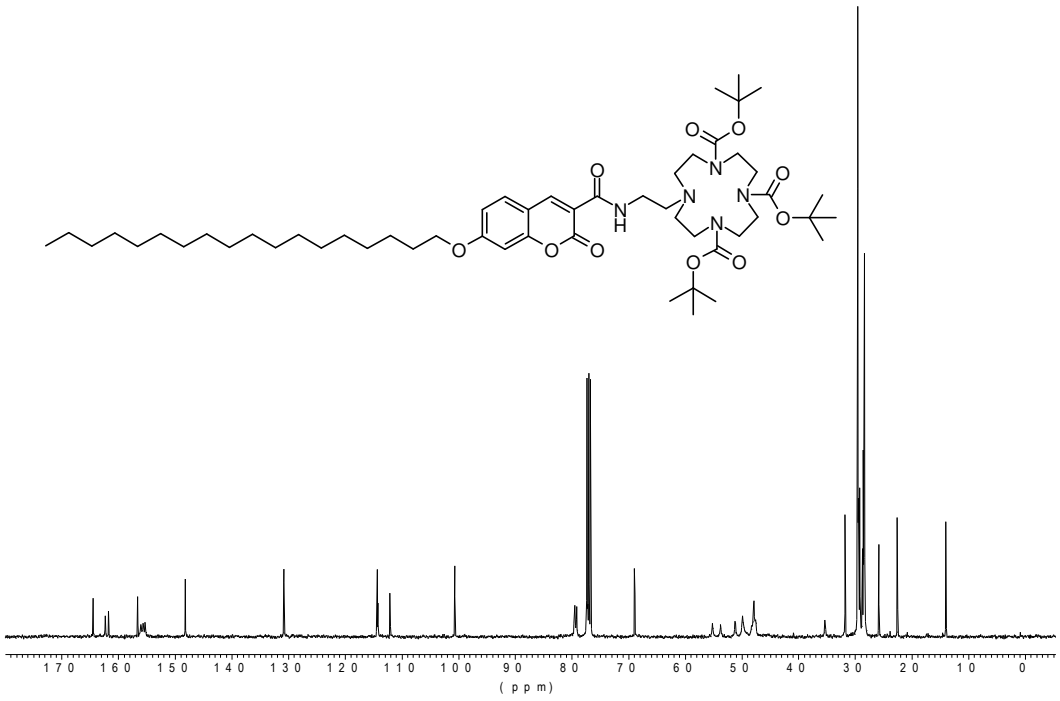
Compound **36** (40 mg, 0.08 mmol) and $Cu_2(OH)_2CO_3$ (8.6 mg, 0.04 mmol) were dissolved in methanol (6 mL). The mixture was stirred at room temperature over night, subsequently for 3 h at 60 h and was filtered immediately. The resulting blue solution was concentrated under reduced pressure, redissolved in water and lyophilized yielding **43** (35 g, 0.06 mmol, 71 %) without further purification as a blue solid.

MP: 173-175 °C. – **IR** (KBr) [cm^{-1}]: $\tilde{\nu}$ = 2925, 2854, 2362, 1724, 1448, 1378, 1240, 1112, 906. – **MS** (ESI(+), H_2O/MeCN): m/z (%) = 462.3 (100) $[A^-]$, 415.3 (35) $[A^- - H_2O - CH_3]$, 374.2 (15) $[A^- - CO_2]$. – **MF**: $C_{19}H_{31}N_2O_7CuH(H_2O)_2 \cdot$ TFA – **FW**: 614.07 g/mol; A^- (without TFA, H_2O, H): 463.01 g/mol

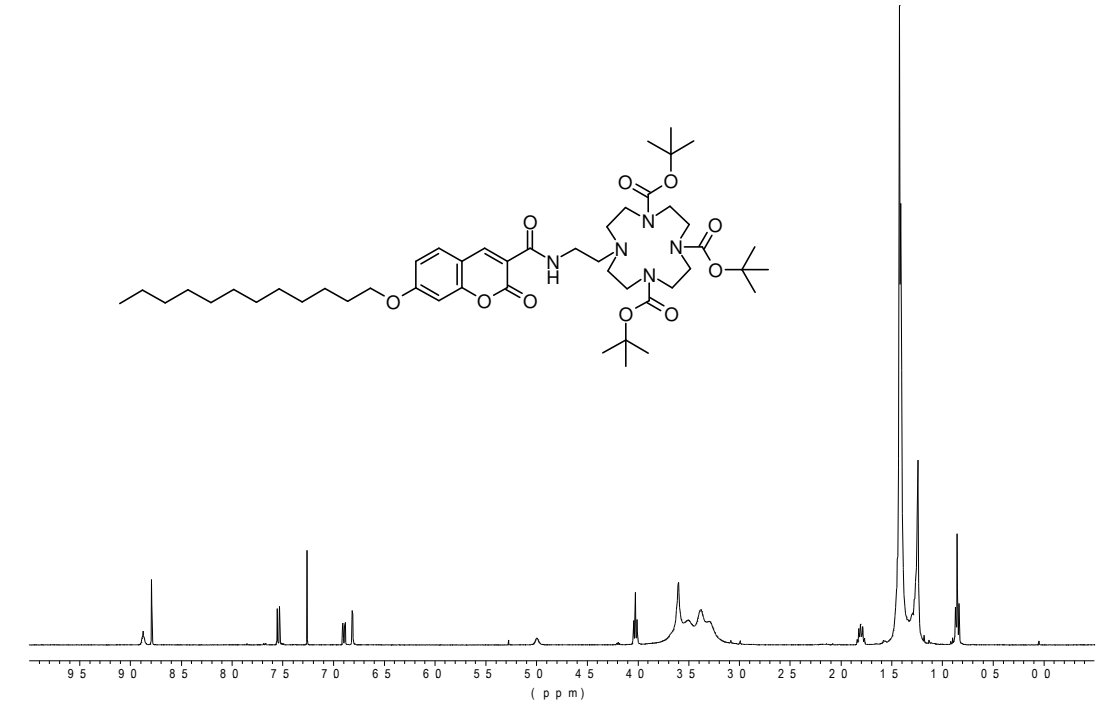
7.4.3 ^{1}H and ^{13}C spectra of synthesized compounds

Compound **20**





Compound **21**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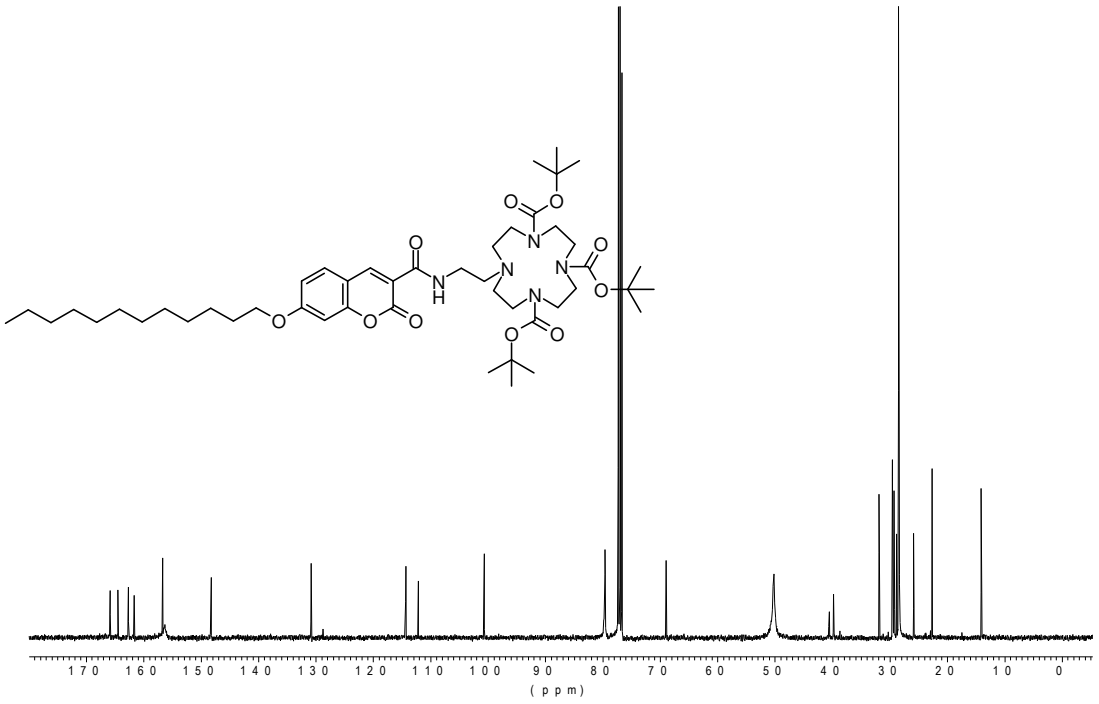
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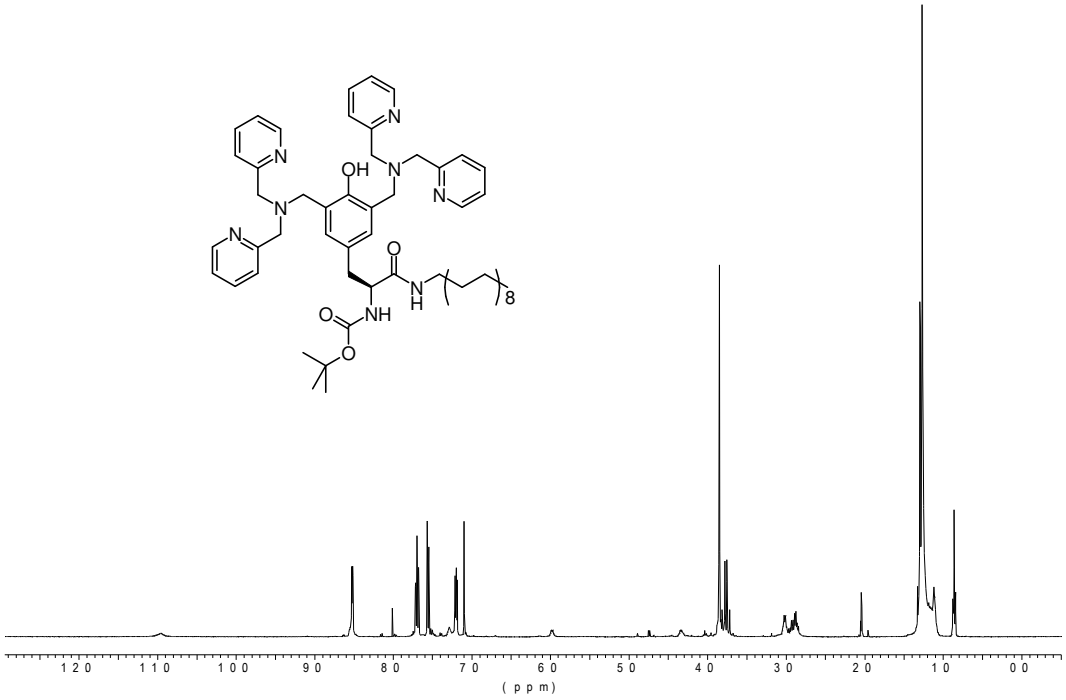
(ppm)

Compound **27**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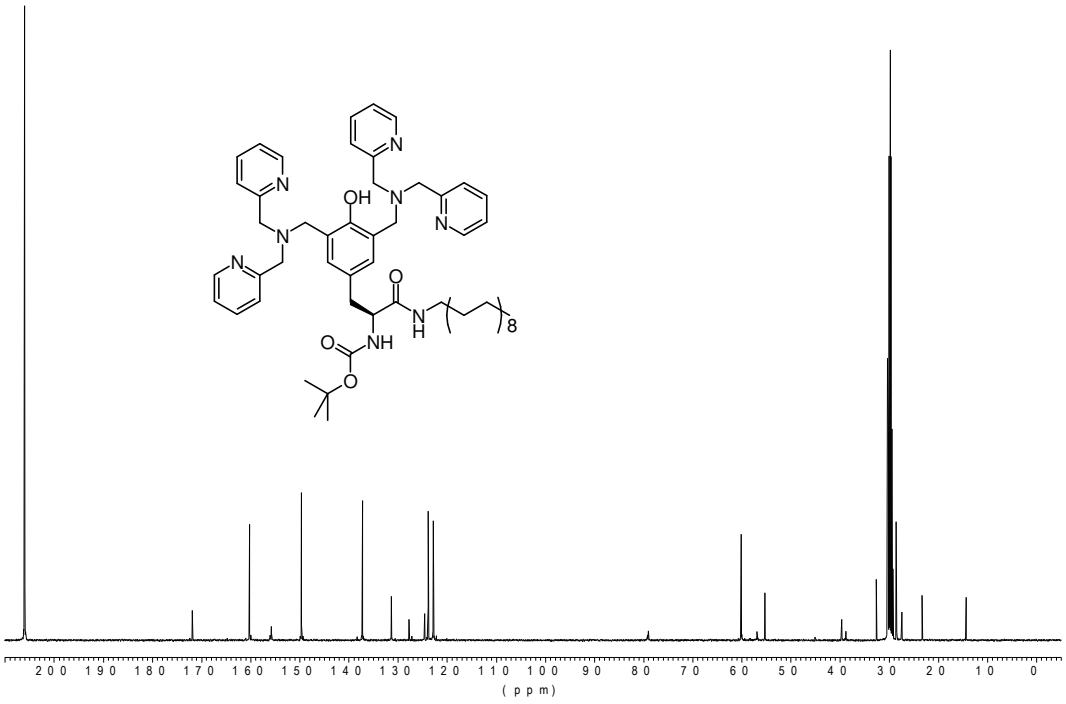
NH
O
O
8
120 110 100 90 80 70 60 50 40 30 20 10 00
(ppm)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O
NH
O
O
8
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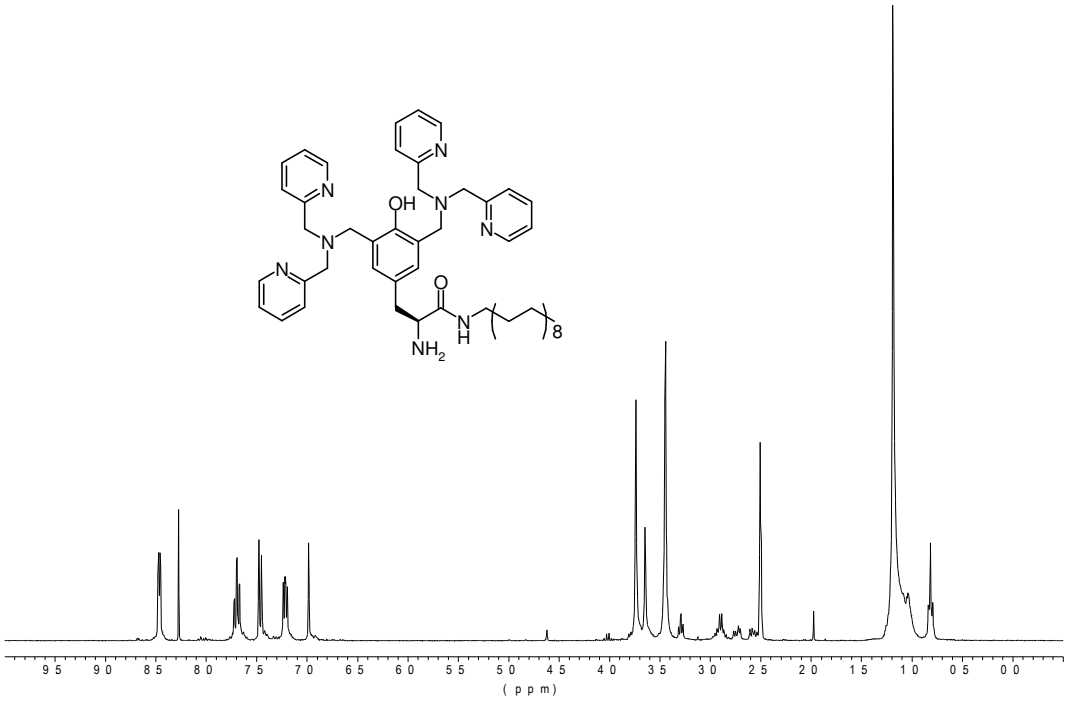
(ppm)

Compound **28**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O
NH2
8
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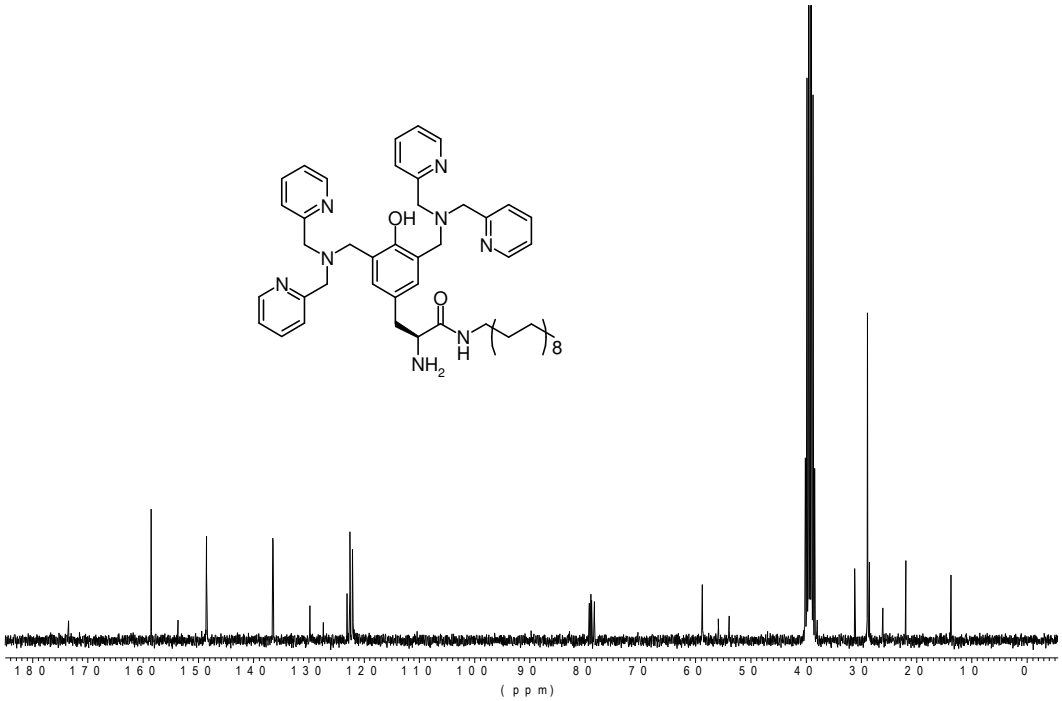
(ppm)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N
O
NH2
8
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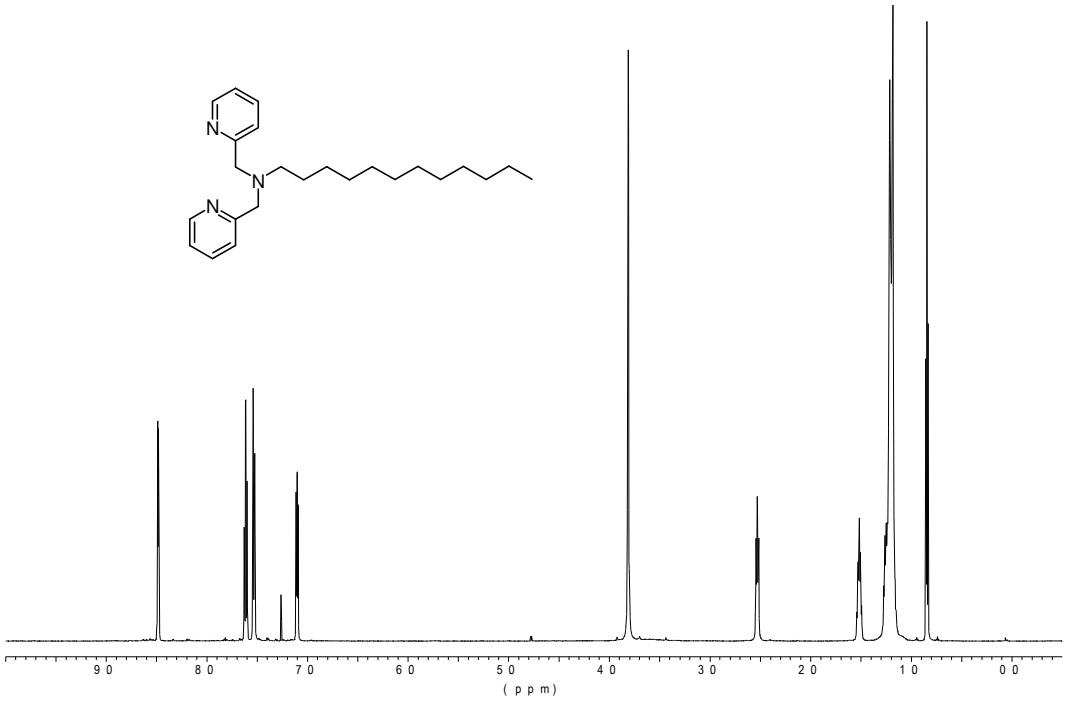
(ppm)

Compound **32**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N
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6 0
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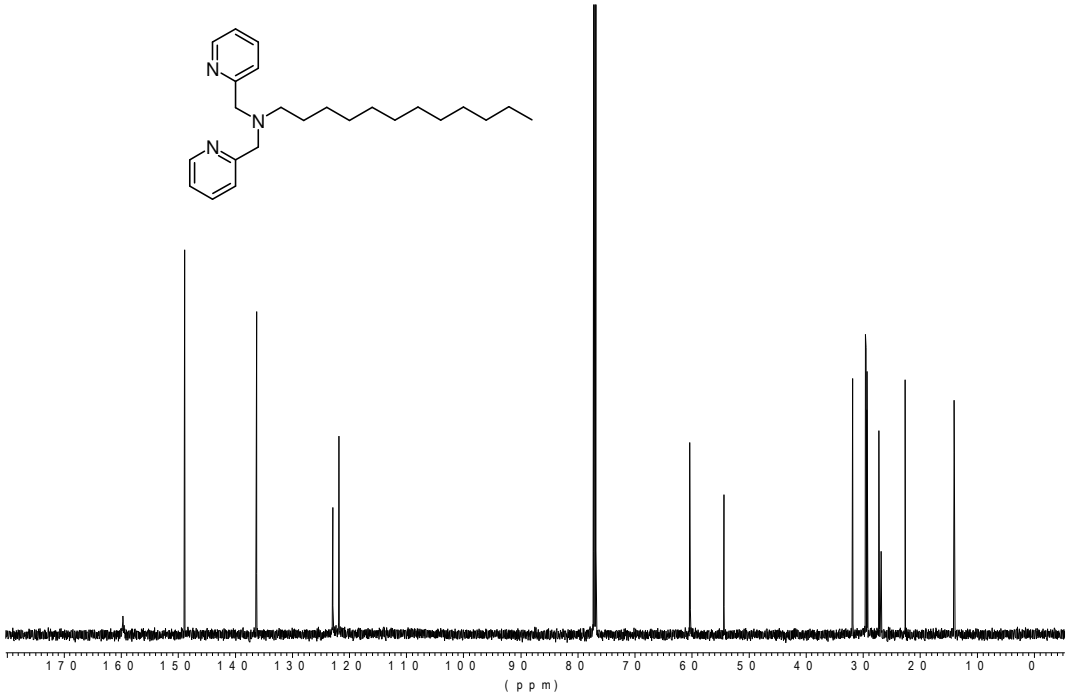
4 0
3 0
2 0
1 0
0 0
(p p m)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1 3 0
1 2 0
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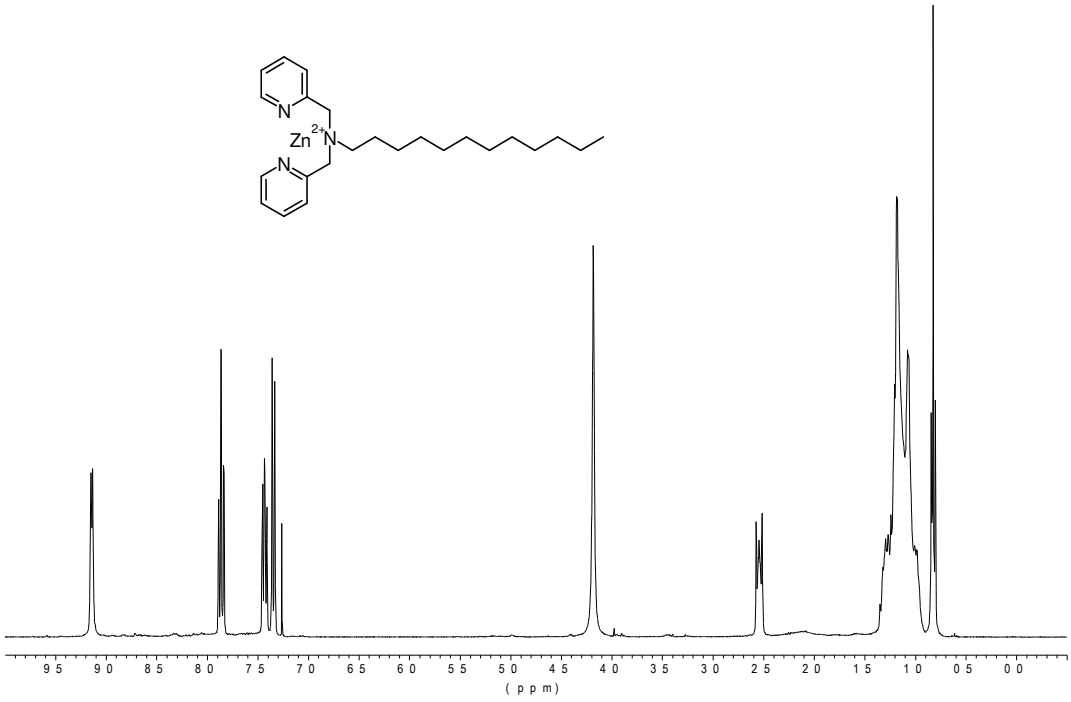
3 0
2 0
1 0
0
(p p m)

Compound **9**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Zn2+
N
N
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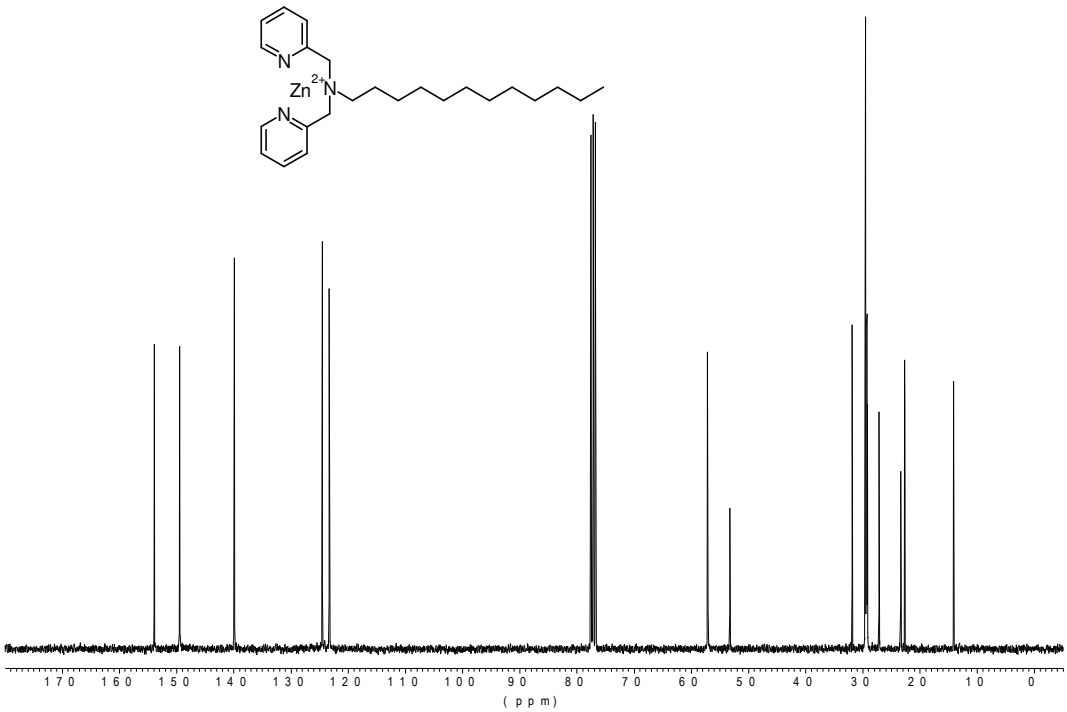
(p p m)


N
Zn2+
N
N
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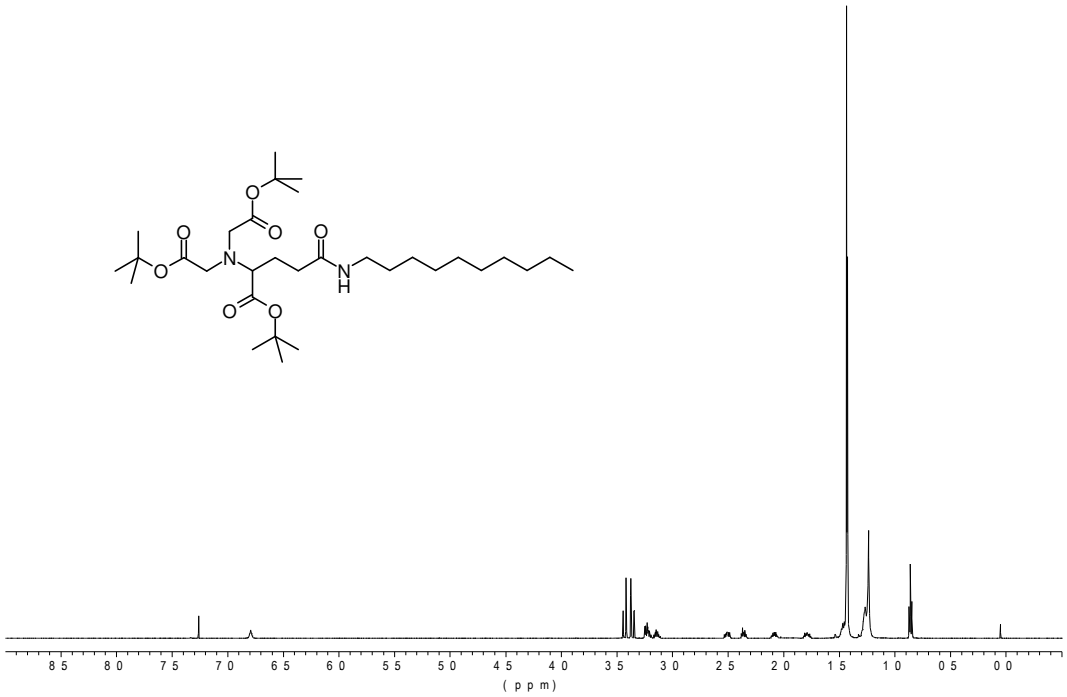
(p p m)

Compound **35**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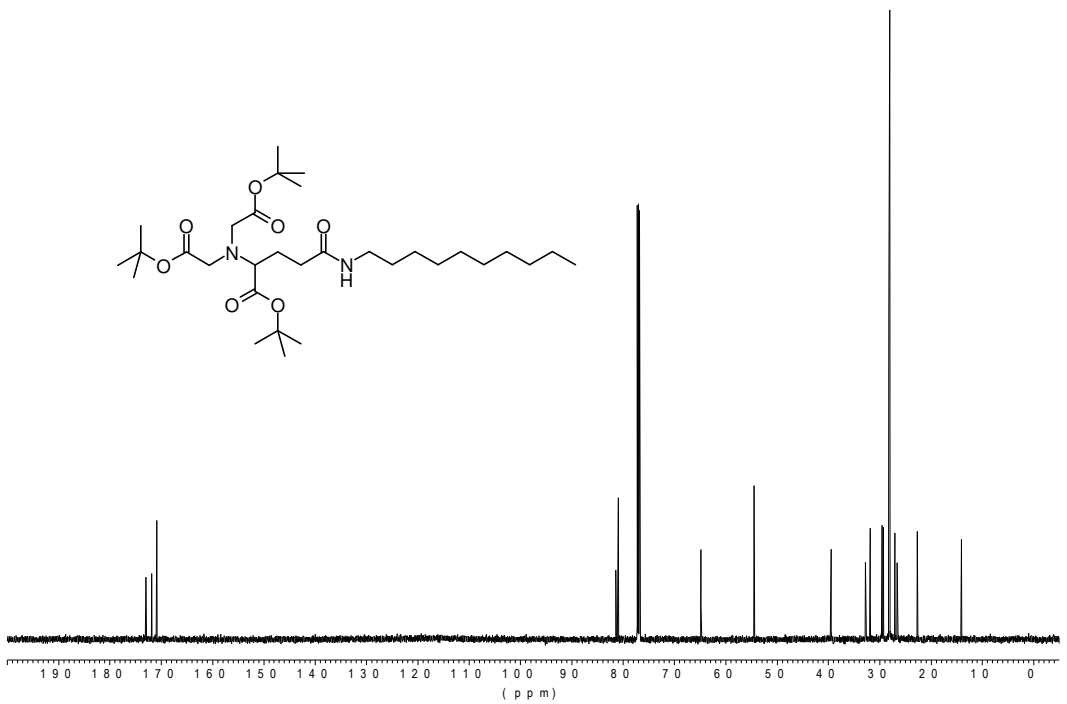
(p p m)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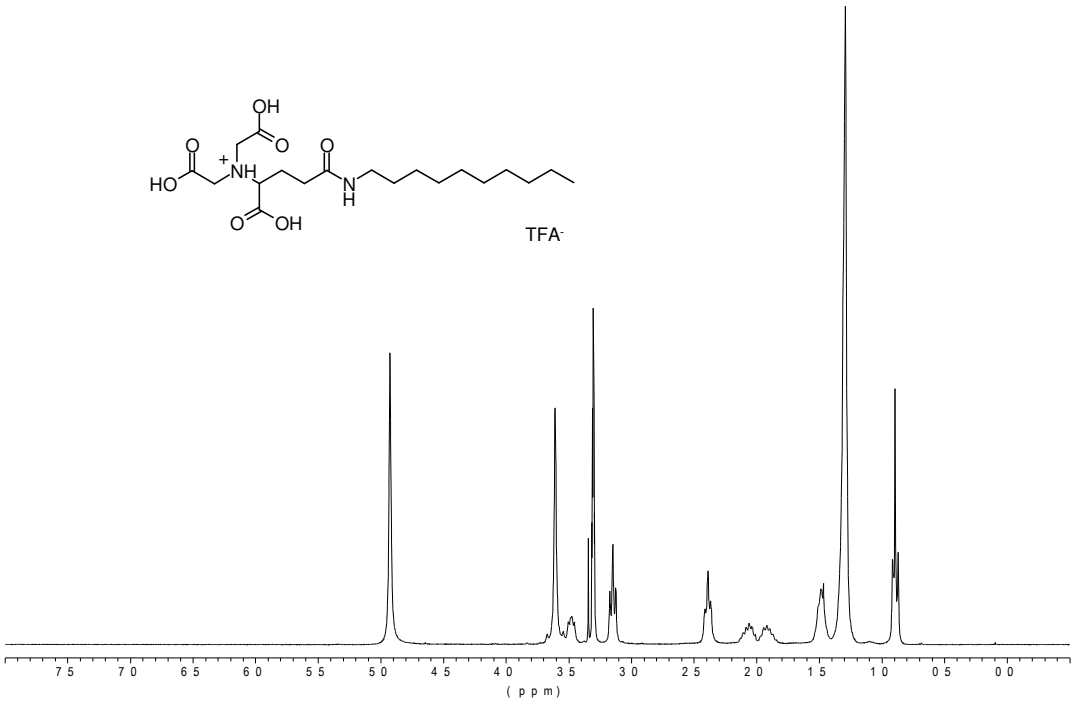
O
O
O
O
N
H
O
O
1 9 0 1 8 0 1 7 0 1 6 0 1 5 0 1 4 0 1 3 0 1 2 0 1 1 0 1 0 0 9 0 8 0 7 0 6 0 5 0 4 0 3 0 2 0 1 0 0
(p p m)

Compound **36**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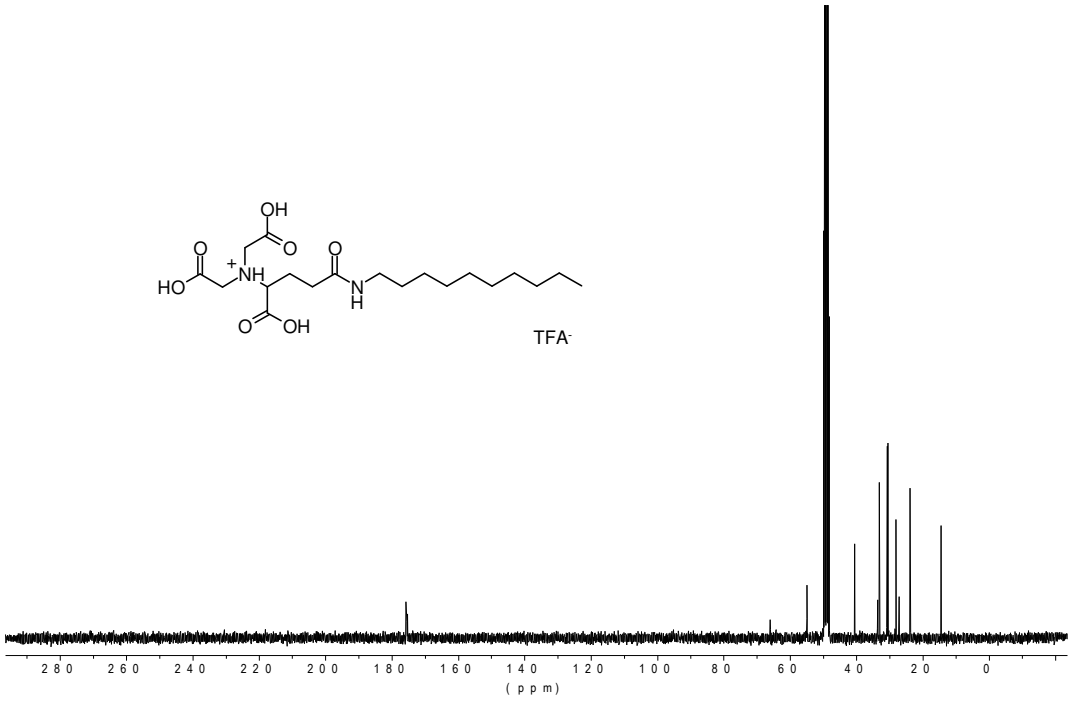
O
O
HO
+
NH
O
N
H
O
OH
TFA-
75 70 65 60 55 50 45 40 35 30 25 20 15 10 05 00
(p p m)


OH
O
O
HO
+
NH
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N
H
O
OH
TFA-
280 260 240 220 200 180 160 140 120 100 80 60 40 20 0
(p p m)

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8. Summary

Reversible coordinative binding of Lewis basic donors to iminodiacetato (IDA) and nitrilotriacetato (NTA) metal complexes is widely used for the design of synthetic receptors binding to peptides, proteins or enzymes at physiological conditions. However, no data on the affinity of M(II)-NTA (M = Cu, Ni, Zn) to a single histidine or imidazole moiety are available. We herein report (chapter 1) the investigation of the binding affinity and thermodynamics of copper(II), nickel(II) and zinc(II) NTA complexes to histidine, imidazole and hen egg white lysozyme, bearing a single surface exposed histidine unit, by isothermal titration calorimetry at physiological conditions. Further, we describe a peptide-metal complex hybrid approach to enhance the binding affinity of Cu(II)-NTA to lysozyme.

In the second part of this work (chapter 2) the rationally design of a selective peptide receptor is described. The combination of copper(II)nitrilotriacetato (NTA) complex with an ammonium-ion sensitive and luminescent benzocrown ether revealed a peptide receptor with a micromolar affinity and selectivity for glycine and histidine containing peptide sequences. This affinity closely resembles that of copper(II) ion peptide binding: The two free coordination sites of the copper(II) NTA complex bind to imidazole and amido nitrogen atoms, retracing the initial coordination steps of non-complexed copper(II) ions. The benzocrown ether recognizes intramolecularly the N-terminal amino moiety and the significantly increased emission intensity signals the binding event, as only if prior coordination of the peptide has taken place, the intramolecular ammonium ion – benzocrown ether interaction is of sufficient strength in water to trigger an emission signal. Intermolecular ammonium ion – benzocrown ether binding is not observed. Isothermal titration calorimetry confirmed the binding constants derived from emission titrations. Thus, as deduced from peptide coordination studies, the combination of a truncated copper(II) coordination sphere and a luminescent benzocrown ether allows for the more rational design of sequence selective peptide receptor.

Suitable combinations of an affinity tag and an artificial probe are useful for non-covalent protein labelling. Several of such peptide tag – probe pairs have been developed and reported in the literature. The most prominent example is the His-tag – Ni(II)-NTA (nitrilotriacetic acid) pair. Recently, the Hamachi group reported a genetically encodable oligo-aspartate sequence (D4-tag) and a corresponding oligonuclear Zn(II) dipicolylamine (Zn(II)-Dpa) complex as new peptide tag – probe pair, which is orthogonal to the His-tag – Ni(II)-NTA pair. We describe in the third part of this work (chapter 3) the preparation of fluorescent 1,4,7,10-tetraazacyclododecane (cyclen) Zn(II) complexes and their application as an alternative artificial probe for the D4-tag system. The binding affinities of the new complexes to the affinity tags were investigated by emission and UV-vis titration. Tetranuclear Zn(II)-cyclen complexes respond to the presence of oligo-aspartate, oligo-glutamate and oligo-aspartate dimers in aqueous solution at micromolar concentrations by a strong spectroscopic change. Based on the high binding affinities due to strong electrostatic interactions and Job´s plot analysis, we propose the formation of receptor–peptide tag aggregates. The results clearly show the potential of Zn(II)-cyclen complexes for applications as non-covalent protein markers, although their optical properties require further optimization for practical use.

The fourth part of this work (chapter 4) deals with the syntheses of new amphiphilic 1,4,7,10-tetraazacyclododecane Zn(II) complexes for a template guided cooperative self-assembly of nucleotides at interfaces fabricated by combination of self-assembly monolayer technique (SAM) and Langmuir Blodgett technique (LB). Three amphiphilic Zn(II)-cyclen complexes were synthesized as binding sites at interfaces prepared by a combination of SAM and LB film approaches or in vesicles. Detailed investigations of the binding properties of surfaces incorporating the new amphiphilic complexes are in progress.

The fifth part of this work (chapter 5) deals with the preparation of self assembled vesicular polydiacetylene (PDA) particles with embedded metal complex receptor sites. The particles respond to the presence of ATP and PPi (pyrophosphate) in buffered aqueous solution by visible changes of their color and emission properties. Blue PDA vesicles of uniform size were obtained upon UV irradiation from mono- and dinuclear zinc(II)-cyclen and iminodiacetato copper [Cu(II)-IDA] modified diacetylenes,

embedded in amphiphilic diacetylene monomers. Addition of ATP and PPi to the PDA vesicle solution induces a color change from blue to red observable by the naked eye. The binding of ATP and PPi changes the emission intensity. Other anions like ADP, AMP, $H_2PO_4^-$, CH_3COO^-, F^-, Cl^-, Br^- and I^- failed to induce any spectral changes. The zinc(II)-cyclen nanoparticles are useful for the facile detection of PPi and ATP in millimolar concentrations in neutral aqueous solutions, while Cu(II)-IDA modified vesicular PDA receptors are able to selectively discriminate between ATP and PPi.

In the sixth chapter (chapter 6) we report a new methodology for the preparation of a artificial phosphate receptors. Phosphate anion probes typically consist of a binding site and a luminescent reporter group. The luminescent moiety is either part of the chemosensor in close proximity of the analyte binding site or in indicator displacement assays non-covalently bound to the binding site and displaced by the analyte. We report here the preparation and binding properties of 80 nm vesicular synthetic receptors, which contain amphiphilic 1,4,7,10-tetraazacyclododecane (cyclen) Zn(II) complexes as phosphate anion binding sites and amphiphilic coumarin derivatives as fluorescent reporter groups. By colocalization of binding sites and reporter groups in the vesicle they respond to the presence of phosphate anions in aqueous solution at micromolar concentrations by a strong emission decrease. The technique avoids the covalent synthesis of labelled analyte binding sites and allows the rapid and versatile preparation of luminescent nanometer size synthetic receptors.

In the last part of this work (chapter 7) the syntheses of new non-fluorescent and fluorescent amphiphilic Lewis acidic metal complexes (metal chelating artificial lipids) based on 1,4,7,10-tetraazacyclododecane Zn(II) complexes, dipicolylamine (Dpa) complexes and a nitrilotriacetato acid (NTA) complex are described. The prepared metal chelating artificial lipids will be used for fabrication of complex self-assembled supramolecular surfaces by one or more different chemosensors for molecular recognition at interfaces.

9. Zusammenfassung

Die reversible koordinative Bindung von Lewis basischen Donoren an Iminodiacetato (IDA) und Nitrilotriacetato (NTA) Metallkomplexe findet eine breite Anwendung für die Entwicklung von synthetischen Rezeptoren für die Bindung von Peptiden, Proteinen oder Enzymen unter physiologischen Bedingungen. Dennoch sind Daten für den Bindungsprozess von M(II)-NTA (M = Cu, Ni, Zn) an einfaches Histidin und Imidazol in der Literatur nicht verfügbar. Deshalb konzentriert sich das erste Kapitel auf die Untersuchung der Bindungsaffinität und der Thermodynamik von Kupfer(II), Nickel(II) und Zink(II) NTA Komplexen an Histidin, Imidazol und Hühnereiweiß Lysozym, das ein oberflächenexponiertes Histidin trägt, mittels Isothermischer Titrationskalorimetrie (ITC) unter physologischen Bedingungen. In einem weiteren Ansatz wurde versucht, die Bindungsaffinität von Cu(II)-NTA an Lysozym durch einen Peptid – Metall Komplex Hybridrezeptor zu steigern.

Im zweiten Kapitel dieser Arbeit wird die rationale Entwicklung eines selektiven Peptidrezeptors beschrieben. Die Kombination aus einem Kupfer(II) nitrilotriacetato (NTA) Komplex und einem Ammoniumionen sensitiven und lumineszenten Benzokronenether, zeigte einen Peptidrezeptor mit mikromolarer Affinität und Selektivität für Peptidsequenzen, die Glycin und Histidin enthalten. Der Bindungsprozess verläuft ähnlich dem bekannter Kupfer(II) Ionen bindender Peptidsequenzen: Zwei der verbleibenden freien Koordinationsplätze am Kupfer(II) NTA Komplex werden durch Imidazol und den Amid Stickstoff besetzt. Dies entspricht in analoger Weise der schrittweisen Koordination freier Kupfer(II) Ionen durch die Peptidesequenz. Der Benzokronenether ist nun in der Lage intramolekular die verbleibende N-terminale Amingruppe des Peptides zu erkennen und der Bindungsvorgang wird durch einen signifikanten Anstieg der Fluoreszenzemission angezeigt. Dies ist jedoch nur möglich, wenn eine Vorkoordination des Peptides an den Metall Komplex erfolgt. Nur unter diesen Bedingungen ist die intramolekulare Ammoniumion – Benzokronenether Wechselwirkung von ausreichender Stärke um unter physiologischen Bedingungen eine Emissionsänderung hervorzurufen. Eine intermolekulare Ammonium Ion – Benzokronenether Wechselwirkung wurde nicht beobachtet. Mittels isothermischer Titrationskalorimetrie (ITC) konnten die Bindungskonstanten, die durch Emissionstitrationen erhalten wurden bestätigt werden. Abgeleitet von Peptid Koordinationsstudien an den Peptidrezeptor, kann gefolgert

werden, dass die Kombination aus einer eingeschränkten Koordinationssphäre am Kupfer(II) Ion und einem lumineszenten Benzokronenether eine weitaus rationelleren Entwicklungsansatz von sequenzselektiven Peptidrezeptoren ermöglicht.

Nichtkovalente Proteinmarkierung durch die Wahl einer geeigneten Kombination aus einem Affinitäts-Tag und einem kleinen artifiziellen Molekül ist äußerst nützlich. Verschiedene derartiger Kombination wurden entwickelt und sind literaturbekannt. Das bekannteste Beispiel hierfür ist die Kombination aus einem His-Tag und einem Ni(II)-NTA Komplex. Hamachi *et al* entwickelten kürzlich eine neue Kombination bestehend aus einer genetisch kodierbaren oligo-Aspartat Sequenz (D4-tag) und einem zweikernigen Zn(II) Dipycolylamin (Zn(II)-Dpa) Komplex, der orthogonal zu oben genanntem System ist. Im dritten Kapitel dieser Arbeit berichten wir die Synthesen von fluoreszenten 1,4,7,10-tetraazacyclododecane (Cyclen) Zn(II) Komplexen und deren Anwendung als alternative artifizielle Moleküle für das D4-System. Die Bindungsaffinitäten an die Affintäts-Tags wurde mittels UV-vis – und Emissions – Titration bestimmt. Nur die vierkernigen Zn(II)-Cyclen Komplexe zeigten eine starke spektroskopische Veränderung ihrer Banden in Anwesenheit von oligo-Aspartat, oligo-Glutamat und einem oligo-Aspartat Dimer bei mikromolarer Konzentration in wässriger Lösung. Basierend auf der hohen Bindungsaffinität, hervorgerufen durch starke elektrostatische Wechselwirkungen, und der Stöchiometrie des Bindungsprozesses ermittelt durch Job´s plot, wird eine Bildung von Peptid-Tag – Rezeptor Aggregaten angenommen. Die Ergebnisse zeigen deutlich das Potential der untersuchten Zn(II)-Cyclen Komplexe für Anwendungen im Bereich zur nichtkovalenten Proteinmarkierung, wenngleich deren optische Eigenschaften für praktische Anwendungen noch Verbesserungsbedarf benötigen.

Das vierte Kapitel dieser Dissertation bezieht sich auf die Synthese neuer amphiphiler 1,4,7,10-tetraazacyclododecan Zn(II) Komplexe zur Templat gesteuerten kooperativen Selbstorganisation von Nucleotiden an Grenzflächen, die mittels einer Kombination aus selbstorganisierten Monoschichten (SAM) und der Langmuir Blodgett Technik (LB) erzeugt werden. Drei amphiphile Zn(II)-Cyclen Komplexe wurden für die Verwendung als Bindungsstellen in Grenzflächen synthetisiert. Genauere Untersuchungen der Bindungseigenschaften von Oberflächen modifiziert mit den hier dargestellten neuen amphiphilen Metall Komplexen sind aktuell noch in Bearbeitung.

Der fünfte Teil dieser Arbeit (Kapitel 5) beschäftigt sich mit der Herstellung von selbstorganisierten vesikulären Polydiacetylen (PDA) Vesikel, in die Lewis saure Metall Komplex Einheiten mit eingebaut wurden. In gepufferter wässriger Lösung zeigen diese Vesikel in Anwesenheit von ATP oder PPi eine erkennbare Farbveränderung und eine Veränderung ihrer Emissionseigenschaften. Die Herstellung von einheitlich großen, blauen PDA Vesikeln erfolgte durch UV Bestrahlung von mono- und dinuklearen Zn(II)-Cyclen und Cu(II)-IDA (iminodiacetato) modifizierten Diacetylenen, welche mit amphiphilen Diacetylen Monomeren gemischt wurden. Die Zugabe von ATP und PPi zu einer solch polymerisierten PDA Vesikel Lösung rief einen Farbwechsel von blau nach rot hervor, der selbst mit bloßem Auge erkennbar war. Nur ATP und PPi erzeugten eine Veränderung der Emissionsintensität. Weitere Anionen wie ADP, AMP, $H_2PO_4^-$, CH_3COO^-, F^-, Cl^-, Br^- and I^- hatten keinen Einfluss auf die Emissionseigenschaften der Partikel. Die Nützlichkeit der dargestellten Zn(II)-Cylen Nanopartikel kann durch eine einfachen Nachweis von PPi und ATP in millimolarer Konzentration in neutraler wässriger Lösung gezeigt werden. Cu(II)-IDA modifizierte vesikuläre PDA Rezeptoren hingegen ermöglichen eine selektive Unterscheidung zwischen ATP und PPi.

Im sechsten Kapitel wird eine neue Methodik zur Herstellung artifizieller Phosphatanionen Rezeptoren vorgestellt. Solche Rezeptoren bestehen üblicherweise aus einer Bindungsstelle und einem lumineszenten Marker. Die lumineszente Einheit kann entweder Bestandteil des Chemosensor sein und befindet sich in kurzer Distanz zur Analyt Bindungsstelle oder wird in einem Indikator Verdrängungs-Assay (IDA) an die Bindungsstelle gebunden und durch den Analyt verdrängt. In diesem Abschnitt berichten wir die Herstellung und die Bindungseigenschaften von 80 nm großen vesikulären synthetischen Rezeptoren, die amphiphilie 1,4,7,10-tetraazacyclododecane (Cyclen) Zn(II) Komplexe als Phosphate Bindungsstellen und amphiphile Coumarin Derivate als fluoreszente Marker beinhalten. Eine Kolokalisierung der Bindungsstellen und der fluoreszenten Marker im Vesikel erlaubt eine Phosphatanionenerkennung in wässriger Lösung im Bereich mikromolarer Konzentrationen durch eine starke Emissionsabnahme. Diese Technik vermeidet die aufwendige Synthese von Bindungsstellen, die kovalent mit fluoreszenten Chromophoren markiert werden und ermöglicht eine schnelle und vielseitig Herstellung von lumineszenten synthetischen Rezeptoren im Nanometerbereich.

Im letzten Kapitel dieser Arbeit wird die Synthese neuer nicht-fluoreszenter und fluoreszenter amphiphiler Lewis azider Metall Komplexe (Metall chelatisierende artifizielle Lipide) basierend auf 1,4,7,10-tetraazacyclododecane (Cyclen) Zn(II) Komplexen, dipicolylamine (Dpa) Metall Komplexen und Nitrilotriacetato (NTA) Metall Komplex vorgestellt. Die dargestellten artifiziellen Metall chelatisierenden Lipide werden für die Herstellung von komplexen selbstorganisierten supramolekularen Oberflächen bestehend aus einheitlichen oder mehreren verschiedenartigen Chemosensoren zur molekularen Erkennung an Grenzflächen verwendet.

10. Abbreviations

Asp (D)	Aspartic acid
Ar	Aryl
Aq	aqueous
ATP	Adenosine triphosphate
Boc	*tert*-Butyloxycarbonyl
c	Concentration
Calcd	Calculated
Cbz	Benzyloxycarbonyl
CI	Chemical Ionisation
cAMP	Cyclic adenosine monophosphate
COSY	Correlated spectroscopy
d	Day(s)
DAD	Diode array detector
DCC	Dicyclohexyl-carbodiimide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EDC	*N*-(3-Dimethylamino-propyl)-*N*'-ethylcarbo-diimide
EI-MS ionization	Electron-impact mass spectrometry
ES-MS	Electrospray ionization mass spectrometry
EE/EtOAc	Ethylacetate
eq	Equivalents
Et	Ethyl
EtOH	Ethanol
FAB	Fast-Atom-Bombardment
h	hours
Glu (E)	Glutamic acid
GTP	Guanosine-5'-triphosphate
HATU	
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
HEPES	*N*-2-Hydroxy-ethylpiperazine-*N*'-2-ethansulfonic acid
His	Histidine
HOBt	Hydroxybenzotriazole
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
ITC	isothermal titration calorimetry
IDA	Iminodiacetic acid
IR	Infrared

J	Coupling Constant
LB	Langmuir Blodgett
Me	Methyl
MF	Molecular formula
MeCN	Acetonitrile
MeOH	Methanol
min	minutes
MP	Melting point
MW	Molecular weight
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser enhancement spectroscopy
NTA	Nitrilotriacetic acid
o/n	over night
PE	Petrol Ether (Hexanes)
Ph	Phenyl
PPi	Pyrophosphate
R_f	Retention factor
ROESY	Rotating frame NOE spectroscopy
RT	Room temperature
SAM	self assembled monolayer
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TRIS	Tris(hydroxymethyl)-aminomethane
UV	Ultraviolett
Vis	Visible
x	Mole Fraction

11. Appendix

11.1 Publications

S. Stadlbauer, B. König
"Investigation on the Binding Affinity of Nitrilotiracetato (NTA) Metal Complexes to Histidine, Imidazole and Lysozyme by Isothermal Titration Calorimetry", *Inorg. Chim. Acta* **2009**, *submitted*.

S. Stadlbauer, M. Bhuyan, A. Grauer, F. Schmidt, H. Nonaka, S. Uchinomiya, A. Ojida, I. Hamachi, B. König
"Fluorescent 1,4,7,10-Tetraazacyclododecane Zn(II) Complexes with high Affinity to D4 and E4 Peptide Sequences", *Chem. Asian. J.* **2009**, *in preparation*.

S. Stadlbauer, B. Gruber, K. Woinaroschy, B. König
"Vesicular Receptors with co-embedded Amphiphilic Zinc Cyclen Complexes and Fluorophors for Phosphate Anion Sensing in Water", *Inorg. Chem.* **2009**, *in preparation*.

A. J. Devadoss, S. Stadlbauer, B. König
"Polydiacetylene based colorimetric self assembled vesicular receptors for biological phosphate ion recognition", *Chem. Eur. J.* **2009**, *manuscript in print.*

A. Riechers, F. Schmidt, S. Stadlbauer, B. König
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S. Miltschitzky, V. Michlova, S. Stadlbauer, B. König
"Synthesis of substituted pyrimidine hydrazine acids (PHA) and their use in peptide recognition", *Heterocycles* **2006**, *67*, 135-160.

11.2 Patent

A. Riechers, F. Schmidt, S. Stadlbauer, B. König, **2009**, EPC 09155910.4 - 2117

11.3 Poster- & Oralpresentations

S. Stadlbauer, A. Riechers, A. Späth, B. König
Poster: *"Utilizing Reversible Copper(II) Peptide Coordination in a Sequence-Selective Luminescent Receptor"*
Vth International Symposium, Supramolecular Systems in Chemistry and Biology 2009, Kiev, Ukraine

S. Stadlbauer, B. König, I. Hamachi
Poster: *"Fluorescent 1,4,7,10-Tetraazacyclododecane Zn(II)-Complexes as Potential Non-Covalent Protein Markers"*
JSPS summer program 2008, The Graduate University of Advanced Science SOKENDAI Shanon Village, Japan

S. Stadlbauer, A. Riechers, A. Späth, B. König
Poster: *"Utilizing Reversible Copper(II) Peptide Coordination in a Sequence-Selective Luminescent Receptor"*
Frontiers in Medicinal Chemistry 2008, Regensburg, Germany

S. Stadlbauer, B. König
Poster: *"Selective binding to Hen Egg White Lysozyme by a nitrilotriacetato (NTA) metal complex linked to a small amino acid epitope of monoclonal antibody Hy HEL 10 FV"*
GdCH Jahrestagung 2007, Ulm, Germany

S. Stadlbauer, B. König

Poster: *"Selective binding to Hen Egg White Lysozyme by a nitrilotriacetato (NTA) metal complex linked to a small amino acid epitope of monoclonal antibody Hy HEL 10 FV"*

1st European Chemistry Congress 2006, Budapest, Hungary

S. Stadlbauer

Talk: *"Peptide recognition in aqueous solution"*

Christmas Colloquium 2006 of the Department of Organic Chemistry, University of Regensburg, Germany

11.4 Curriculum vitae

Stefan Peter Stadlbauer

*16.11.1979, Cham i. d. Opf.

Education

02/2006-06/2009	Dissertation: "*Coordination Chemistry in Molecular Recognition*" University of Regensburg
04/2005-01/2006	Diploma thesis: *"Selective binding to Hen Egg White Lysozyme by a nitrilotriacetato (NTA) metal complex linked to a small amino acid epitope of monoclonal antibody Hy HEL 10 FV"*, University of Regensburg
10/2000-04/2005	Studies of Chemistry, University of Regensburg
07/1999-07/2000	Civilian Service at the Fachklinik Furth im Wald für Abhängigkeitserkrankungen und Psychosomatik
09/1990-07/1999	Abitur (A-level) at the Joseph-von-Fraunhofer Gymnasium Cham i. d. Opf.

Research Experience

02/2006-current	Graduate student (PhD), Institute of Organic Chemistry, University of Regensburg (advisor: Professor Burkhard König)
06/2008-08/2008	Research collaboration with Prof. Dr. Itaru Hamachi, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University /Japan

Teaching Experience

2005 –	Teaching assistant in laboratory courses for chemistry, biology and biochemistry students, supervisor for bachelor, master and diploma students

Fellowships

06/2007-08/2008	Research fellowship of the Japanese Society for Promotion of Science (JSPS)
09/2006	Travel grant of the Karl-Ziegler Stiftung for the 1st EuCheMS Congress, Budapest, Hungary