# Cu(I)-Catalyzed „Click-Chemistry“

## Design of a Chemical Photomultiplier

## Target-Guided Synthesis of Bidentate Metal-Complex Receptors

**Dissertation**

Zur Erlangung des Doktorgrades der Naturwissenschaften
(Dr. rer. nat.)
an der Fakultät für Chemie und Pharmazie
der Universität Regensburg


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**Stefan Ritter**

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The experimental part of this work was carried out between January 2004 and December 2006 at the Institute for Organic Chemistry at the University of Regensburg under the supervision of Prof. Dr. B. König.


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*Man braucht im Leben nichts zu fürchten,*

*man muss nur alles verstehen.*

Marie Curie

*FÜR ANNE,*

*MEINE FAMILIE*

*UND TOBIAS*

# Table of Contents

# 1. Amplification and Transduction of a Light Signal by Regulated Photo-Activated Catalysis[a]

## 1.1. Introduction

### 1.1.1. Photoinitiated Reactions

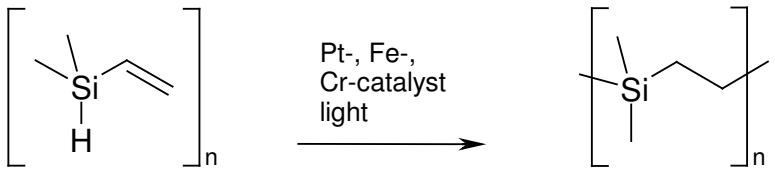
The most well-known example of photoinitiated reactions in nature is the neuronal response triggered by a light signal in retinal cells. The photoreceptor Rhodopsin is composed of the 40-kDa apoprotein opsin and its chromophore, 11-*cis*-retinal, a derivative of vitamin A that acts as an inverse agonist in the rhodopsin ground state. Photon absorption by rhodopsin isomerizes the 11-*cis* chromophore to all-*trans*, which triggers a series of conformational changes in opsin and leads to an active state of rhodopsin.[1] This means that retinal is the allosteric group of rhodopsin as its conformational change activates rhodopsin without taking place at the active site.[2] The active state of rhodopsin is responsible for binding the heterotrimeric G-protein of the rod cell, transducin (Gt), and for the catalysis of the uptake of guanosine triphosphate by the α-subunit of Gt. Thereby it initiates the enzymatic cascade that leads to vision.[3,4]

This signal amplification by allosteric catalysis (SAAC) is also achieved by several artificial systems to mimic biological signal transduction processes, in which a molecular recognition event of an external signal or analyte to a receptor is transduced into catalytically amplified chemical information. Using engineered enzymes,[5] ribozymes[6] or adjustable organic catalysis[7,8] this concept was applied by several groups for analytical purposes.[9] Artificial SAAC systems reported so far converted external chemical signals into amplified chemical output. This mimics the signal transduction pathway, for example of a membrane bound receptor with subsequent amplification of the response.

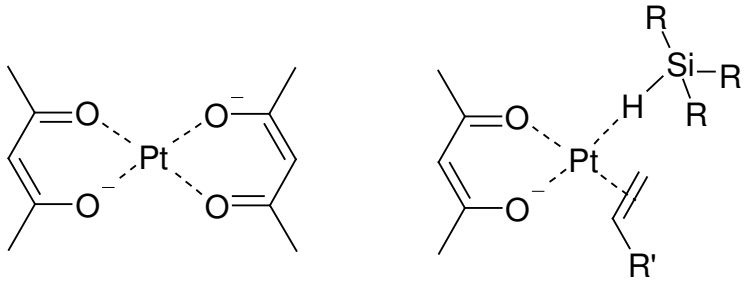
Visual response, in which light stimulates a photoreceptor to activate a signal transduction cascade resulting in amplified chemical output, is another biological model for signal amplifying catalysis. Examples of artificial light-induced signal amplifying catalysis are the classical photographic process (autocatalytic signal amplification),[10]

[a] Results of this chapter have been published: S.C. Ritter, B. König, *Chem. Commun.* **2006**, *45*, 4694.

and the hydrosilylation of alkynes, catalyzed by Pt(II)-complexes after initial irradiation[11] or by Fe(II)- or Cr(III)-complexes under continuous irradiation (scheme 1).[12] Such photoactivated catalysts include $Pt(acac)_2$, ($\eta^2$-Cyclopentadienyl) trialkylplatinum and iron- and chromium-carbonyl complexes. These catalysts are activated by irradiation with UV-light. Activation takes place as one of the ligands is lost in the presence of silanes (scheme 2).




Scheme 1: Light-activated hydrosilylation of alkynes.




Scheme 2: Pt(II)-catalyst in its inactive form and after activation with UV-light.

However, due to uncontrolled polymerisation, these systems are not able to transform the initial light-signal into a specific chemical output signal, similar to photo-initiated radical reactions. Further, it is still uncertain if a definite amount of light forms a corresponding amount of active catalyst.

Another approach to use light to control chemical reactions is the light-dependent azobenzene isomerisation. This system mimics the function of rhodopsin, as its photoactivation is also based on a change of conformation. It has been used to design photo-adjustable catalysts and enzymes[13,14] (fig. 1), which can be switched "on" and "off" as the *cis*- and the *trans*-conformer of azobenzene-catalysts show different absorption behaviour and also different catalytic activity.

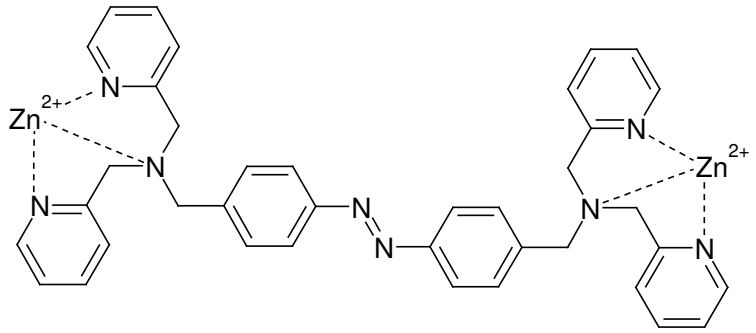



Figure 1: *Trans*-isomer of a photo-adjustable bis-Zn(II)-BPA-complex for non-enzymatic hydrolisis of RNA (additional ligands in the coordination-sphere of $Zn^{2+}$ are missing for better clarity).

While the *trans*-isomer is absorbing in the UV-region between 300 nm and 400 nm, the *cis*-isomer shows absorption of visible light at about 450 nm.[14,15] These systems show allosteric behaviour in their catalytic activity, but are also not able to amplify the light-signal, which can be called the allosteric effector, in a specific way.

### 1.1.2. Scope

To design an artificial system which is able to amplify and transduct an initial light signal into chemical output information, four fundamental conditions have to be fulfilled:

1) A light-sensitive element (a chromophore) has to use the initial photo-signal to activate a catalytic system. This could be achieved by a photochemical reaction, by a change of structure, or by a change of redox-potential. Further, the chromophore should show a high molar extinction coefficient ($\log \varepsilon > 4$) and a high quantum yield ($\Phi$ nearly 1) to enable efficient exploitation of light energy.
2) The amount (concentration) of active catalyst formed by the irradiated chromophore has to depend on the amount of light (number of photons) in order to guarantee reproducable results. Naturally a linear relationship between both values is desirable. Short-listed catalysts include enzymes and transition-metal complexes such as ruthenium-, palladium-, platinum- or copper-complexes.

3) The catalyst has to show a high turnover number to enable a large amplification of the initial light-signal.

4) The catalyzed reaction has to depend on the concentration of catalyst (a direct proportional relationship is desirable). Besides, the catalysis has to be a mono- or bi-molecular reaction to guarantee a fast and definite reaction-process. Further, it should proceed as quickly as possible, without formation of by-products, and enable a simple read-out to allow for quick evaluation. Polymerisation reactions as well as multi-component reactions (MCR) must be excluded, as the former proceed uncontrolled, and the latter are too complex and possibly proceed too slowly.

Although these requirements are quite demanding, the combination of the light-driven reduction of flavines (fulfills **1)**) with the Cu(I)-catalyzed "click-chemistry" (fulfills **3)** and **4)**) promises to be a good approach to design such a "chemical photo-multiplier".

Fig. 2 summarizes the general signalling pathway. Initial excitation of the flavine-chromophore in the presence of an electron donor leads to its photoreduction. The reduced chromophore allows for the conversion of an inactive catalyst precursor ($Cu^{2+}$) into an active catalyst ($Cu^{+}$). The active catalyst then amplifies and converts the signal into a chemical output. The number of received photons is correlated with the amount of active catalyst generated, which is translated into the reaction rate of the conversion from substrate to product.

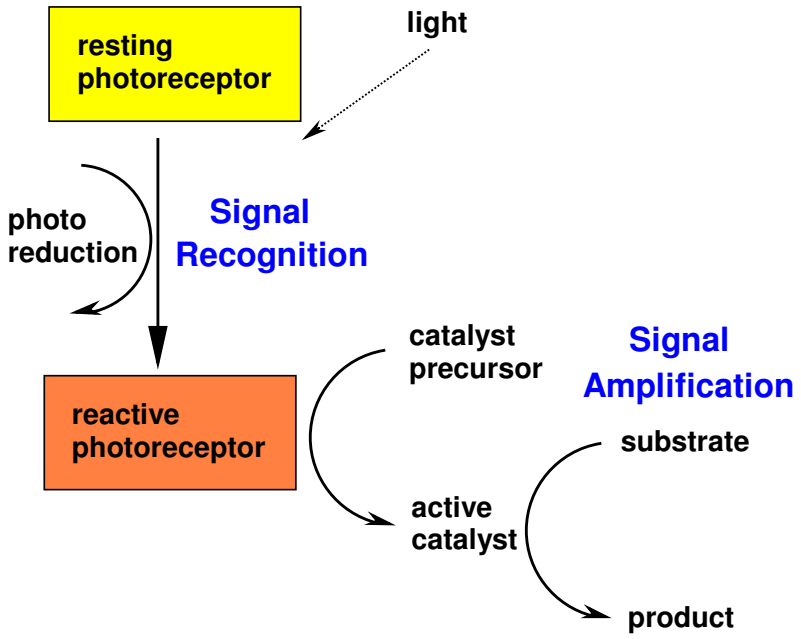



Figure 2: General signal-transduction pathway of a photoreceptor showing allosteric behaviour.

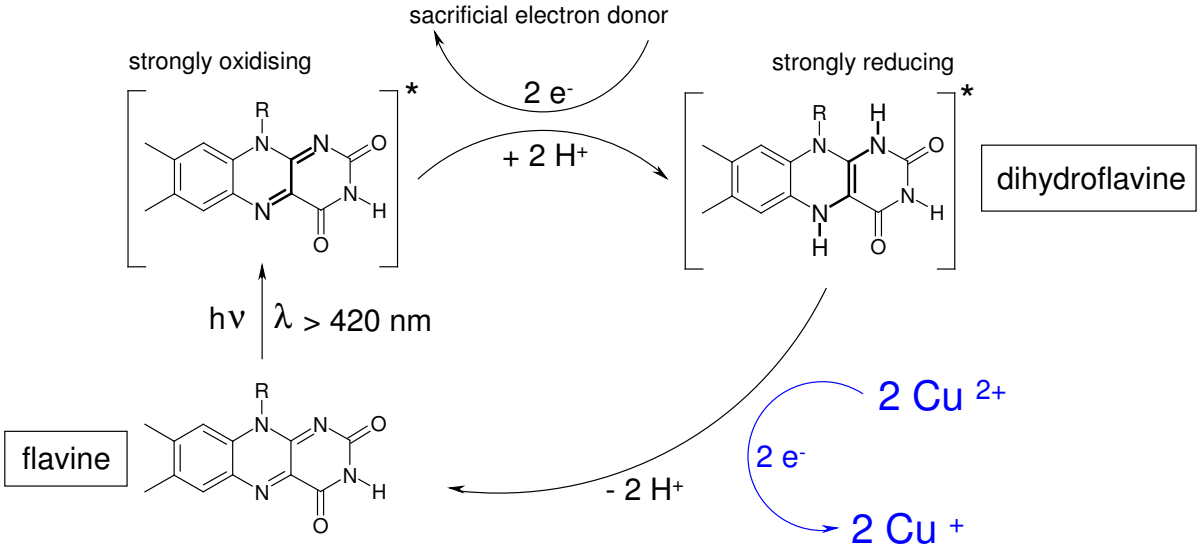
Riboflavine tetraacetate (**1**)[16] was used as redoxactive chromophore receiving the light signal. In the presence of a sacrificial electron donor, e.g. benzyl alcohol or triethylamine, excited flavin **1*** undergoes photoreduction to give **1-H$_2$** (scheme 3).[17,24] The quantum yield of this well-known process is in the order of 0.4.[18] Under the experimental conditions dihydroflavin (**1-H$_2$**) is expected to have a sufficient redox potential to reduce $Cu^{2+}$ to $Cu^{+}$.[19] Finally, the Cu(I)-catalyzed cycloaddition of an alkyne with an azide produces the chemical output of the cascade.

## 1.2. Main Part

### 1.2.1. The Light-Driven Flavine Redox System

Upon irradiation with visible light ($\lambda > 420$ nm), flavines are excited from the ground-state into the n,$\pi^*$-triplet-state,[20,21,22] which is involved with a large change in redox-potential.[23] In the presence of a suitable reducing agent (sacrificial electron donor), like benzyl alcohols,[22,24,25] amines,[26] and thiols,[27] the excited flavine is reduced to dihydroflavine (scheme 3). The presence of oxygen must be strictly excluded to avoid

formation of flavin-hydroperoxides and reoxidation of **1-H$_2$** to **1**.[21,24,28] These processes would, in the end, prevent the reduction of $Cu^{2+}$ by **1-H$_2$**.




Scheme 3: Reversible flavine-redoxsystem enabling the photo-regulated formation of $Cu^{+}$.

The photoreduction of **1** by several amines ($NEt_3$, $HNEt_2$ and $BuNH_2$) and benzyl alcohol in different solvents ($H_2O$, MeCN, $^tBuOH/H_2O$ 2:1 and $DMSO/H_2O$ 1:1) was confirmed experimentally by UV/Vis-spectroscopy as the absorption spectrum of **1** significantly differs from **1-H$_2$** (fig. 3).

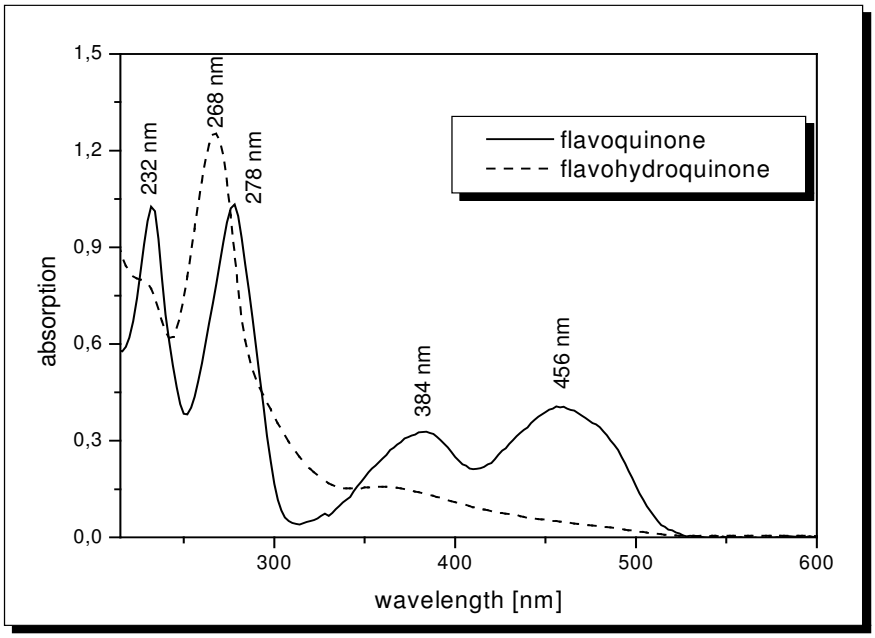


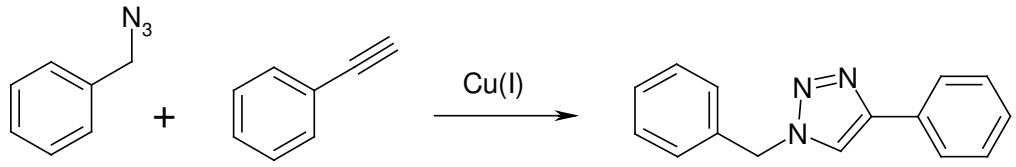

Figure 3: UV/Vis-spectra of **1** and **1-H$_2$** in $H_2O$ ($c = 5 \cdot 10^{-5}$ mol/l). **1-H$_2$** was generated by irradiation with visible light in the presence of benzyl alcohol.

While **1** shows a characteristic peak at 456 nm, the absorption of **1-$H_2$** in the visual region is rather insignificant. Also, the absorption at 384 nm becomes weaker after reduction. These results match the spectroscopic behaviour of similar flavines.[29]

Results of photoreduction-reactions with **1** showed that **1-$H_2$** is formed in aqueous solutions (100 % $H_2O$, $^tBuOH/H_2O$ 2:1 and DMSO/$H_2O$ 1:1) in the presence of amines ($NEt_3$ and $HNEt_2$) as well as benzyl alcohol. Carrying out the reaction in MeCN, photoreduction of **1** was observed only in the presence of amines ($NEt_3$, $HNEt_2$ and $BuNH_2$), but not with benzyl alcohol.

## 1.2.2. Generation of the Cu(I)-Catalyst

In order to find out if **1-$H_2$** is able to reduce $Cu^{2+}$ to $Cu^+$, mixtures of **1**, various sacrificial electron donors and different Cu(II)-salts in four solvents ($H_2O$, MeCN and mixtures of DMSO/$H_2O$ 1:1 and $^tBuOH/H_2O$ 2:1) were irradiated with visible light (see experimental section, general procedure (**GP**) **1**). As formation of $Cu^+$ cannot be observed directly in solution, it is detected indirectly using its catalytic activity in the [2+3] dipolar cycloaddition reaction. A “click-reaction” that can easily be followed is the formation of triazole **4** from phenylacetylene (**2**) and benzylazide (**3**).




**2** **3** **4**

Scheme 3: Cu(I)-catalyzed triazole formation.

To monitor the conversion, **4** can either be isolated from the reaction mixture (**GP 2**), or the resonance shift of the benzylic $CH_2$-signal in the $^1$H-NMR-spectrum is used; the $CH_2$-group of **2** shows a singlet at 4.33 ppm, whereas the corresponding signal of the $CH_2$-group of the triazole-product appears at 5.55 ppm. Both peaks are unaffected by the signals of other components or by-products, neither at the beginning nor at the end of the reaction (fig. 4, 5). The conversion can then be determined from the peak areas (**GP 3**).

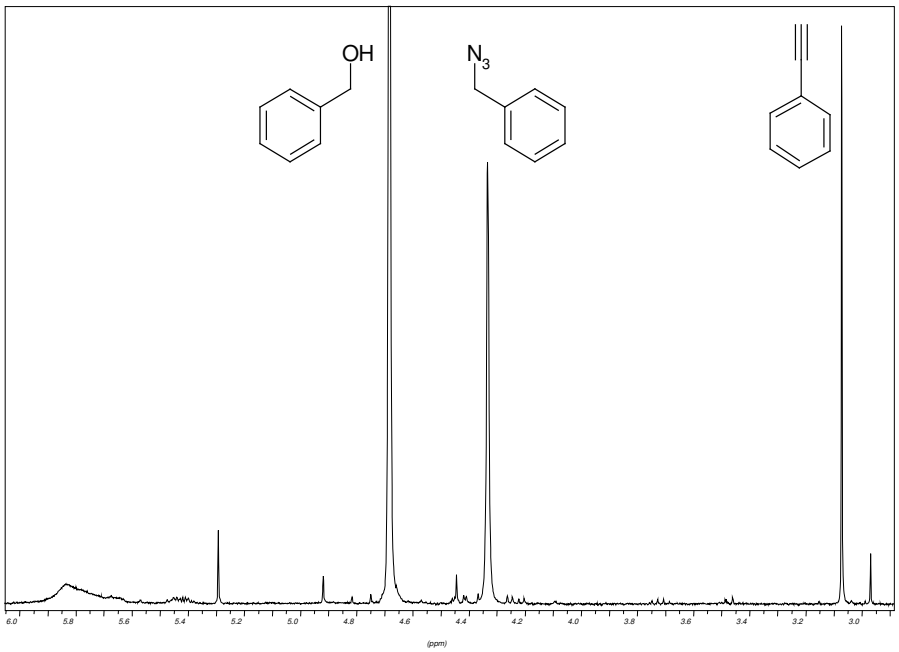



Figure 4: $^1$H-NMR ($CDCl_3$) spectrum of a mixture of **1**, **2**, **3**, BnOH and $CuSO_4$ x 5 $H_2O$ without irradiation. Assignment of main signals is shown.

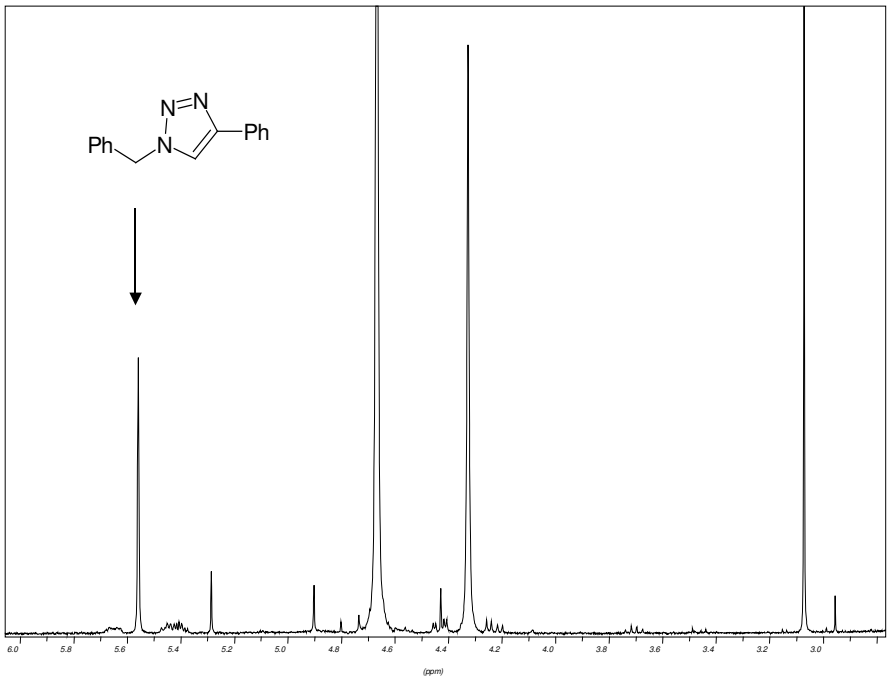



Figure 5: $^1$H-NMR-spectrum ($CDCl_3$) of the reaction mixture after 280 min of irradiation with visible light. The arrow marks the growing resonance signal of the $CH_2$-group of **4**.

In aqueous solution as well as in DMSO/$H_2O$ 1:1 and $^tBuOH/H_2O$ 2:1, the formation of **4,** and therefore formation of Cu(I), was observed for mixtures of **1** with BnOH, $NEt_3$ or $HNEt_2$ as sacrificial electron donors and $CuSO_4$ x 5 $H_2O$ or $CuCl_2$ as Cu(II)-sources (following **GP 1** and **GP 2**). No reaction was observed if $Cu(ClO_4)_2$ x 6 $H_2O$ was used. A reason for this might be that the perchlorate-anion has a sufficient oxidation potential to oxidize either **1-$H_2$** or $Cu^+$.

In acetonitrile solution, the number of Cu(II)-salts that can be tested is quite limited, as only $CuCl_2$ and $Cu(ClO_4)_2$ x 6 $H_2O$ are soluble in MeCN. Results were the same as in aqueous solutions: only $CuCl_2$ allowed formation of $Cu^+$, but not the perchlorate salt. Sacrificial electron donors $NEt_3$, $HNEt_2$, $BuNH_2$, ethylenediamine, BnOH, p-methoxy-benzylalcohol and thiophenol were tested, but the formation of **4** could only be observed with $NEt_3$.[30] One possible explanation for this result is that the $Cu^{2+}/Cu^+$ redox potential strongly depends on the available ligands for copper complexation. Further, chelating ligands like EDTA or ethylenediamine cannot be used, as they stabilize $Cu^{2+}$ and therefore shift the $Cu^{2+}/Cu^+$ redox potential to more negative values.[8,31]

Knowing the fundamental conditions for the photo-regulated “click-reaction”, further parameters can be investigated:

- How does the process of the reaction influence the time of irradiation and, therefore, the amount of light (= number of photons)?
- How does the concentration of Cu(II)-salt and sacrificial electron donor influence the process of the reaction?
- What is the influence of the solvent on the reaction?

### 1.2.3. Light-Depending “Click-Reaction” in Aqueous Solution

The dependence of the conversion on the concentration of $Cu^{2+}$ and on the duration of irradiation was investigated. Mixtures of **1**, **2**, **3**, BnOH and $CuSO_4$ x 5 $H_2O$ (10, 25 and 50 mol%) in $^tBuOH/H_2O$ 2:1 were irradiated for up to 9 h following **GP 1**. To determine the amount of **4** formed after a certain reaction time, the triazole was

isolated from the reaction mixture (**GP 2**). Results shown in diagram 1 are average values obtained after three repititions.

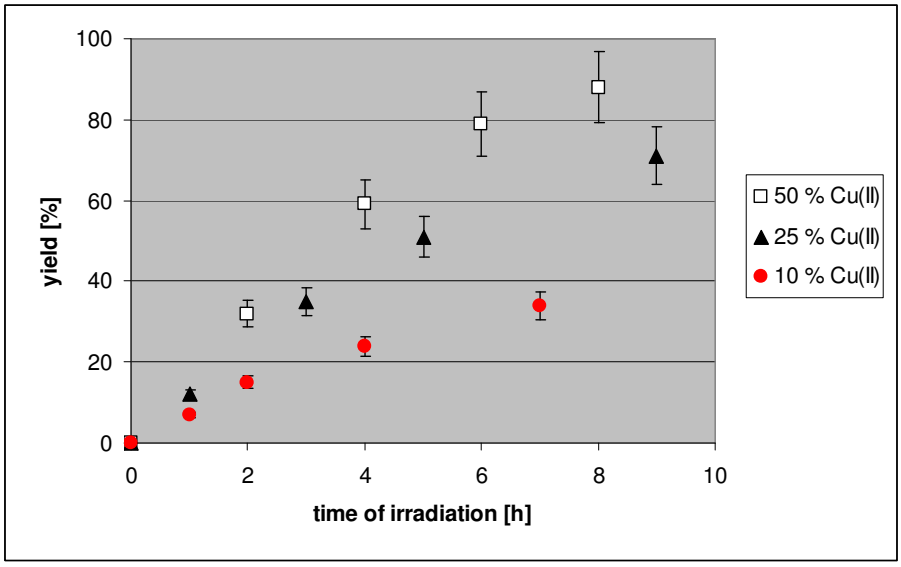



Diagram 1: Formation of **4** depending on time of irradiation and amount of $CuSO_4$ x 5 $H_2O$.

Diagram 1 clearly shows a dependence of formation of **4** on irradiation-time and concentration of $Cu^{2+}$, but errors are too large to allow a quantitative interpretation. It should be noted that **GP 2** is not a suitable method to obtain reliable data. For this reason the conversion in all subsequent experiments was monitored using **GP 3**.

In a new experiment, the progress of the reaction in alternating periods of irradiation and stirring in the dark was investigated. The behaviour of the reaction in these conditions is more of interest, as $Cu^{+}$ is not stable in aqueous solutions in the absence of any stabilizing ligands.[32] For this reason, the reaction is expected to stop when irradiation is switched off.

The reaction mixture (**GP 1**) was irradiated for 90 min, then stirred in the dark for 130 min, irradiated again for 70 min and finally stirred in the dark for 130 min. Diagram 2 gives the reaction conversion versus time. Values were derived from threefold repitition.

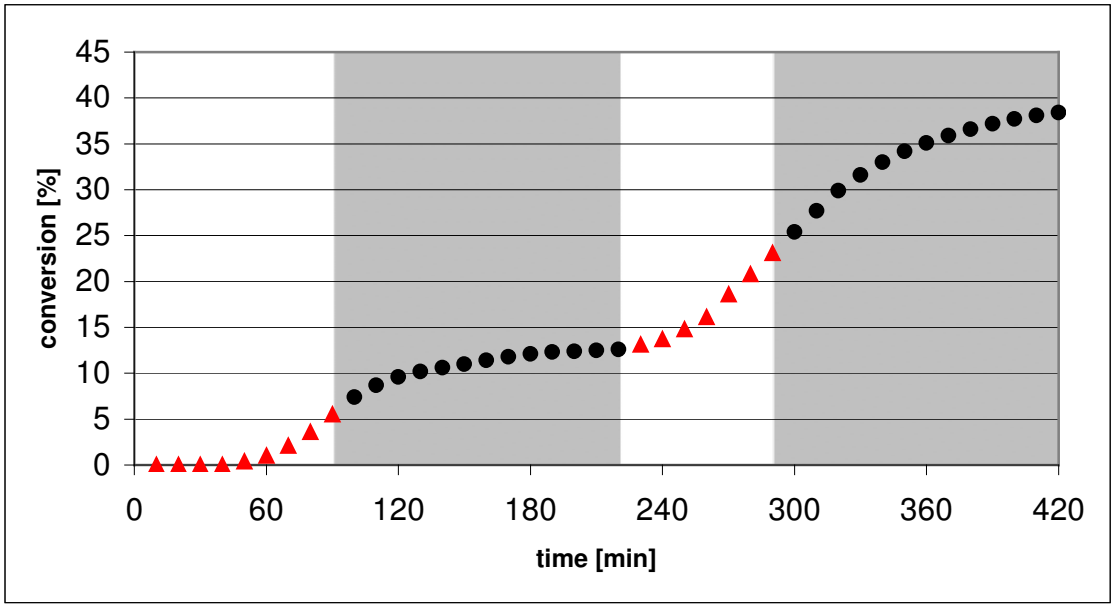



Diagram 2: Progress of reaction conversion during periods of irradiation (red triangles, white background) and dark periods (black dots, grey background).

The reaction clearly shows an induction period of approx. 50 min, a significant increase in the reaction rate upon irradiation and ceasing in the dark. These results confirm the previous expections. Thus, performed in aqueous media the reaction cascade shows light-dependent behaviour: the cycloaddition reaction proceeds only under continuous irradiation due to the instability of $Cu^{+}$ in aqueous media. Therefore, this approach cannot be used to develop a system that amplifies an initial light signal.

### 1.2.4. Light-Initiated “Click-Reaction” in Acetonitrile Solution

Amplification of the initial light signal requires the produced Cu(I) to be active and stable for some time. The choice of solvent is therefore crucial. In acetonitrile the photochemically produced Cu(I) is sufficiently stable to catalyze the cycloaddition for extended periods after short irradiation times (15 s to 180 s). To facilitate the monitoring of the Cu(I)-catalyzed cycloaddition, a bimolecular reaction between suitable dyes was used, showing a change in the absorption- or emission-spectrum after triaziole formation. This approach will enable a quick and easy readout.

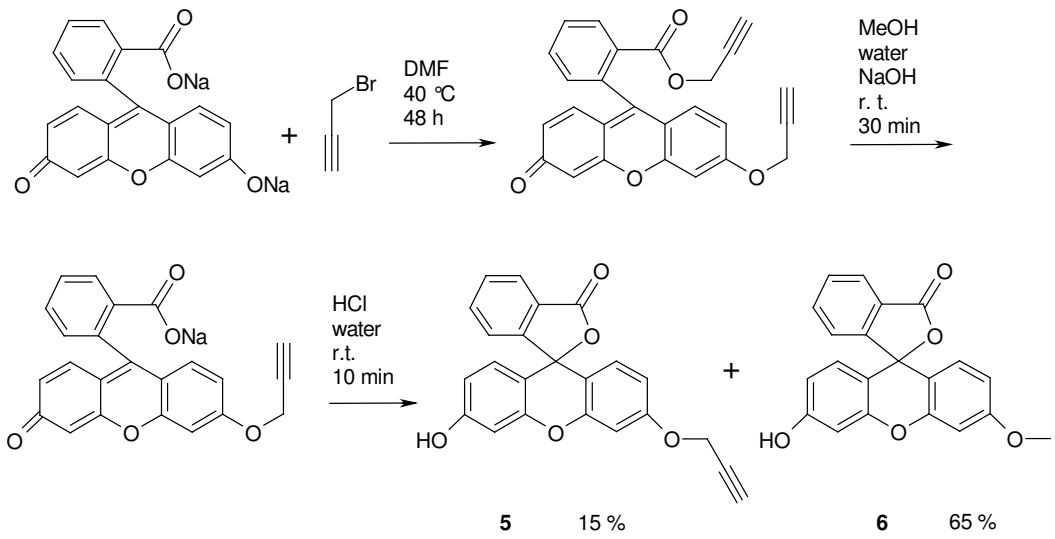
### *1.2.4.1. Synthesis and Spectroscopic Characteristics of Fluorescent Dyes for Cu(I)-Catalyzed Cycloaddition Reaction*

One possible approach is to use a fluorescent resonance energy transfer (FRET) between two covalently connected fluorophores. Anslyn *et al.* reported about the application of this idea to determine the concentration of Pb(II)-cations in analytical probes. The reported system used a FRET occuring after the cycloaddition reaction between a coumarin-azide and an anthracene-alkyne.[8]

In order to design a similar system with simple and cheap fluorescent dyes, alkyne- and azide-substituted fluorescein- and dansyl-derivatives were used. Fluorescein can easily be functionalized with electrophiles at one of its phenol groups, forming phenolethers.[33] Dansyl-derivatives can easily be derived from the sulfonic acid chloride, using amines or alcohols. Fluorescence spectra of corresponding substituted fluoresceins show emission in polar solvents, like MeOH, between 520 nm and 550 nm, when excited between 470 nm and 490 nm. Substituted dansyl-sulfonamides show emission in non-polar solvents, like $CHCl_3$, at about 480 nm after excitation at about 330 nm. Thus, if a FRET between both dyes occurs, then the excitation of the dansyl unit at 330 nm should result in the emission signal of the fluorescein unit at $\lambda > 520$ nm.

The alkyne-substituent was coupled to the fluorescein moiety in a $S_N2$ reaction using propargylbromide and fluorescein di-sodium salt. In order to cleave the formed propargyl ester without affecting the ether, the raw product was treated with aqueous NaOH in MeOH. Finally, the lactone was formed by addition of hydrochloric acid. The raw product had to be purified by column chromatography. Surprisingly, this led to a mixture of the desired compound **5** and the corresponding methyl ether **6**. Separation of **5** from **6** was not possible using chromatographic methods. However, as **5** was soluble in diethylether while **6** was not, separation of both compounds was achieved. **5** was obtained in a low yield of 15 %, whereas **6** could be obtained in a yield of 65 % (scheme 4).

The mechanism of the formation of **6** could not be clarified. The use of MeOH as solvent during synthesis should not be the reason, as its replacement by THF or propargyl alcohol gave exclusively compound **6**.




Scheme 4: Synthesis of alkyne-substituted fluorescein dye **5**.

Spectroscopic characteristics of **5** were as expected. Figure 6 shows absorption and emission spectrum of **5** in MeOH.

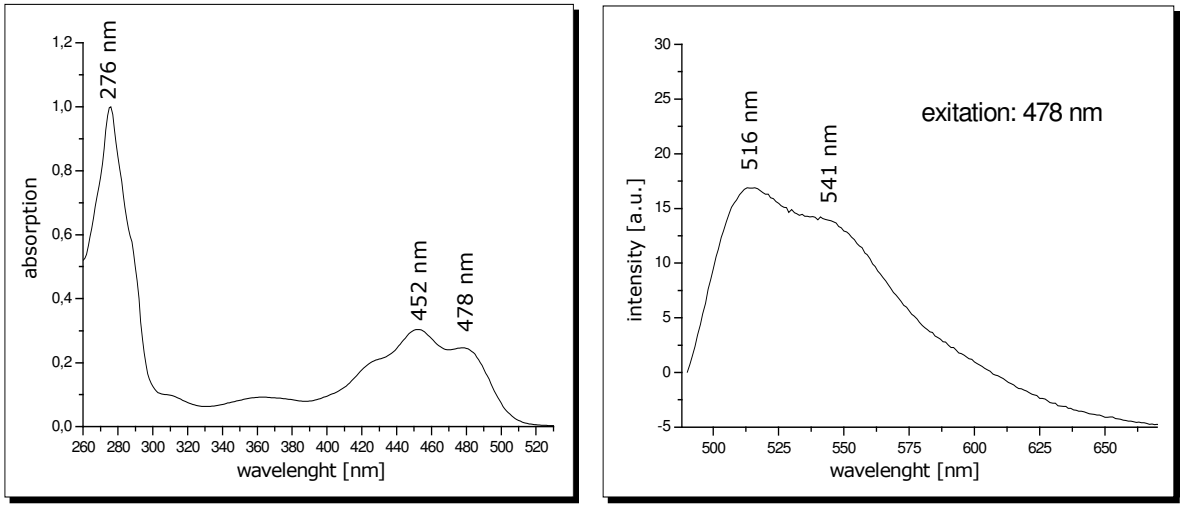
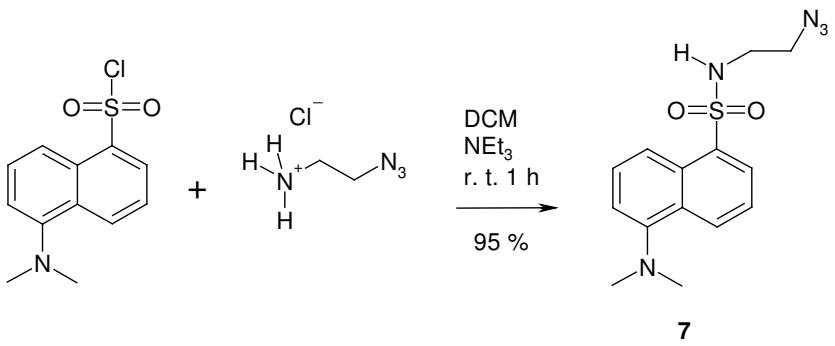



Figure 6: Absorption and emission spectrum of **5** in MeOH (c = 1.30 x $10^{-4}$ mol/L).

In order to synthesize an azide-substituted dansyl derivative, dansyl chloride was reacted with 2-azido-ethylamine hydrochloride under basic conditions using $NEt_3$. After a short workup, the desired dansyl derivative **7** could be obtained in almost quantitative yield (scheme 5).




**7**

Scheme 5: Synthesis of dansyl azide **7**.

Spectroscopic characteristics of **7** in MeOH were ***not*** as expected. The fluorescence spectrum showed a strong red-shift of 39 nm of the emission wavelength compared to the value in $CHCl_3$ (fig. 7). The absorption spectrum of **7** showed no considerable differences in MeOH or $CHCl_3$.

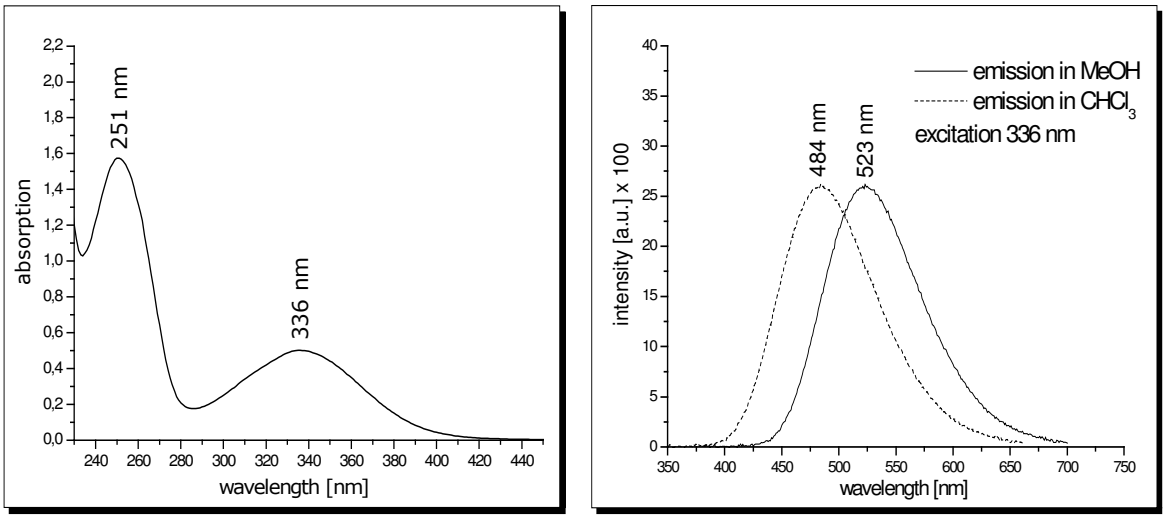
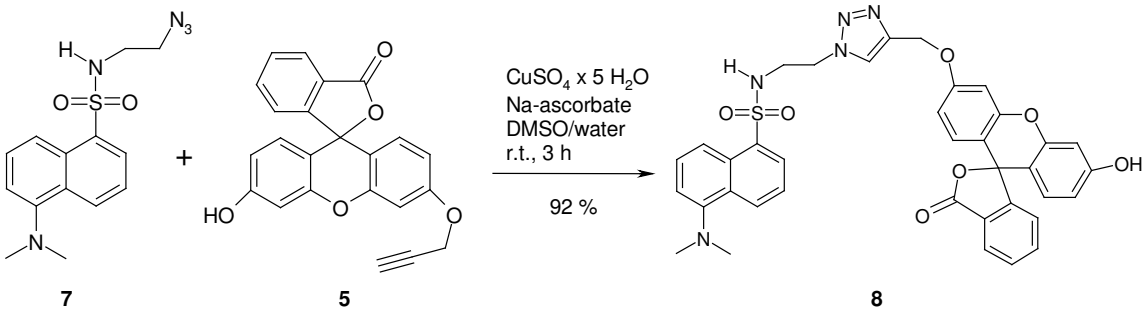



Figure 7: Absorption spectrum of **7** in MeOH and emission spectrum in MeOH and $CHCl_3$ (c = 1.13 x $10^{-4}$ mol/L). For better clarity the differences in the emission intensity are not taken into consideration.

Because of the shift of the emission signal of **7** to longer wavelengths in MeOH, a FRET between both dyes could not occur. The usage of non-polar solvents, like $CHCl_3$, was not possible, as fluorescein derivatives show fluorescence only in polar media. To verify this hypothesis, the triazole product **8**, from **5** and **7**, was synthesized in a typical “click-reaction” (scheme 6).




**7** **5** **8**

Scheme 6: Synthesis of triaziole **8**.

As expected, the triazole **8** showed no FRET when excited at 336 nm – neither in MeOH nor in $CHCl_3$. Spectral characteristics of **8** were simply the same as a equimolar mixture of **5** and **7** (fig. 8). This proved that there was no affection on the spectra of either dye after covalent connection.

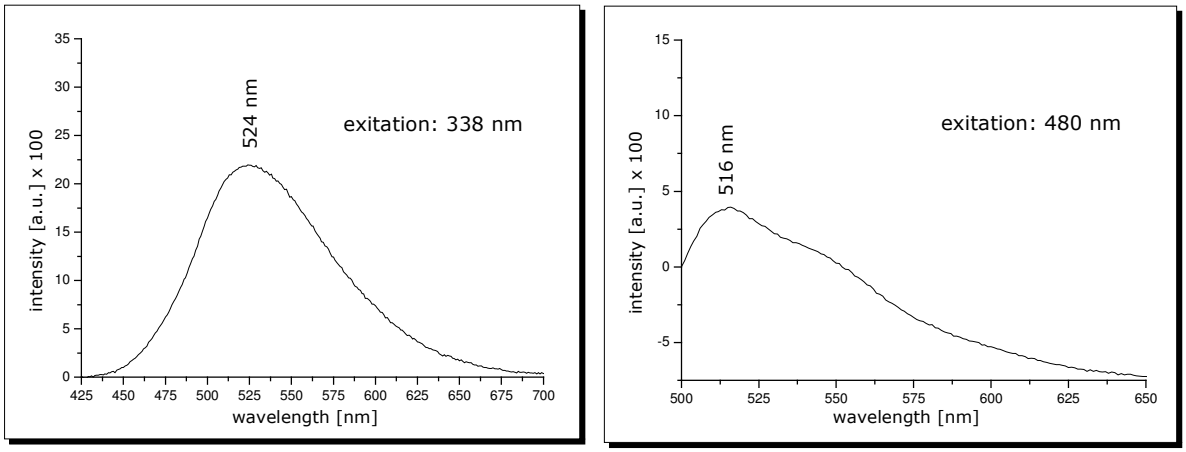
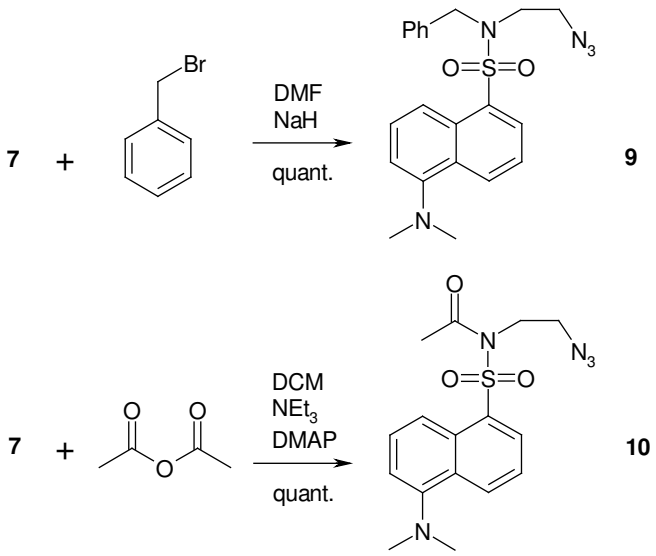



Figure 8: Fluorescence spectra of **8** in MeOH (c = 1.13 x $10^{-4}$ mol/L).

In order to check if an additional substituent at the sulfonamide nitrogen shifts the emission signal of **7** to shorter wavelengths, an electron-pushing and an electron-withdrawing group were introduced at this position. As sulfonamides can be deprotonated quite easily ($pK_a$ (water)= 10 – 11.5),[34] new derivatives are accessible by reaction with suitable electrophiles like acetylanhydride or benzyl bromide.

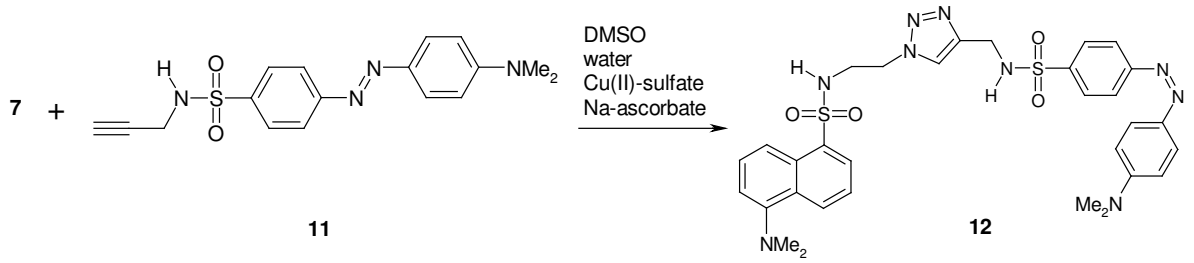



Scheme 7: Synthesis of two further dansyl derivatives **9** and **10**.

Scheme 7 shows the synthesis of the dansyl derivatives **9** and **10**. Compound **10** was synthesized using $NEt_3$, which is a sufficiently strong base to deprotonate **7**. The following reaction with acetanhydride was catalyzed using DMAP. In order to introduce the benzyl group, a non-nucleophilic base, like sodium hydride, had to be used. In both cases, the desired compounds were obtained in quantitative yields.

Fluorescence spectra of **9** and **10** showed that neither the introduction of a benzyl group nor the acetylation gave the desired effect of a blue shift of the emission signal. The emission maximum of **9** is at 520 nm and therefore almost identical to that of **7**. Further, the introduction of an acetyl group (compound **10**) yielded exactly the opposite effect: the emission maximum was shifted to 560 nm.

In the end the approach of generating a FRET between a fluorescein and a dansyl derivative was not successful. In order to use some of the synthesized compounds to monitor the process of a “click-reaction”, a different system was used. The working group of Finn used the intramolecular quenching of the fluorescence signal of a dansyl group by a dabsyl dye[35] to determine the conversion of a Cu(I)-catalyzed cycloaddition reaction.[36] Compound **7** could be used in a corresponding approach. After the “click-reaction” with the dabsyl derivative **11** (scheme 8) the triazole (**12**) did not show any fluorescence. Compounds **11** and **12** were synthesized as previously described.[36]




Scheme 8: Cu(I)-catalyzed cycloaddition used as indicator reaction.

### *1.2.4.2. Kinetic Measurements of the Light-Initiated Cu(I)-Catalyzed Cycloaddition Reaction*

The reaction mixture of **7**, **11** (each 0.4 mmol), **1** (4 mol%), $NEt_3$ (1 eq) and $CuCl_2$ (8 mol%) in MeCN was irradiated under identical conditions (see **GP 4**) for various lengths of time (from 0 to 180 sec) and the reaction rate of the initiated Cu(I)-catalyzed cycloaddition in the dark was monitored (diagram 3). The quenching of the fluorescence of **7** can even be followed with bare eyes (fig. 9).

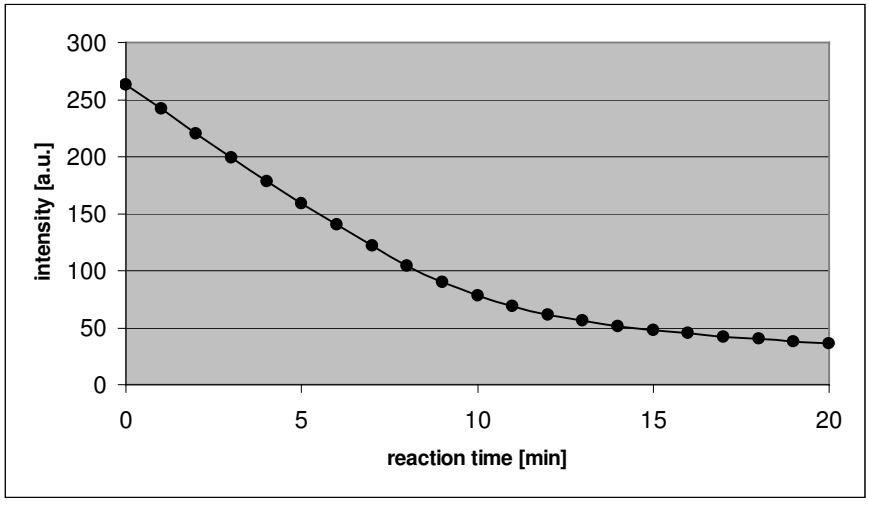



Diagram 3: Decrease of emission intensity over 20 min of reaction time exemplary for one reaction (initial irradiation time: 180 s). Values were derived from 3 repititions.

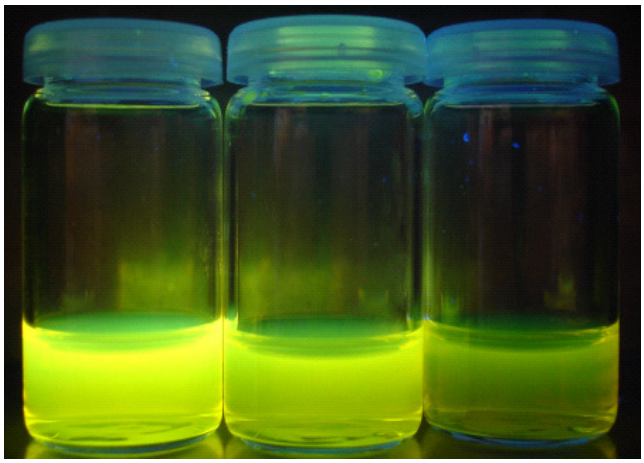


Figure 9: Emission fading during the photoinitiated cycloaddition; initial irradiation time was 90 s; from left to right: emission of the solution after 1 min, 10 min and 20 min reaction time in the dark.

A slow background reaction was observed without irradiation due to the spontaneous reduction of $Cu^{2+}$ to $Cu^{+}$ by $NEt_3$. Experimental results showed that after addition of 1 eq $NEt_3$, enough $Cu^{+}$ was generated from $CuCl_2$ to start the cycloaddition reaction. However, within irradiation times of 30 to 180 s, a significant increase of the reaction rate was observed (diagram 4).

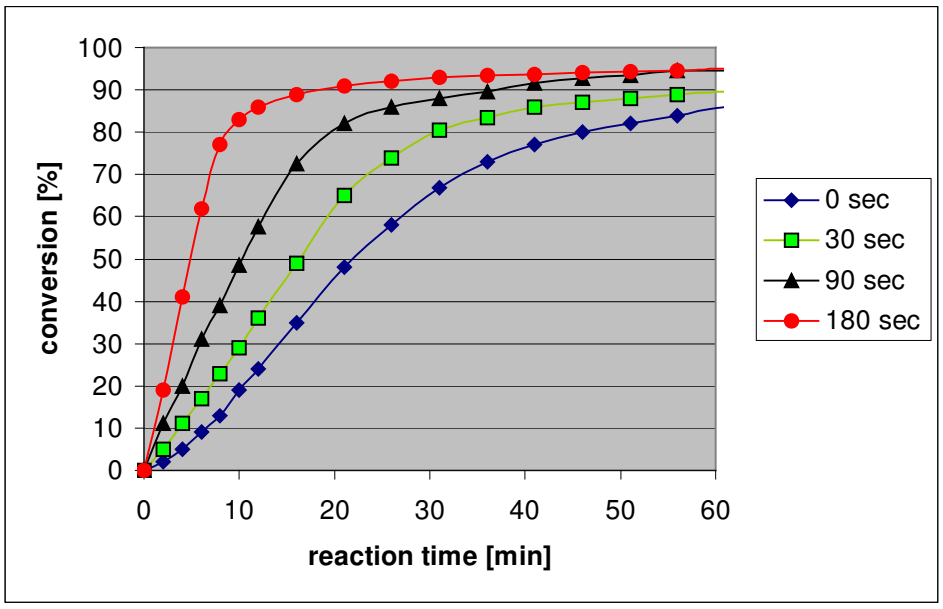



Diagram 4: Conversion vs. reaction time depending on time of initial irradiation.

Of course, this background reaction is not desired. In order to slow this reaction down or even stop it completely, the dependence of this “dark reaction” on the concentration of $NEt_3$ was investigated. Diagram 5 shows the reaction process

exemplary for two reactions with 1 eq and 0.1 eq $NEt_3$. There is a significant drop of the reaction rate if catalytic amounts of sacrificial electron donor are used.

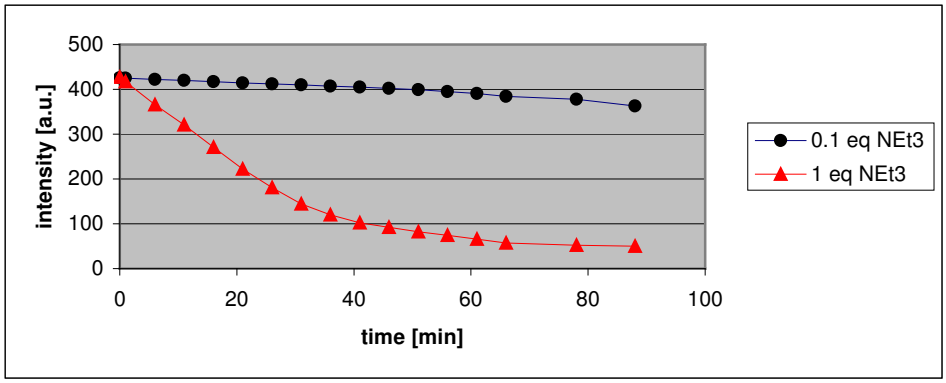



Diagram 5: Decrease of fluorescence of reaction batch with 1 eq and 0.1 eq $NEt_3$.

Considering that an equimolar relation between $NEt_3$ and **1** is sufficient to enable formation from **1-H$_2$**, and the following reduction of Cu(II), the use of 0.1 eq $NEt_3$ should not slow down the light initiated reaction. Experimental results showed otherwise: the use of lower amounts of $NEt_3$ slowed the light-initiated reaction more than the "dark reaction". When 0.15 eq $NEt_3$ or less was used, the light initiated reaction stopped completely (diagram 6).

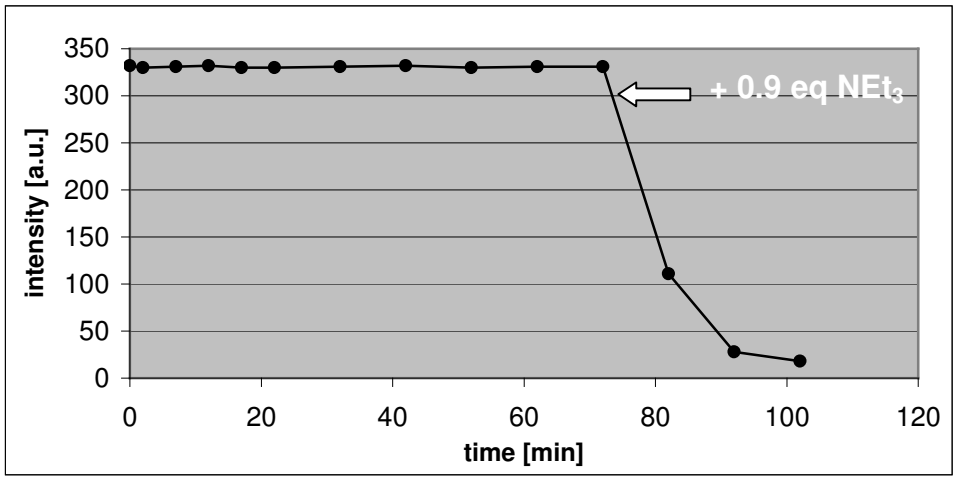



Diagram 6: Fluorescence vs. reaction time after initial irradiation of 180 s with 0.1 eq $NEt_3$. The arrow marks the addition of further 0.9 eq $NEt_3$ after 72 min.

However, after the addition of further 0.9 eq $NEt_3$, the reaction started immediately, without any further irradiation. The reaction proceeded only insignificantly lower than the corresponding one with 1 eq $NEt_3$. This means that upon irradiation, almost all sacrificial electron donor is used up, and formation of **1-H$_2$** takes place. **1-H$_2$** is stable

in the reaction mixture for at least 70 min, but the dihydroflavine itself is not able to reduce $Cu^{2+}$ to $Cu^{+}$. Thus, the presence of $NEt_3$ is essential, as it forms a Cu(II)-amine complex. Exceptionally $[Cu(NEt_3)_4]^{2+}$ can be reduced by **1-$H_2$**. This confirms the prior assumption in chapter [1.2.2.]. Thus, for all subsequent experiments 1 eq of $NEt_3$ was used.

To estimate the efficiency of the light to catalyst conversion of the reaction, the dependence of the reaction rate of formation of **12** on the Cu(I) concentration under the experimental conditions, was determined (**GP 5**).

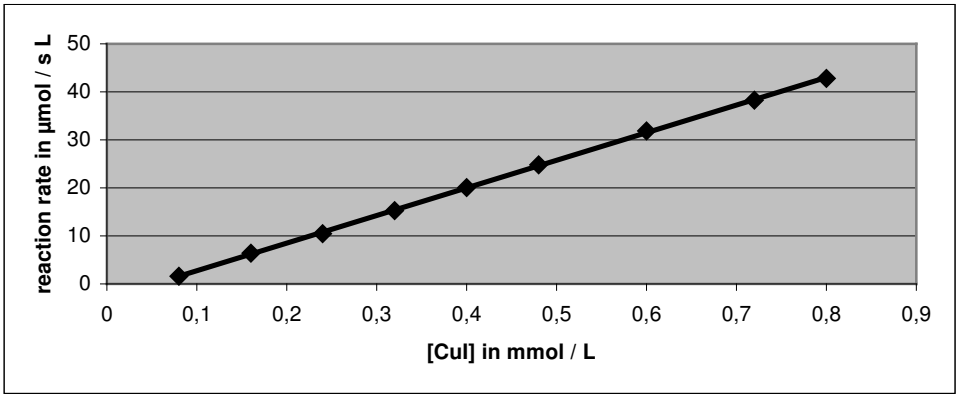



Diagram 7: Dependence of the cycloaddition reaction rate on the Cu(I)-concentration.

Diagram 7 shows the reaction rate with respect to the concentration of copper iodide. Reaction rates were calculated from linear region in conversion vs. time diagram (up to 40 % conversion). Results clearly show a linear dependence for the experimental conditions employed (MeCN as solvent; $NEt_3$ as additive; low CuI-concentration of $10^{-4}$ to $10^{-3}$ mol/L). Thus, the reaction rate is first order in Cu(I). For catalytic reactions in aqueous solution and concentrations > $10^{-2}$ mol/L, a binuclear Cu(I)-complex was previously proposed.[37]

Now with information from diagram 4 and diagram 7, the dependence of the rate enhancement, as well as the dependence of the surplus Cu(I) formation on the amount of photons, can be determined (diagram 8).

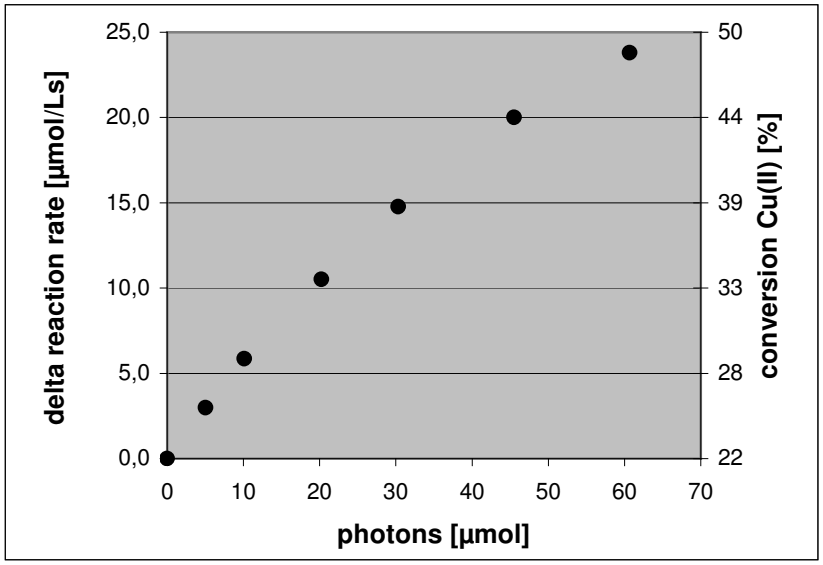



Diagram 8: Acceleration of the initial reaction rate and conversion of Cu(II) to Cu(I) depending on the amount of light (data points are for irradiation-times of 0, 15, 30, 60, 90, 135 and 180 s).

Results show that even without irradiation, already 22 % of Cu(II) is converted. Further, with short irradiation times (< 30 s), the background reaction becomes important, while longer irradiation (> 100 s) leads to a non-linear response of the photoreceptor due to bleaching of **1-H$_2$**,[17b] the formation of side-products instead of **1-H$_2$**,[21] and decreasing concentrations of the sacrificial electron donor and $CuCl_2$ (related flavin photoreactions showed a similar behaviour).[22,24]

In order to improve the performance of the photoreceptor, concentrations of **1** and $CuCl_2$ were varied. Under better reaction conditions, the course of the graph in diagram 8 should become steeper. In particular, the efficiency of the Cu(II)-conversion is rather low – between 20 % and 60 %. Theoretically, larger concentrations of **1** (up to 15 mol%) should not only lead to more Cu(II)-conversion (and therefore a wider linear range), but also to shorter irradiation times. In contrast, experimental results showed that larger concentrations of **1** lead primarily to an acceleration of the background reaction. The reason for this is unknown. Use of lower amounts of **1** or varying the concentration of Cu(II) also showed no improvement in performance. Thus, reaction conditions with **1** eq $NEt_3$, 4 mol% **1** and 8 mol% of $CuCl_2$ were found to be optimal.

Results from diagram 8 give a quantum yield of the Cu(I) generation of 0.2 and an overall quantum yield of triazole-formation (which can also be called the **overall**

**amplification factor** of the reaction cascade) of up to 15 after 20 min reaction time. This corresponds to a turnover number of 70 for $Cu^+$ after that time.

## 1.3. Summary and Conclusions

In summary, a simple catalytic system, which is able to translate a light signal into a chemical output with amplified response, was developed. The overall amplification factor is 15 after a reaction time of 20 min. Although its fidelity in terms of sensitivity and amplification is far from biological models, the results show that coupling optical and chemical processes allows for the processing and amplification of information.

Mechanistic details of the reaction cascade like efficiency of formation of **1-$H_2$**, and Cu(I), as well as the dependence of the cycloaddition reaction on the Cu(I)-concentration, were determined. With this information, reaction conditions were optimized to achieve best possible performance of this photoreceptor system.

Reaction process was monitored using a literature-known system, which allowed a quick and easy optical readout.

New fluorescent dyes containing alkyne- and azide-groups were synthesized. These compunds allow modular connection to a lot of substrates, such as labelling of proteins.

The catalytic system also works in aqueous media, where the reaction cascade shows light-depending behaviour. This enables control of the reaction by simply turning a light-source “on” or “off”.

## 1.4. Outlook

Theoretically, if the quantum yield of the formation of dihydroflavine was 1, every photon would be able to generate up to two copper(I)-cations (scheme 3). The resulting quantum yield of Cu(I)-formation would be 2, if the reduction of Cu(II) took place without any undesired background reaction. Then the reported catalytic system would show an amplification factor up to 1000, if the turnover number of the Cu(I)-

catalyst was also raised (larger concentration of substrates, longer reaction time). The main improvement to be made is to switch off the background reaction. This could be achieved by using different complexing ligands, which allow for the easy reduction of $Cu^{2+}$ without participating in a redox reaction. Polydentate thio-ether ligands could fulfill these creteria.[38] Another possibility is the use of a different amine base, e.g. diethylamine. To compensate the resulting change in the redox potential of the $Cu^{2+}/Cu^{+}$-system, a dihydroflavine with a more negative redox potential has to be used. This can be achieved by the introduction of an electron-pushing substituent at the 7- or 8-position of the isoalloxazine system.[39] A further approach would be the change of the solvent to e.g. DMSO or THF, which allow for solvation of $CuCl_2$.

## 1.5. Experimental Part

### 1.5.1. General Information

All reactions were performed under an inert atmosphere using standard Schlenk techniques. Acetonitrile (HPLC-grade, Baker) was freshly distilled over sodium hydride and $P_4O_{10}$.

For irradiation a commercial available Osram® daylight lamp (200 W, 220 V) was used. To exclude irradiation with UV-light a LOT-Oriel filter was used. To compensate fluctuations in electricity and, therefore, in the intensity of emitted light, experiments were repeated twice and each day at the same time.

#### *1.5.1.1. Spectroscopy*

**Emission Spectroscopy**

Fluorescence measurements were done in acetonitrile (UV-grade, Baker or Merck) at 25 °C in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer. The excitation wavelength was 337 nm for all measurements and the internal photomultiplier voltage was adjusted to 700 V. The intensity of fluorescence of (**7**) was measured in a range of 420 nm to 650 nm. Concentration of (**7**)/(**11**) was 4 µmol/L or lower to avoid intermolecular quenching of fluorescence, which was observed at concentrations higher than 20 µmol/L. To determine the conversion of the reaction the decrease of intensity of the maximum fluorescence at 524 nm was observed.

**Absorption Spectroscopy**

Varian Cary BIO 50 UV/VIS/NIR Spectrometer. Use of a 1 cm quartz cell (Hellma) and Uvasol solvents (Merck or Baker).

**NMR Spectra**

Bruker Avance 600 ($^1$H: 600.1 MHz, $^{13}$C: 150.1 MHz, T = 300 K), Bruker Avance 400 ($^1$H: 400.1 MHz, $^{13}$C: 100.6 MHz, T = 300 K), Bruker Avance 300 ($^1$H: 300.1 MHz, $^{13}$C: 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, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Assignment of signals in $^{13}$C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for $CH_3$ or CH, (-) for $CH_2$ and ($C_{quat}$) for quaternary C. Error of reported values: chemical shift: 0.01 ppm for $^1$H-NMR, 0.1 ppm for $^{13}$C-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.

*1.5.1.2. Analysis*

**Cyclic Voltammetry**

Measurements were carried out under an inert atmosphere of argon in degassed and dry MeCN. The electrodes used were platinum (counter electrode), glassy carbon (working electrode) and Ag/AgCl (reference). Scan rate: 20 mV/s; electrolyte: tetrabutylammonium hexafluorophosphate (c = 0.2 mol/L). Concentrations of 1 and Cu(II) amine complexes were 2.5 mmol/L. $E_0$ values were determined versus $Fc/Fc^+$ as internal reference ($[E_0] = ([E_a]+[E_c])/2$).

*1.5.1.3. Synthesis*

Melting Points were determined on a Tottoli micro melting point apparatus and are uncorrected. 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 was used. For preparative column-chromatography, Merck Geduran SI 60 silica gel was used. Commercially available solvents of standard quality were used. If otherwise stated, purification and drying was done according to accepted general procedures.[40] Elemental analyses were carried out by the Center for Chemical Analysis of the Faculty of Natural Sciences of the University Regensburg.

## 1.5.2. General Procedures

**GP 1** for reactions in water, DMSO/water 1:1, $^{t}$BuOH/water 2:1:

Under an atmosphere of argon 1000 mg of benzyl azide (7.50 mmol), 766 mg of phenyl acetylene (7.50 mmol), the organic solvent and 15.0 mmol (2 eq) of sacrificial electron donor (benzyl alcohol: 1620 mg, 1545 µl; $NEt_3$: 1518 mg, 2091 µl; $HNEt_2$: 1097 mg, 1552 µl) were added into a Schlenk-flask. Then a solution of 0.75 mmol (10 mol%) Cu(II)-salt ($CuSO_4$ x 5 $H_2O$: 187 mg; $CuCl_2$: 101 mg, $Cu(ClO_4)_2$ x 6 $H_2O$: 278 mg) in water was added. The total volume of solvent (-mixture) added was 22 ml. The mixture was degassed and saturated with argon three times (using the "pump-and-freeze method"). After warming to 25 °C 163 mg of **1** (0.30 mmol, 4 mol%) were added and the mixture was stirred well. To keep the reaction temperature constant at 25 °C the Schlenk-flask was placed into a thermostated water-bath. The mixture was irradiated for various lengths of time.

**GP 1** for reactions in MeCN:

Under an atmosphere of argon 1000 mg of benzyl azide (7.50 mmol), 766 mg of phenyl acetylene (7.50 mmol), 15.0 mmol (2 eq) of sacrificial electron donor (benzyl alcohol: 1620 mg, 1545 µl; $NEt_3$: 1518 mg, 2091 µl; $HNEt_2$: 1097 mg, 1552 µl,

$BuNH_2$: 1097 mg, 1483 µl; ethylenediamine: 902 mg, 1002 µl; p-methoxy-benzyl alcohol: 2072 mg, 1862 µl; thiophenol: 1653 mg, 1540 µl) and 12 ml of dry MeCN were added into a Schlenk-flask. Then a solution of 0.75 mmol (10 mol%) Cu(II)-salt ($CuCl_2$: 101 mg; $Cu(ClO_4)_2$ x 6 $H_2O$: 278 mg) in 10 ml MeCN was added. The mixture was degassed and saturated with argon three times (using the "pump-and-freeze method"). After warming to 25 °C, 163 mg of **1** (0.30 mmol, 4 mol%) were added and the mixture was stirred well. To keep the reaction temperature constant at 25 °C, the Schlenk-flask was placed into a thermostated water-bath. The mixture was irradiated for various time.

**GP 2**:

After irradiation the mixture was diluted with 30 ml water and extracted three times with 100 ml EE each time. The organic phase was dried over $Na_2SO_4$, concentrated at reduced pressure and well dried in vacuum. The remaining yellow solid was eluted over a short silica gel column with pure EE to seperate **4** from **1**. The obtained solution was concentrated and dried in vacuum. This yielded **4** as a colourless crystalline solid.

**GP 3**:

Aliquots of 40 µl were taken every 10 minutes from the mixture and diluted with 0.8 ml $CDCl_3$. 40 µl of 30 % aqueous $H_2O_2$ were added to stop the reaction. After phase separation, a NMR-spectrum was recorded.

**GP 4**:

Azide **2** (127.8 mg, 400 µmol) was dissolved in a schlenk-tube in 5 ml of MeCN. Alkyne **3** (137.0 mg, 400 µmol) was dissolved in a schlenk-flask in 15 ml of MeCN. Both solutions were degassed and saturated with argon. Under exclusion of light, **1** (8.7 mg, 16 µmol, 4 mol %), $CuCl_2$ (4.3 mg, 32 µmol, 8 mol %) and 55.4 µl of $NEt_3$ (40.5 mg, 400 µmol) were added in this order to the azide-solution. The solution was stirred in the dark (for 120 s to 300 s) and then irradiated (for 0 s to 180 s). After irradiation the alkyne solution was added. The mixture was stirred well in the dark and aliquots of 20 µl were taken every minute. The samples were added to a solution

of 20 µl of 30 % aqueous $H_2O_2$ in 2000 µl MeCN to stop the reaction. Samples of 60 µl of this solution were diluted with 3000 µl MeCN and emission spectra were recorded.

To prevent reduction of the light intensity required for the photoreduction process, the alkyne compound was added after irradiation. Compound **11** showed a strong absorption signal in the blue-light region ($\lambda_{max}$ (log $\varepsilon$) = 442 nm (4.496), in acetonitrile solution) and therefore competed with **1**, which showed a similar absorption behaviour between 400 and 500 nm ($\lambda_{max}$ (log $\varepsilon$) = 449 nm (3.953), in aqueous solution). The azide compound did not influence neither the absorption behaviour of **1** nor the photoreduction process, as it showed no absorbance in the relevant region above 400 nm.

**GP 5**:

Azide **7** (63.9 mg, 200 µmol) was dissolved in a Schlenk tube in different amounts of MeCN (approx. 2.0 – 2.9 ml, depending on the volume of CuI solution, which was added later). Alkyne **11** (68.5 mg, 200 µmol) was dissolved in a Schlenk flask in 7.0 ml of MeCN. Both solutions were degassed and saturated with argon. Then, 27.7 µl of $NEt_3$ (20.2 mg, 200 µmol) and various amounts (approx. 100 µL to 1000 µL) of a stock solution of 38.1 mg of CuI (200 µmol) in 20 ml degassed acetonitrile were added to the azide solution to ensure a constant overall volume. Finally, the alkyne solution was added. The mixture was stirred well and aliquots of 20 µl were taken every minute. The samples were added to a solution of 20 µl of 30 % aqueous $H_2O_2$ in 2000 µl acetonitrile to stop the reaction. Samples of 60 µl of this solution were diluted with 3000 µl of acetonitrile to determine the emission spectra.

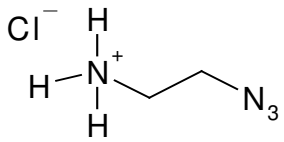
### 1.5.3. Synthesis of New Compounds

**Safety**

Sodium azide is toxic and can generate the extremely hazardous hydrazoic acid (volatile, toxic, explosive) if it gets in contact with acids. Organic azides with a saturated carbon:azide ratio of < 6 (such as azidoethylamine or azidopropylamine) may be heat and shock sensitive and should be handled carefully. Phenylacetylene is suspected to cause cancer.

**Chemicals**

Dansyl chloride, fluorescein di-sodium salt, 2-bromoethylamine hydrobromide, 3-bromopropylamine hydrobromide, propargylamine and phenyl acetylene were obtained from Fluka Chemicals. Benzyl azide was synthesized from benzyl bromide and sodium azide.

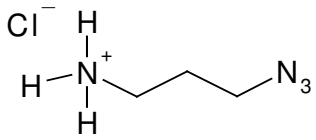


2-azidoethylamine hydrochloride:

The hydrobromide (2.0 g, 9.8 mmol) was dissolved in a saturated aqueous solution of $NaN_3$ (2.6 g, 39.4 mmol, 4 eq) and heated to 90 °C for 16 h. **CAUTION!** as the solution is acidic this causes the formation of volatile and toxic $HN_3$! After cooling the solution to room temperature NaOH was added to make the solution basic (pH > 10). This produces the free amine which was separated from all inorganic compounds by distillation under reduced pressure (100 mbar, bp: 50 – 60 °C). The distillate was an aqueous solution of the amine which was afterwards acidified with 6 N hydrochloric acid. Lyophilisation of this solution gave the desired compound as a colourless, hygroscopic solid (1.10 g, 92 %).

**$^1$H-NMR** (300 MHz, MeOD): $\delta$ = 3.12 (t, $^3J$ = 5.6 Hz, 2 H), 3.75 (t, $^3J$ = 5.6 Hz, 2 H); **$^{13}$C-NMR** (75.5 MHz, MeOD): $\delta$ = 40.1 (–, 1 C), 49.5 (–, 1 C); **MS** (ESI, $H_2O$/MeCN):

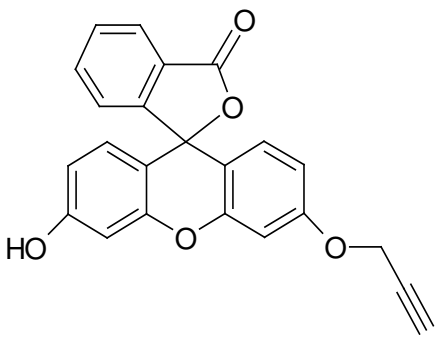
m/z (%) = 87.0 [$MH^+$] (100), 127.9 [$MH^+$+MeCN] (17); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3530, 2988, 2145, 1605, 1498, 1276, 1148; **MF**: $C_2H_7N_4Cl$; **MW** = 122.56 g/mol;

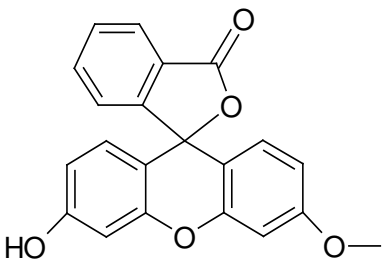


3-azidopropylamine hydrochloride:

The hydrobromide (2.0 g, 9.8 mmol) was dissolved in a saturated aqueous solution of $NaN_3$ (2.6 g, 39.4 mmol, 4 eq) and heated to 90 °C for 16 h. **CAUTION!** as the solution is acidic this causes the formation of volatile and toxic $HN_3$! After cooling the solution to room temperature NaOH was added to make the solution basic (pH > 10). This produces the free amine which was separated from all inorganic compounds by distillation under reduced pressure (100 mbar, bp: 50 – 60 °C). The distillate was an aqueous solution of the amine which was afterwards acidified with 6 N hydrochloric acid. Lyophilisation of this solution gave the desired compound as a colourless, hygroscopic solid (1.1 g; 92 %).

**$^1$H-NMR** (300 MHz, MeOD): $\delta$ = 1.97 (tt, $^3J$ = 6.6 Hz, $^3J$ = 7.4 Hz, 2 H), 3.01 – 3.06 (m, 2 H), 3.52 (t, $^3J$ = 6.6 Hz, 2 H); **$^{13}$C-NMR** (75.5 MHz, MeOD): $\delta$ = 28.0 (–, 1 C), 38.5 (–, 1 C), 49.6 (–, 1 C); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 101.0 [$MH^+$] (100), 142.0 [$MH^+$+MeCN] (13); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3532, 2987, 2147, 1604, 1497, 1278, 1149; **MF**: $C_3H_9N_4Cl$; **MW** = 136.58 g/mol;





Propargylfluorescein (**5**) and Methylfluorescein (**6**):

Fluorescein-disodium-salt (3.8 g, 10 mmol) was suspended in 100 ml DMF and 8.6 ml of a solution of propargyl bromide in toluene (80 %, 11.9 g, 80 mmol) was added.

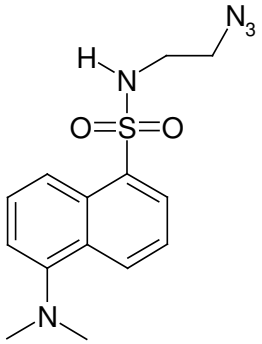
The mixture was stirred at r.t. for 24 h, then a reflux condenser was put on and the suspension was heated to 40 °C for further 24 h. After addition of 150 ml water, a precipitate fell out of solution. The mixture was extracted twice with 300 ml EE each time. In order to redissolve the precipitate completely the mixture had to be heated to approx. 40 °C. The organic layer was washed with 100 ml water, 50 ml brine and dried over $Na_2SO_4$. Removal of the solvent at reduced pressure and drying in vacuum gave a red solid which was suspended in 160 ml MeOH. After addition of 25 ml aqueous NaOH (c = 2 mol/L) the solid dissolved slowly. The mixture was stirred at 40 °C for 4 h. The MeOH was evaporated at reduced pressure and 50 ml water was added. The aqueous solution was washed with diethylether (50 ml, twice). The organic layers were discarded. The aqueous layer was acidified to pH 2 with hydrochloric acid (c = 10 mol/L) to precipitate the product. The suspension was stirred at r.t. for 1 h and filtered. The residue was dissolved in 300 ml EE. The solution was washed with 100 ml water, 50 ml brine and dried over $Na_2SO_4$. Removal of the solvent and drying in vacuum gave a red solid which was purified by column chromatography (diethylether:PE = 2:1). This yielded a mixture of compound **5** and **6** in a ratio of 15:65 (determined by $^1$H-NMR). To separate both compounds the yellow solid is treated with small amounts of diethyl ether several times. Solutions were checked by TLC (eluent: diethylether:PE = 2:1, Rf (**5**) = 0.38, Rf (**6**) = 0.35). Pure solutions of **5** were combined. Removal of the solvent and drying in vacuum finally yielded 555 mg of **5** (15 %, 1.5 mmol).

Compound **5**:

**Mp**: 125 °C; **$^1$H-NMR** (300 MHz, DMSO-d6): δ = 3.64 (t, $^4$J = 2.2 Hz, 1 H, CH), 4.89 (d, $^4$J = 2.2 Hz, 2 H, $CH_2$), 6.57 (s, 1 H), 6.58 (s, 1 H), 6.66 – 6.77 (m, 3 H), 7.00 (d, $^4$J = 2.5 Hz, 1 H), 7.30 (d, $^3$J = 7.7 Hz, 1 H), 7.72 (dt, $^3$J = 7.4 Hz, $^4$J = 1.1 Hz, 1 H), 7.80 (dt, $^3$J = 7.5 Hz, $^4$J = 1.2 Hz, 1 H), 8.01 (d, $^3$J = 7.1 Hz, 1 H), 10.21 (bs, 1 H, OH); **$^{13}$C-NMR** (75.5 MHz, DMSO-d6): δ = 55.7 (–), 78.7 (+, alkyne-CH), 78.8 ($C_{quat}$, alkyne), 82.5 ($C_{quat}$), 101.7 (+), 102.1 (+), 109.3 ($C_{quat}$), 111.6 ($C_{quat}$), 112.3 (+), 112.8 (+), 123.9 (+), 124.6 (+), 125.9 ($C_{quat}$), 128.9 (+), 129.0 (+), 130.1 (+), 135.6 (+), 151.6 ($C_{quat}$), 151.6 ($C_{quat}$), 152.3 ($C_{quat}$), 158.7 ($C_{quat}$), 159.5 ($C_{quat}$), 168.6 ($C_{quat}$); **MS** (ESI, MeOH): m/z (%) = 371.1 [$MH^+$] (100); **UV** (MeOH) $\lambda_{max}$ (log ε) = 453 nm (3.370), 478 nm (3.280); **MF**: $C_{23}H_{14}O_5$; **MW** = 370.36 g/mol;

Compound **6**:

**Mp**: 135 °C; **$^1$H-NMR** (300 MHz, DMSO-d6): $\delta$ = 3.83 (s, 3 H, $CH_3$), 6.57 (s, 1 H), 6.58 (s, 1 H), 6.66 – 6.77 (m, 3 H), 6.93 (d, $^4J$ = 2.5 Hz, 1 H), 7.29 (d, $^3J$ = 7.7 Hz, 1 H), 7.71 (dt, $^3J$ = 7.4 Hz, $^4J$ = 1.1 Hz, 1 H), 7.79 (dt, $^3J$ = 7.5 Hz, $^4J$ = 1.2 Hz, 1 H), 8.00 (d, $^3J$ = 7.1 Hz, 1 H), 10.22 (bs, 1 H, OH); **$^{13}$C-NMR** (75.5 MHz, DMSO-d6): $\delta$ = 55.5 (+), 82.5 ($C_{quat}$), 100.7 (+), 102.1 (+), 109.3 ($C_{quat}$), 110.8 ($C_{quat}$), 112.3 (+), 112.8 (+), 123.9 (+), 124.6 (+), 125.9 ($C_{quat}$), 128.9 (+), 129.0 (+), 130.1 (+), 135.6 (+), 151.6 ($C_{quat}$), 151.6 ($C_{quat}$), 152.3 ($C_{quat}$), 159.5 ($C_{quat}$), 160.9 ($C_{quat}$), 168.6 ($C_{quat}$); **MS** (ESI, MeOH): m/z (%) = 347.1 [$MH^+$] (100); **UV** (MeOH) $\lambda_{max}$ (log $\varepsilon$) = 453 nm (3.366), 478 nm (3.275); **MF**: $C_{21}H_{14}O_5$; **MW** = 346.34 g/mol;

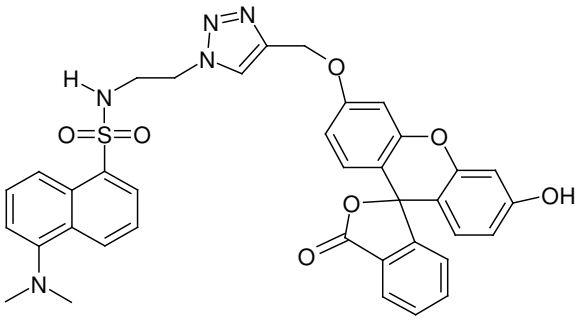



5-Dimethylamino-naphtalene-1-sulfonic acid (2-azido-ethyl)-amide (**7**):

Dansyl chloride (864 mg, 3.2 mmol) was dissolved in 10 ml DCM. To this solution 1.78 ml of $NEt_3$ (1296 mg, 12.8 mmol, 4 eq) and 2-azido-ethylamine hydrochloride (785 mg, 6.4 mmol, 2 eq) were added. The mixture was stirred 1 h at room temperature and diluted with 40 ml DCM as TLC showed almost 100 % conversion. The solution was washed once with 50 ml of saturated aqueous solution of $NaHCO_3$, dried over $Na_2SO_4$, filtered and concentrated at reduced pressure. The crude product was purified by column chromatography over a short silica gel column, eluting with EE:PE = 7:3. This gave the dansyl derivative **7** as light yellow oil, which crystallized after drying in vacuum (972 mg, 95 %). $R_f$ (EE:PE = 1:3) = 0.30.

**$^1$H-NMR** (300 MHz, $CDCl_3$): $\delta$ = 2.88 (s, 6 H, 2 $CH_3$), 3.05 (dt, $^3J$ = 5.8 Hz, $^3J$ = 6.3 Hz, 2 H, $CH_2$), 3.28 (t, $^3J$ = 5.8 Hz, 2 H, $CH_2$), 5.43 (t, $^3J$ = 6.3 Hz, 1 H, NH), 7.18 (dd, $^3J$ = 7.7 Hz, $^4J$ = 1.0 Hz, 1 H), 7.48 – 7.58 (m, 2 H), 8.24 (dd, $^3J$ = 7.3 Hz, $^4J$ = 1.3 Hz, 1 H), 8.29 (d, $^3J$ = 8.8 Hz, 1 H), 8.55 (dt, $^3J$ = 8.5 Hz, $^4J$ = 1.1 Hz, 1 H); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): $\delta$ = 41.3 (–), 44.4 (+), 49.8 (–), 114.3 (+), 117.5 (+),

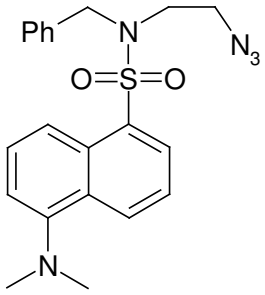
122.1 (+), 127.6 (+), 128.4 ($C_{quat}$), 128.5 (+), 128.8 ($C_{quat}$), 129.7 (+), 133.5 ($C_{quat}$), 150.9 ($C_{quat}$); **MS** (ESI, DCM/MeOH): m/z (%) = 320.2 [$MH^+$] (100), 639.4 [$2M+H^+$] (20); **EA** ($C_{14}H_{17}N_5SO_2$) calc.: C 52.65 H 5.37 N 21.93, found: C 52.71 H 5.44 N 21.79; **UV** (MeOH) $\lambda_{max}$ (log $\varepsilon$) = 336 nm (3.684); **MF**: $C_{14}H_{17}N_5SO_2$; **MW** = 319.38 g/mol;



(5-Dimethylamino-naphtalene-1-sulfonic acid [2-(4-methyl-[1,2,3]triazol-1-yl)-ethyl]-amidyl)-fluorescein (**8**):

The azide **7** (51 mg, 0.16 mmol) and the alkyne **5** (59 mg, 0.16 mmol) were dissolved in 4 ml EtOH and a solution of 63 mg sodium ascorbate (0.32 mmol, 2 eq) in 1 ml water was added. Finally a solution of 40 mg $CuSO_4$ x 5 $H_2O$ (0.16 mmol, 1 eq) in 0.5 ml water was added and the mixture was stirred at r.t. for 3 h. TLC after that time showed complete conversion. The mixture was diluted with 20 ml water and extracted with EE (50 ml, twice). The organic phases were combined, dried over $Na_2SO_4$ and concentrated at reduced pressure. The resulting orange solid was purified by column chromatography (DCM:MeOH = 98:2 to 95:5). This gave **8** as yellow solid in a yield of 101 mg (92 %, 0.15 mmol). $R_f$ (DCM:MeOH = 97:3) = 0.2.

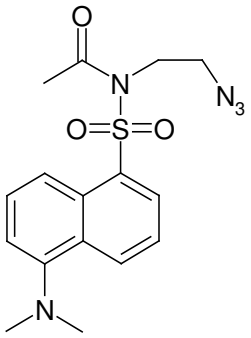
**$^1$H-NMR** (300 MHz, acetone-d6): $\delta$ = 2.87 (s, 6 H, 2 $CH_3$), 3.44 – 3.50 (m, 2 H, $CH_2$), 4.80 (t, $^3J$ = 5.9 Hz, 2 H, $CH_2$), 5.13 (s, 2 H, $CH_2$), 6.62 – 6.81 (m, 5 H), 6.99 – 7.01 (m, 1H), 7.17 (t, $^3J$ = 6.2 Hz, 1 H, NH), 7.23 – 7.28 (m, 2 H), 7.50 – 7.62 (m, 2 H), 7.68 – 7.80 (m, 2 H), 7.84 (s, 1 H, triazole), 7.97 – 8.01 (m, 1 H), 8.21 (dd, $^3J$ = 7.4 Hz, $^4J$ = 1.1 Hz, 1 H), 8.32 (d, $^3J$ = 8.8 Hz, 1 H), 8.57 (d, $^3J$ = 8.5 Hz, 1 H), 9.15 (bs, 1 H, OH); Due to low solubility of **8** in all organic solvents and overlapping of too many signals $^{13}$C-NMR spectrum is not evaluable; **MS** (ESI, DCM/MeOH): m/z (%) = 690.2 [$MH^+$] (100); **UV** (MeOH) $\lambda_{max}$ (log $\varepsilon$) = 336 nm (3.688), 453 nm (3.375), 478 nm (3.285); **MF**: $C_{37}H_{31}N_5SO_7$; **MW** = 689.74 g/mol;




5-Dimethylamino-naphtalene-1-sulfonic acid (2-azido-ethyl)-benzyl-amide (**9**):

The dansyl derivative **7** (200 mg, 0.63 mmol) was dissolved in 3 ml dry DMF. The solution was cooled to 0 °C in an ice-bath. After addition of NaH (60 % suspension in paraffine, 28 mg, 0.69 mmol, 1.1 eq) 150 µl of benzyl bromide (214 mg, 1.25 mmol, 2 eq) was added dropwise. The ice-bath was removed and the solution was stirred for 6 h while warming to room temperature. TLC after that time showed complete conversion. Water (20 ml) is added and the resulting mixture is extracted with EE (30 ml, twice). The organic layer is dried over $Na_2SO_4$ and solvent is removed at reduced pressure. This gave a brown oil which was purified by column chromatography (EE:PE = 8:2). Drying in vacuum yielded compound **9** as greenish yellow oil (254 mg, 0.62 mmol, 99 %). $R_f$ (EE:PE = 1:3) = 0.37.

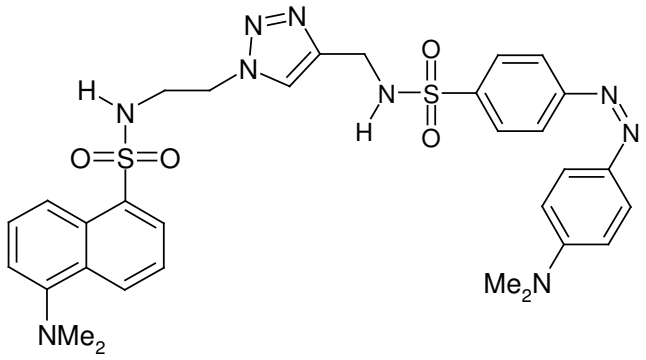
**$^1$H-NMR** (300 MHz, $CDCl_3$): $\delta$ = 2.90 (s, 6 H, 2 $CH_3$), 3.20 (t, $^3J$ = 6.6 Hz, 2 H, N-$CH_2$), 3.38 (t, $^3J$ = 6.6 Hz, 2 H, $CH_2$), 4.53 (s, 2 H, Bn-$CH_2$), 7.17 – 7.28 (m, 6 H, arom. CH), 7.50 – 7.63 (m, 2 H), 8.26 (dd, $^3J$ = 7.3 Hz, $^4J$ = 1.2 Hz, 1 H), 8.38 (d, $^3J$ = 8.8 Hz, 1 H), 8.58 (dt, $^3J$ = 8.5 Hz, $^4J$ = 1.1 Hz, 1 H); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): $\delta$ = 45.5 (+), 45.6 (–, N-$CH_2$), 49.8 (–), 52.3 (–, Bn), 115.4 (+, 1 C), 119.3 (+, 1 C), 123.2 (+, 1 C), 128.1 (+, 1 C), 128.4 (+, 1 C), 128.6 (+, 2 C), 128.8 (+, 2 C), 130.1 (+, 1 C), 130.1 ($C_{quat}$, 1 C), 130.1 ($C_{quat}$, 1 C), 130.8 (+, 1 C), 134.6 ($C_{quat}$, 1 C), 135.7 ($C_{quat}$, 1 C), 151.9 ($C_{quat}$); **MS** (ESI, DCM/MeOH): m/z (%) = 410.1 [$MH^+$] (100), 819.5 [$2M+H^+$] (5); **EA** ($C_{21}H_{23}N_5SO_2$) calc.: C 61.59 H 5.66 N 17.10, found: C 61.72 H 5.74 N 17.00; **UV** (MeOH) $\lambda_{max}$ (log $\varepsilon$) = 337 nm (3.694); **MF**: $C_{21}H_{23}N_5SO_2$; **MW** = 409.51 g/mol;



5-Dimethylamino-naphtalene-1-sulfonic acid acetyl-(2-azido-ethyl)-amide (**10**):

The sulfonamide **7** (281 mg, 0.88 mmol) was dissolved in 12 ml dry DCM. After addition of 818 µl $NEt_3$ (594 mg, 3.52 mmol, 4 eq), 333 µl acetanhydride (359 mg, 3.52 mmol, 4 eq) and 11 mg DMAP (0.09 mmol, 10 mol%) the solution was stirred at room temeprature for 2 h. TLC after that time showed complete conversion. The solution was diluted with 200 ml DCM and washed with 100 ml sat. aqueous $NaHCO_3$ and 100 ml brine. The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. The resulting brown oil was purified by column chromatography (PE:EE = 8:2) to yield 314 mg of **10** as a yellow oil (0.87 mmol, 99 %). $R_f$ (EE:PE = 1:3) = 0.40.

**$^1$H-NMR** (300 MHz, $CDCl_3$): $\delta$ = 2.32 (s, 3 H, $CH_3$), 2.86 (s, 6 H, 2 $CH_3$), 3.56 (t, $^3J$ = 6.6 Hz, 2 H, $CH_2$), 4.09 (t, $^3J$ = 6.6 Hz, 2 H, N-$CH_2$), 7.18 (dd, $^3J$ = 7.7 Hz, $^4J$ = 1.0 Hz, 1H), 7.51 – 7.60 (m, 2 H), 7.99 (d, $^3J$ = 8.5 Hz, 1 H), 8.18 (dd, $^3J$ = 7.4 Hz, $^4J$ = 1.4 Hz, 1 H), 8.60 (dt, $^3J$ = 8.5 Hz, $^4J$ = 1.1 Hz, 1 H); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): $\delta$ = 25.3 (+), 45.2 (–), 45.4 (+), 49.8 (–), 115.7 (+), 117.8 (+), 123.1 (+), 129.2 (+), 129.5 ($C_{quat}$), 129.8 (+), 130.0 ($C_{quat}$), 132.0 (+), 134.2 ($C_{quat}$), 152.3 ($C_{quat}$); **MS** (ESI, DCM/MeOH): m/z (%) = 362.1 [$MH^+$] (100); **EA** ($C_{16}H_{19}N_5SO_3$) calc.: C 53.17 H 5.30 N 19.38, found: C 53.31 H 5.41 N 19.18; **UV** (MeOH) $\lambda_{max}$ (log $\varepsilon$) = 350 nm (3.577); **MF**: $C_{16}H_{19}N_5SO_3$; **MW** = 361.42 g/mol;



5-Dimethylamino-naphtalene-1-sulfonic acid [2-(4-{[4-(4-dimethylamino-phenylazo)-benzenesolfonylamino]-methyl}-[1,2,3]triazol-1-yl)-ethyl]-amide (**12**):

In a 500 mL round-bottomed flask compounds **7** (192 mg, 0.60 mmol) and **11** (206 mg, 0.60 mmol) were dissolved in 60 mL DMSO. Water (30 mL) was added, followed by aqueous sodium ascorbate (0.2 mL of 1 M stock solution) and aqueous copper sulfate (0.6 mL of 100 mM stock solution). The reaction was followed by TLC, eluting with 1:1 PE:EE. After stirring overnight, the reaction was found to be complete, and 150 mL water was added to precipitate the product. After standing open to air for several hours, the orange solid was filtered and washed with water. The product was dried to give 314 mg of triazole **12** (0.48 mmol, 79 %). The orange solid was recrystallized from acetonitrile to give red crystals.

**$^1$H-NMR** (300 MHz, DMSO-d6): $\delta$ = 2.79 (s, 6 H, 2 $CH_3$), 3.06 (s, 6 H, 2 $CH_3$), 3.22 (t, $^3J$ = 6.0 Hz, 2 H, $CH_2$), 4.00 (s, 2 H, $CH_2$), 4.32 (t, $^3J$ = 6.0 Hz, 2 H, $CH_2$), 6.83 (d, $^3J$ = 9.3 Hz, 2 H), 7.21 (d, $^3J$ = 7.4 Hz, 1 H), 7.54 – 7.62 (m, 2 H), 7.76 (s, 1 H), 7.83 (d, $^3J$ = 9.1 Hz, 2 H), 7.88 – 7.95 (m, 4 H), 8.08 (dd, $^3J$ = 7.3 Hz, $^4J$ = 1.0 Hz, 1 H), 8.21 – 8.24 (m, 3 H), 8.44 (d, $^3J$ = 8.5 Hz, 1 H); **$^{13}$C-NMR** (75.5 MHz, DMSO-d6): $\delta$ = 37.9 (–), 39.7 (+), 42.25 (–), 44.9 (+), 48.9 (–), 111.5 (+), 115.0 (+), 118.85 (+), 122.0 (+), 123.4 (+), 123.51 (+), 125.3 (+), 127.8 (+), 128.3 (+), 128.8 ($C_{quat}$), 128.9 ($C_{quat}$), 129.5 (+), 135.2 ($C_{quat}$), 140.0 ($C_{quat}$), 142.5 ($C_{quat}$), 142.9 ($C_{quat}$), 151.2 ($C_{quat}$), 153.0 ($C_{quat}$), 154.4 ($C_{quat}$); **MS** (ESI, DCM/MeOH): m/z (%) = 662.3 [$MH^+$] (100); **EA** ($C_{31}H_{35}N_9S_2O_4$) calc.: C 56.26 H 5.33 N 19.05, found: C 56.34 H 5.38 N 19.19; **UV** (MeCN) $\lambda_{max}$ (log $\varepsilon$) = 337 nm (3.679), 443 nm (4.478); **MF**: $C_{31}H_{35}N_9S_2O_4$; **MW** = 661.80 g/mol;

## 1.6. References


[1] See e.g. O.P. Ernst, F.J. Bartl, *ChemBioChem* **2002**, *3*, 968.

[2] Allosteric is defined as: A term to describe proteins that have two or more receptor sites, one of which (the active site) binds the principal substrate, whereas the other(s) bind(s) effector molecules that can influence its biological activity. [www.nature.de]

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# 2. Target Guided Synthesis of Bidentate Metal Complex Receptors

## 2.1. Introduction

### 2.1.1. Target Guided Synthesis

During the last 20 years there has been an immense progress in drug discovery. While at the beginning only small numbers of natural products and compounds, derived from classical organic synthesis, were investigated for their pharmaceutical application, nowadays large combinatorial libraries are tested by high-throughput screening.[1] These developments are of course bound to as well huge improvements in methods for producing, handling, and screening large numbers of compounds.[2,3,4,5] Despite these achievements, there are still challenges, related to the synthesis, purification, and diversity of compound libraries as well as the pharmacological properties of their members.[6,7] Further, combinatorial chemistry still does not exhaust its possibilities completely.[8,9] In a given screen usually less than 1 % of all compounds in a library emerge to be active, which makes the search time consuming, expensive and thus inefficient. Therefore, methods for producing just the active compounds are highly desirable. Target-guided synthesis (TGS) seeks to address this challenge by using the target molecule itself for forming its own inhibitors from a collection of building block reagents.

The approach is that only building blocks which interact with the separate binding sites on the protein's surface will react with each other, if their reactive groups are in close proximity. This will result in highly potent protein-effectors as the resulting polydentate receptor simultaneously accesses multiple binding pockets within the protein. The newly formed inhibitors usually display much higher binding affinities for their biological targets than the individual components, since they simultaneously engage in multiple binding interactions.[10] These target-guided approaches avoid the classical screening of large compound libraries. The hit identification can be as simple as determining whether a given combination of building blocks has resulted in a product. The following tests for determining the potency, bioavailability and toxicity of the effector, as well as the development of structure-activity relationships (SAR)

can then be limited to a small number of compounds. This means a huge improvement of the efficiency of the discovery process.

The pioneer in TGS was Rideout *et al.*, who observed a marked synergism between the cytotoxic effects of decanal and *N*-amino-guanidines, which was proposed to be due to the selfassembly of cytotoxic hydrazones inside cells.[11,12] Since then, several approaches to target-guided synthesis have been explored which use different effects of the target on the receptor-formation:

I. dynamic combinatorial chemistry [13 – 22]
II. stepwise target-guided synthesis [23]
III. kinetically controlled TGS [24 – 32]

I.) Dynamic combinatorial chemistry

The dynamic combinatorial chemistry approach was introduced by Lehn et al.[13] It is based on the principle of shifting a thermodynamical equilibrium. Building blocks, which react *reversibly* with each other, form a *thermodynamically controlled* mixture of products. In the presence of the target molecule, the equilibrium is shifted toward the compounds that show the highest affinity towards the effector. In order to identify all reaction products, the equilibrium has to be "frozen" (for example, by hydride reduction or by lowering the pH). Then, the analysis by HPLC, LC-MS or MS can be performed. Disadvantages are: the equilibrium can be affected not only by the target molecule, a complex mixture of several products has to be analyzed and the additional reaction step to "freeze" the equilibrium might be limiting the number of building blocks due to incompatible functional groups.

II.) Stepwise target-guided synthesis

The stepwise TGS makes only indirect use of the target molecule for effector synthesis and consists of three steps.[23] First, a library of building blocks is screened to identify candidates that bind to the target. Then, the building blocks with the highest affinity are linked together, using conventional combinatorial chemistry approaches. The resulting library of "divalent" effectors is then screened to find receptors with a high binding constant, using traditional assays. Disadvantages are:

two screenings of possibly a lot of compounds have to be done and the combination of two high-affinity building blocks will not necessarily result in the best performing bidantate receptor.

III.) Kinetically controlled TGS

The kinetically controlled approach is based on three principles in which it fundamentally differs from 1): an *irreversible* reaction between *pairs* of building blocks is influenced by the target molecule, which acts like a catalyst, leading to the formation of the *kinetic product*. The target accelerates most the formation of the product that best fits its binding pockets, as it lowers the energy of its transition state. In principle, hit discovery becomes independent of the actual function of the target because it relies solely on its ability to bind reagents and hold them in close proximity until they become connected through an “arranged” chemical reaction.

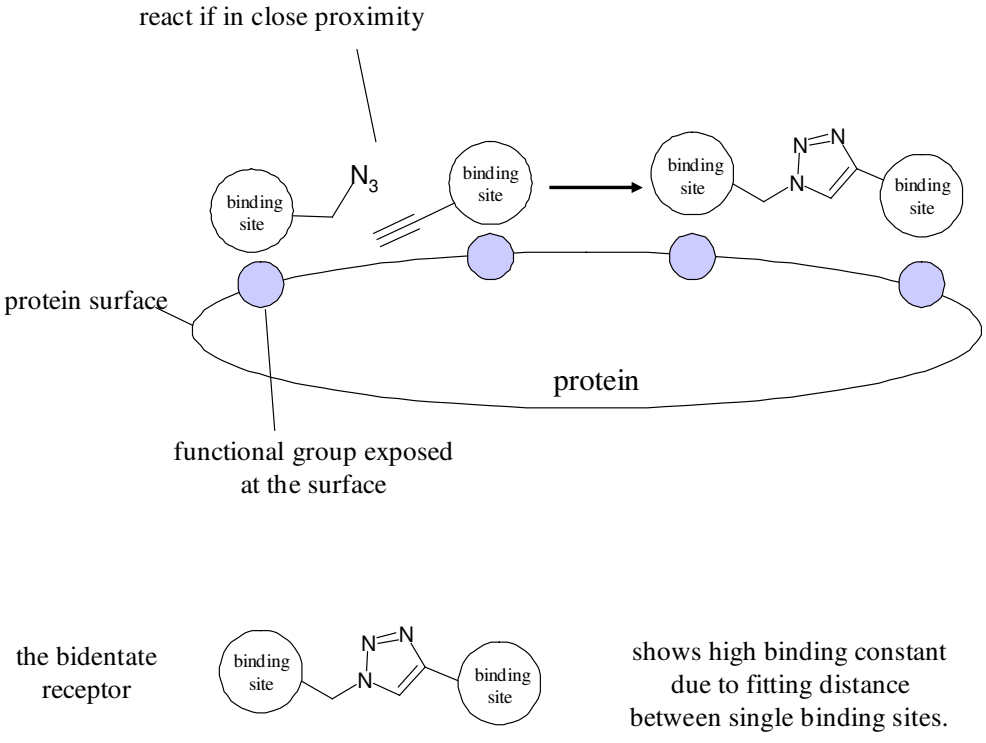



Figure 1: Model to synthesize high affinity receptors using the click-chemistry approach and the target molecule as template.

So far, several successful applications of the kinetically controlled approach to TGS have been reported. For example, Benkovic and Boger have developed multisubstrate adduct inhibitors (MAI) of the enzyme glycinamide ribonucleotide transformylase (GAR Tfase). In this case, the enzyme-templated alkylation of one of its substrates with a folate-derived electrophile led to highly potent inhibitors.[26,27] Recently, Huc reported about a similar approach, in which inhibitors of carbonic anhydrase were generated by alkylation of a thiol with R-chloroketones in the presence of the Zn(II)-enzyme.[28] The template-effect of the enzyme on the reaction favoured the formation of the alkylation product with the highest affinity for the target, which was revealed by competition-experiments.

Nicolaou and co-workers have utilized the kinetically controlled variant of TGS in combinatorial synthesis to develop dimeric derivatives of vancomycin.[29] Appropriately functionalized monomeric vancomycin derivatives were connected via olefin metathesis or disulfide formation in the presence of vancomycin's target, Ac-D-Ala-D-Ala or $Ac_2$-**L**-Lys-**D**-Ala-**D**-Ala. This resulted in the formation of highly potent dimers.

The scope of most TGS methods is rather limited, because highly reactive reagents (strong electrophiles or nucleophiles, metathesis catalysts etc.) are used. As high reactivity usually goes hand in hand with low selectivity, those reagents can react in several pathways, including ones that even destroy the protein target. In contrast, the recently developed in situ click-chemistry approach to kinetically controlled TGS[24] uses bioorthogonal reactions and reagents, for example, the [1,3]-dipolar cycloaddition reaction[33] between azides and acetylenes. For four reasons this system is especially well-suited for TGS:

1) The reaction is extremely slow at room temperature (activation enthalpy of approx. 25 kcal/mol), despite the very high driving force that makes it irreversible (reaction enthalpy > 50 kcal/mol).[34]
2) Certain catalysts strongly stabilize the transition state and therefore dramatically lower the activation enthalpy.[35]
3) It does not involve components that might disturb the binding sites (external reagents, catalysts, byproducts).[36]
4) The reactants are inert to biological molecules.

Mock et al. had previously provided proof-of-concept by demonstrating that the triazole formation is accelerated by 4 to 5 orders of magnitude by the synthetic receptor cucurbituril to give exclusively the *anti*-triazole regioisomer.[37]

### 2.1.2. The "Click-Chemistry" Approach in Target-Guided Synthesis

The method to synthesize high affinity receptors is based on the well known Huisgen reaction. The 1,3-dipolar cycloaddition between azides and alkynes leads to 1,4- and 1,5-substituted triazoles.[33] The groups of Sharpless and Meldal separately discovered the Cu(I)-catalyzed variation of this reaction, which allows very fast and efficient formation of exclusively 1,4-triazoles at mild reaction conditions.[38] This reaction can be used to obtain well fitting receptors. Sharpless and Finn *et al*. obtained inhibitors acetylcholineesterase (AChE) in the presence of the enzyme as template.[39,40,41] The reaction between receptor precursors was accelerated when the reactive groups (azide and alkyne) were in close proximity to each other. Therefore, this reaction corresponds to the selection of receptors from a virtual library of substances.[42]

The working groups of Sharpless and Kolb managed to go one step further by using the target molecule itself as the catalytic species in a kinetically controlled TGS-approach to yield high affinity inhibitors of AChE (mouse or electric eel AChE).[30] They synthesized a building block library of azides and acetylenes based on the known site-specific inhibitor tacrine (binding to the active site) and phenylphenanthridinium (PP) or phenyltetrahydro-isoquinolines (PIQ) (binding to the peripheral site). A series of binary mixtures of these reagents was incubated with the enzyme as template (several hours at room temperature) in order to test, whether it would combine selected pairs of complementary reagents to synthesize its "divalent" inhibitors (figure 2).[24] Analysis of the crude reaction mixtures by desorption/ionization on silicon mass spectrometry (DIOS-MS)[43] revealed only one product for the combination of tacrine with PP and two for the combination of tacrine with PIQ. All inhibitors showed dissociation constants in the femtomolar region.

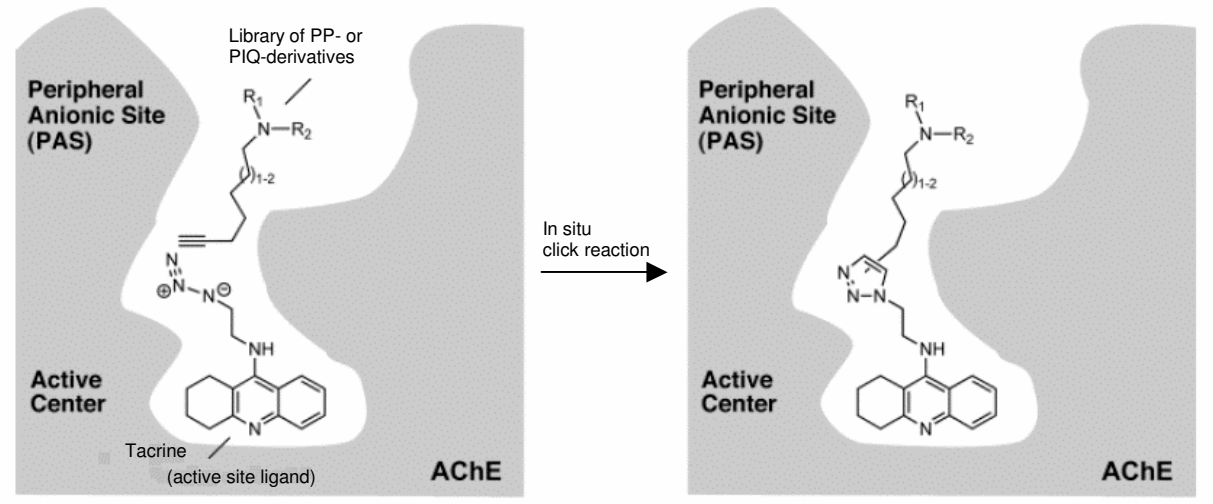



Figure 2: In situ click chemistry between tacrine (active site binder) and PP or PIQ derivatives to screen for highly potent AChE-inhibitors.

A more interesting result was, that in all 3 cases HPLC showed only the 1,5-disubstituted triazole ("*syn*-triazole"). The corresponding 1,4-isomers turned out to be less active by two orders of magnitude. Further, studies of X-ray structures revealed that the triazole unit, created by the azide/alkyne cycloaddition, engages in hydrogen bonding and stacking interactions with amino acid residues in the wall of the gorge.[44] The important conclusion that can be drawn from this observation is that triazoles are not just passive linkers, but rather active pharmacophores that may contribute significantly to protein binding.

This approach was also used to obtain inhibitors for bovine carbonic anhydrase II (bCA II).[31] The building blocks to derive divalent receptors were 4-acetylene-benzenesulfonamide (binding to bCA II with nanomolar affinity) and several azide substituted alkanes, aminoalkanes, carbonic acids, stilbenes and benzenes. The choice of building blocks was related to the work of Whitesides.[45] Results of incubation experiments with the enzyme as template showed that 11 of 24 possible triazoles were formed. Analysis by LC-MS revealed that all of them were 1,4-triazoles. In contrast to AChE, the enzyme-catalyzed reaction by bCA II was highly *anti*-selective. Results of binding experiments showed an increase for all 11 "hits". Compared to 4-acetylene-benzenesulfonamide, binding affinities were higher up to two orders of magnitude ($K_D$ = 0.2 – 7.1 nM).

Dervan et al. demonstrated that this method is not limited to proteins.[32] The azide/acetylene cycloaddition was used to explore the double-stranded DNA-templated interconnection of hairpin polyamides in the minor groove. This gave hairpin dimers in site-specific fashion, which are capable of targeting longer DNA-sequences.

### 2.1.3. Scope

The aim of this chapter is the synthesis of new bidentate metal-complex receptors with high binding affinities for peptides and small proteins. The synthesis is based on the Cu(I)-catalyzed cycloaddition and shall be done following two different approaches:

- the kinetically controlled TGS at physiological conditions using the target molecule as template. Hit detection will be done in situ, using mass spectroscopy. Binding constants will be determined using standard spectroscopical methods like NMR or UV/Vis.
- the stepwise TGS in order to build up bidentate receptors which cannot be derived from the kinetically controlled TGS. Hit detection and determination of binding constants will be done in a screening assay.

The following receptor molecules with binding sites for certain functional groups of peptides shall be synthesized: Zinc(II)-cyclen complexes for imide groups,[46] bis-zinc(II)-cyclen complexes for phosphate groups,[47] copper(II)- and zinc(II)-nitrilotriaceticacid (NTA) complexes for (N-terminal) histidines,[48] guanidines for carboxylate anions [49] and porphyrins for non polar regions on the protein surface.[50] The receptors as well as the precursors have to be soluble in water. Every receptor precursor has to contain either an azide or an alkyne group.

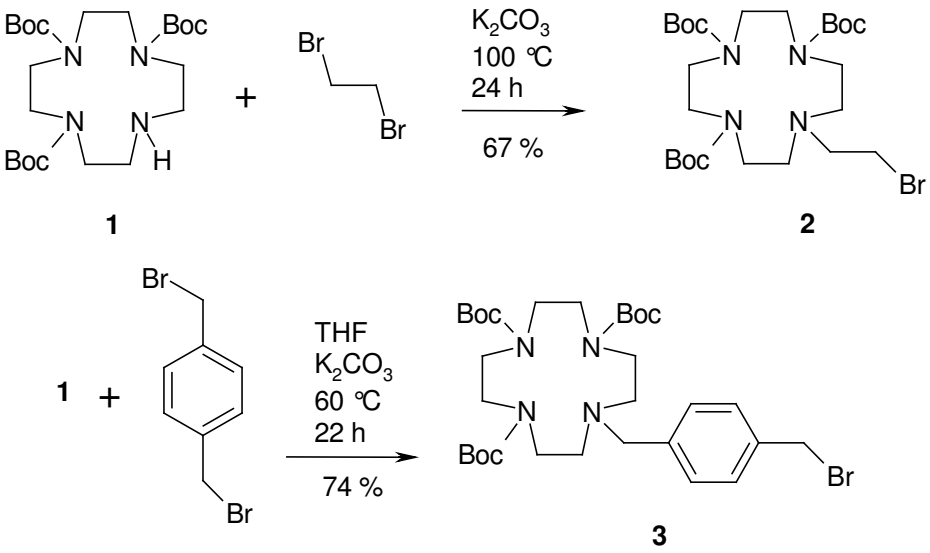
## 2.2. Kinetically Controlled Target Guided Synthesis

After synthesis of the receptor precursors, different types of precursors were connected using the template effect of the target molecule. Synthesis was done following standard peptide coupling-, $S_N$- and complexation-reactions. The target was a biologically active pentapeptide [51] with following AA-sequence: H-LHis-LLeu-LLeu-LVal-LPhe-OLi. In order to detect the in situ formed high affinity receptors, a simple mixture of receptor precursors and the peptide in buffered solution was analyzed with mass spectroscopy.

### 2.2.1. Synthesis of Receptor Building Blocks

#### *2.2.1.1. Synthesis of Zn(II)-Cyclen Complexes*

The synthesis of Zn(II)-Cyclen complexes with an azide functionality started as usual with threefold Boc-protected cyclen (**1**). Compound **1** was reacted with 1,3-Dibromoethane, or α,α-Dibromo-paraxylol, in a typical $S_N$-reaction. The use of potassium iodide would yield a mixture of bromides und iodides and, therefore, cannot be used. In order to avoid twofold substitution, the bromides were used in large excess. The reactions gave the desired bromides in acceptable yields of 67 % for **2** and 74 % for **3** (scheme 1). The corresponding bis-Cyclen compounds, formed after twofold substitution, were isolated as side products.

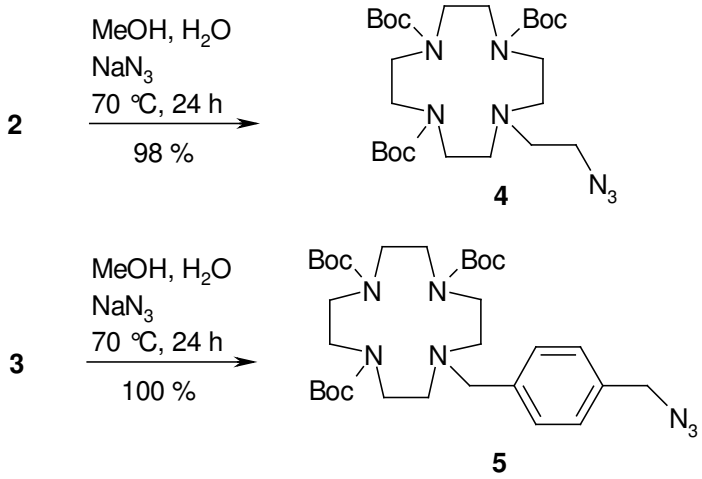



Scheme 1: Synthesis of substituted Boc-cyclen compounds **2** and **3**.

The next reaction step was the transformation of the bromides into azides. This was achieved in two different ways.

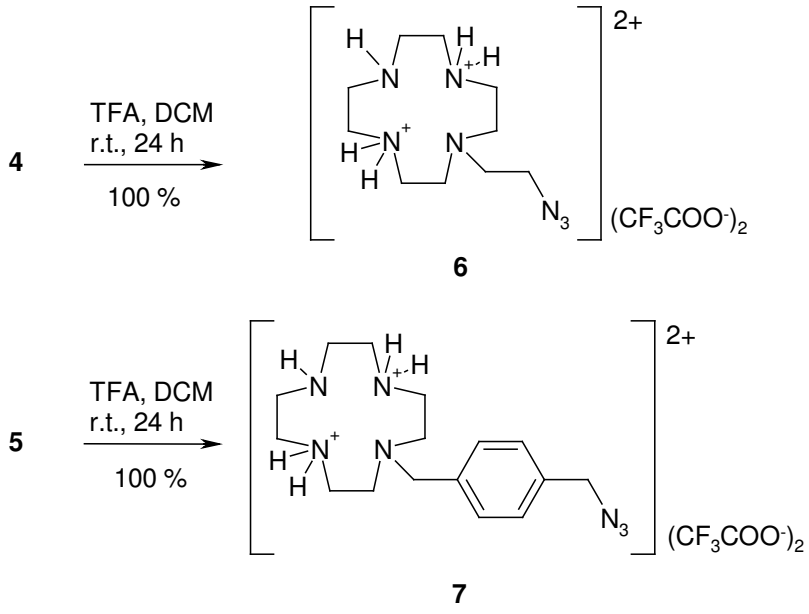
The first one was the use of a strongly basic anion exchanger, which was loaded with azide ions. The general advantage of this method is that a suitable bromide dissolved in any organic solvent simply has to be shaken with an according amount of ion exchanger for some hours at room temperature. After the reaction, the exchanger is filtered and washed. Repeating this procedure several times, a quantitative conversion can be achieved without using large excesses of ion exchanger. However, experimental results showed that compounds **2** and **3** were adsorbed by the polymer material of the exchanger beads. Further, organic solvents partly dissolved unidentified compounds from the exchanger material, which were detected as impurities in the NMR spectra of the raw products. For this reason, purification with column chromatography was necessary. This gave the azides **4** and **5** in yields of 92 %.

The second method proved to be the better one. The bromides **2** and **3** were dissolved in MeOH and a saturated solution of $NaN_3$ was added. After a reaction time of 24 h and a very simple workup (extraction and drying in vacuum), the desired azides were obtained in nearly quantitative yields and in sufficient purity. Compounds **4** and **5** were not stable at room temperature and decomposed slowly while becoming yellow. Storage in the freezer is recommended but will not stop the decomposition process.



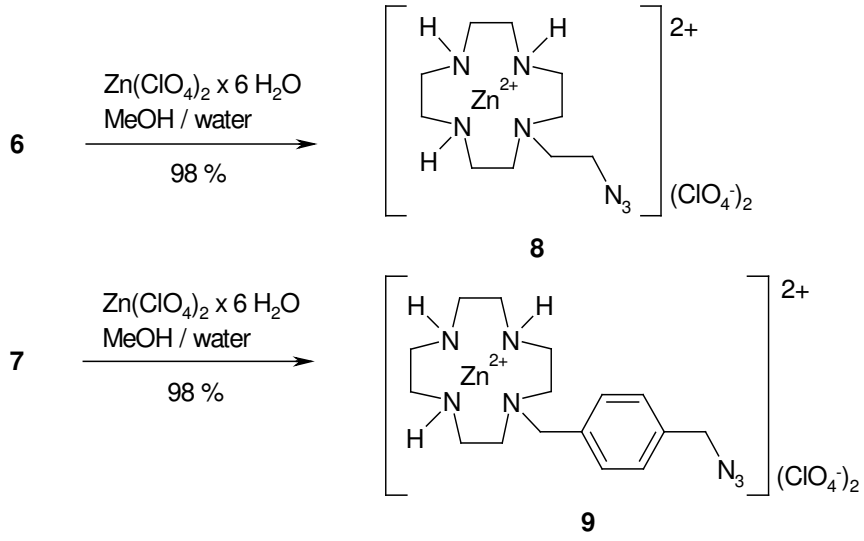

Scheme 2: Synthesis of azides **4** and **5**.

After the introduction of the desired azide functionality the Boc-protecting groups had to be cleaved. This was achieved with TFA in DCM, which yielded the trifluoro acetate salts **6** and **7** as yellow oils in sufficient purity. Both compounds decomposed slowly as well as the precursors.



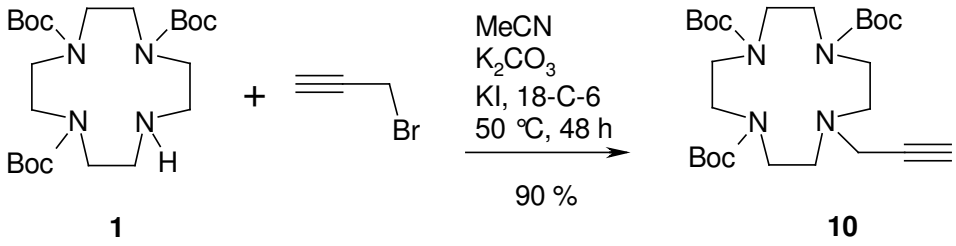

Scheme 3: Cleavage of the Boc-protecting groups with TFA in DCM.

The last two reaction steps were deprotonation of the cyclen ligand with a strongly basic anion exchanger (loaded with $OH^-$) and the following complexation with $Zn(ClO_4)_2$ x 6 $H_2O$ in a mixture of MeOH/water. In order to achieve nearly quantitative precipitation of the complexes **8** and **9**, EtOH was added to the reaction mixture.



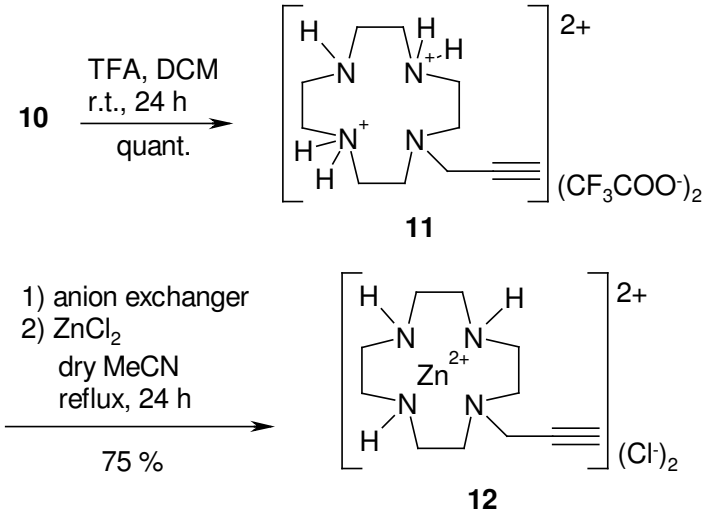

Scheme 4: Formation of the Zn(II)-cyclen complexes **8** and **9**.

The synthesis of a Zn(II)-cyclen complex with an alkyne functionality started with **1** and propargyl bromide. As propargyl bromide is quite volatile the reaction temperature was limited to 50 °C. In order to support reaction conversion, catalytic amounts of potassium iodide and 18-C-6 were added to the reaction mixture. The alkyne **10** was obtained in a good yield of 90 %.



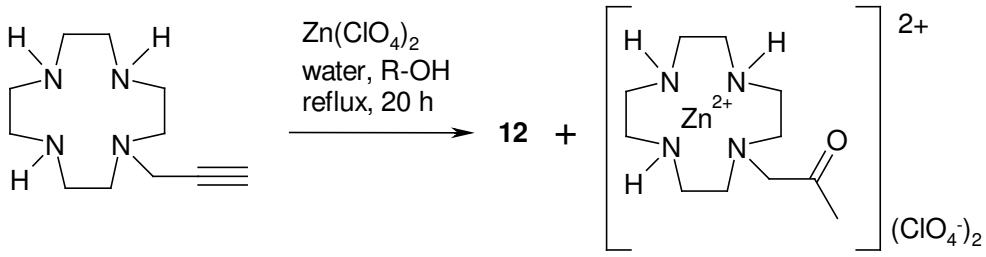

Scheme 5: Synthesis of alkyne **10**.

Following, the Boc-protecting groups were cleaved using TFA in DCM. This gave compound **11** in quantitative yield. Recrystallisation of the raw product from MeCN gave colourless crystals of the TFA salt (see exp. section for structure). Deprotonation was achieved with a strongly basic anion exchanger, loaded with $OH^-$. For complexation of $Zn^{2+}$ water-free reaction conditions had to be chosen. Therefore, the reaction was carried out in dry acetonitrile using $ZnCl_2$. This gave the Zn(II)-cyclen complex **12** in a yield of 75 %.




Scheme 6: Cleavage of the Boc-protecting groups with TFA and complexation of $Zn^{2+}$ under water-free conditions in dry MeCN.

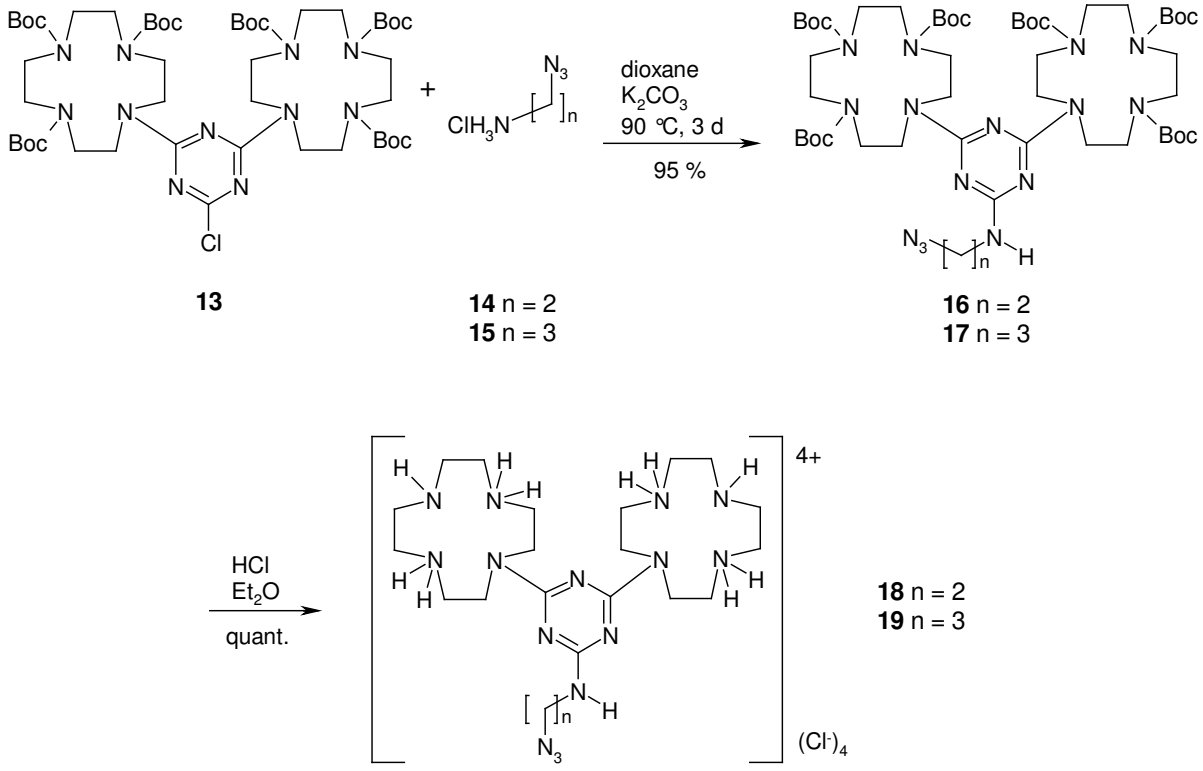
When the complexation reaction was carried out in aqueous solutions of MeOH or EtOH, the already formed **12** catalyzed the addition of water to the triple bond, in analogy to the mercury catalyzed reaction leading to enoles which tautomerize to ketones. [52]




Scheme 7: Complexation in aqueous solutions led to a ketone, due to $Zn^{2+}$ catalyzed addition of water to the triple bond.

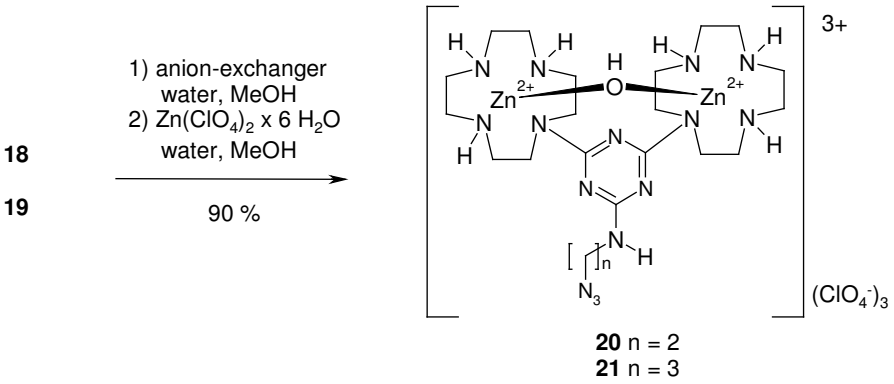
### *2.2.1.2. Synthesis of Bis-Zn(II)-Cyclen Complexes*

For the synthesis of bis-Zn(II)-cyclen-complexes the Boc-protected Bis-cyclen **13** was used as starting compound. A nucleophilic aromatic substitution reaction with the ammonium-salt **14** or **15** introduced the desired azide-group. Afterwards the Boc-groups were cleaved using a saturated solution of HCl in diethylether.




Scheme 8: Synthesis of Boc-protected Bis-cyclen-compounds **16** and **17** with azide-group and following cleavage of the Boc-groups.[a]

Following the treatment of **18** and **19** with a strongly basic anion-exchanger (loaded with $OH^-$) in aqueous solution and the reaction of the free amines with zinc perchlorate in methanol gave the desired compexes **20** and **21** in good yields.

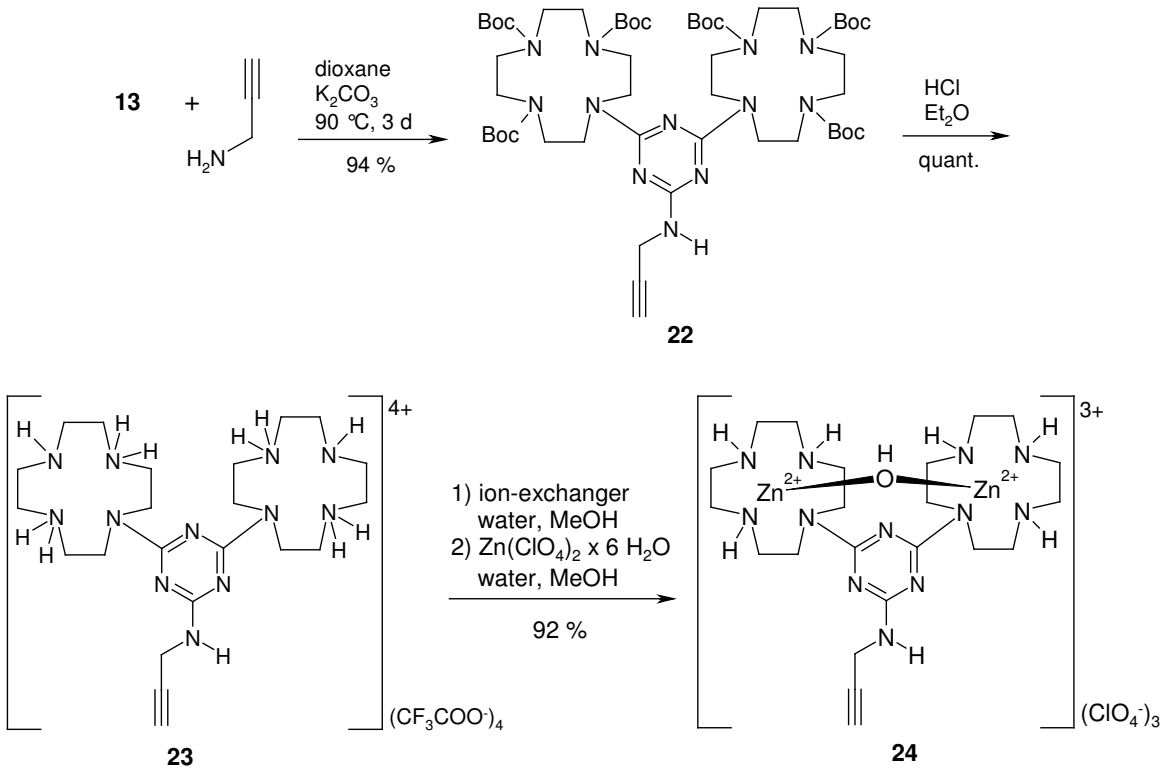



Scheme 9: Synthesis of Zn(II)-complexes **20** and **21**.

The synthesis of the complex **24** was done in the same way as the synthesis of **20** and **21**, but using propargylamine in the first reaction-step. It is remarkable that this time the triple bond was not attacked by water. This proves that only mono-Zn(II)-

[a] For details about the synthesis of **14** and **15** please see experimental part of chapter 1.

cyclen comlexes are able to catalyze this reaction, but neither uncomplexed $Zn^{2+}$ nor bis-Zn(II)-cyclen-complexes.



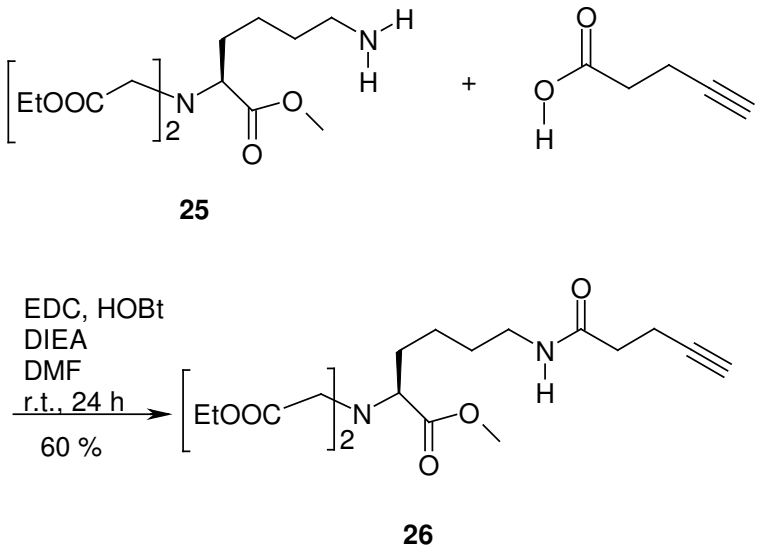

Scheme 10: Synthesis of bis-Zn(II)-cyclen-complex **24**.

### *2.2.1.3. Synthesis of NTA-Complexes*

Natural amino-acids are suitable precursors for the synthesis of NTA-complexes. The choice which one to use depends on the functional group one wants to connect to the side-chain and on the way it should be introduced. As it is easy to synthesize carbonic acids or amines, functionalized with an alkyne- or azide-group (some are even commercially available), it makes sense to use lysine (Lys), aspartic acid (Asp) or glutamic acid (Glu) and peptide-coupling reactions to build the desired NTA-complexes. However, Asp always forms imides in peptide-couplings, so its use is senseless.

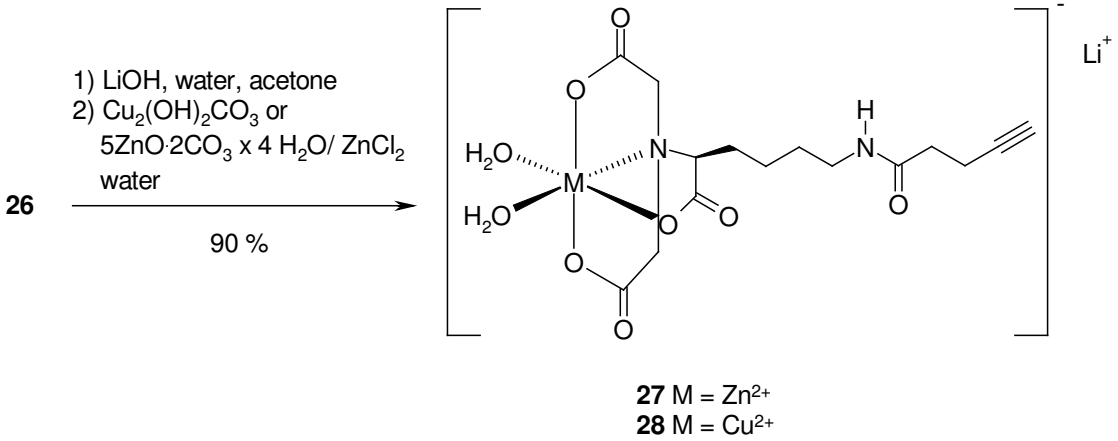
For the synthesis of lysine based NTA-complexes, bearing an alkyne or azide group, the twofold alkylated lysine methylester **25** was used. After a peptide-coupling

reaction with 4-pentynoic acid the amide **26** was obtained in moderate, but acceptable yield.




Scheme 11: Synthesis of peptide **26** using 4-pentynoic acid and standard coupling reagents.

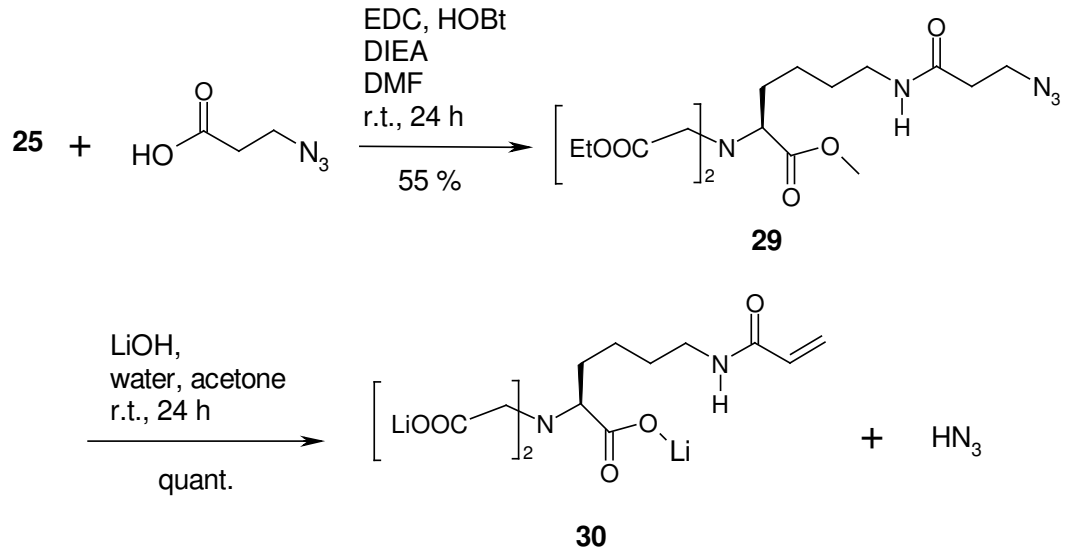
The following reaction step was the cleavage of the ester-protection-groups using LiOH in a mixture of water and acetone. This gave the free carbonic acid without purification in quantitative yield. In order to form the desired metal-complexes **27** and **28** either $Cu_2(OH)_2CO_3$ or a mixture of $ZnCl_2$ and $5ZnO{\cdot}2CO_3$ x 4 $H_2O$ was used in aqueous solution.




Scheme 12: Synthesis of the NTA-complexes **27** and **28**.

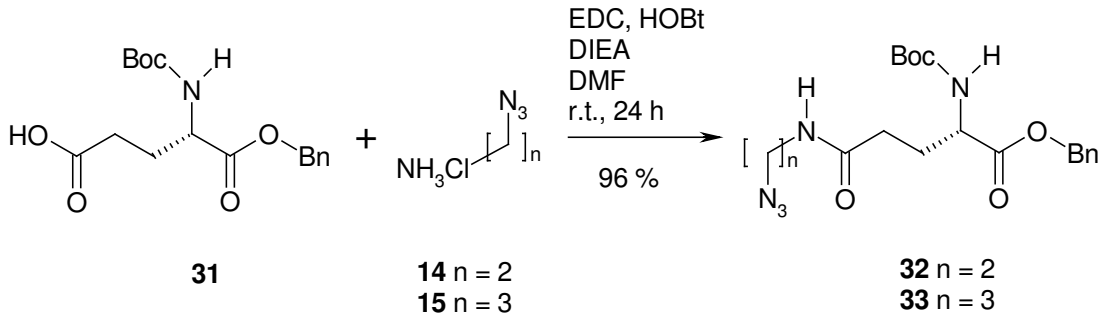
In the same way another NTA-complex, bearing an azide group, should be synthesized, simply using 3-azido-propionic acid instead of pentynoic acid. A peptide-coupling led to **29** in comparable yield, but the cleavage of the ester-groups yielded the elimination-product **30** instead of the desired azido compound. For this reason no

Lys based NTA-complex bearing an azide group was synthesized. Further the use of Boc-Glu-OBzl as building block for various NTA-complexes proved to be the better choice.




Scheme 13: Synthesis of **29** and failed synthesis after cleavage of the ester-groups and simultanous elimination of $HN_3$.

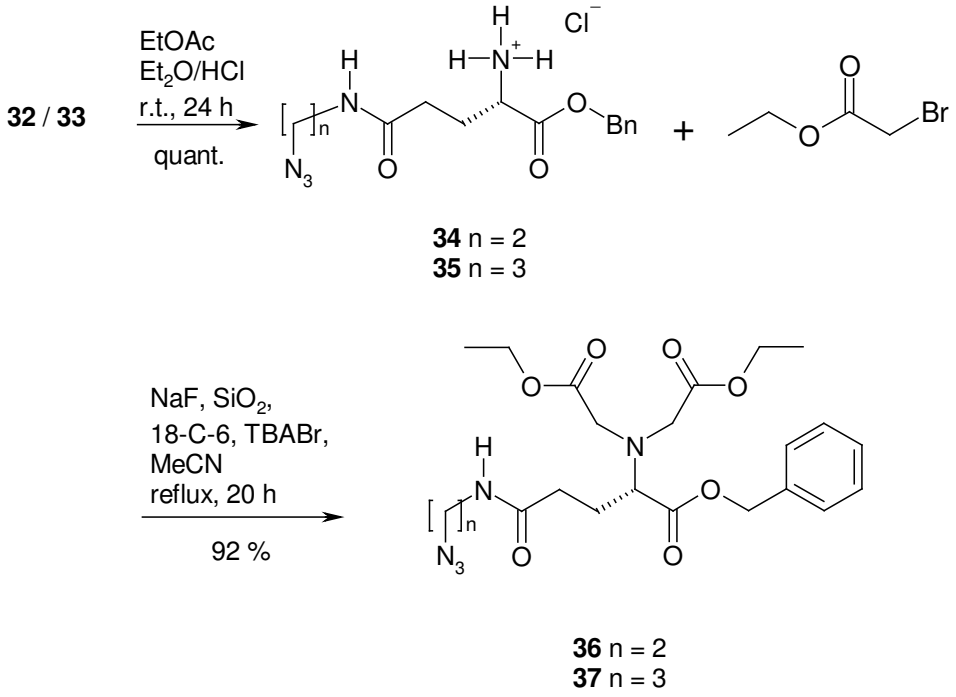
The protected Glu **31** is commercial available and a suitable compound to start the synthesis of NTA-complexes. In order to introduce an azide group the amines **14** and **15** were used in peptide-coupling reactions using common coupling reactants and reaction conditions. This gave the desired amides **32** and **33** in excellent yields after precipitation from the reaction solution with water. It was even possible to use the raw products in the following reaction step without any further purification.




Scheme 14: Synthesis of amides **32** and **33**.

The cleavage of the Boc-group was achieved by using a saturated solution of HCl in diethylether. This gave **34** and **35** in quantitative yields. Alternatively, this reaction was performed with TFA in DCM, but the resulting ammonium-salt could not be used in the following alkylation reaction with bromo-ethylacetate.

Compounds **34** and **35** were not stable at basic conditions (like any other comparable glutamic acid compound),[53] as the free amino-group formed a five-membered lactame by attacking the amide-C. On the other hand it was not possible to alkylate the $NH_2$-group when it was protonated. Therefore it was necessary to adjust a suitable pH-value to make this reaction possible. This was achieved by using NaF as a weak base. $SiO_2$ was added to react with the HF that was formed in the acidic solution. Phase transfer catalysts for cations and anions, 18-crown-6 and $TBA^+Br^-$ were also essential for this reaction. Despite the quite complex reaction mixture, the desired products **36** and **37** were obtained in very good yields.



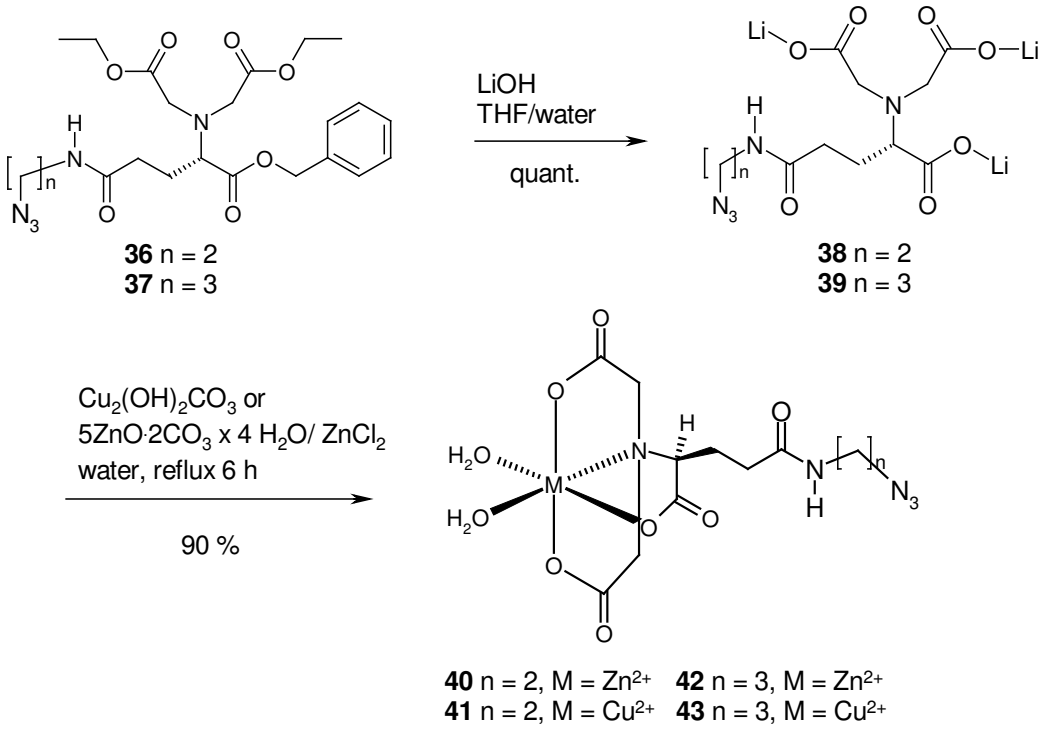

Scheme 15: Cleavage of the Boc-group gave compounds **34** and **35**. The following alkylation at slightly acidic conditions yielded the ester-protected NTA-building blocks **36** and **37**.

Table 1 shows the dependence of the yield on the amount of NaF used. It should also be noted that a longer reaction time than 20 h will cause a lower yield (estimated 3 % per 24 h).

| base (eq) | $K_2CO_3$ | NaF (1) | NaF (2.5) | NaF (7.5) | NaF (9) |
|---|---|---|---|---|---|
| yield | 0 % | 13 % | 30 % | 81 % | 92 % |

Table 1: Dependence of the yield on the amount of NaF.

The subsequent reaction steps were the cleavage of the ester-groups and the complexation of the metal cation. Both steps were carried out in the same way as already described for compounds **27** and **28**. This gave the desired zinc(II)-complexes **40** and **41** or the corresponding copper(II)-complexes **42** and **43** in good yields.



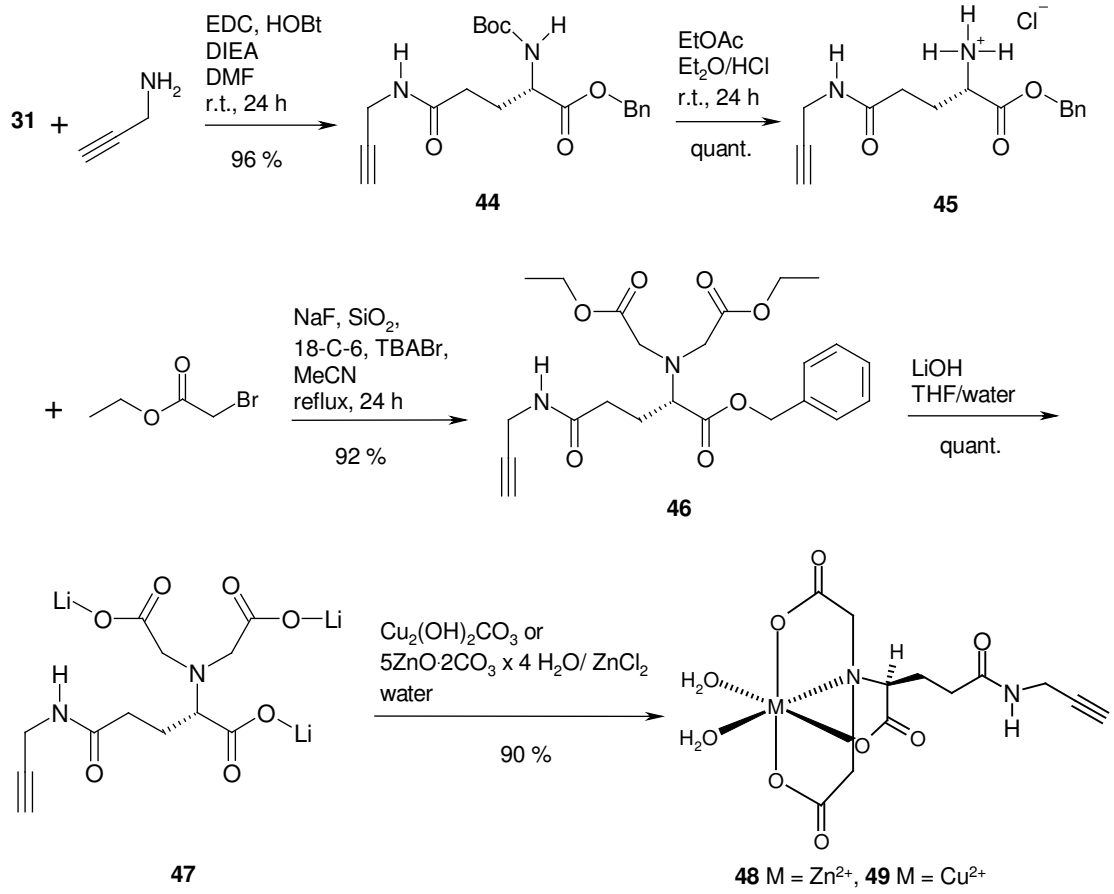

**36** n = 2
**37** n = 3

**38** n = 2
**39** n = 3

**40** n = 2, M = $Zn^{2+}$ **42** n = 3, M = $Zn^{2+}$
**41** n = 2, M = $Cu^{2+}$ **43** n = 3, M = $Cu^{2+}$

Scheme 16: Synthesis of the complexes **40**, **41**, **42** and **43**.

The synthesis of the complexes (**48**) and (**49**) worked as well as described above. Propargylamine was used in the first reaction-step.



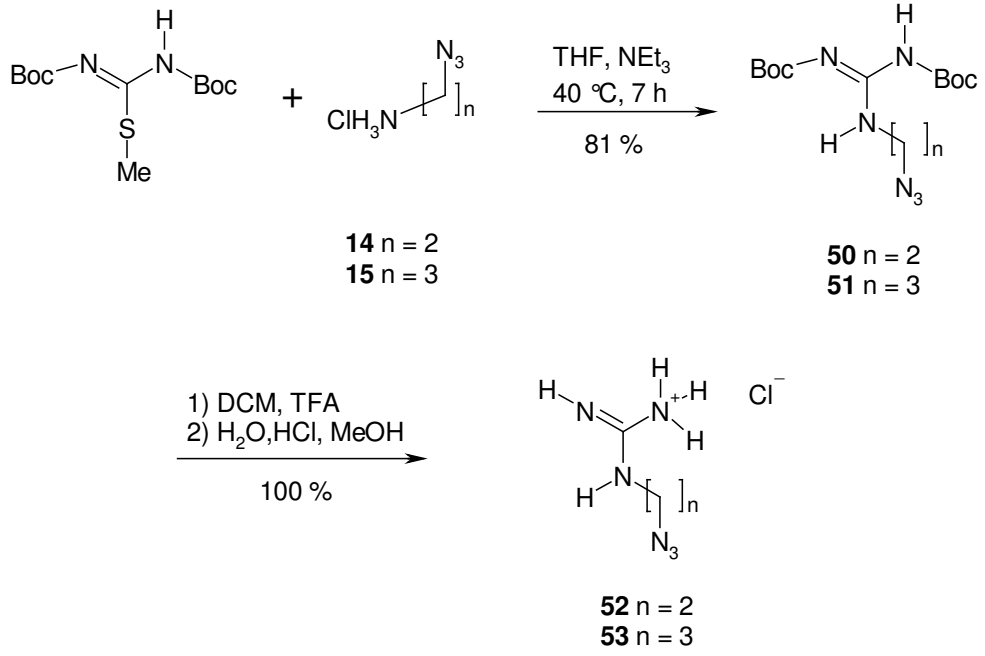

Scheme 17: Synthesis of NTA-complexes **48** and **49**.

### *2.2.1.4. Synthesis of Guanidines*

The Synthesis of the Boc-protected guanidinium precursors **50** and **51** started with N,N'-Di-Boc-S-methyl-isothiourea and the ammonium salts **14** and **15**. Triethylamine was used in excess, in order to liberate the azido-amines from their hydrochloride salts. The nucleophilic substitution yielded **50** and **51** in acceptable 81 %. As **50** and **51** were not stable under the reaction conditions, the reaction had to be stopped after 7 h, what caused a lower yield.

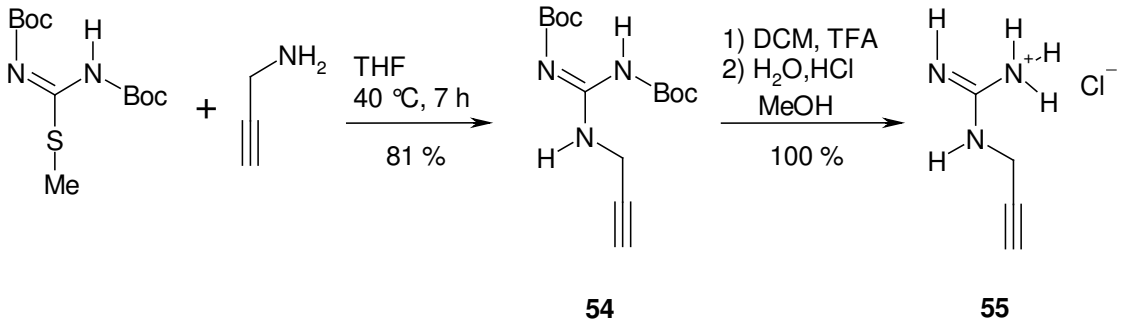
The deprotection was done with TFA in DCM. In order to remove surplus TFA and transform the resulting TFA-salts into hydrochlorides the crude product was treated with 36% aqueous HCl and methanol (to form volatile methyl-trifluoroacetate). This gave the azido-substituted guanidines **52** and **53** in quantitative yields.

**Caution!** As the share of nitrogen in these compounds is almost 50 %, they have to be handled with care! Heating or shaking might result in an *explosive* decomposition!




Scheme 18: Synthesis of guanidines **52** and **53**.

The synthesis of the guanidinum salt **55** worked as described for **52**/**53**. Propargylamine was used without supplementary base in the first reaction-step.




Scheme 19: Synthesis of alkyne substituted guanidine **55**.

*2.2.1.5. Synthesis of Zn(II)-Porphyrins*

The basic structure of a porphyrin consists of four pyrrole units, which are connected to a cyclic conjugated $C_{20}$-skeleton (fig. 3). This skeleton is a plane aromatic macrocycle with 22 π-electrons of which only 18 belong to the perimeter of a 1,16-diaza[18]annulene (according to Hückel rule: 4n + 2 = 18 π electrons [n = 4]). This also explains why chlorins and bacteriochlorins are aromatic as well (fig. 3).[54]

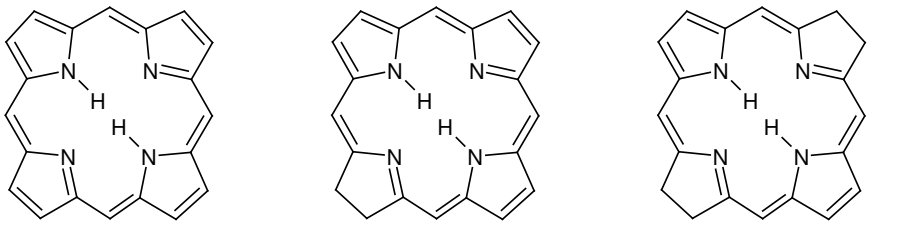


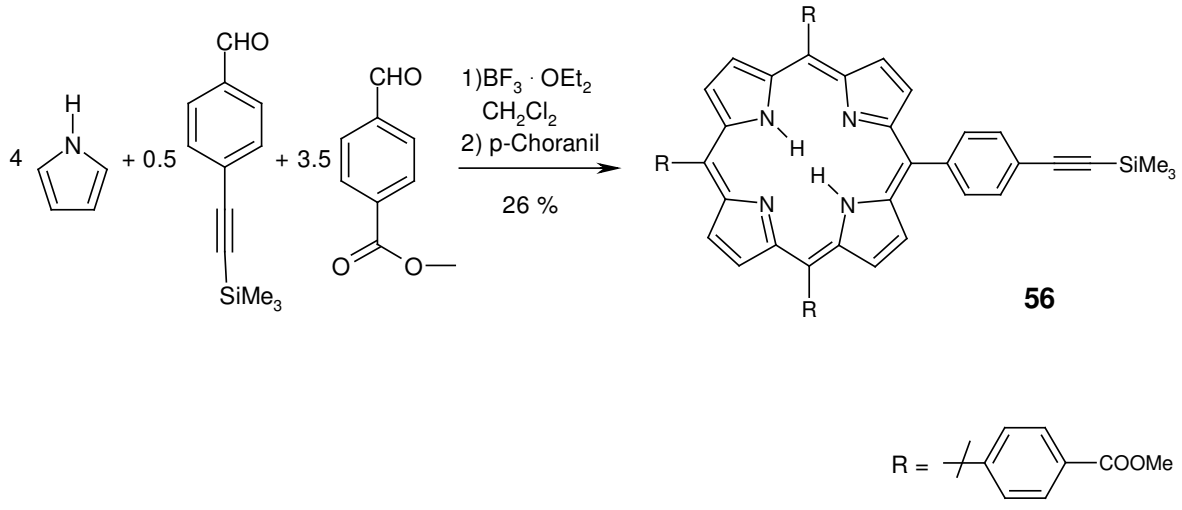

Figure 3: Structures of a porphin, a chlorine and a bacteriochlorin and number of π-electrons.

Porphyrins are formed by oxidation of porphyrinogenes, which can be derived from an acid catalyzed cascade reaction between pyrrol and aldehydes. Acid catalysts can be strong inorganic or organic acids like HCl and TFA, or even Lewis acids like $BF_3 \cdot OEt_2$. Entropic effects favour the ring closure after the 4$^{th}$ step of the cascade leading to the non-aromatic porphyrinogen. Otherwise this approach would only lead to poly-pyrrols, which can also be isolated from the reaction mixture. The oxidation of porphyrinogens can be done with weak oxidisers as the resulting porphyrin is an aromatic system. The common oxidants for this reaction are either molecular oxygen ($O_2$) or electron poor chinones like 2,3,5,6-tetrachloro chinone (p-chloranil).

Due to the extended π -system porphyrins are coloured (red to purple, depending on substituents) and show a very strong absorption at approx. 400 nm (lg ε = 5.0 – 6.0) which is called the “B-band” or “soret-band”. The absorption spectrum shows 3 further absorption bands between 500 nm and 600 nm (called Q-bands [lg ε = 3.5 – 4.5]) which are responsible for the colour. When excited at the soret band, porphyrins show an intensive fluorescence at approx. 600 nm. This makes identification of porphyrins in the crude reaction mixture with TLC very easy and therefore enables separation from other coloured side products.

Synthesis of symmetric porphyrins is easy, as a 1:1 mixture of pyrrole and aldehyde leads to exceptional one porphyrin. However, synthesis of asymmetric substituted porphyrins with 2 – 4 different substituents is more difficult, as the reaction will yield a statistical mixture of all possible combinations. In order to favor the formation of one porphyrin, the right stoichiometric relation between pyrrole and the two different aldehydes has to be chosen. Further, the statistic mixture of porphyrins and isomers has to be purified.

Porphyrin **56** was synthesized from pyrrol, 4-((trimethylsilyl)-ethinyl)-benzaldehyde and 4-(methoxy-carbonyl)-benzaldehyde in a stoichiometric relation of 4:0.5:3.5 (scheme 20). This relation was chosen as it revealed to give **56** in a higher yield (related to the TMS-ethinyl-benzaldehyde). The reaction led to the formation of a mixture of 6 possible porphyrins (0 – 4 methoxy groups). Due to differences in polarity, purification with column chromatography was possible. Tertiary mixtures of PE/DCM/EE proved to be suitable eluents. DCM was necessary to guarantee solubilty of the porphyrins in the mixture and EE was necessary to raise polarity. The porphyrin with 4 TMS groups revealed to be the most non-polar one. Even the two isomers **57** and **58** could be separated and identified via the shape of signals in the $^1$H-NMR spectrum (figure 4).




Scheme 20: Synthesis of asymmetric alkyne-substituted porphyrin **56**.

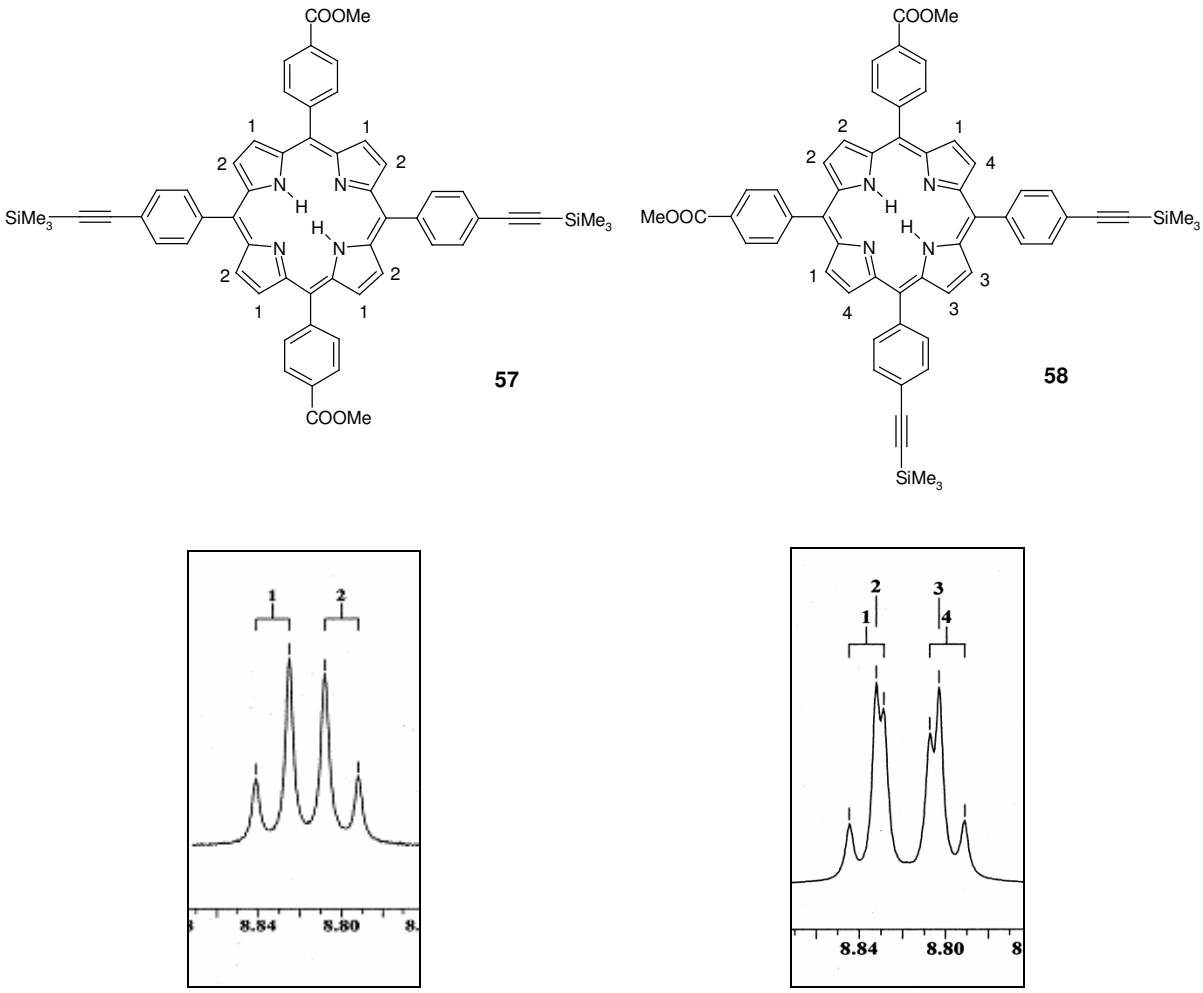
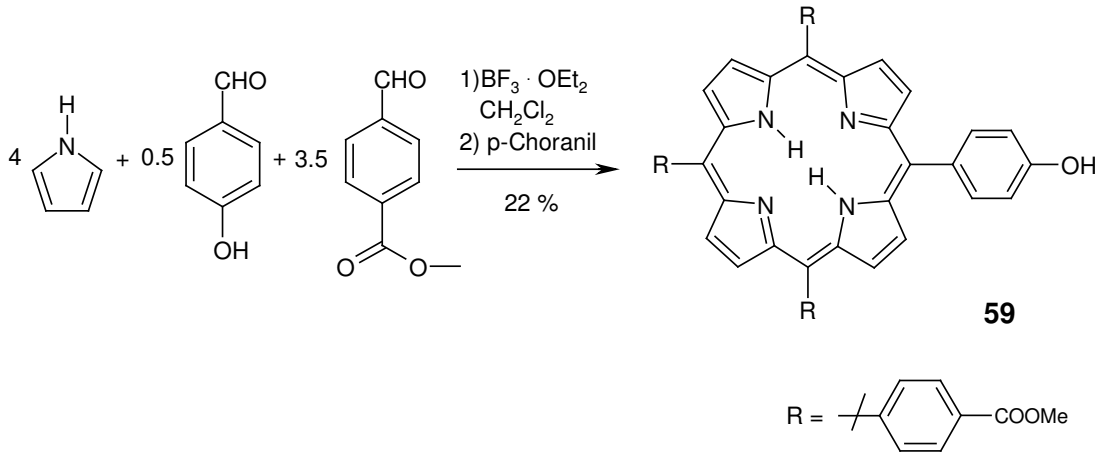


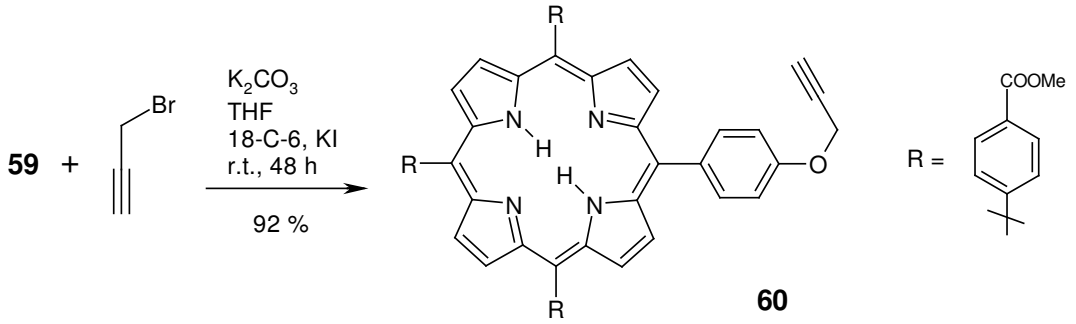

Figure 4: Identification of isomers **57** ($\alpha,\gamma$ "trans") and **58** ($\alpha,\delta$ "cis") via the shape of the signals of the hydrogens of the porphyrin-core in the $^1$H-NMR spectrum. Assignment of signals to corresponding hydrogens 1 – 4 is given.

Porphyrin **59** was synthesized in the same way like **56**. However, 4-hydroxy-benzaldehyde was used instead of 4-((trimethylsilyl)ethinyl)-benzaldehyde in order to introduce a phenolic OH-group which enables further functionalisation (scheme 21). This OH-group also increased the polarity of the product (compared to **56** and side-products). Therefore, the porphyrin with 4 methoxy groups revealed to be the most non-polar one of the mixture of 6, followed by **59**.



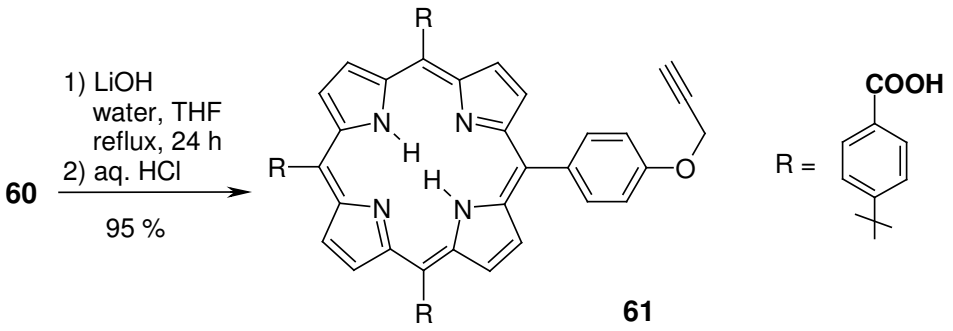

Scheme 21: Synthesis of asymmetric OH-substituted porphyrin **59**.

In order to introduce an alkyne group into **59**, the phenol was deprotonated with potassium carbonate and reacted with propargyl bromide. Catalytic amounts of potassium iodide and 18-C-6 were essential for this reaction. This gave the phenol ether **60** in a high yield of 92 %.




Scheme 22: Functionalisation of **59** with propargyl bromide.

For binding affinity studies the porphyrin needed to be soluble in water. This was achieved by cleaving the methyl-ester groups, which yielded the threefold carboxylic acid **61** (scheme 23). In order to achieve complete cleavage of all ester groups, LiOH had to be used in an excess of 10 eq per ester function. Further, the reaction mixture had to be heated. As **61** was not soluble in water at a pH lower than 5, the product could be obtained directly from the reaction solution by precipitation of the carboxylate with aq. HCl. Despite the high polarity of **61** a further purification by column chromatography with mixtures of $CHCl_3$/MeOH/AcOH was possible (see exp. section). Although this procedure caused a loss of product, **61** was obtained in almost quantitative yield.




Scheme 23: Cleavage of methyl-esters to obtain the water soluble threefold carboxylic acid **61**.

The 2 NH-groups in the porphyrin-core can act as weak bases and therefore can be protonated ($pK_{a1} \approx 7$, $pK_{a2} \approx 4$) resulting in the so called *1H-* and *2H*-form. This causes a change of the colour from red to green.

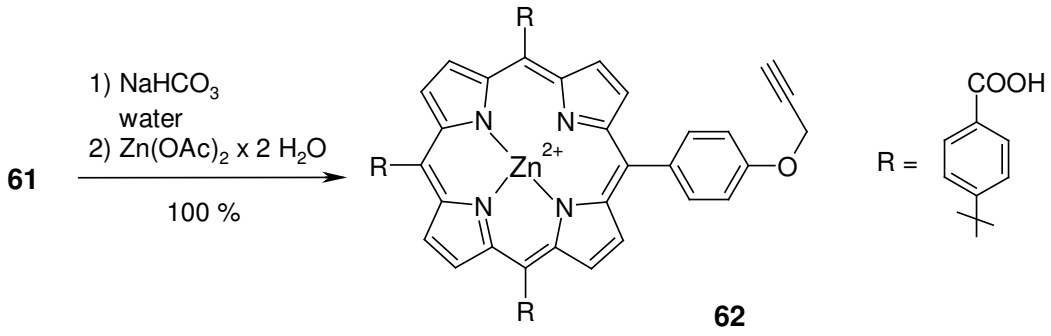
The NH-protons also react acidic and can be replaced by metal-cations. As the 2 further pyrrole-N-atoms also participate as electron donating ligands in binding interactions, this leads to a tetra-dentate chelate ligand. For this reason, porphyrins show high binding constants for a lot of metal cations. Especially $Fe^{2+/3+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ are bound tightly by porphyrines and form biological relevant complexes. Removal of these cations is only possible by treatment with strong acids like TFA or $H_2SO_4$.[54]

Therefore, connection of **61** to another binding site building block (bearing an azide group) via the Cu(I)-catalyzed click-reaction was only possible when an over-stoichiometric amount of Cu(II)-salt was used.[55]

The incorporation of a metal cation also causes changes in the spectroscopical characteristics. The Q-bands are reduced from 3 to 2, whereas new absorption bands (N,M,L-bands) arise between 210 nm and 330 nm. Diamagnetic cations like $Zn^{2+}$ show almost no effect on the intensity of the fluorescence, while paramagnetic cations like $Cu^{2+}$ show an efficient quenching of the porphyrin emission. Thus, a Cu(II)-porphyrin complex cannot be used to follow the binding process to a protein via fluorescence titration, though it still is a suitable receptor for non-polar regions in a polar medium. This problem can be solved by blocking the porphyrin core with $Zn^{2+}$. The zinc cation is bound strongly enough to prevent replacement by $Cu^{2+}$ and therefore works like a protecting group. Further, it does not influence the fluorescence.

For this reason, the Zn(II)-complex of **61** (**62**) was synthesized as this avoided complexation of $Cu^{2+}$ and therefore enabled click-reactions with catalytic amounts of a Cu(II) salt and the investigation of binding processes with fluorescence spectroscopy.

For complexation of $Zn^{2+}$ the choice of Zn(II)-salt and adjustment of the right pH value was important. First, **61** was transformed into the sodium carboxylate, in order to achieve solubility in water. This was done by addition of a saturated solution of $NaHCO_3$ to a suspension of **61** in water until the acid was dissolved completely. The pH-value of the solution was 7. Then, an aqueous solution of zinc acetate (pH 7) was added. The complexation of $Zn^{2+}$ by **61** resulted in a lowering of the pH to 4.5 as the 2 protons of the porphyrin core were replaced by the metal cation. This caused the precipitation of **62** as the carboxylate was reprotonated. Centrifugation of the suspension gave the Zn(II)-porphyrin complex **62** in quantitative yield (scheme 24). If an excess of $NaHCO_3$ was used, this resulted in precipitation of $Zn(OH)_2$ due to the high pH. If a more acidic Zn(II)-salt like $ZnCl_2$, $Zn(NO_3)_2$ or $Zn(ClO_4)_2$ was used, this caused only precipitation of **61**, as the reprotonation reaction was much faster than the complexation and as there was no buffering effect.

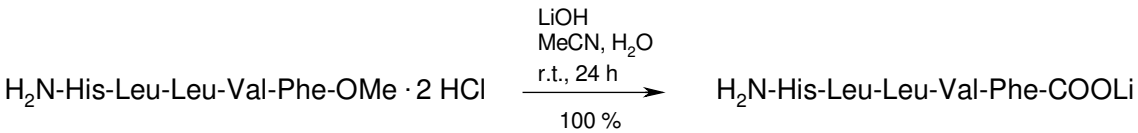



Scheme 24: Synthesis of Zn(II)-complex **62**.

The Zn(II)-porphyrin complex **62** can be used in any kind of click-reaction with suitable azides to obtain bidentate receptors with high binding affinities to non-polar areas on protein-surfaces in polar media.

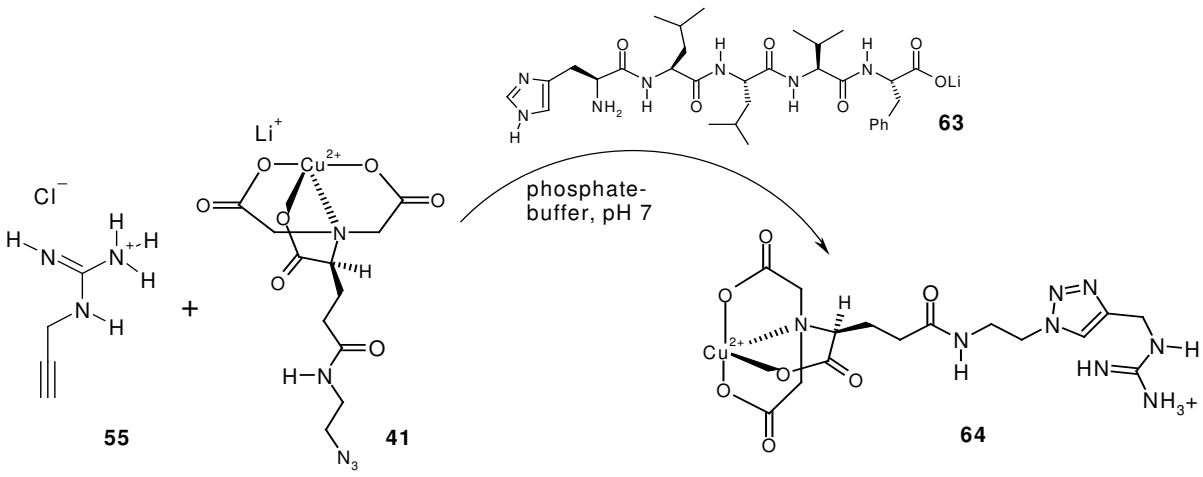
## 2.2.2. Induced Fit "Click-Reaction" to Identify High Affinity Receptors

In order to test if the synthesized single binding site receptors can be used in kinetically controlled TGS a suitable combination of receptors and target molecule had to be used. As histidine (His) and carboxylate groups are quite common functionalities on protein surfaces, receptors for these two groups – NTA-complexes and guanidines – were the first choice. The target which was chosen was a pentapeptide with the following sequence: H-LHis-LLeu-LLeu-LVal-LPhe-OLi (**63**). As this peptide bears a terminal histidine and a carboxylate group separated by a not too flexible spacer in a defined distance, this seemed to be a suitable target. It was synthesized from the corresponding methyl ester, which was commercially available and shows biological activity (scheme 25).[51]




Scheme 25: Synthesis of the target molecule **63** from the commercially available methyl ester.

In a simple screening assay the synthesized NTA-complexes **27**, **28**, **40**, **41**, **42**, **43**, **48**, **49** were mixed with the guanidines (G) **52**, **53**, **55** and the target **63** in buffered solution (phosphate buffer, pH 7, c = 5 mM) in all possible combinations (see table 2). In order to guarantee reproducable and comparable mixtures, stock solutions of each compound in buffer were prepared (approx. 3 mM solutions), of which aliquots of 300 µl were taken and mixed. This gave equimolar mixtures of target, azide and alkyne with a concentration of each compound of approx 1 mmol/l. The mixtures were shaken at r.t. for 24 h and then analyzed by mass spectroscopy (pos. and neg. ESI in mixtures of MeOH/water/$NH_4OAc$) to check, whether the triazole was formed or not (scheme 26).




Scheme 26: Exemplary reaction mixture of guanidine **55**, NTA-complex **41** and the peptide **63** in phosphate buffer, leading to **64** which was analyzed by mass spectroscopy.

This method is very sensitive and allows detection of even very small amounts. However, it is just a qualitative method and does not give information about the reaction conversion or quantities. Results are given in table 2. MS-spectra of a positive tested mixture (**63** + **41** + **55**) and a negative tested one (**63** + **43** + **55**) are shown in figure 5.

| G \ NTA | 27 | 28 | 40 | 41 | 42 | 43 | 48 | 49 |
|---|---|---|---|---|---|---|---|---|
| **52** | O | O | ✗ | ✗ | ✗ | ✗ | O | ✓ |
| **53** | O | O | ✗ | ✗ | ✗ | ✗ | O | ✓ |
| **55** | ✗ | ✗ | O | ✓ | O | O | ✗ | ✗ |

Table 2: Hit detection via MS-spectroscopy of 12 possible combinations of NTA-complexes and guanidines. Hits are marked with a tick. Fields marked with a cross are impossible combinations (azide + azide / alkyne + alkyne). Fields marked with a circle are negative tested combinations. Blue filled fields mark Cu(II)-NTA-complexes.

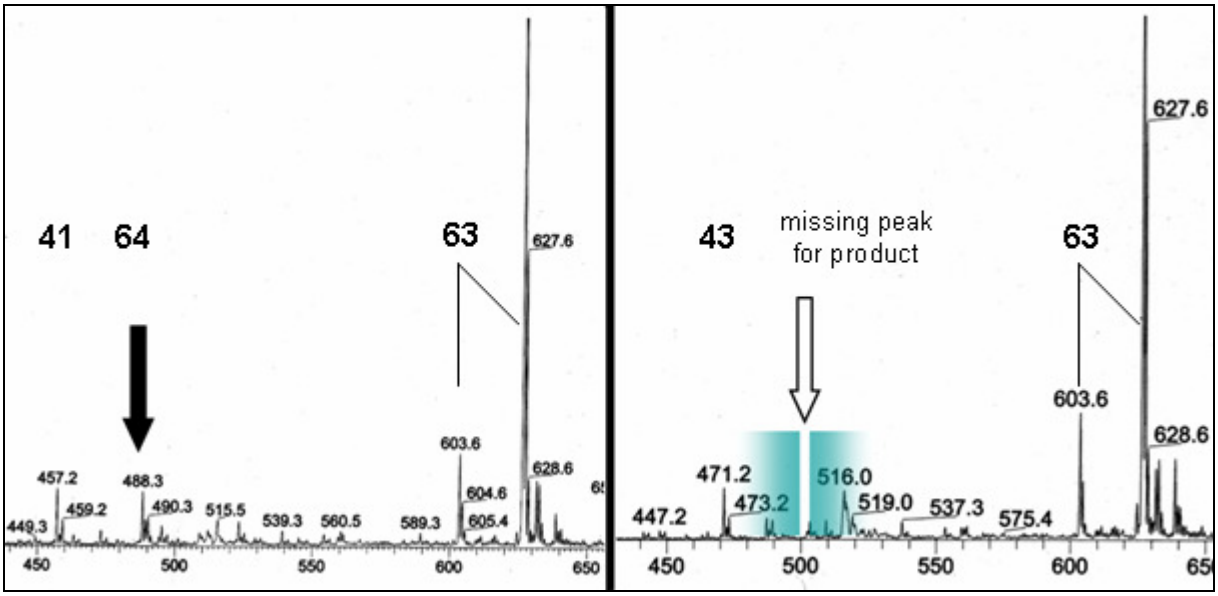



Figure 5: MS-spectrum (neg. ESI) of a positive tested mixture (**63** + **41** + **55**) on the left side and a negative tested one (**63** + **43** + **55**) on the right. The black arrow on the left marks the signal of the formed triazole **64** (m/z = 488.3), whereas the white arrow on the right marks the area where the signal for the triazole (which was not formed) is missing.

In both MS-spectra (neg. ESI) the major peaks could be assigned: The peaks with m/z = 603.6 and 627.6 were assigned to the peptide **63**. The peak with m/z = 457.2 (left side) was assigned to [(**41** + LiOAc)$^-$]. Peak with m/z = 471.2 (right side) was assigned to [(**43** + LiOAc)$^-$]. Both peaks show the typical characteristic isotope pattern of compounds containing copper. The same is true for the peak with m/z = 488.3 (left side) which was assigned to the triazole **64**. However, on the right side the corresponding peak for the triazole-anion was expected to arise at m/z = 502, but the spectrum clearly shows no peak there.

Results presented in table 2 show that only 3 of 12 possible combinations led to the formation of the triazole. First conclusions that could be drawn from these results were:

- only Cu(II)-NTA-complexes formed triazoles. This was expected, as Cu(II)-NTA-complexes show higher binding affinities towards terminal histidines than Zn(II)-NTA-complexes.[48]
- the triazole formation was not catalyzed by the Cu(II)-complexes themselves, as 2 of 4 complexes (**28** and **43**) showed no triazole-formation.[56] The two complexes which were reactive, were tested again (see table 3).

- the formed triazoles were the shortest ones that were possible. Neither the Lys-based Cu(II)-NTA-complex **28** showed reactivity nor the longer Glu-based **43**. This suggests that there is a relation between reactivity and structure. Only the formation of the short and less flexible receptor was induced by the pentapeptide **63**.

In a simple control experiment it was tested whether the triazole-formation was also possible when the pentapeptide was not present in the mixture. Only the Cu(II)-NTA-complexes were tested (table 3). The experimental procedure was the same as described on page 67, except adding 300 µl buffer instead of peptide solution.

| G \ NTA | 28 | 41 | 43 | 49 |
|---|---|---|---|---|
| 52 | O | | | ✓ |
| 53 | O | | | ✓ |
| 55 | | O | O | |

Table 3: Hit detection via MS-spectroscopy of 8 possible combinations of Cu(II)-NTA-complexes and guanidines **without 63**. Hits are marked with a tick.

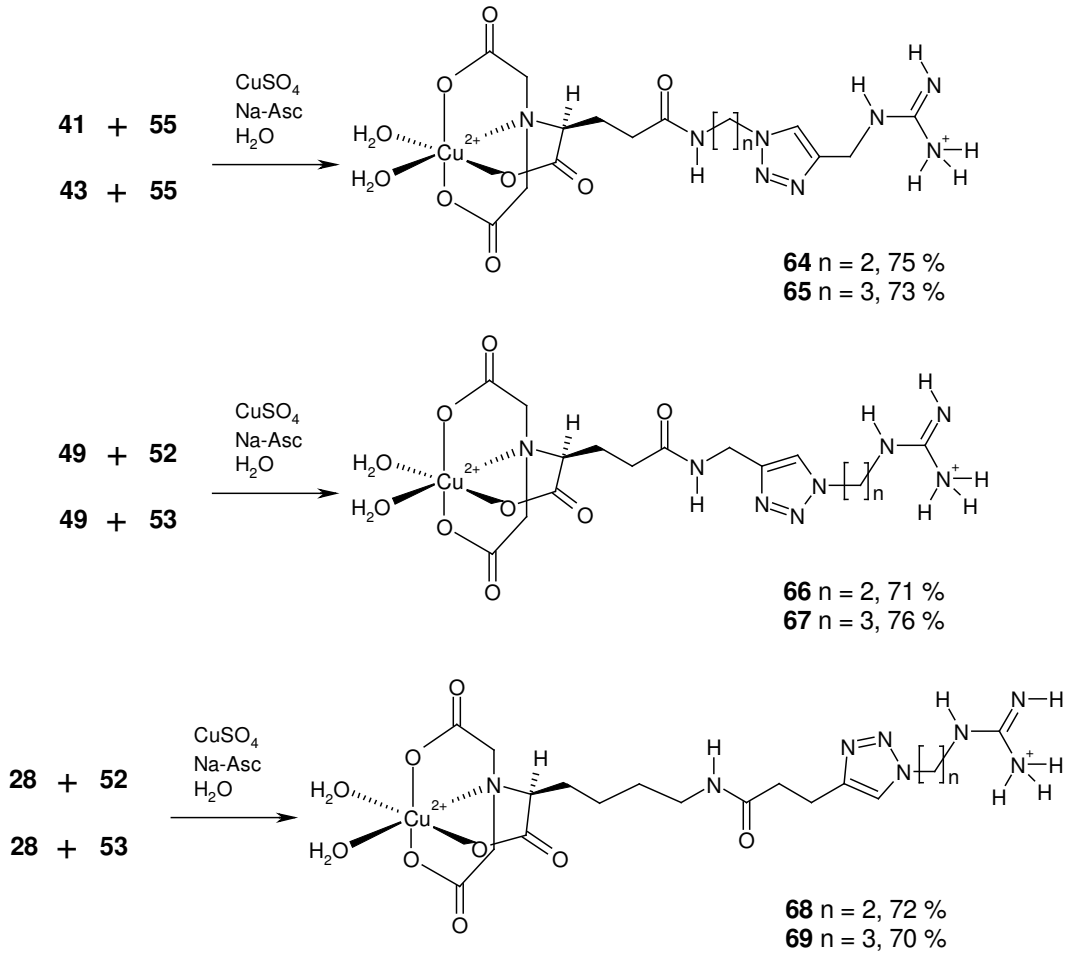
MS-spectroscopy of the mixtures revealed that the Cu(II)-complex **49** shows triazole-formation even in the absence of **63**. A possible explanation for this is that the alkyne group of **49** is coordinated to the metal cation and therefore is already activated for the cycloaddition. As the colour of **49** is brown and not blue or green like complexes **28**, **41**, **43** this observation strengthens this assumption.

In order to check if the remaining hit **64** really shows the highest binding affinity towards the target **63** and if there is a relation beween structure and affinity, a fluorescence-assay was established.

## 2.2.3. Binding Affinities of Receptors

### *2.2.3.1. Investigation of the Binding Process with $^1$H-NMR Spectroscopy*

First, the 6 triazoles from **41**+**55**, **43**+**55**, **49**+**52**, **49**+**53**, **28**+**52** and **28**+**53** were synthesized using a common click-reaction procedure (scheme 27): To an aq. solution of the alkyne and the azide (1:1 relation) catalytic amounts of $CuSO_4$ x 5 $H_2O$ and sodium ascorbate were added. The resulting triazoles **64** – **69** were precipitated from solution with EtOH (see exp. section **GP 8** for details).




Scheme 27: Synthesis of Cu(II)-NTA-guanidine-complexes **64** – **69**.

Neither the receptors, nor the target bear functional groups, which allowed direct visualisation of the binding process via UV/Vis or fluorescence spectroscopy. Further, it was not possible to observe a change in the structure of **63** with CD-spectroscopy, when one of the receptors was added. The reason for this is that **63** is too small to assume a definite shape and therefore cannot give defined signals in the CD-spectrum. Visualisation of the binding process with $^{1}$H-NMR-spectroscopy was only possible via the interaction of the His – sidechain residue with the paramagnetic $Cu^{2+}$. However, this allowed only a qualitative conclusion and no determination of a binding constant. According to Arnesano *et al.* who investigated type II copper(II) proteins (CopC from *Pseudomonas Syringae*), the characterisation of such proteins represents a challenge, as Cu(II) has a long electron relaxation time. This causes all NMR-signals of nuclei, which are in close proximity to the metal ion, to broaden.[57] The nuclear longitudinal realxation rate ($R_1$) of Cu(II)-complexes strongly depends on the electronic relaxation time ($\tau$) which is expressed by the Solomon Bloemenberg equation:

$$R_1 = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left[\frac{\tau_c}{1+(\omega_I-\omega_S)^2\tau_c^{\,2}}+\frac{3\tau_c}{1+\omega_I^{\,2}\tau_c^{\,2}}+\frac{6\tau_c}{1+(\omega_I+\omega_S)^2\tau_c^{\,2}}\right]+\frac{2}{3}S(S+1)\left(\frac{A}{\hbar}\right)^2\frac{\tau_e}{1+\omega_S^{\,2}\tau_e^{\,2}}$$

Solomon-Bloemenberg equation: dependence of the nuclear longitudinal realxation rate ($R_1$) on the electronic relaxation time ($\tau$) and the distance ($r$) between the NMR-active nucleus and the electron of the paramagnetic metal center.

Using this equation, it is possible to calculate the distance between the NMR-active nucleus and the Cu(II)-center after determination of $R_1$. Figure 6 shows the spherical areas, within which NMR-active nuclei cannot be detected due to signal-broadening.[b] According to Aresano *et al.* signal-broadening in $^{1}$H-NMR occurs, when the distance ($r$) between the Cu(II) center and a hydrogen is smaller than 110 pm. Hetero-nuclei like $^{13}$C, $^{15}$N or $^{31}$P cannot be detected within a spherical area of 120 pm ($r$ = 60 pm) around the $Cu^{2+}$.

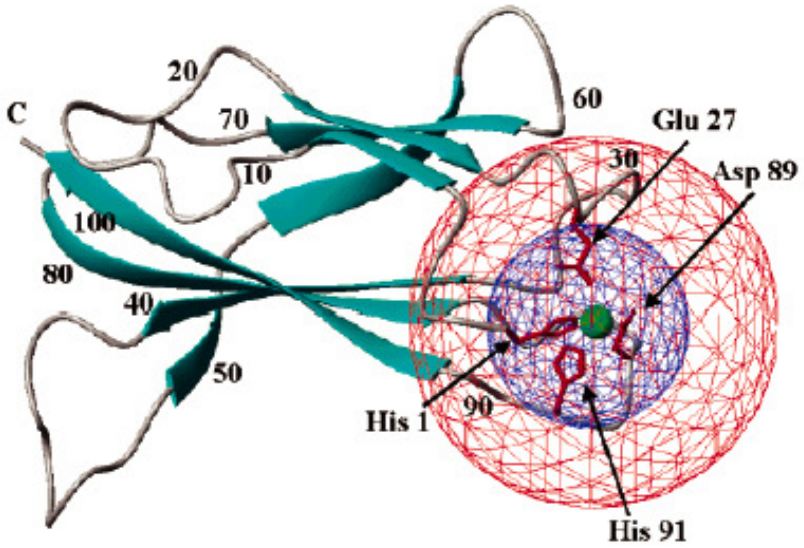



Figure 6: Detection limits through NMR are shown as concentric spheres centered on the copper ion in the Cu(II)-CopC stucture. The red sphere ($r$ = 110 pm) represents the region in which detection of $^{1}$H signals is not possible. Broadening of signals of hetero-nuclei is restricted to the blue sphere ($r$ = 60 pm).

A $^{1}$H-NMR investigation of a 2:1 mixture of **63** and **66** in buffered aqueous solution confirmed theses results (see figure 7 and 8).

[b] Figure 6 and calculation of distances were taken from: Arnesano *et al.* in *JACS* **2003**, *125*, 7200.

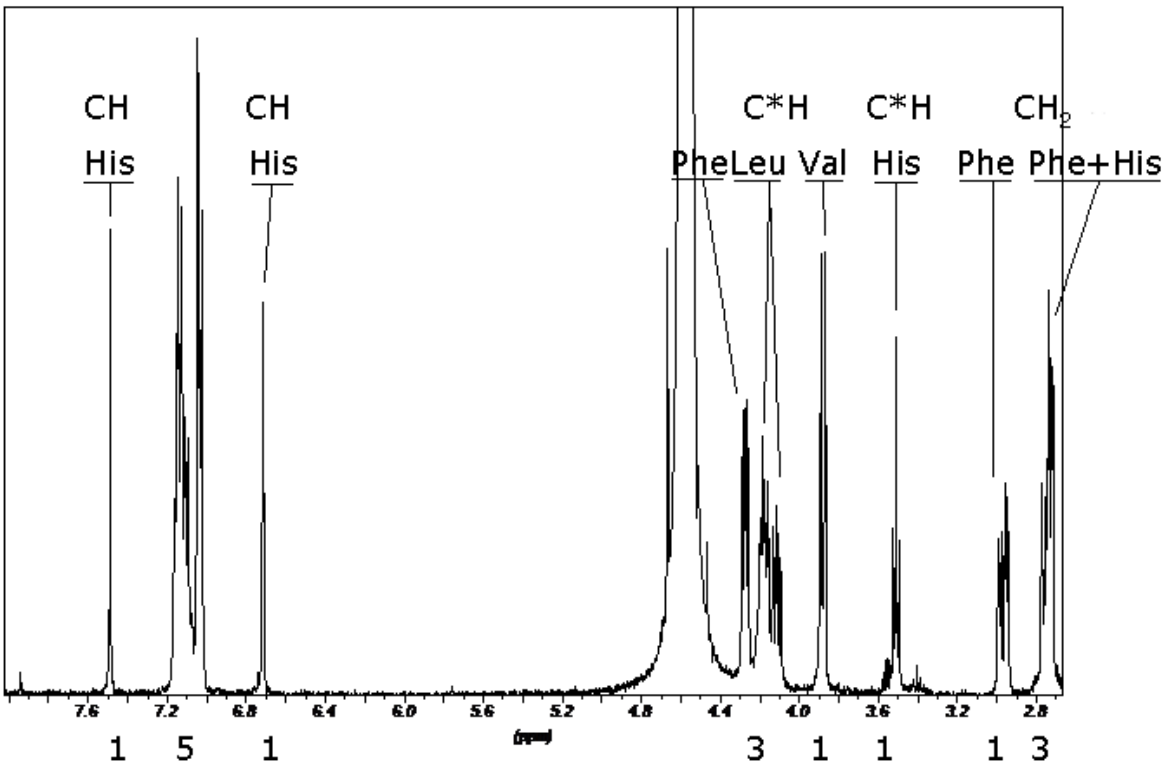



Figure 7: $^{1}$H-NMR spectrum of **63** in aq. borate buffer pH 9.2. Assignments and integrals of signals are given.

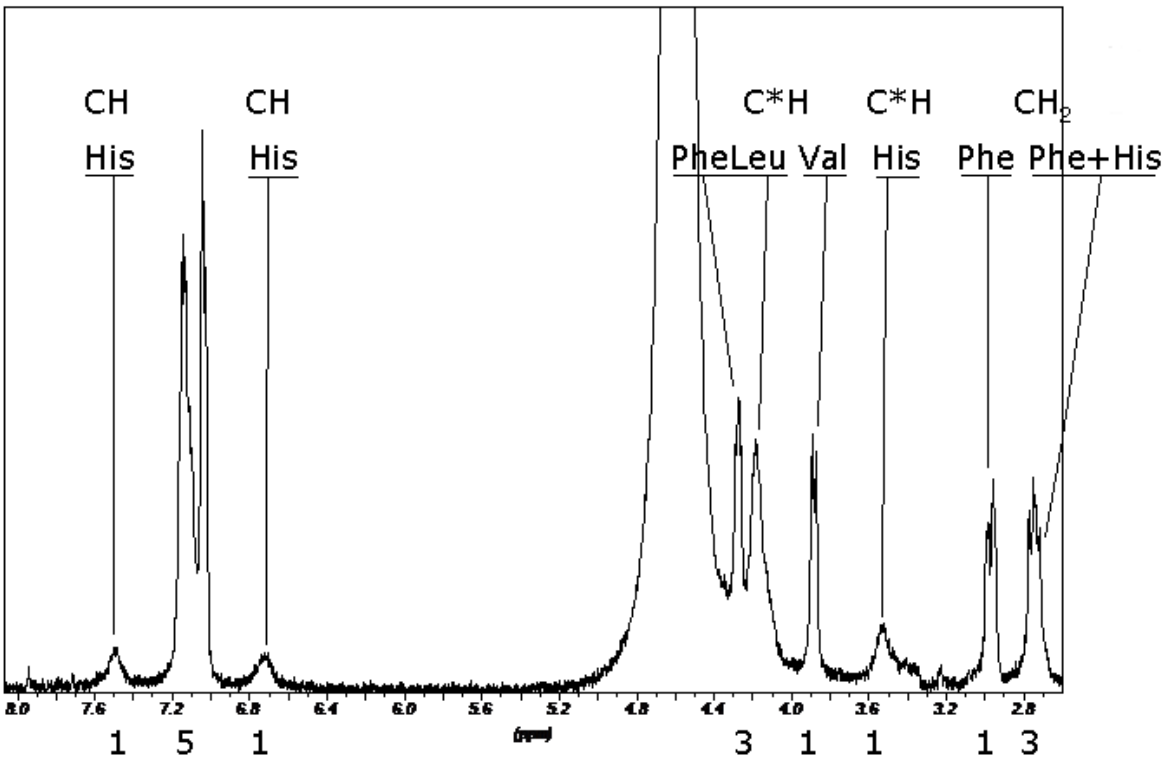



Figure 8: $^{1}$H-NMR spectrum of a mixture of **63** and 0.5 eq **66** in aq. borate buffer pH 9.2. Assignments and integrals of signals are given. A strong broadening of all His-signals and a weaker broadening of Leu-signals was observed due to interaction with $Cu^{2+}$.

Figure 7 shows the $^{1}$H-NMR spectrum (between 2.7 and 8.1 ppm) of **63** in borate buffer at pH 9.2. The assignments of the His-hydrogens are given, as well as the assignment of all chiral hydrogens (C*H), and the hydrogens of the benzylic $CH_2$ group of Phe. Figure 8 shows the same detail of the $^{1}$H-NMR spectrum of **63** after addition of 0.5 eq **66**. It was observed that all signals broaden under the influence of the present $Cu^{2+}$. However, the signals of the His-hydrogens, as well as the Leu-hydrogens, showed a stronger broadening than the other signals. This was also valid for the signals of the sidechain hydrogens of Leu between 0.6 and 0.8 ppm, which are not shown in figure 7/8 for better clarity.

This specific signal-broadening can only be caused by the closer proximity to $Cu^{2+}$, due to binding interactions. Distance-calculations with Spartan confirmed that only the $H_2N$-His-Leu-Leu- moiety of the pentapeptide is inside an area of 10 Å around the Cu(II)-cation, while the -Val-Phe-OH residue is outside. The conformer with the lowest energy was calculated. This revealed a distance between $Cu^{2+}$ and the Val-C*H of 10.4 Å and a distance of 11.1 Å between the Phe-C*H and the copper center. All hydrogens of the His-Leu-Leu- moiety were within an area of 8.5 Å (fig. 9).

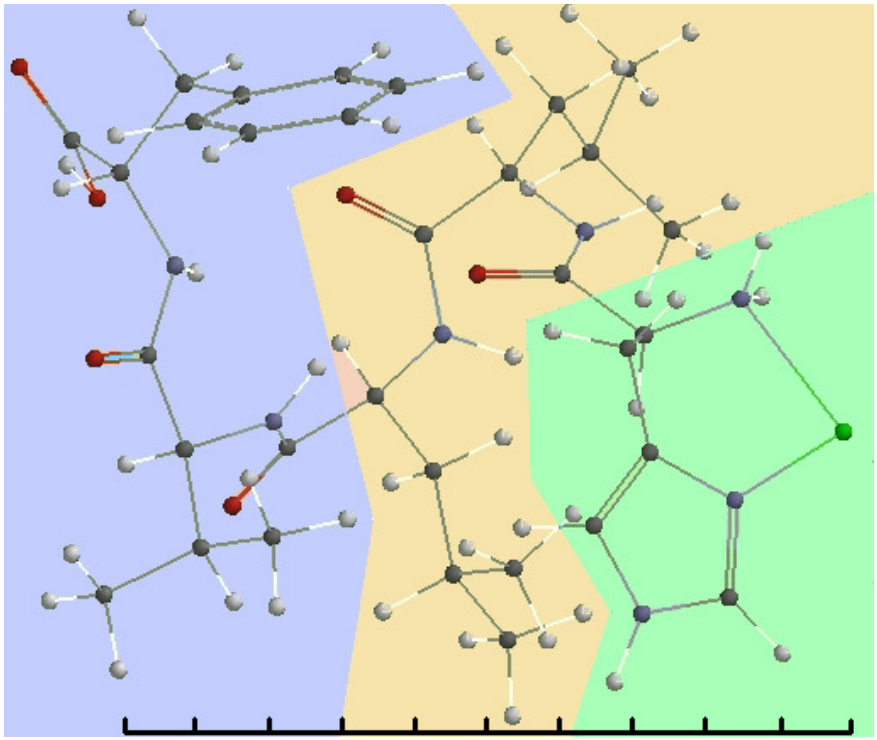


Figure 9: Spartan-calculation (lowest energy conformer) of the structure of a Cu(II)-complex of **63** in the gas-phase. Colors of elements: Cu (green), C (black), H (grey), N (violet), O (red). The green background marks the His, the orange background marks the two Leu and the blue background marks the –Val-Phe-OH residue. Ticks of the scale at the bottom represent 1 Å.

Figure 9 clearly shows that the amino acids His, Leu-Leu and Val-Phe are arranged like shells with distances of approx. 3.5 Å, 7.5 Å and 10.5 Å. This corresponds to the different signal broadening in the $^1$H-NMR spectrum.

### *2.2.3.2. Investigation of the Binding Process with Mass Spectroscopy*

Aqueous buffered solutions (phosphate buffer, pH 8.0, c = 5 mM) of the receptors **64** – **69** (c = 4 mM) and the peptide **63** (c = 4 mM) were prepared and mixed in a 1:1 ratio. The mixtures were investigated by MS-spectroscopy. The used method was electro spray injection (ESI) with a mixture of MeCN/$H_2O$. The detection of cations gave too complex spectra, due to multipile exchange of protons by $Na^+$ or $K^+$. Therefore, only negative ESI-spectra were evaluated.

Each spectrum showed peaks for the peptide, the receptor and the formed complex between both. Assignments and intensities of signals (relative abundance, all anions were single negatively charged) were:

1. deprotonated peptide **63**; int.: 100 %
2. deprotonated receptors **64** – **69**; int.: 5 – 10 %
3. deprotonated **complex of peptide+receptor**; int.: < 0.5 %

No further anions were detected.

These results confirm binding interactions between all receptors and the peptide target. A quantification of the binding strength from mass spectroscopic results was not possible.

## 2.2.4. Fluorescent Labelled Histidines

As the direct determination of the binding constant between receptors **64** – **69** and the target **63** was not possible, an indirect one had to be used. One promising approach was the visualisation of binding process in an indicator displacement assay. This was already done with a similar system by Anslyn *et al.*[58] The competitive binding of a guanidinium to a receptor and 5-carboxyfluorescein was followed via the changes in the absorption spectrum of the fluorophore. However, a titration of the receptors **64** – **69** with 5-carboxyfluoresceine showed no changes in the absorption spectra.

In order to visualize the binding process of the NTA-moiety in a competitive fluorescence binding assay fluorescent labelled N-terminal histidines were synthesized. The principle of this assay is depicted in in figure 10.

The left side shows a mixture of the indicator (active fluorophore = yellow ellipse) and one of the receptors **64** – **69**. Due to the binding between both, the fluorescence is quenched (grey ellipse) by $Cu^{2+}$ (middle). After addition of the target **63** the fluorescence returns as the receptor shows a higher binding affinity towards the target (2 binding interactions) than towards the indicator (one interaction).

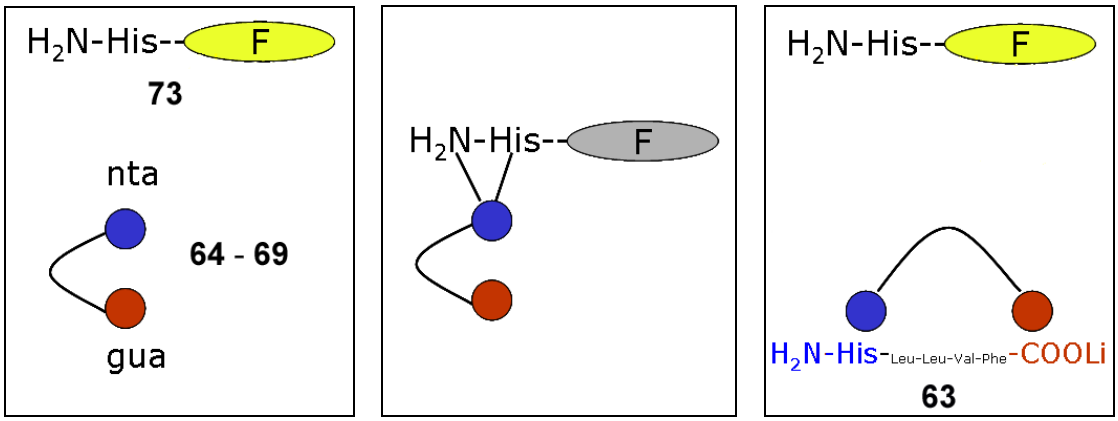


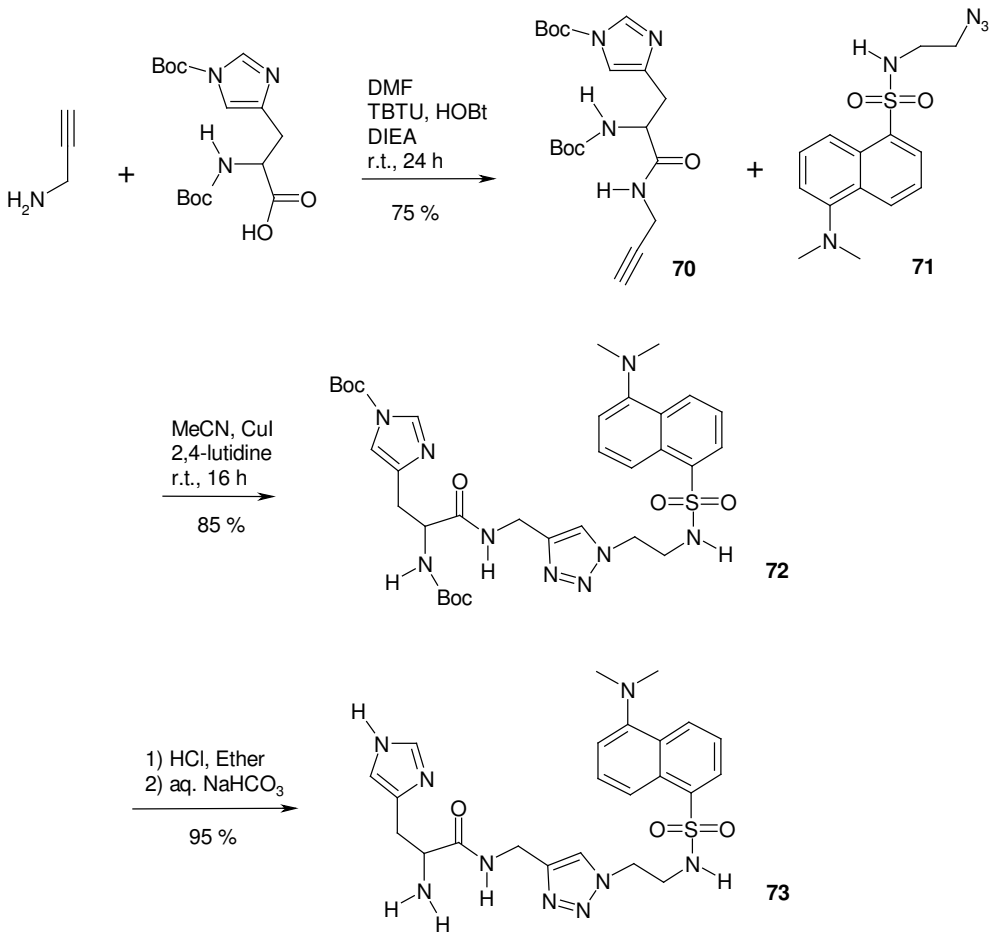

Figure 10: Principle of the competitive fluorescence assay with target **63**, receptors **64** – **69**, and fluorescent indicator **73**. The blue circle represents the Cu(II)-NTA-complex, the red one the guanidine moiety. The active fluorophore (F) is represented by a yellow ellipse, whilst the inactive is grey.

*2.2.4.1. Synthesis of Fluorescent Indicators*

Two different fluoropheres – a dansyl- and a fluoresceine-unit – were connected to the C-terminus of a histidine. A literature known procedure could be used to synthesize a dansyl-functionalized histidine.[59] However, an alternative synthesis using the click-chemistry approach was used, as the necessary dansyl-building block **71** was already synthesized (scheme 28).[c]

The synthesis of the dansyl-labelled His started with a peptide coupling of propargyl amine to twofold Boc-protected His (di-cyclohexylamine salt). Coupling reagents were TBTU and HOBt. The use of EDC instead of TBTU did not lead to the formation of **70**. After column chromatography (CC) **70** was obtained in acceptable yield. The connection of **70** to **71** was done in MeCN with CuI as direct $Cu^+$-source and 2,4-lutidine as base. Purification with CC beared problems, due to the different behaviour of **72** on TLC-plates and on the column. The last step of synthesis was the cleavage of the Boc-groups. This was achieved using HCl-saturated diethyl ether. As it was not possible to determine the degree of protonation of the ammonium salt of **73** it was necessary to deprotonate the ammonium salt to obtain the free base. This was done with aq. $NaHCO_3$ and extraction with methylene chloride. As **73** showed amphiphile behaviour, this resulted in a lower yield.

[c] For details about the synthesis of **71** please see experimental part of chapter 1, compound **7**.




Scheme 28: Synthesis of the dansyl labelled N-terminal histidine **73**.

UV/Vis and fluorescence spectra of **73** were as expected. Merely the emission maximum was shifted to longer wavelengths (from 523 nm to 561 nm) compared to **71** (figure 11). Explanations for this observation are a more polar solvent (aq. buffer) and a possible interaction of the naphtalene system with the triazole.

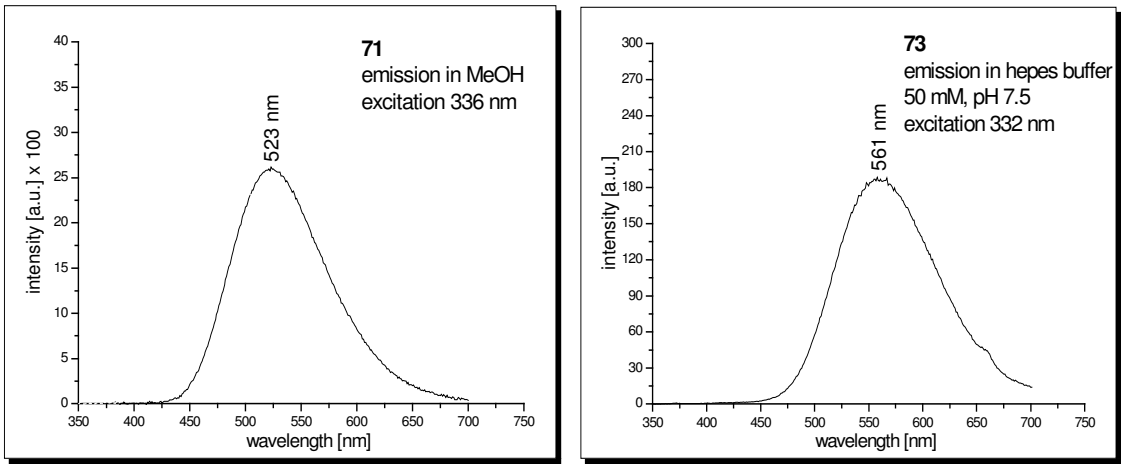
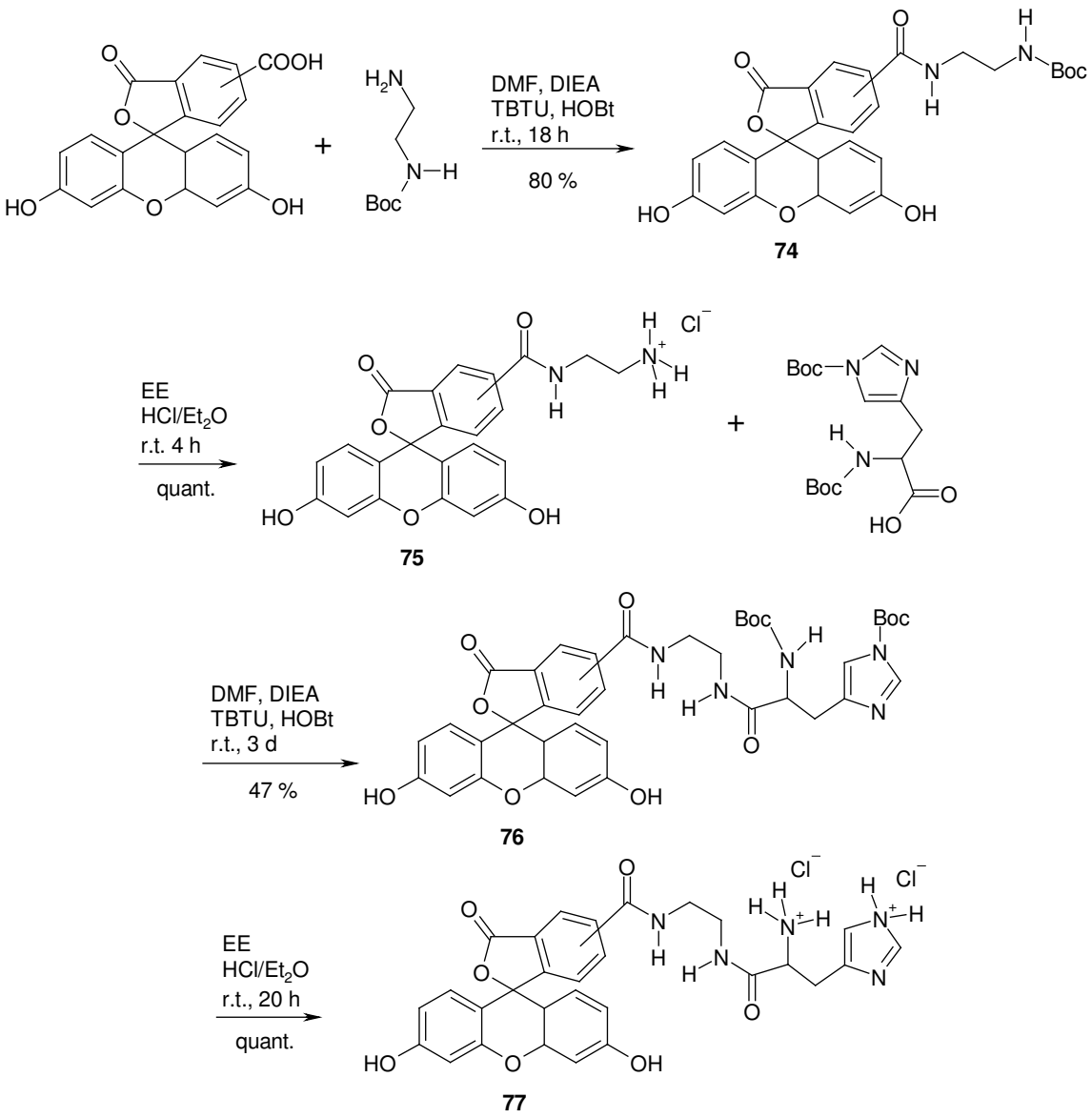



Figure 11: Fluorescence spectrum of **71** in MeOH (c = 1.13 x $10^{-4}$ mol/L) on the left with the maximum at 523 nm and fluorescence spectrum of **73** in aq. buffer (hepes [pH 7.5, 50 mM], c = 2.69 x $10^{-4}$ mol/L) on the right. Different intensities of emissions were due to different settings of the spectrometer's internal photomultiplier.

In order to connect a fluorescein group to the C-terminus of His, first an isomeric mixture of 5- and 6-carboxy fluorescein was reacted with Boc-protected ethylene diamine. After cleavage of the protection group, **75** was coupled to $N_{\alpha}$,$N_{im}$-di-Boc-L-histidine (di-cyclohexylamine salt). Following, the Boc-groups of **76** were removed with HCl saturated diethyl ether. This yielded a mixture of isomers of the fluorescein labelled N-terminal His hydrochloride salt **77** in an overall yield of 38 % (scheme 29). The degree of protonation of **77** was considered to be 2 and therefore the HCl-salt was not converted into the corresponding free amine.




Scheme 29: Synthesis of the fluorescein labelled N-terminal histidine **77**.

UV/Vis and fluorescence spectrum of **77** are given in figure 12. Compound **77** showed a very intensive emission signal ($\lambda_{Ex}$= 488 nm), which was still detectable at a concentration of 10 nM.

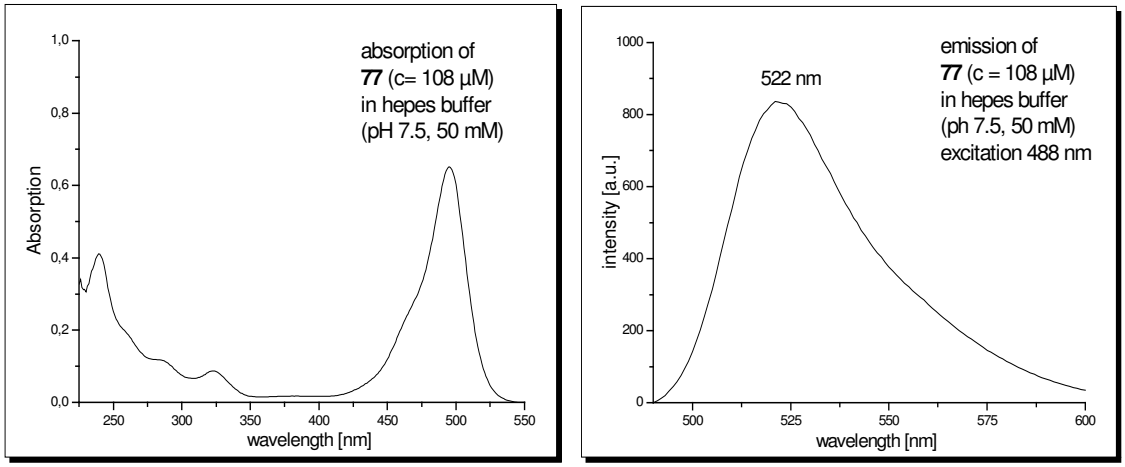
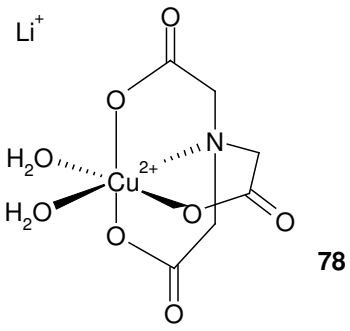



Figure 12: On the left: Absorption spectrum of **77** (c = 1.08 x $10^{-4}$ mol/L) in hepes buffer (pH 7.5, c = 50 mM) with the maximum absorbance at 495 nm. On the right: corresponding fluorescence spectrum ($\lambda_{Ex}$= 488 nm).

## *2.2.4.2. Binding Properties of Fluorescent Indicators*

In order to verify that a Cu(II)-NTA unit is able to quench the fluorescence of the dansyl labelled His, the emission of **73** was measured in a titration experiment with the not functionalized Cu(II)-NTA complex **78**.




**78**

Figure 13: Cu(II)-NTA (**78**) which was used in a first titration experiment with **73**.

Figure 14 shows the decrease of the fluorescence of **73** (c = 26.9 µM) upon addition of **78** (c = 2.69 mM) in buffered solution (50 mM hepes buffer, pH 7.5). The steep decrease after addition of 0.3 eq of **78** is remarkable and suggests a 2:1 stoichiometry of **73**:**78**. This was confirmed by a Job's plot (figure 15) and a manual fitting of the titration curve (figure 16). The latter gave an unexpected high binding

constant of 2.1 (± 0.2) x $10^{10}$ $L^2/mol^2$. However, a 2:1 stoichiometry between **73** and **78** seems not possible, as **78** can bind only 1 further bidentate ligand.

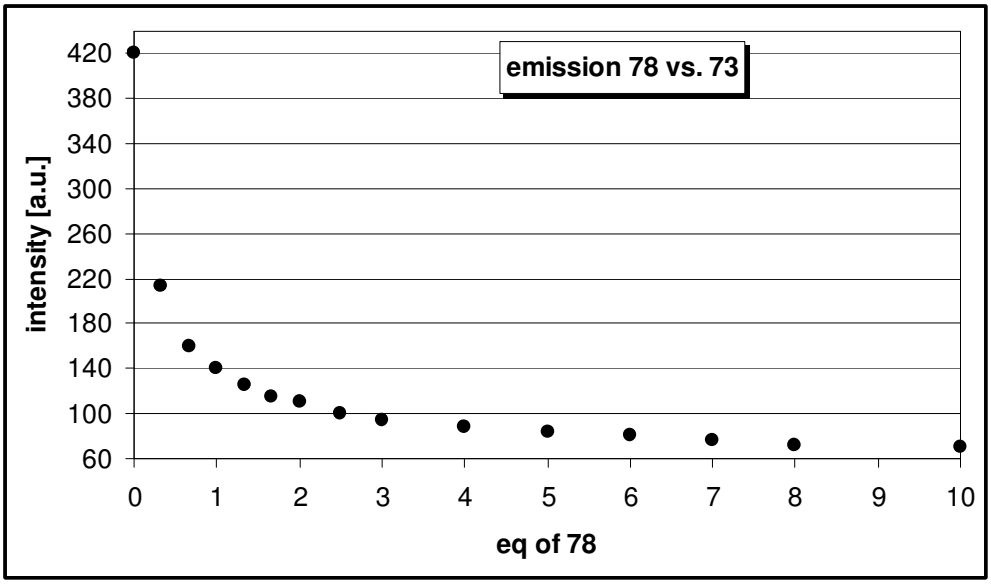



Figure 14: Quenching of fluorescence of **73** by **78**.

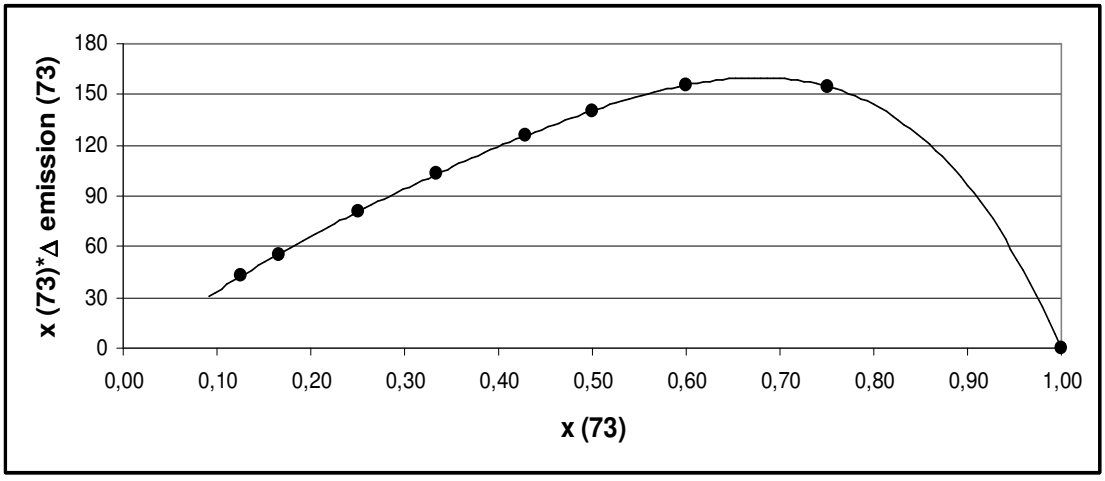



Figure 15: Job's plot of titration of **73** with **78**.

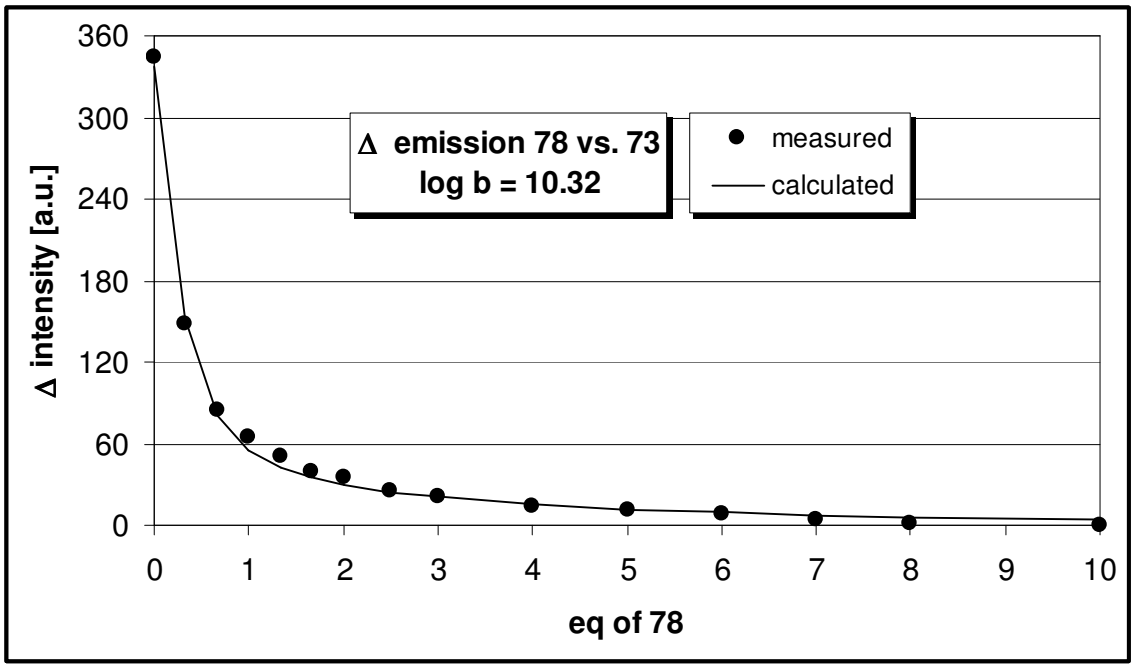



Figure 16: Measured and calculated curve of titration of **73** with **78**.

One possible explanation for this observation is that **73** shows a comparable high binding affinity towards $Cu^{2+}$ like **78**, therefore partially decomplexes **78** and forms a 2:1 complex with $Cu^{2+}$. This assumption was confirmed by MS-spectroscopy of a mixture of **73** and **78**. The spectrum showed besides a 1:1 complex of **73**:**78** also a 2:1 complex of **73**:$Cu^{2+}$.

Due to these observations, the binding affinities of **73** towards $Cu^{2+}$, $Zn^{2+}$ and $Ni^{2+}$ were investigated with fluorescence titrations. Figure 17 shows the results of the fluorescence titration for $Cu^{2+}$ and figure 18 for $Zn^{2+}$. Table 4 gives the association constants ($K_a$) and the stoichiometries.

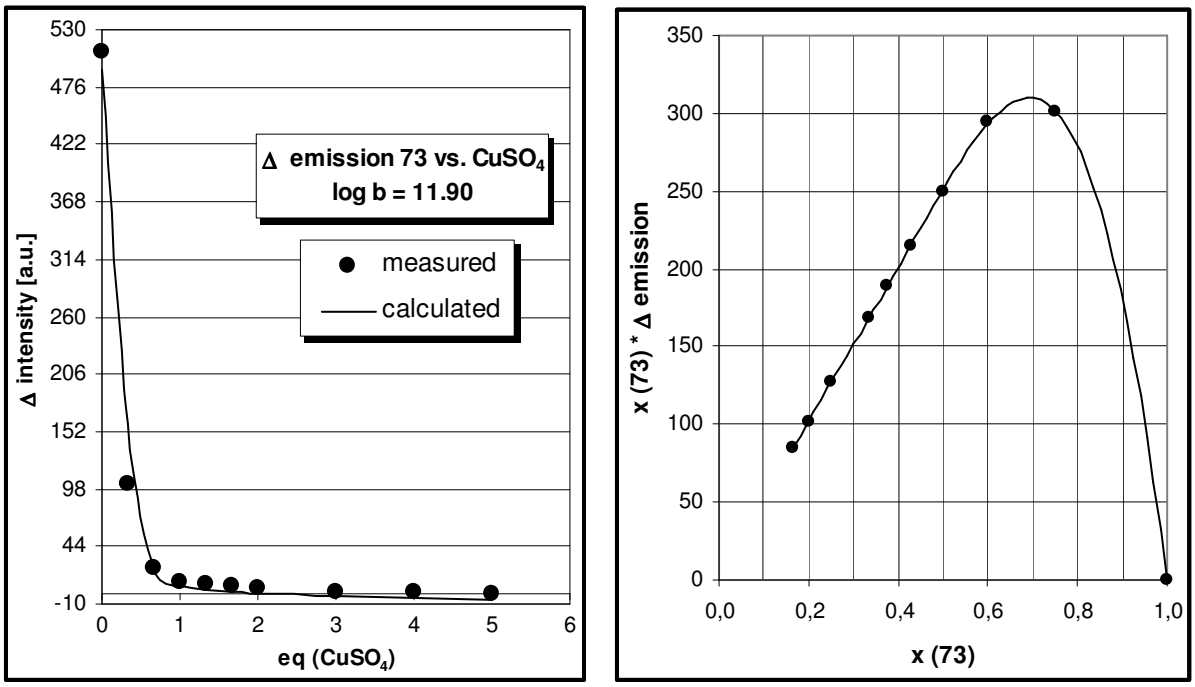



Figure 17: Measured and calculated curve of titration of **73** with $CuSO_4$ x 5 $H_2O$ in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 2:1 stoichiometry (right).

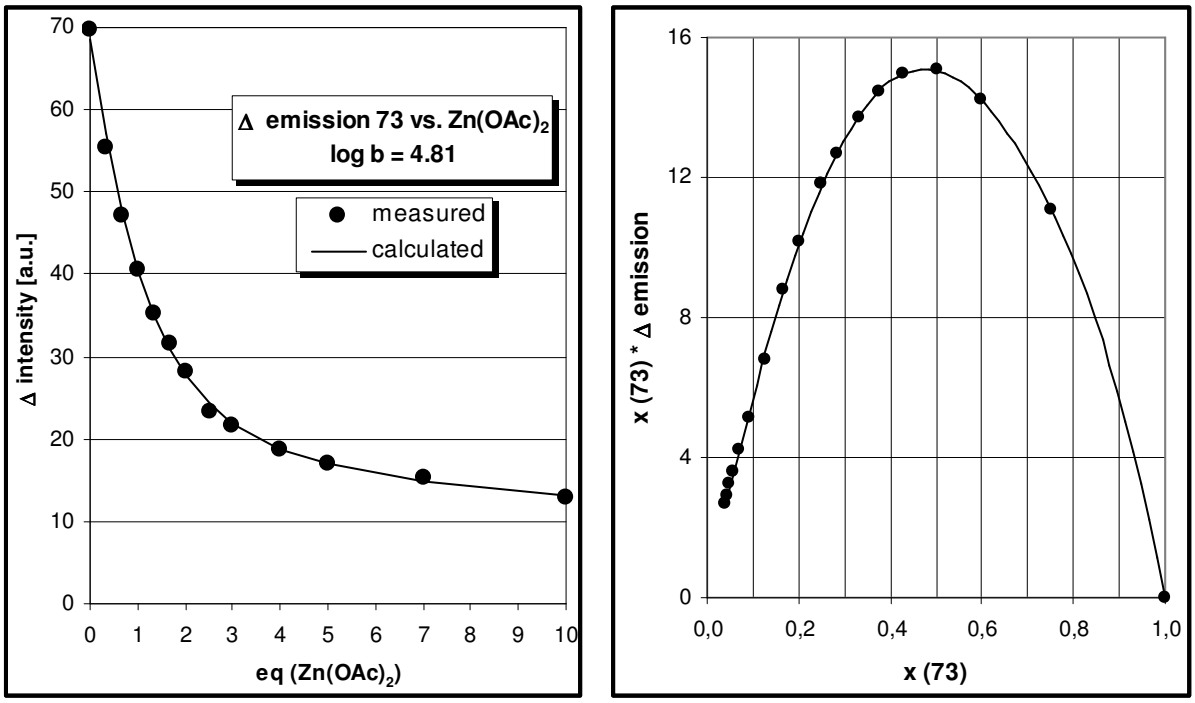



Figure 18: Measured and calculated curve of titration of **73** with $Zn(OAc)_2$ x 2 $H_2O$ in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 1:1 stoichiometry (right).

| **73** + cation from | log b (±) | $K_a$ (±) | **73** : $K^{2+}$ |
|---|---|---|---|
| $CuSO_4$ x 5 $H_2O$ | 11.9 (± 0.2) | 7.9 (± 2.5) x $10^{11}$ $L^2/mol^2$ | 2:1 |
| $Zn(OAc)_2$ x 2 $H_2O$ | 4.8 (± 0.1) | 6.5 (± 0.7) x $10^4$ L/mol | 1:1 |
| $NiCl_2$ x 6 $H_2O$ | 7.1 (± 0.3) | 1.4 (± 1.0) x $10^7$ $L^2/mol^2$ | 2:1 |

Table 4: Binding affinities of **73** towards cations $Cu^{2+}$, $Zn^{2+}$ and $Ni^{2+}$. Stoichiometry of complexes was derived from job's plots.

The results showed a much lower affinity of **73** towards $Ni^{2+}$ and $Zn^{2+}$. Further, the Job's plots of the titrations gave different stoichiometries for $Zn^{2+}$ and $Cu^{2+}$ or $Ni^{2+}$. A 1:1 stoichiometry of **73**:$Zn^{2+}$ was also confirmed by MS-spectroscopy. The spectrum of an equimolar mixture of **73** and zinc(II) acetate showed besides the free ligand the corresponding complex.

Similar dansyl labelled peptide systems containing His are reported in literature which show comparable behaviour towards heavy metal cations.[60]

Ligand **73** bears up to 5 possible donor groups: an imidazole, a primary amine, an amide, a triazole and a sulfonamide. Ligand quality ranking of these groups is estimated to be: amine > imidazole > triazole > sulfonamide > amide.[61] Due to these multiple binding interactions **73** is a suitable chelate ligand for metal cations and further shows high binding affinities towards Cu(II)-NTA complexes. However, it is unsuitable in an indicator displacement assay.

In comparison with these results, the fluorescein labelled His **77** was investigated in equal experiments. First, the binding of $Cu^{2+}$ by **77** was measured (fig. 19).

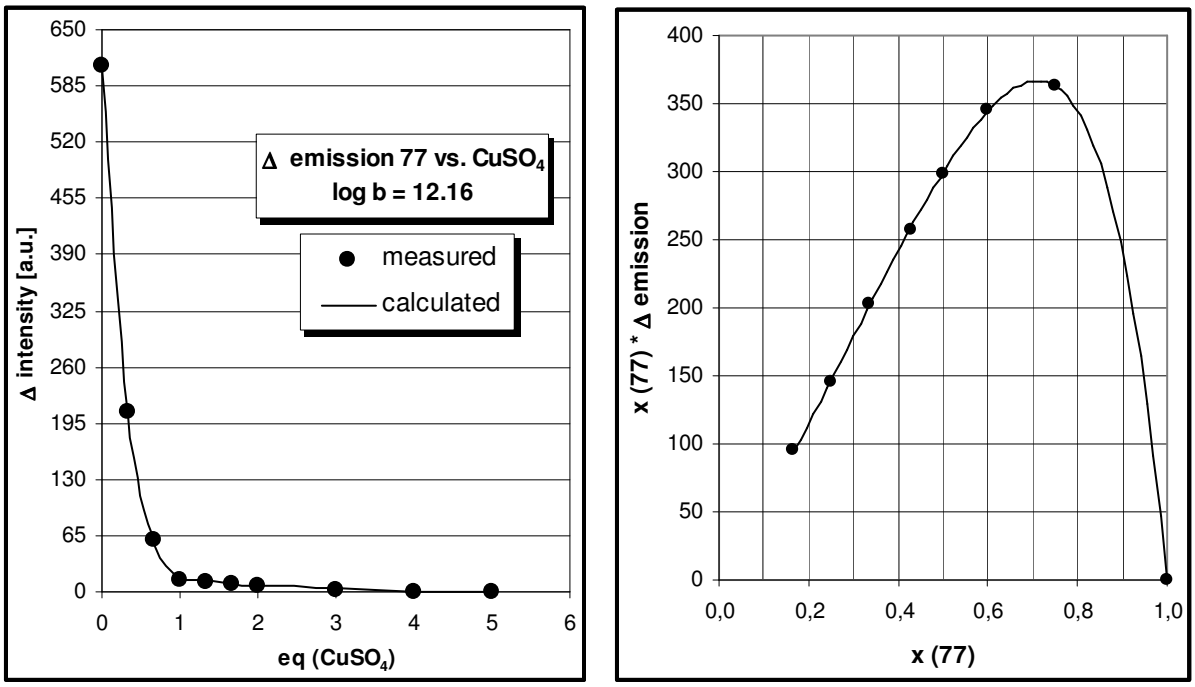



Figure 19: Measured and calculated curve of titration of **77** (c = 10.8 µM) with $CuSO_4$ x 5 $H_2O$ (c = 1.08 mM) in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 2:1 stoichiometry (right).

The measurements revealed a binding affinity of 1.45 x $10^{12}$ $L^2/mol^2$ and a stoichiometry of 2:1. This value is only insignificant higher than the association constant of **73** towards $Cu^{2+}$.

Further titration experiments were performed with **77** and the Cu(II)-NTA complexes **78** and **67** (fig. 20 and 21). The titration of **77** with **78** gave an association constant of 3.89 x $10^{12}$ $L^2/mol^2$. This value is even a hundred times higher than the binding constant between **73** and **78** (2.1 x $10^{10}$ $L^2/mol^2$). The binding constant between indicator **77** and the bidentate receptor **67** was a little bit lower, but in the same range. Measurements revealed a value of 7.2 x $10^{11}$ $L^2/mol^2$.

Mass-spectroscopy of a 1:1 mixture of **77** and **78** gave the same result as for indicator **73**. The spectrum showed besides a 1:1 complex of **77**:**78** also a 2:1 complex of **77**:$Cu^{2+}$.

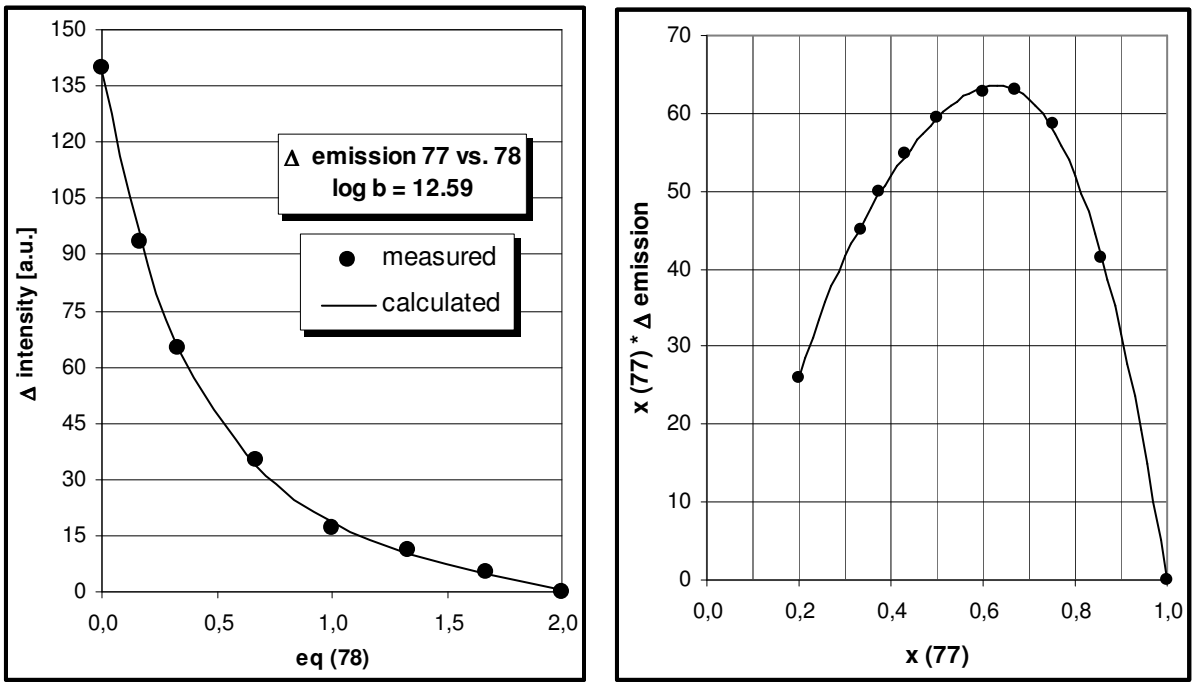



Figure 20: Measured and calculated curve of titration of **77** (c = 1.08 µM) with **78** (c = 108 µM) in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 2:1 stoichiometry (right).

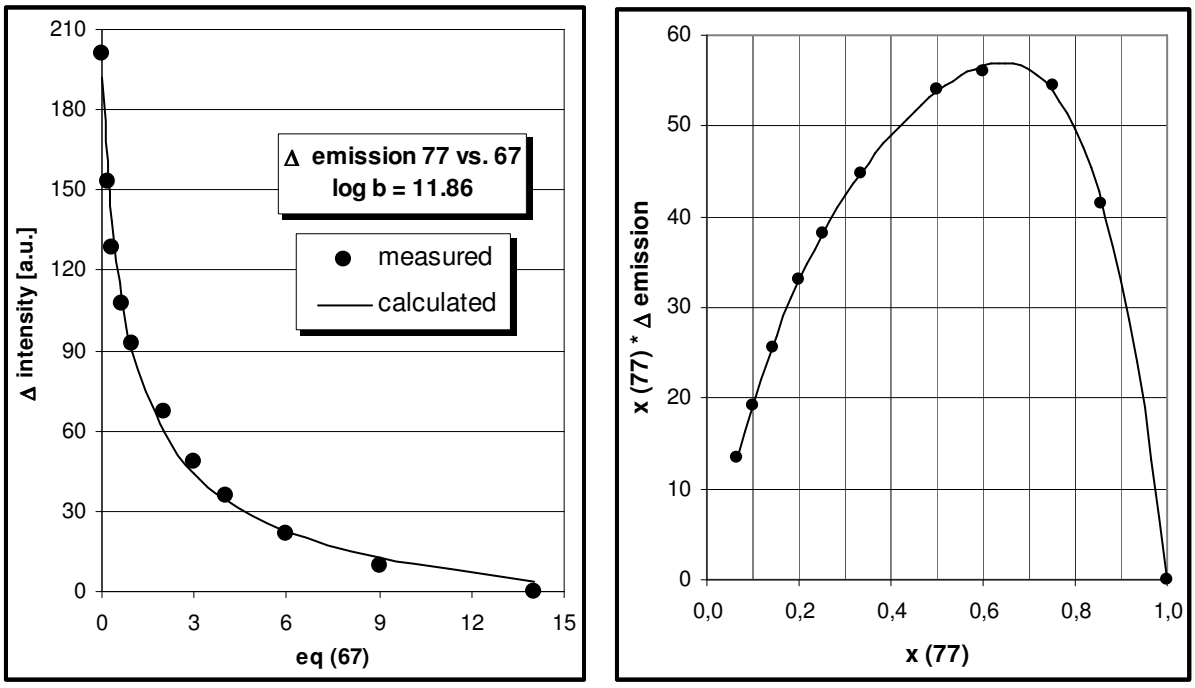



Figure 21: Measured and calculated curve of titration of **77** (c = 1.08 µM) with **67** (c = 108 µM) in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 2:1 stoichiometry (right).

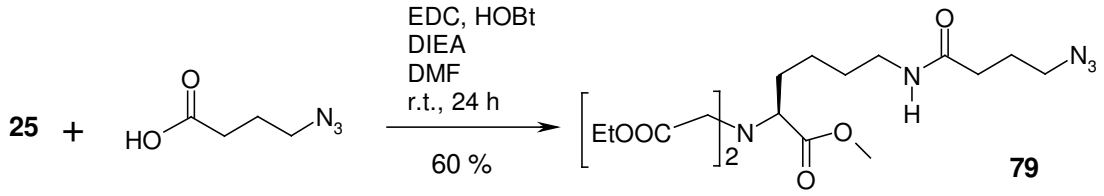
Although the synthesized fluorescent indicators **73** and **77** prooved to be unsuitable in an indicator displacement assay, these compounds can be used for the detection of $Cu^{2+}$ and Cu(II)-NTA complexes in aqueous solution. Especially **77** would allow detection of Cu(II) even at nano molar concentrations, due to its intensive fluorescence.

## 2.3. Stepwise Target Guided Synthesis

### 2.3.1. Synthesis of Bidentate Receptors

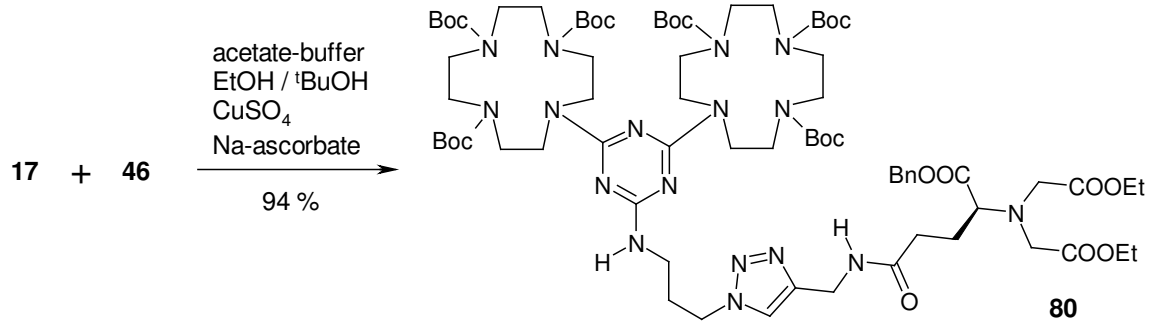
#### *2.3.1.1. Synthesis of Zn(II)-NTA-Bis-Zn(II)-Cyclen Complexes*

For the synthesis of compounds **80**, **81**, **82**, **83**, **84** the NTA-building block precursors **26**, **36**, **37**, **46**, **79** and the cyclen building block precursors **16**, **17**, **22** were used. **79** was synthesized from **25** and γ-azido butyric acid (scheme 30) like **26** and **29**.




Scheme 30: Synthesis of NTA-building block precursor **79**.

The precursors were connected in a common Cu(I) catalyzed "click-reaction" with sodium ascorbate and $CuSO_4$ x 5 $H_2O$.[62] Scheme 31 shows exemplary the synthesis of **80**, the following schemes show the subsequent reaction steps. Table 5 gives the yield for the synthesis of compounds **80** – **84**. For details about carrying out reactions and the structures of these compounds, as well as their subsequent steps, please see experimental section *chapter 2.5.3.2*.



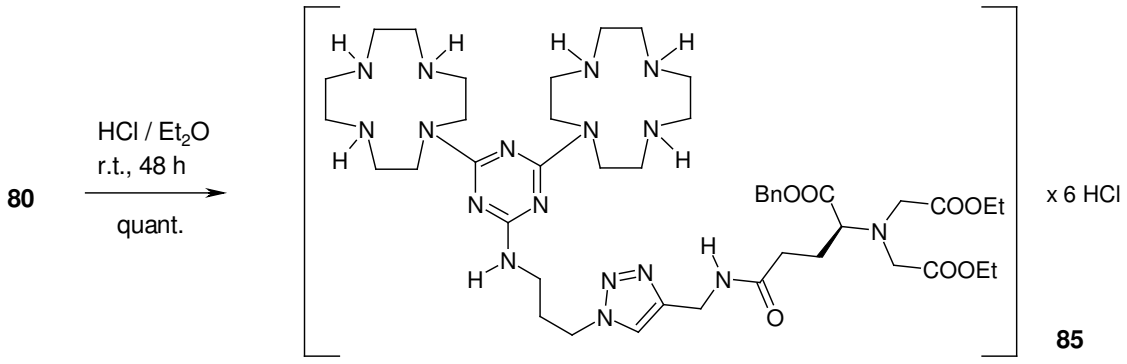

Scheme 31: Connection of precursors **17** and **46** using the "click-chemistry" approach.

| Precursors | Triazole | Yield |
|---|---|---|
| **17** + **46** | **80** | 94 % |
| **22** + **36** | **81** | 96 % |
| **22** + **37** | **82** | 93 % |
| **16** + **26** | **83** | 91 % |
| **22** + **79** | **84** | 90 % |

Table 5: Combination of precursors and resulting triazole.

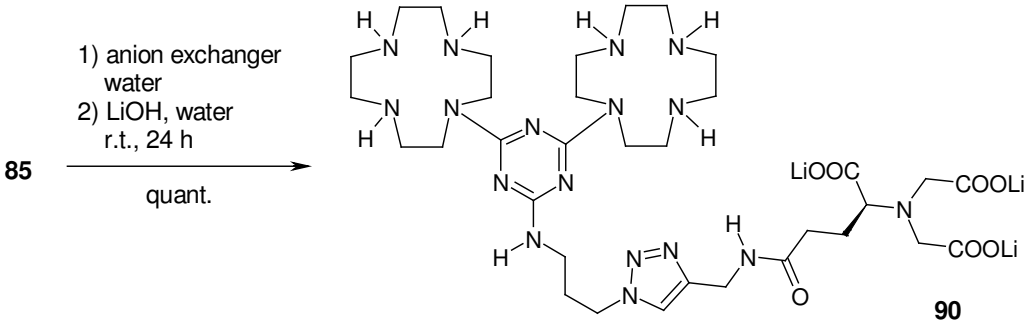
The copper as well as the ascorbate salt were not used in catalytic amounts as this proved to give higher yields after shorter reaction times. As the the reduction of Cu(II) by sodium ascorbate causes a lowering of the pH-value, the reaction was carried out in buffered solution (acetate buffer, pH 5, c = 500 mM). Further, a simple workup with aqueous solutions of $H_2O_2$ and EDTA was necessary, due to complexation of $Cu^{2+}$ by the formed triazole.[63] This gave the triazoles **80** – **84** in high yields (table 5, for details see experimental section chapter *2.5.3.1* **GP 9**).

Following, the Boc-protecting groups were cleaved with a saturated solution of HCl in diethylether. Compounds **85**, **86**, **87**, **88** and **89** precipitated from solution quantitatively and were used directly in the next reaction step (see experimental section **GP 10**). Scheme 32 shows exemplary the synthesis of **85**. Compound **86** was derived from **81**, **87** from **82**, **88** from **83** and **89** from **84**.



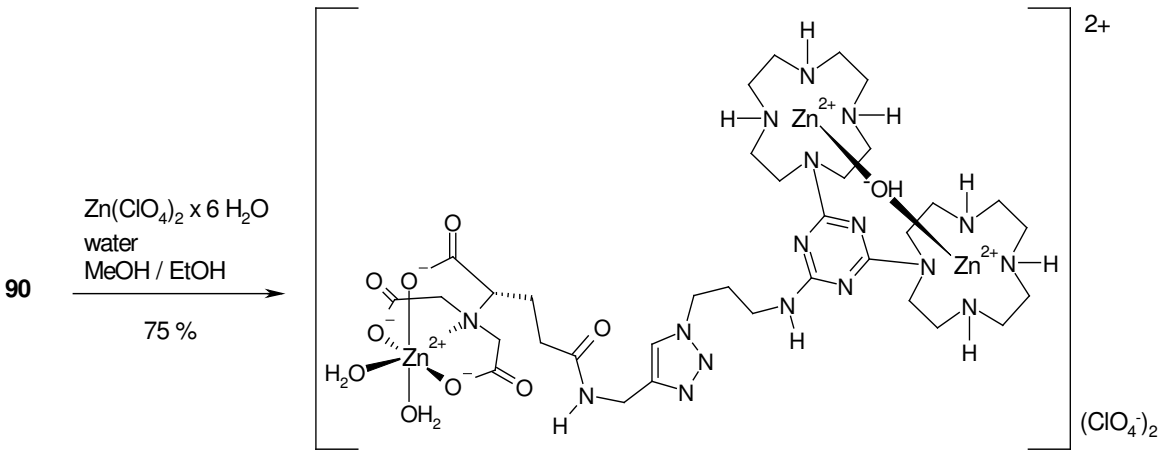

Scheme 32: Removal of Boc-protecting groups with HCl saturated diethyl ether.

The ammonium salts **85** – **89** were deprotonated using a strongly basic anion exchanger in its $OH^-$-form. Cleavage of the ester groups during this procedure was not observed. The obtained aqueous solutions of the free amines were treated with a stoichiometric amount of LiOH in order to cleave the ester functionalities (Bn-ester as well as Me- and Et-ester, see experimental section **GP 11**). Lyophilisation of these solutions gave the polydentate ligands **90**, **91**, **92**, **93**, **94** (derived from **85**, **86**, **87**, **88**, **89** in this sequence) in quantitative yield, ready for complexation with $Zn^{2+}$ (scheme 33).




Scheme 33: Deprotonation of the ammonium salt **85** with a strongly basic anion exchanger ($OH^-$-form) and cleavage of ester groups with LiOH.

Complexation was carried out in a refluxing mixture of MeOH and water. Important was the control of the pH value, which had to be kept at approx. 8. This was necessary in order to prevent protonation of the ligand by the lewis acidic Zn(II)-solution or precipitation of polymeric $Zn(OH)_2$ by the basic solution of the amine ligand. Complexation at stable pH value was achieved by simultaneous addition of methanol solutions of ligands **90** – **94** and $Zn(ClO_4)_2$ x 6 $H_2O$ to water at 80 °C. Further, zinc perchlorate was used only in a small excess. Precipitation after addition of EtOH gave the threefold Zn(II)-complexes **95**, **96**, **97**, **98**, **99** (derived from **90**, **91**, **92**, **93**, **94** in this sequence) in good yields of 75 % as colourless solids (scheme 34, see experimental section **GP 12**).



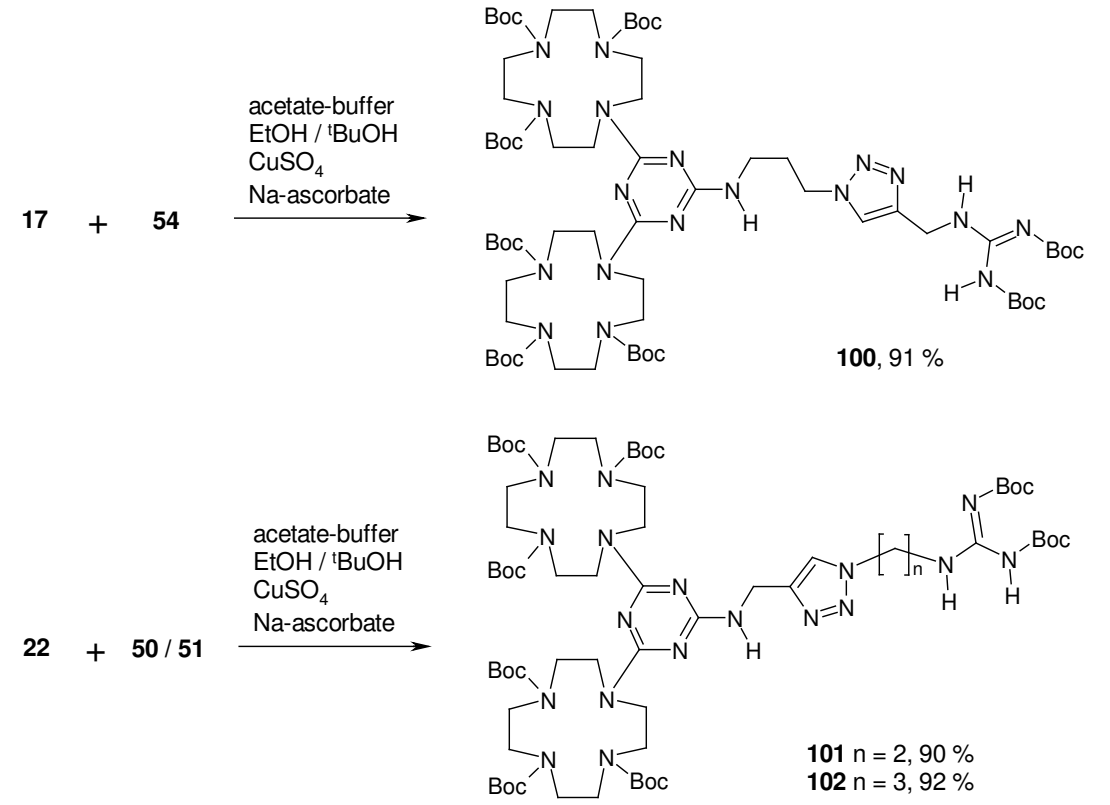

**95**

Scheme 34: Complexation of $Zn^{2+}$ yields the bidentate receptor **95**.

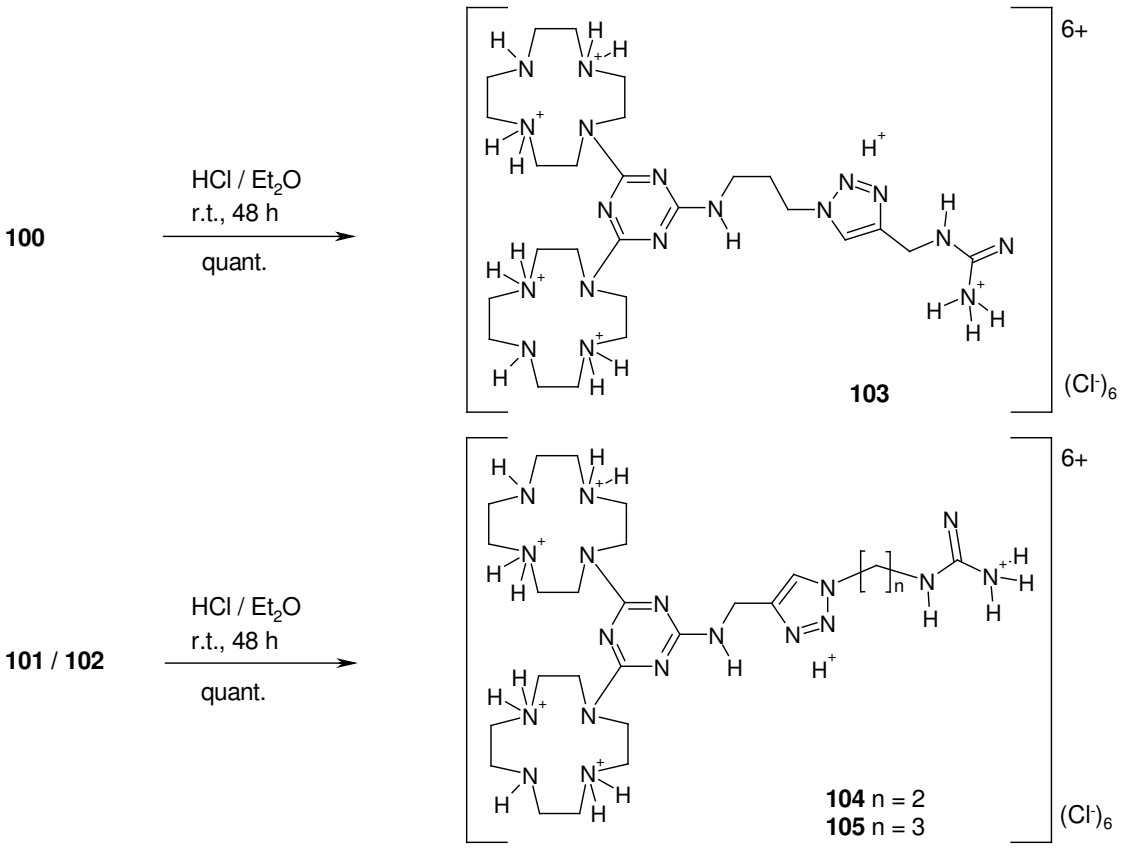
## *2.3.1.2. Synthesis of Guanidine-Bis-Zn(II)-Cyclen Complexes*

For the synthesis of compounds **100**, **101** and **102** the guanidine precursors **50**, **51**, **54** and the cyclen building block precursors **16**, **17**, **22** were used. The precursors were connected in a common Cu(I) catalyzed "click-reaction" according to the synthesis of compounds **80** – **84** (**GP 13**, scheme 35).

Subsequently, the Boc-protecting groups were cleaved using the established procedure with HCl saturated diethyl ether (**GP 10**, scheme 36). This yielded quantitatively the ammonium salts **103**, **104** and **105**.

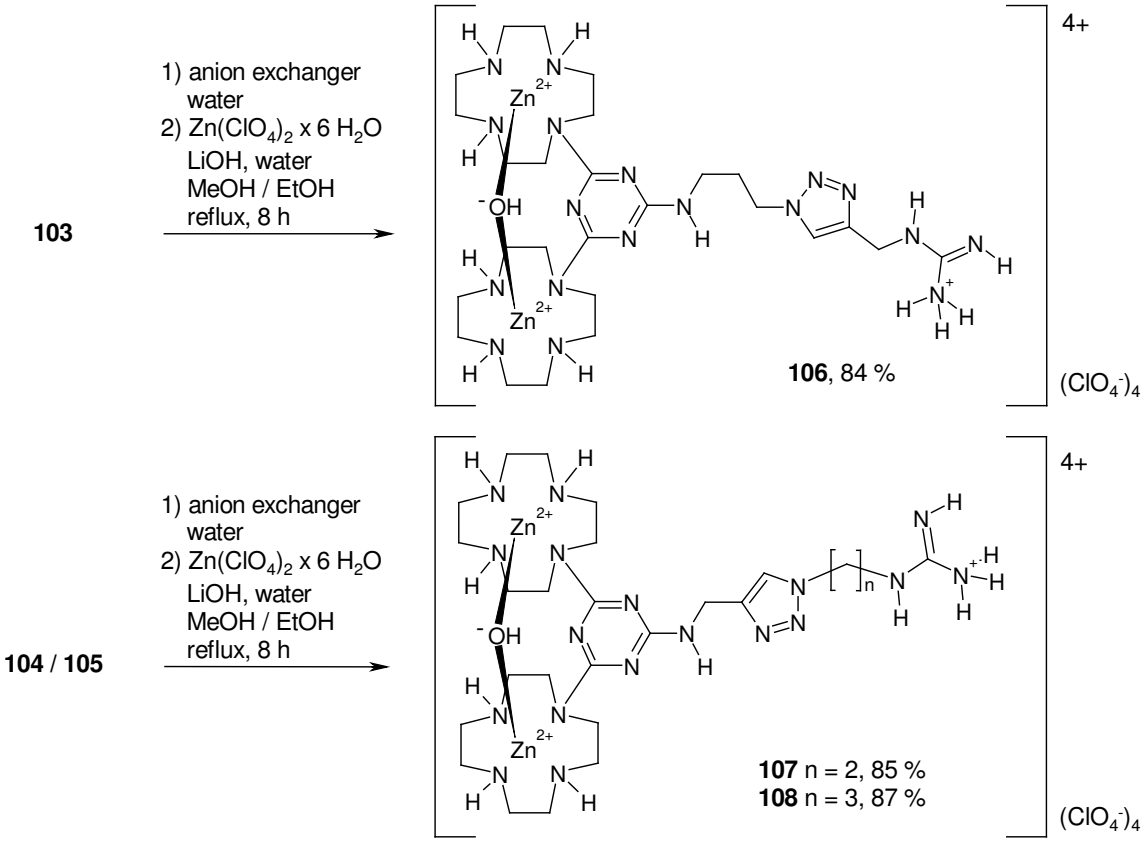



Scheme 35: Connection of the precursors using the Cu(I) catalyzed “click”-reaction.




Scheme 36: Cleavage of the Boc-groups with HCl saturated diethyl ether.

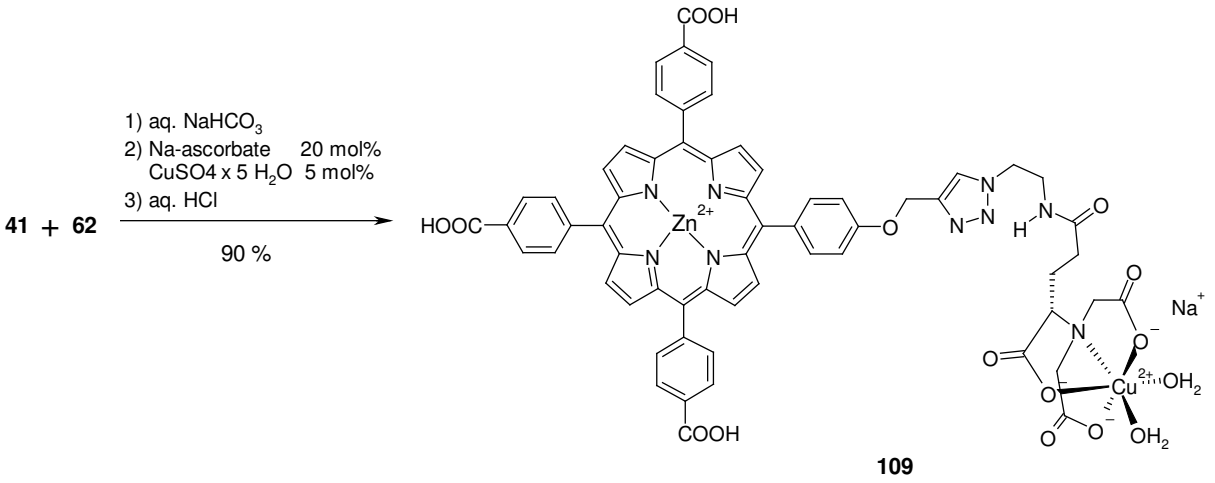
After deprotonation with a strongly basic anion exchanger ($OH^-$-form), the resulting amines were ready for complexation of $Zn^{2+}$. The synthesis of Zn(II)-complexes **106**, **107** and **108** was done like the synthesis of complexes **95** – **99**, however with one major difference: As the amine-ligands tended to precipitate after protonation, the pH value of the reaction mixture had to be kept as basic as possible. This was achieved by addition of 0.1 M aq. LiOH to the reaction mixture until $Zn(OH)_2$ began to precipitate (scheme 37, for details see experimental section **GP 14**).




Scheme 37: Complexation of $Zn^{2+}$ in basic solution using $Zn(ClO_4)_2$ x 6 $H_2O$ and LiOH.

*2.3.1.3. Synthesis of a Cu(II)-NTA-Zn(II)-Porphyrin Complex*

The tetraphenyl-porphyrin (TPP) unit is well-suited for protein surface recognition due to its large hydrophobic surface area of more than 300 $Å^2$. More importantly, TPP derivatives are highly fluorescent and can show emission intensity changes on binding to a protein target. In order to synthesize a bidentate receptor with a porphyrin and a NTA-moiety, compounds **62** and **41** were connected in a typical "click-reaction". The Cu(II)-NTA complex **41** was used in slight excess of 1.1 equivalents. As **62** was soluble only in basic aqueous solutions it was first treated with sat. aq. solution of $NaHCO_3$. After addition of the NTA-complex **41**, aliquots of aq. stock solutions of copper sulfate and sodium ascorbate were added to generate the essential $Cu^+$ in situ. The $Zn^{2+}$ in **62** worked like a protecting group and avoided complexation of $Cu^{2+}$. After 24 h stirring at room temperature, the reaction was expected to be complete. Thus, the solution was acidified with 1 N HCl to precipitate the product in pure form. All other compounds like copper- and ascorbate salts, as well as remaining **41** stayed in solution. This gave **109** in a high yield of 90 %.




**109**

Scheme 38: Connection of the Cu(II)-NTA complex **41** and the Zn(II)-porphyrin **62** in a typical "click-reaction".

The use of an asymmetric substituted tetra-phenyl-Zn(II)-porphyrin like **62** in "click-reactions" bears two major advantages:

- a water-soluble fluorescent receptor with a high affinity for non-polar binding sites can be connected easily to any suitable substrate.
- as its solubility strongly depends on the pH, this enables a simple purification by precipitation and therefore allows the use of an excess of reaction partner which results in high reaction yields.

### 2.3.2. Binding Affinities of Receptors

The binding affinities of receptors **95** – **99** and **106** – **108** towards different phosphorylated peptides were investigated.[d] The used peptides had following sequences:

- 5-Carboxyfluorescein-Gly-pTyr-Asp-Lys-Pro-His-Val-Leu-OH (**P1**)
- 5-Carboxyfluorescein-Gly-Phe-Asp-pThr-Tyr-Leu-Ile-Arg-Arg-OH (**P2**)
- 5-Carboxyfluorescein-Gly-pTyr-Glu-Glu-Ile-Pro-OH (**P3**)

These peptide sequences revealed to be potent binding partners for proteins of the STAT – family.[64] Signal tranducers and activators of transcription (STATs) are latent cytoplasmic transcription factors which transmit signals from the cell membrane to the nucleus.[65] STAT3, for example, is active in a lot of primary human tumors and tumor-derived cell lines. Further, it seems to be an essential mediator of the abberant activity of upstream tyrosine kinases.[66] Inhibition of active STAT3 results in growth inhibition and apoptosis of tumor cells.[67] In common with other STAT proteins, the STAT3 SH2 domain shows binding interactions to phosphotyrosine domains in two processes: first in the binding of the activated upstream kinase (prior to phosphorylation) and then in the dimerisation of two phophorylated STAT3 molecules. Therefore, small molecules like peptides **P1** – **P3** are potential inhibitors of STAT1, STAT3 and other members of this protein family.

[d] Peptides were synthesized by Dr. F. Freudenmann. Binding investigations were performed by Bianca Sperl under the supervision of Dr. Thorsten Berg (Max Planck Institute of Biochemistry, Martinsried).

The spectroscopical method which was used to investigate the binding between the Peptides **P1** – **P3** and different STAT proteins was a homogenous fluorescence polarisation assay.[64] This method is well suited for the investigation of binding between small fluorescence labelled molecules and a significantly larger binding partner.[68]

Complexes **95** – **99** are potential receptors for **P1** as the peptide provides two complementary binding sites: the phosphorylated tyrosine for the bis-Zn-cyclen moiety and the imidazole ring of the histidine sidechain for the Cu-NTA moiety.

Whereas, complexes **106** – **108** are potential receptors for all three peptides, as each provides one or two carboxylate groups (Asp, Glu) in proximity to the phosphorylated amino acid (Tyr, Thr), which enables binding interactions with the guanidinium moiety.

### *2.3.2.1. Binding Affinities of Zn(II)-NTA-Bis-Zn(II)-Cyclen Complexes*

The influence of the metal complex receptors **95** – **99** on the binding between **P1** and STAT1 was investigated by detection of the polarisation of the fluoresceine emission. The diagrams in figures 23 and 24 show the dependence of the peptide – protein binding on the concentration of added receptor. The y-axis shows the ratio of **P1**-STAT1-complex in %, the x-axis shows the receptor concentration in µmol/L. The bis-Zn(II)-cyclen **110** (fig. 22) was used as reference compound. It provides no second binding site and therefore should show an influence on the **P1** – STAT1 binding at higher concentrations compared to the bidentate receptors **95** – **99**.

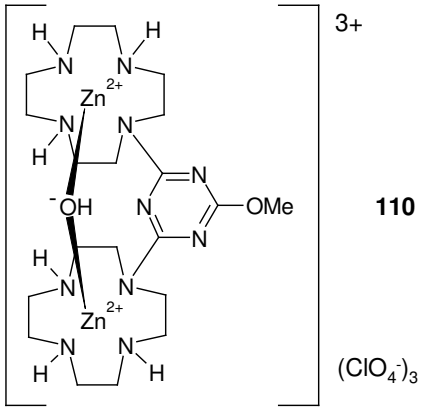



Figure 22: bis-Zn(II)-cyclen **110** which was used as reference compound in titration experiments.

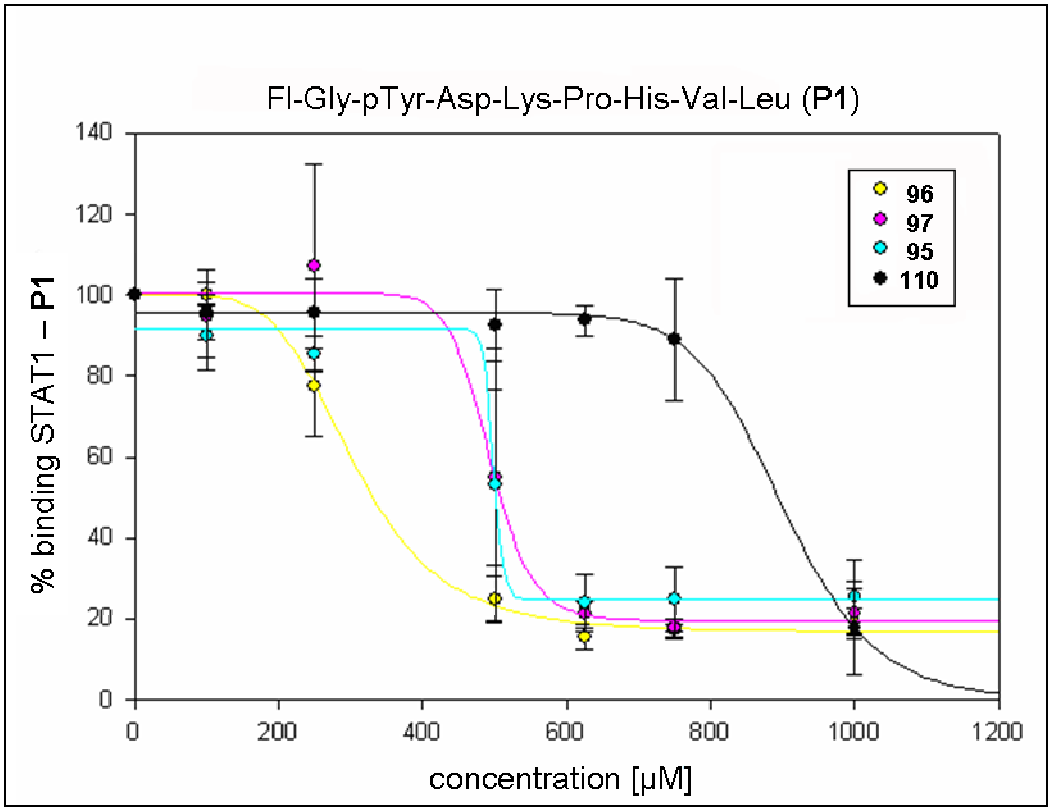



Figure 23: Titration curves of the receptors **95** – **97** and the reference compound **110** in the fluorescence polarisation assay with the **P1**-STAT1-complex.

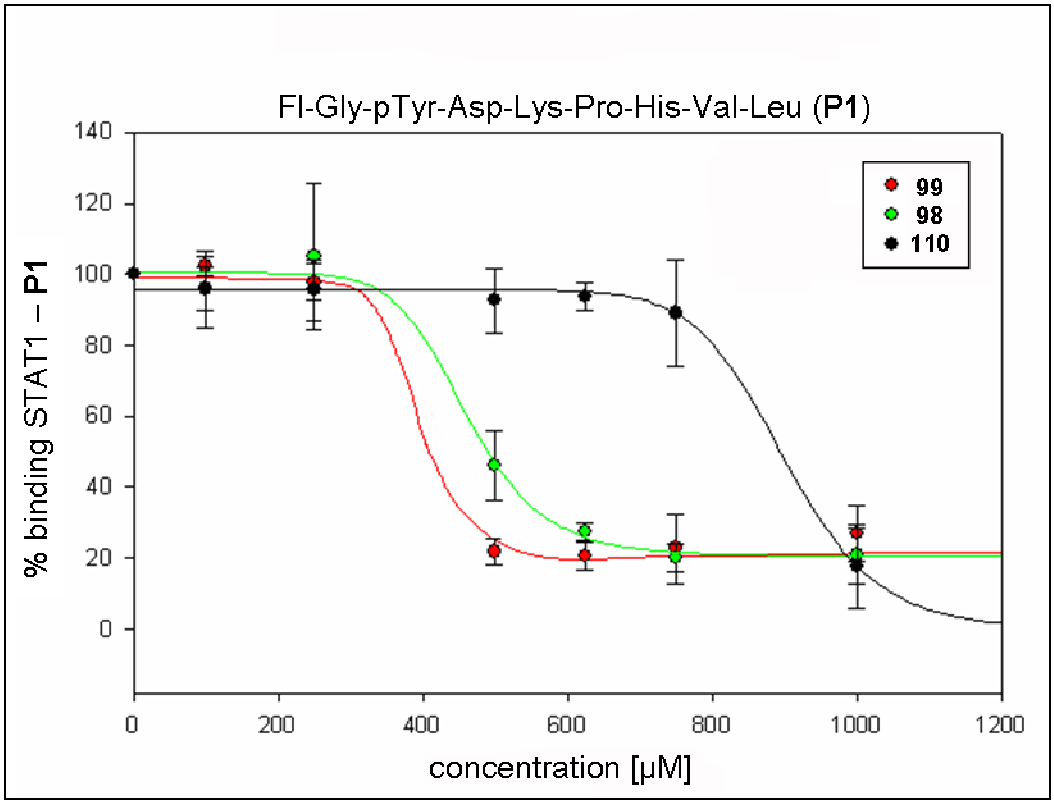



Figure 24: Titration curves of the receptors **98**, **99** and the reference compound **110** in the fluorescence polarisation assay with the **P1**-STAT1-complex.

Measurements revealed that receptors **95** – **99** influence the STAT1 – **P1** binding at a concentration of approx. 450 µmol/L while the reference compound **110** shows the same effect at approx. 900 µmol/L. Further, all bidentate receptors show more or less the same binding behaviour. This indicates that the different structural chracteristics like the distance between the binding sites or the position of certain functionalities (triazole, amide groups) have no influence on the binding properties

Titration experiments of receptors **95** – **97** with peptide **P2** showed nearly the same results as for **P1**. The bidentae receptors showed an influence on the STAT3 – **P2** binding at half concentration (300 µmol/L) compared to **110** (600 µmol/L). This was unexpected, as **P2** beared no His in its sequence and therefore provided no second binding site for the receptors.

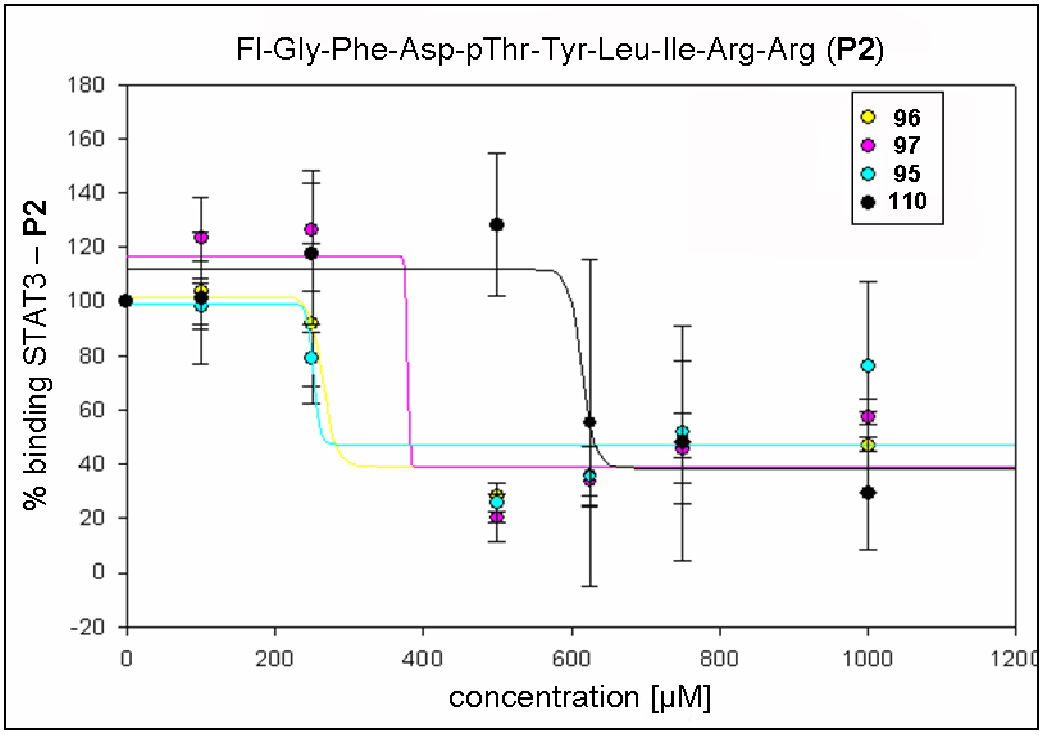



Figure 25: Titration curves of the receptors **95** – **97** and the reference compound **110** in the fluorescence polarisation assay with the **P2**-STAT3-complex.

These results showed that not the additional NTA-binding site increased the binding affinity by a factor of 2, but the whole substituent at the triazine ring in **95** – **99**. The insignificantly higher binding affinity of the bidentate receptors compared to **110** is likely due to unspecific interactions.

*2.3.2.2. Binding Affinities of Guanidine-Bis-Zn(II)-Cyclen Complexes*

The titration experiments with bidentate receptors **106** – **108** and the peptides **P1** – **P3** were performed in analogy to the investigations with complexes **95** – **99** described in the previous chapter *2.3.2.1*. First, the influence of the guanidines on the binding between GST-Lck and peptide **P3** was determined (fig. 26).

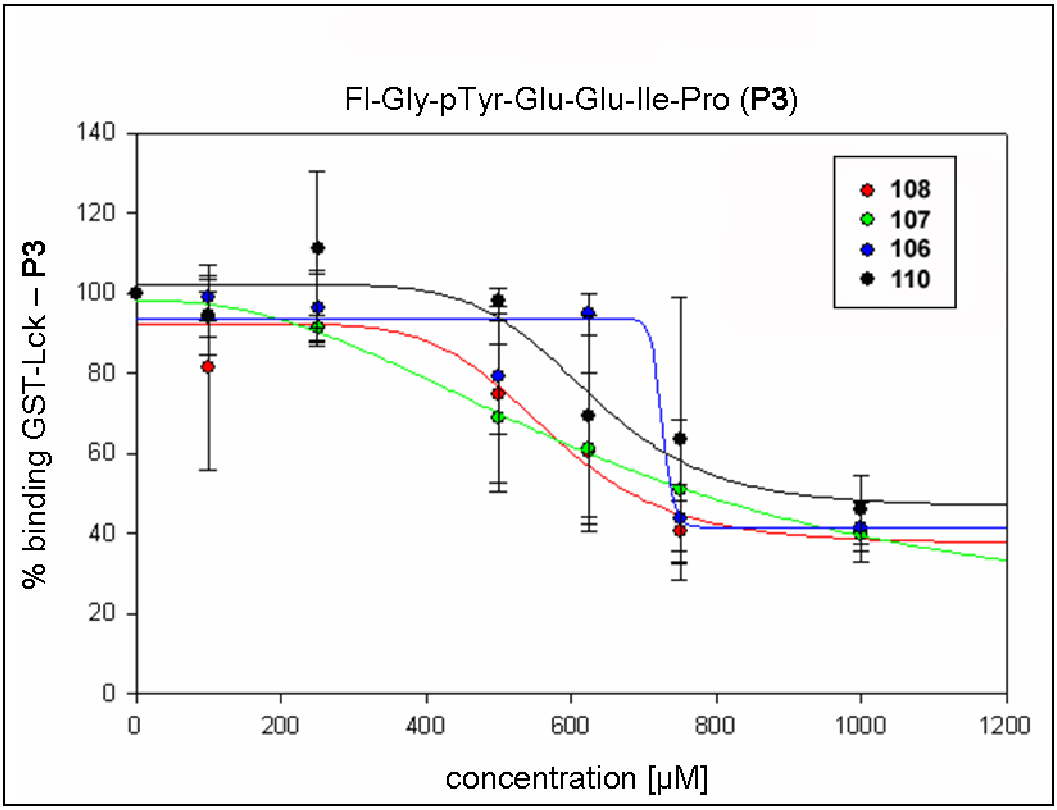



Figure 26: Titration curves of the receptors **106** – **108** and the reference compound **110** in the fluorescence polarisation assay with the **P3**-GST-Lck-complex.

First, peptide **P3**, bearing two carboxylate groups in proximity to the phosphorylated Tyr and a further one at the free C-terminus, was used. Consequently, it was expected to be a suitable binding partner for the receptors **106** – **108**. However, the measurement revealed almost no increase of the binding affinity of the bidentate receptors, compared to **110**.

Additional measurements were performed with the peptides **P1** and **P2**. Both contain a carboxylate group next to the phosphorylated Tyr- or Thr-sidechain. However, a more or less distinct effect on the protein-peptide binding can only be detected for STAT1 - **P1** (fig. 27 and 28).

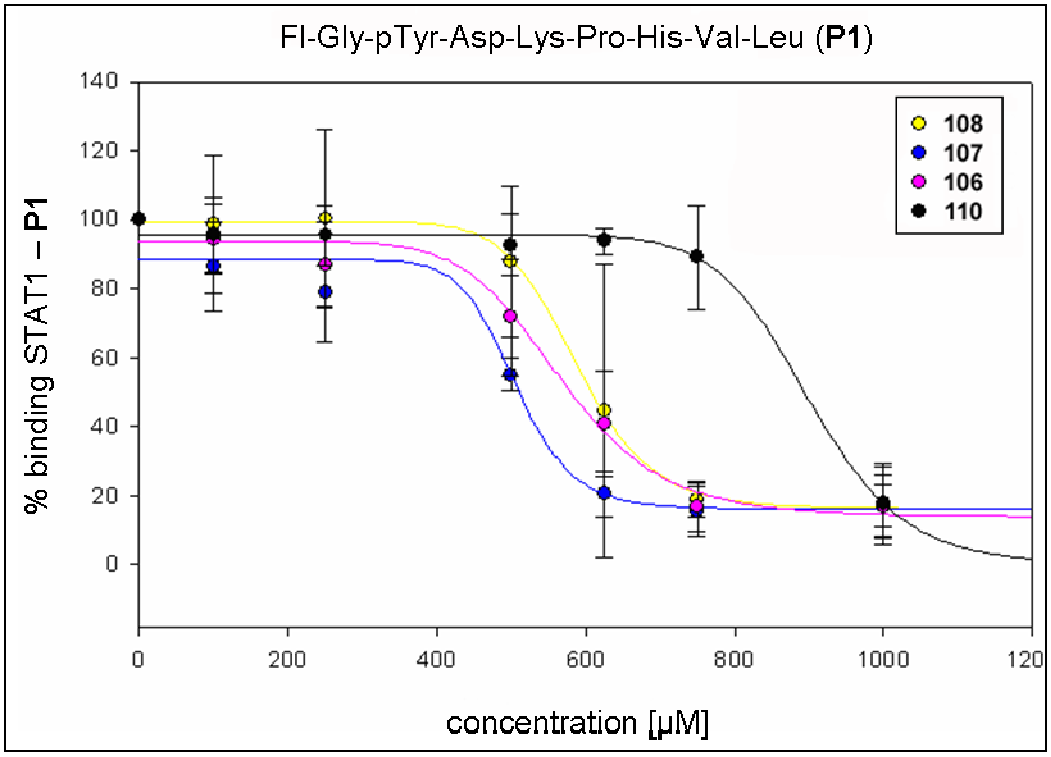



Figure 27: Titration curves of the receptors **106** – **108** and the reference compound **110** in the fluorescence polarisation assay with the **P1**-STAT1-complex.

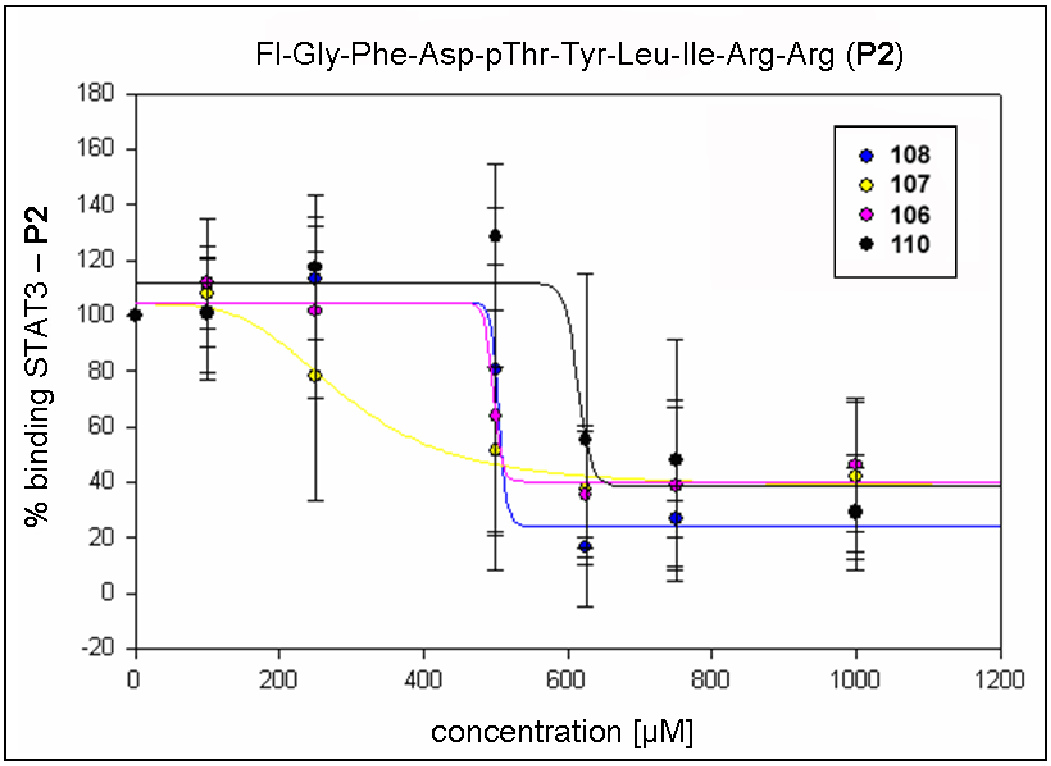



Figure 28: Titration curves of the receptors **106** – **108** and the reference compound **110** in the fluorescence polarisation assay with the **P2**-STAT3-complex.

In conclusion the results of the titration experiments showed that the bidentate receptors **95** – **99** and **106** – **108** show an insignificant higher binding affinity than the reference compound **110**. Further, no specific binding or any relationship between the structure of the receptors and the binding affinity could be determined.

However, one aspect should be taken into consideration: all measurements were based on the influence of the receptor molecules on the interactions between the peptides **P1** – **P3** and their natural binding partners (STAT1, STAT3 and GST-Lck). Consequently, the binding interactions between the receptors and the peptide targets were investigated in an indirect way. Furthermore, the inhibition of peptide-protein interactions by artificial receptors is still a very challenging task. Therefore it would be quite possible that the synthesized bidentate receptors show a high and specific binding towards suitable peptide targets if the binding interactions were investigated directly.

#### *2.3.2.3. Binding Affinities of a Cu(II)-NTA-Zn(II)-Porphyrin Complex*

As the porphyrin moiety of **109** showed a strong fluorescence, which was expected to change during the binding process,[69] first the spectroscopic characteristics of **109** were investigated and compared with **62**. The UV/Vis-spectrum of **109** did not show any significant changes compared to **62** – the soret band was neither shifted nor was there a change of the extinction coefficient. However, the fluorescence spectrum of **109** showed a decrease of emission intensity by nearly 50 %, compared to **62**. This was due to the Cu(II)-NTA moiety which quenched the emission of the Zn(II)-porphyrin moiety (fig. 29).

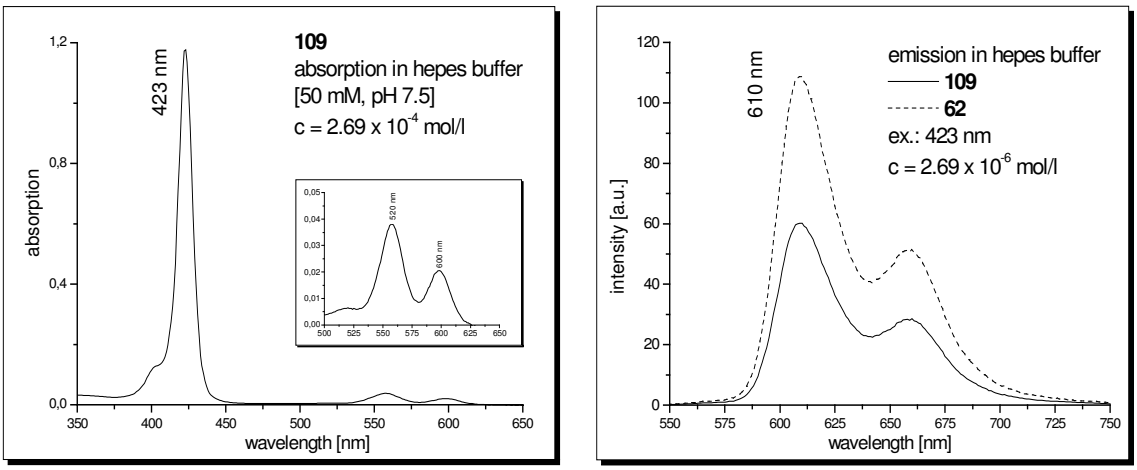



Figure 29: UV/Vis spectrum of **109** in buffer (hepes [pH 7.5, 50 mM], c = 2.69 x $10^{-4}$ mol/l) on the left with the strong absorption maximum (B-band) at 423 nm and 2 weak absorption bands (Q-bands) at 520 nm and 600 nm (enlarged). Fluorescence spectrum of **109** and **62** in buffer (hepes [pH 7.5, 50 mM], c = 2.69 x $10^{-6}$ mol/l) on the right.

1) Binding affinities to single amino acids:

In a first series of experiments, the binding of single amino acids to **109** was investigated. The tested amino-acids were: H-His-OMe, Boc-His-OH, H-Lys-OH, H-Gly-OH, H-Ser-OH. Experiments were performed in hepes buffer pH 7.5 (c = 50 mmol/L). Results revealed that only H-His-OMe was bound by **109** with an association constant of $K_a$ = 1.5 x $10^5$ L/mol (1:1 stoichiometry).[70] This was expected as Cu(II)-NTA complexes selectively bind terminal histidines, whereas Zn(II)-porphyrins show only a weak binding of amines by the zinc cation.[71] During the titration of **109** (c = 2.7 µM) with H-His-OMe (c = 270 µM) the fluorescence increased and reached in the end almost the intensity of **62** (fig. 30).[72] This showed that, after binding of a terminal histidine, the copper-NTA complex no longer quenched the fluorescence of the zinc porphyrin. Thus, the Zn(II)-porphyrin moiety also can be used to investigate binding-events of the Cu(II)-NTA moiety.

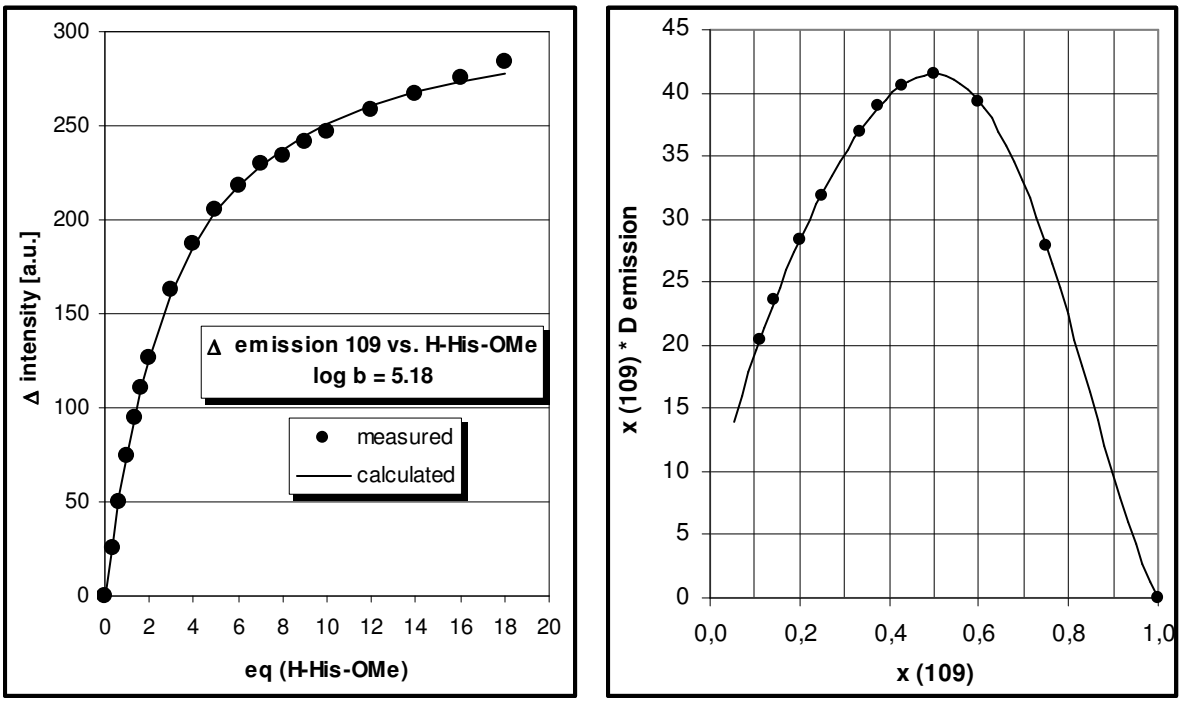



Figure 30: Measured and calculated curve of titration of **109** with H-His-OMe in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 1:1 stoichiometry (right).

2) Binding affinity to pentapeptide **63**:

In order to check the influence of a hydrophobic peptide residue, connected to a N-terminal His, on the binding to **109**, the binding affinity of the pentapeptide **63** to **109** was investigated. It was expected that **63** shows a higher binding affinity than H-His-OMe because of interactions between the non-polar amino acid sequence –Leu-Leu-Val-Phe-OH and the porphyrin.

The fluorescence titration of **109** with **63** in hepes buffer was performed under the same conditions like the titration of **109** with H-His-OMe. However, results revealed only an insignificant higher binding affinity of 2.1 x $10^5$ L/mol (1:1 stoichiometry). Therefore, the influence of the non-polar Leu-Leu-Val-Phe-OH moiety has to be considered rather low.

Maybe, a longer non-polar peptide residue with more aromatic side chain functionalities (π- π interactions with the porphyrin) would result in a significant stronger binding to **109**. However, it has to be noted that an elongation of the non-polar peptide moiety is restricted due to decreasing solubility in aqueous solution.

3) Binding affinity to Proteins:

Porphyrin **109** was synthesized in order to achieve selective bindings to non-polar regions on a protein surface with a nearby exposed histidine. Proteins which fulfill these criteria are for example hen eggwhite lysozyme (HEL) or myoglobine (horse muscle myoglobine, Myo). Figure 31 shows the titration curve of **109** vs. HEL and figure 32 shows the titration of **109** with Myo. Results revealed in both cases a decrease of fluorescence, a 1:1 binding of the receptor to the protein and high binding affinities of 1.7 x $10^5$ L/mol for HEL respectively 4.1 x$10^5$ L/mol for Myo.

Compared to HEL, the decrease of fluorescence of **109**, after binding to Myo, was stronger, because Myo contains a paramagnetic $Fe^{II}$ in its active center, which quenches the fluorescence more effectively.

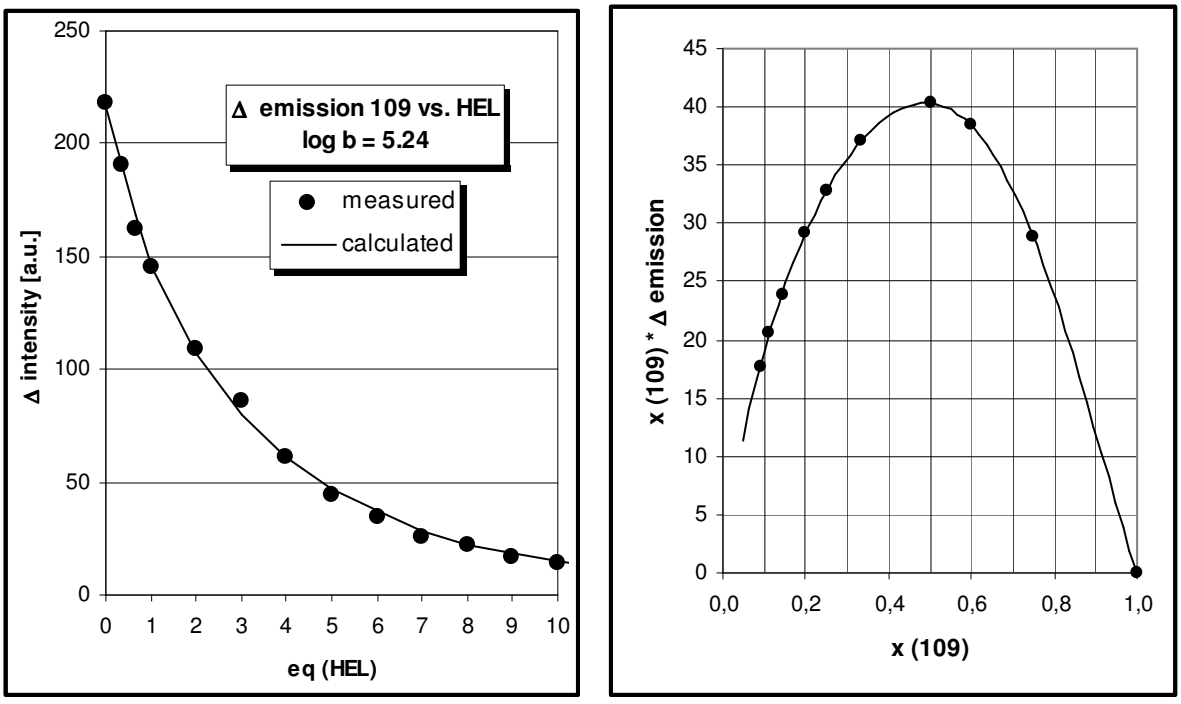



Figure 31: Measured and calculated curve of titration of **109** with HEL in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 1:1 stoichiometry (right).

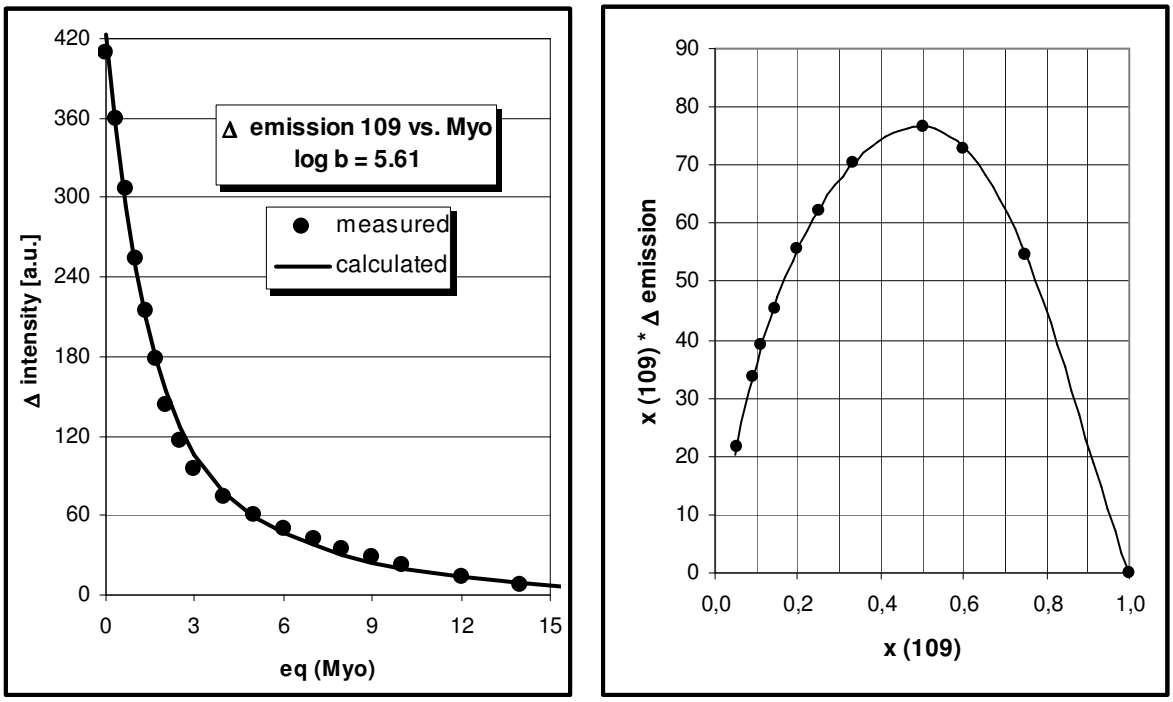



Figure 32: Measured and calculated curve of titration of **109** with Myo in 50 mM hepes buffer, pH 7.5 (left). Job's plot of titration showed a 1:1 stoichiometry (right).

As both, HEL and Myo, have got several hydrophobic areas on their surface a non-specific binding of the porphyrin moiety was expected. An enhancement of binding affinity as well as specificity should be achieved by the additional binding site of the Cu(II)-NTA moiety. Figure 33 shows a 3D illustraion of HEL with depiction of the polarity on its surface. One can see the predominant hydrophobic areas. Only a few polar amino acid side chain residues (e.g. of Lys [basic, blue] or Asp [acidic, red]) increase the polarity. A comparison with **109** with matching scale shows that, after binding of the Cu(II)-NTA moiety to His 15, there are several non polar areas, where the porphyrin moiety can bind to.

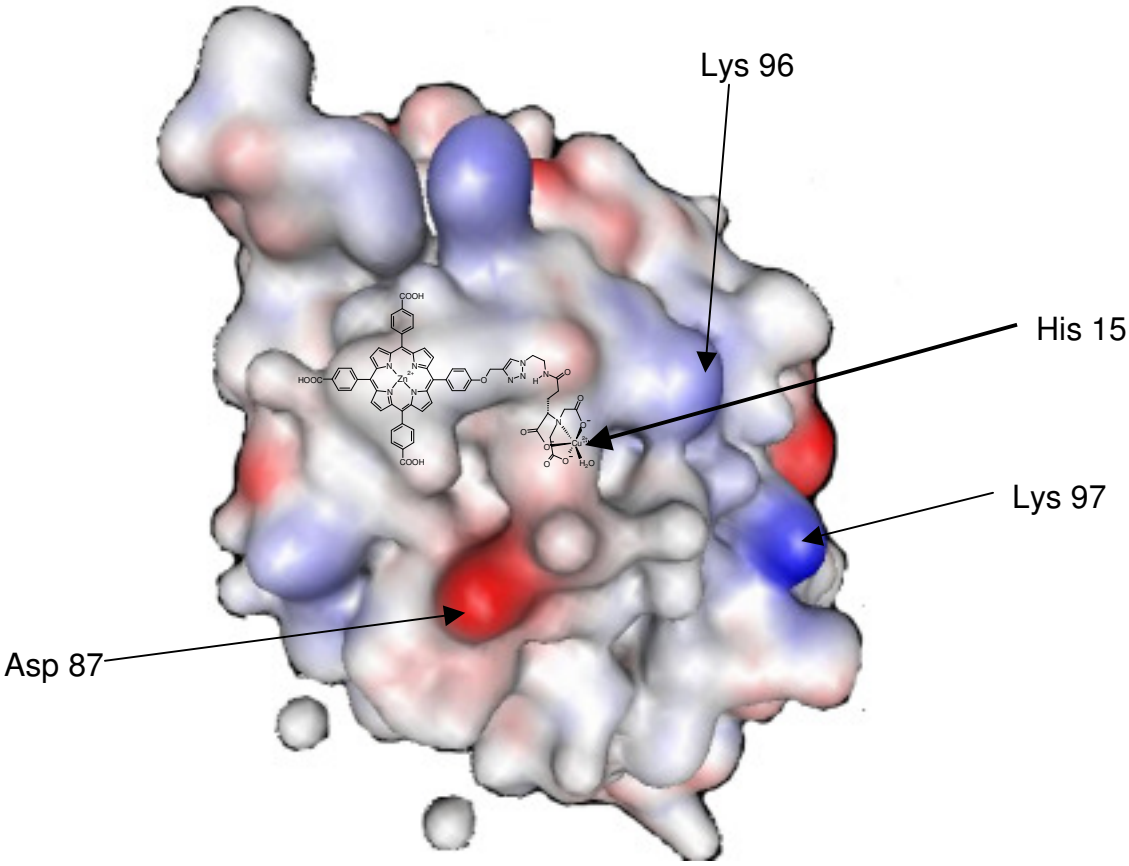



Figure 33: Electrostatic representation of HEL. Hydrophobic patches are represented in white, acidic patches in red and basic patches in blue. Locations of amino acid residues of His 15 and nearby Asp 87, Lys 96 and Lys 97 are assigned. A depiction of **109** with matching scale is added in overlaid fashion.

In order to confirm that the additional binding site in **109** results in an enhancement of binding affinity, the titration experiments were repeated with **62**. The measurements had to be performed in solutions saturated with Ar.[73] Otherwise it was not possible to obtain constant values of fluorescence intensity.[74] A possible explanation for this is the formation of singlet oxygen by **62**.[75] This was not observed for **109**, as the copper complex quenches the energy of the excited zinc-poprphyrin.[76]

However, the binding constants of **62** to HEL or Myo did not differ significantly from values obtained for **109**. The association constant ($K_a$) for **62** to HEL was 1.9 x $10^5$ L/mol, for **62** to Myo $K_a$ was 4.9 x $10^5$ L/mol. A possible explanation for the slight increase of binding affinity of **62** to the proteins compared to **109** is the higher hydrophilicity of **109**, due to the copper-NTA moiety. The final conclusion that can be drawn from this observation is that the additional Cu(II)-NTA binding site results in no enhancement of binding affinity towards the tested proteins.

## 2.4. Summary and Conclusions

Novel receptor building blocks for different binding motifs have been synthesized. Zn(II)-cyclen complexes for imide groups, bis-Zn(II)-cyclen complexes for phosphate groups, Zn(II) and Cu(II) nitrilo-triacetic acid complexes for N-terminal histidines, guanidines for carboxylic acids and a Zn(II)-porphyrin for non-polar regions on a protein surface. All compounds bear either an azide or an alkyne function and therefore can be connected in any combination using the Cu(I)-catalyzed cycloaddition.

A biological relevant pentapeptide, with a histidine and a carboxyl-group as possible binding motifs, has been used as target in a kinetically controlled target guided reaction. In order to identify a bidentate receptor with a high binding constant for the target, combinations of Zn(II)- and Cu(II)-NTA complexes and guanidines have been tested in this reaction. Hit-detection was performed in situ with MS-spectroscopy. Results revealed one possible high affinity receptor out of 12 combinations.

Six bidentate receptors have been synthesized and their binding interactions with the peptide target have been investigated with NMR- MS- and fluorescence-spectroscopy. All methods confirmed binding interactions.

Results of NMR-investigations allowed a prediction of the structural relations of the formed complex between target and receptor. This prediction was confirmed by Spartan-calculations.

Further, the association constant of this complex was determined with fluorescence spectroscopy to be in the micro molar range. However, a definite determination, as well as a comparison of the association constants of these six receptors was not possible.

Fluorescent labelled N-terminal histidines, with a dansyl- or a fluorescein group have been synthesized. The binding interactions between the histidine-moiety and heavy metal cations as well as Cu(II)-NTA complexes has been investigated with fluorescence spectroscopy. Results revealed high association constants ($> 10^{11}$ $L^2/mol^2$) of these indicators towards $Cu^{2+}$ and Cu(II)-NTA complexes. Therefore, these indicators can be used in very sensitive detection assays for Cu(II) and corresponding complexes.

Using the Cu(I)-catalyzed cycloaddition, several bidentate receptor molecules with different binding sites have been synthesized. Combinations of receptor building blocks were: bis-Zn(II)-cyclen-complexes with Zn(II)-NTA-complexes or guanidine compounds and further a Cu(II)-NTA- with a Zn(II)-porphyrin complex.

The binding interactions between the bis-Zn(II)-cyclen compounds and phosphorylated peptides were investigated with a fluorescence polarisation assay. Results confirmed a slight increase of the binding affinity (factor 2) compared to a monodentate bis-Zn(II)-cyclen reference compound.

The binding interactions between the Zn(II)-porphyrin-Cu(II)-NTA complex and amino acids, a pentapeptide and two His-containing proteins (HEL and Myo) were investigated with fluorescence titrations. Results showed a specific binding of histidine as well as of peptides with an N-terminal His. Binding affinities were in the micromolar range. The binding affinities of the porphyrine receptor towards His-containing proteins revealed to be also in the micromolar range, but unspecific.

These results not only show the possibilities, but also emphasize the importance of the Cu(I)-catalyzed “click-reaction” for the “Target Guided Synthesis”-approach in the field of drug-discovery.

Further, this work shows that the Cu(I)-catalyzed “click-reaction” is a valuable tool to connect diverse kinds of building blocks in any way, which enables the easy synthesis of polydentate receptors with increased affinity for various target molecules.

## 2.5. Experimental Part

### 2.5.1. General Information

All reactions were performed under an inert atmosphere of $N_2$ using standard Schlenk techniques if not otherwise stated.

#### *2.5.1.1. Spectroscopy*

**Emission Spectroscopy**

Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) at 20 °C in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer.

**Absorption Spectroscopy**

Varian Cary BIO 50 UV/VIS/NIR Spectrometer. Use of a 1 cm quartz cell (Hellma) and Uvasol solvents (Merck or Baker).

**NMR Spectra**

Bruker Avance 600 ($^1$H: 600.1 MHz, $^{13}$C: 150.1 MHz, T = 300 K), Bruker Avance 400 ($^1$H: 400.1 MHz, $^{13}$C: 100.6 MHz, T = 300 K), Bruker Avance 300 ($^1$H: 300.1 MHz, $^{13}$C: 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 $^{13}$C-spectra was determined with DEPT-technique (pulse angle: 135 °) and given as (+) for $CH_3$ or CH, (-) for $CH_2$ and ($C_{quat}$) for quaternary C. Error of reported values: chemical shift: 0.01 ppm for $^1$H-NMR, 0.1 ppm for $^{13}$C-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.

*2.5.1.2. Synthesis*

Melting Points were determined on a Tottoli micro melting point apparatus and are uncorrected. 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. For preparative column-chromatography, Merck Geduran SI 60 silica gel was used. Commercially available solvents of standard quality were used. If otherwise stated, purification and drying was done according to accepted general procedures.[77] Elemental analyses were carried out by the Center for Chemical Analysis of the Faculty of Natural Sciences of the University Regensburg.

## 2.5.2. Synthesis of Building Blocks for Kinetically Controlled TGS

*2.5.2.1. General Procedures*

**GP 1** – Conversion of bromides into azides using an ion-exchanger loaded with $N_3^-$:

The bromide was dissolved in MeCN and 4 eq of the $N_3^-$ - loaded anion-exchanger was added. The mixture was shaken 18 h at 40 °C. The ion-exchanger was filtered and washed with MeCN and MeOH. After evaporation of the solvents the remaining oil was treated a second time with the ion-exchanger in the same way. This gave the crude product as a yellowish oil.

**GP 2** – Conversion of alkyl halides into azides in aqueous methanol solution:

The bromide was dissolved MeOH and a saturated aqueous solution of $NaN_3$ was added. Further addition of MeOH was necessary to give a clear solution. A reflux condenser was put on and the solution was heated to 70 °C for 24 h. After cooling to r.t. 50 ml water were added and the MeOH was evaporated at reduced pressure. The remaining suspension was extracted with EE (100 ml, twice). The organic layers were combined, washed with brine (50 ml), dried over $NaSO_4$ and filtered. Removal of the solvent yielded the azide in sufficient purity of > 98 % (determined with $^1$H-NMR).

**GP 3** – Cleavage of Boc protecting-groups with TFA in DCM:

The azide was dissolved in DCM and TFA was added (30 eq). The solution was stirred at r.t. for 24 h. The solvent was removed at reduced pressure and 50 ml of MeOH were added. All volatile compounds were removed at reduced pressure and the remaining oil was dried in vacuum over KOH.

**GP 4** – Cleavage of Boc protecting-groups with HCl-saturated diethyl ether:

The Boc-protected compound was dissolved in diethyl ether (20 ml per mmol). A saturated solution of HCl in diethyl ether was added (1 ml per 0.15 mmol Boc). After a few minutes a white precipitate appeared. The mixture was stirred at r.t. under an atmosphere of $N_2$ for 48 h. The solvent was removed at reduced pressure and the remaining colourless solid was dried in vacuum.

**GP 5** – Synthesis of bis-Zn(II)-cyclen complexes:

The ammonium-salt was dissolved in water and eluted over a strongly basic anion-exchanger ($OH^-$ -form, loading: 0.9 mmol/ml, 12 eq). The ion-exchanger resin was washed well with water. After lyophilisation of the aqueous solution, the amine was obtained as a colourless frothy solid. The amine was dissolved in as much methanol as necessary and added dropwise to a 0.15 M solution of $Zn(ClO_4)_2$ x 6 $H_2O$ (2 – 3 eq) in aqueous MeOH (MeOH:$H_2O$ = 25:1). After a few minutes, the product precipitated from the solution. The suspension was stirred at r.t. for 14 h and then heated to reflux for 4 h. If necessary, boiling water was added dropwise, until the

solid dissolved completely. Then, boiling ethanol was added until a precepitate began to form. In order to achieve complete precipitation the suspension was cooled to - 20 °C for 16 h. The solid was filtered, washed with EtOH and dried in vacuum.

**GP 6** – Alkylation of glutamines under acidic conditions:

The ammonium chloride was dissolved in acetonitrile (4 ml per mmol). Following, bromo-ethylacetate (60 eq), NaF (9 eq) and $SiO_2$ (5 eq) were added. Further, phasetransfer-catalysts TBABr und 18-crown-6 (each approx. 100 mg) were added. The mixture was refluxed under an atmosphere of $N_2$ for 20 h. After cooling to r.t. potassium carbonate (1 eq) was added. After stirring the suspension at r.t. for 1 h and filtration, the residue was washed thoroughly with EE. The solvents were evaporated at reduced pressure and the excess bromo-ethylacetate was removed in vacuum. This gave the crude product as dark brown oil, which was purified by column chromatography twice. First EE:PE in a ration of 2:3 and then DCM:MeOH 98:2.

**GP 7** – Synthesis of asymmetric porphyrins:

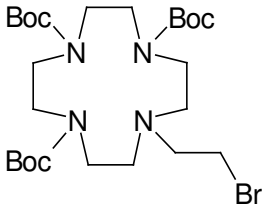
Freshly distilled Pyrrol (280 µl, 268 mg, 4.00 mmol, 8 eq) was dissolved in 900 ml DCM in a 1000 ml round bottom flask under an atmosphere of $N_2$. After addition of the two different benzaldehydes, a catalytic amount of $BF_3 \cdot OEt_2$ was added and the solution changed its colour to red. The mixture was stirred in the dark at r.t. for 20 h and concentrated to 500 ml at reduced pressure. In order to oxidise the formed porphyrinogen, p-chloranil was added and the mixture was refluxed for 1 h. After concentration and drying in vacuum, the dark brown solid was treated with DCM in order to separate all insoluble polymeric compounds. The suspension was filtered and the filtrate was concentrated at reduced pressure to perform purification by CC.

**GP 8** – Synthesis of bidentate Cu(II)-NTA-guanidine-complexes:

The Cu(II)-NTA-complex and the guanidinium chloride were dissolved in water (10 ml per mmol). Aqueous solutions (0.5 ml each) of catalytic amounts of $CuSO_4$ x 5 $H_2O$ (5 mol%) and sodium ascorbate (20 mol%) were added. The mixture was stirred at r.t. for 16 h and then heated to boiling. No reflux condenser was put on, in order to

concentrate the solution to a volume less than 1 ml. Boiling EtOH was added very slowly until the product started to precipitate. After cooling to r.t. the suspension was cooled to – 20 °C for 3 h and the suspension was filtered. The residue was washed with EtOH and dried in vacuum.

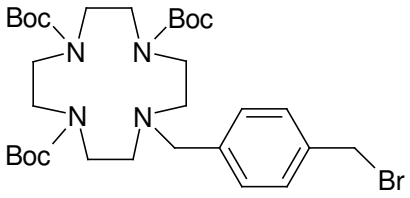
*2.5.2.2. Synthesis of New Compounds*




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

The dibromide (4.01 ml, 8.74 g, 46.55 mmol, 22 eq), $K_2CO_3$ (1170 mg, 8.46 mmol, 4 eq) and **1** (1000 mg, 2.12 mmol) were heated to 100 °C for 24 h. TLC control after that time showed almost complete conversion of **1** and one side product which could be identified as the two fold substitution product. The suspension was filtered and the residue was washed thoroughly with EE. After evaporation of the EE and removement of the surplus 1,2-dibromo ethane in vacuum the remaining oil was purified with CC (EE:PE = 3:2). This gave **2** as colourless solid in a yield of 823 mg (1.42 mmol, 67 %). $R_f$ (EE:PE = 1:1) = 0.67.

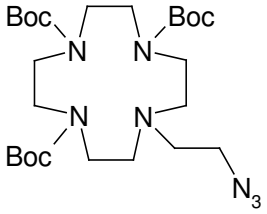
**Mp**: 84 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (s, 18 H, Boc-$CH_3$), 1.40, (s, 9 H, Boc-$CH_3$), 2.64 – 2.72 (m, 4 H, 2 cyclen-$CH_2$), 2.95 (t, $^3$J = 7.5 Hz, 2 H, $CH_2$), 3.23 – 3.48 (m + t, 14 H, 6 cyclen-$CH_2$ + $CH_2$, δ [ppm] = 3.37, $^3$J = 7.4 Hz); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 27.6 (–, 1 C), 28.4 (+, 6 C, Boc-$CH_3$), 28.6 (+, 3 C, Boc-$CH_3$), 47.6 (–, 1 C), 47.9 (–, 2 C), 48.2 (–, 1 C), 50.0 (–, 2 C), 53.7 (–, 1 C), 54.6 (–, 1 C), 54.9 (–, 1 C), 79.3 ($C_{quat,}$ 1 C, Boc), 79.6 ($C_{quat,}$ 1 C, Boc), 79.6 ($C_{quat,}$ 1 C, Boc), 155.3 ($C_{quat,}$ urethane), 155.7 ($C_{quat,}$ urethane), 156.1 ($C_{quat,}$ urethane); **MS** (CI, $NH_3$): m/z (%) = 579.3 [$MH^+$] (100); **EA** ($C_{25}H_{47}N_4O_6Br$) calc.: C 51.81, H 8.17, N 9.67, found: C 51.42, H 8.21, N 9.39; **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 2972, 2931, 1687, 1462, 1416, 1171; **MF**: $C_{25}H_{47}N_4O_6Br$; **MW** = 579.58 g/mol.




10-(4-Bromomethyl-benzyl)-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester (**3**):

The α,α'-Dibrom-paraxylol (870 mg, 3.30 mmol, 6 eq) was dissolved in 14 ml dry THF at 50 °C. Following, potassium carbonate (456 mg, 3.30 mmol, 6 eq) and **1** (260 mg, 0.55 mmol) were added. The suspension was refluxed for 22 h. All inorganic compounds were filtered off and washed thoroughly with MeCN. The solution was concentrated at reduced pressure. The resulting oil was purified with column chromatography (gradient of PE:EE from 4:1 to 3:2). This yielded 267 mg of **3** as colourless solid (0.41 mmol, 74 %) and 26 mg of **1** (0.06 mmol, 8 %). $R_f$ (EE:PE = 1:1) = 0.78.

**Mp**: 101 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 1.39 (s, 18 H, Boc-$CH_3$), 1.45 (s, 9 H, Boc-$CH_3$), 2.54 – 2.60 (m, 4 H, cyclen-$CH_2$), 3.25 – 3.38 (m, 8 H, cyclen-$CH_2$), 3.52 – 3.58 (m, 4 H, cyclen-$CH_2$), 3.74 (s, 2 H, Bn-$CH_2$), 4.56 (s, 2 H, Bn-$CH_2$), 7.25 (d, $^3$J = 7.8 Hz, 2 H, arom.), 7.36 (d, $^3$J = 7.8 Hz, 2 H, arom.); **$^{13}$C-NMR** (100.1 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 6 C, Boc-$CH_3$), 28.7 (+, 3 C, Boc-$CH_3$), 33.2 (–, Bn), 47.5 – 48.0 (–, 4 C, cyclen), 49.9 (–, 2 C, cyclen), 54.9 (–, 1 C, cyclen), 55.8 (–, 1 C, cyclen), 56.8 (–, 1 C, Bn), 79.4 ($C_{quat,}$ 1 C, Boc), 79.4 ($C_{quat,}$ 1 C, Boc), 79.5 ($C_{quat,}$ 1 C, Boc), 128.9 (+, 2 C, arom.), 130.6 (+, 2 C, arom.), 136.8 ($C_{quat}$, 1 C, arom.), 137.3 ($C_{quat}$, 1 C, arom.), 155.4 ($C_{quat,}$ urethane), 155.7 ($C_{quat,}$ urethane), 156.1 ($C_{quat,}$ urethane); **MS** (FD, $CH_2Cl_2$): m/z (%) = 654.2 [$M^{+\cdot}$] (100); **EA** ($C_{31}H_{51}N_4O_6Br$) calc.: C 56.79, H 7.84, N 8.54, gef.: C 56.38, H 7.92, N 8.24; **UV/Vis** ($CH_3CN$): λ (lg ε) = 238 nm (3.945); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3025, 2974, 2930, 2810, 1688, 1460, 1416, 1172, 797; **MF**: $C_{31}H_{51}N_4O_6Br$; **MW** = 655.67 g/mol.



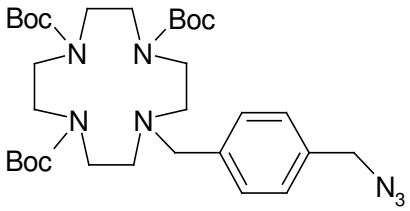

10-(2-Azido-ethyl)-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester (**4**):

Synthesis followed **GP 1** using bromide **2** (388 mg, 0.67 mmol), 10 ml of MeCN and 5.96 ml (0.9 mmol/ml, 5.36 mmol) of the $N_3^-$ - loaded anion-exchanger. The raw product was purified with CC (EE:PE = 2:3). This gave the azide **4** as colourless oil in a yield of 335 mg (0.61 mmol, 92 %). $R_f$ (EE:PE = 1:1) = 0.66.

Alternative synthesis followed **GP 2** using bromide **2** (500 mg, 0.86 mmol), 5 ml of MeOH and $NaN_3$ (1402 mg, 21.57 mmol, 25 eq). This yielded **4** as colourless oil (457 mg, 0.84 mmol, 98 %).

The azide was not stable at r.t. and decomposed slowly. Storage in the freezer is recommended.

**$^1$H-NMR** (300 MHz, $CDCl_3$): $\delta$ [ppm] = 1.38 (s, 18 H, Boc-$CH_3$), 1.40 (s, 9 H, Boc-$CH_3$), 2.60 – 2.64 (m, 4 H, 2 cyclen-$CH_2$), 2.68 (t, $^3J$ = 6.4 Hz, 2 H, $CH_2$), 3.22 – 3.51 (m + t, 14 H, 6 cyclen-$CH_2$ + $CH_2$, $\delta$ [ppm] = 3.39, $^3J$ = 6.4 Hz); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): $\delta$ [ppm] = 27.5 (+, 6 C, Boc-$CH_3$), 27.7 (+, 3 C, Boc-$CH_3$), 46.5 - 47.2 (–, 5 C), 49.0 (–, 2 C), 50.6 (–, 1 C), 53.3 (–, 1 C), 54.3 (–, 1 C), 78.3 ($C_{quat,}$ 1 C, Boc), 78.6 ($C_{quat,}$ 1 C, Boc), 78.7 ($C_{quat,}$ 1 C, Boc), 154.4 ($C_{quat,}$ urethane), 154.7 ($C_{quat,}$ urethane), 155.2 ($C_{quat,}$ urethane); **MS** (ESI, $CH_2Cl_2$/MeOH): m/z (%) = 542.3 [$MH^+$] (100); **MS-HR** (EI): [$M^\cdot$] calc.: 541.3588, found: 541.3587 ($\pm$ 0.54 ppm); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 2975, 2935, 2147, 1686, 1460, 1415, 1172; **MF**: $C_{25}H_{47}N_7O_6$; **MW** = 541.69 g/mol;



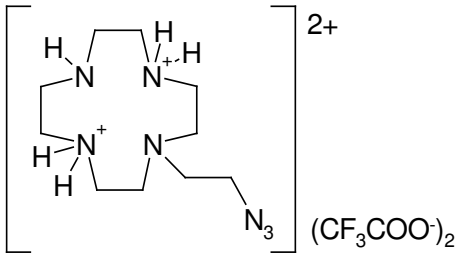

10-(4-Azidomethyl-benzyl)-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester (**5**):

Synthesis followed **GP 1** using bromide **3** (544 mg, 0.83 mmol), 12 ml of MeCN and 7.38 ml (0.9 mmol/ml, 6.64 mmol) of the $N_3^-$ - loaded anion-exchanger. The raw product was purified with CC (EE:PE = 2:3). This gave the azide **5** as colourless solid in a yield of 472 mg (0.76 mmol, 92 %). $R_f$ (EE:PE = 1:1) = 0.68.

Alternative synthesis followed **GP 2** using bromide **3** (1081 mg, 1.65 mmol), 5 ml of MeOH and $NaN_3$ (1072 mg, 16.49 mmol, 10 eq). This yielded **5** as colourless solid (1020 mg, 1.65 mmol, 100 %).

The azide was not stable at r.t. and decomposed slowly. Storage in the freezer is recommended.

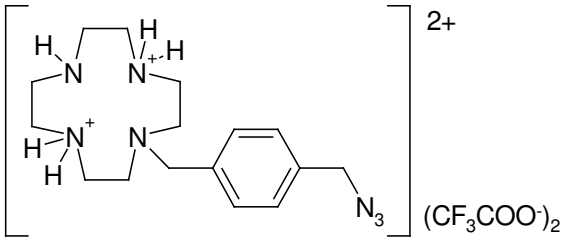
**Mp**: 99 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.36 (s, 18 H, Boc-$CH_3$), 1.40 (s, 9 H, Boc-$CH_3$), 2.55 – 2.63 (m, 4 H, cyclen-$CH_2$), 3.16 – 3.51 (m, 12 H, cyclen-$CH_2$), 3.66 (s, 2 H, Bn-$CH_2$), 4.24 (s, 2 H, Bn-$CH_2$), 7.17 (d, $^3$J = 8.2 Hz, 2 H, arom.), 7.21 (d, $^3$J = 8.2 Hz, 2 H, arom.); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 6 C, Boc-$CH_3$), 28.7 (+, 3 C, Boc-$CH_3$), 47.4 – 48.1 (–, 4 C), 49.9 (–, 2 C), 54.5 (–, 1 C, Bn), 55.1 (–, 1 C), 56.0 (–, 1 C), 57.0 (–, 1 C), 79.4 ($C_{quat,}$ 1 C, Boc), 79.5 ($C_{quat,}$ 2 C, Boc), 128.1 (+, 2 C, arom.), 130.7 (+, 2 C, arom.), 134.3 ($C_{quat}$, 1 C, arom.), 137.2 ($C_{quat}$, 1 C, arom.), 155.4 ($C_{quat,}$ urethane), 155.7 ($C_{quat,}$ urethane), 156.1 ($C_{quat,}$ urethane); **MS** (ESI, MeCN/MeOH): m/z (%) = 618.4 [$MH^+$] (100); **MS-HR** (EI): [$M^{\cdot}$] calc.: 617.3901, found: 617.3897 (± = 0.73 ppm); **UV/Vis** ($CH_3CN$): λ (lg ε) = 238 nm (3.943); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3305, 2977, 2932, 2817, 2100, 1694, 1460, 1366, 1251, 1172, 772; **MF**: $C_{31}H_{51}N_7O_6$; **MW** = 617.79 g/mol.



4-(2-Azido-ethyl)-4,10-diaza-1,7-diazonia-cyclododecane di-trifluoro acetate (**6**):

Synthesis followed **GP 3** using azide **4** (360 mg, 0.66 mmol), 10 ml of DCM and TFA (1.53 ml, 19.94 mmol). This yielded 310 mg of **6** (0.66 mmol, 100 %) as yellowish oil.

**$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.67 (t, $^3J$ = 5.6 Hz, 2 H, $CH_2$), 2.84 – 2.95 (m, 8 H, cyclen-$CH_2$), 3.05 – 3.11 (m, 8 H, cyclen-$CH_2$), 3.60 (t, $^3J$ = 5.6 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 42.9 (–, 2 C, cyclen), 43.3 (–, 2 C, cyclen), 45.3 (–, 2 C, cyclen), 49.3 (–, 2 C, cyclen), 49.8 (–, 1 C), 51.5 (–, 1 C); **MS** (ESI, $H_2O/CH_3CN$): m/z (%) = 242.1 [$MH^+$] (100); **MF**: $[C_{10}H_{25}N_7]^{2+}(CF_3COO^-)_2$ = $C_{14}H_{25}N_7O_4F_6$; **MW** = 469.39 g/mol.

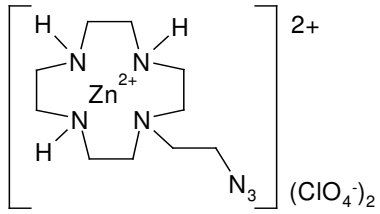


4-(4-Azidomethyl-benzyl)-4,10-diaza-1,7-diazonia-cyclododecane di-trifluoro acetate (**7**):

Synthesis followed **GP 3** using azide **5** (853 mg, 1.38 mmol), 10 ml of DCM and TFA (2.26 ml, 29.59 mmol). This yielded 360 mg of **7** (1.38 mmol, 100 %) as yellowish oil.

**$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.81 – 3.13 (m, 16 H, cyclen-$CH_2$), 3.82 (s, 2 H, Bn-$CH_2$), 4.38 (s, 2 H, Bn-$CH_2$), 7.32 – 7.36 (m, 4 H, arom. CH); **$^{13}$C-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 43.1 (–, 2 C, cyclen), 43.2 (–, 2 C, cyclen), 45.5 (–, 2 C, cyclen), 48.9 (–, 2 C, cyclen), 54.9 (–, 1 C, Bn), 57.2 (–, 1 C, Bn), 129.7 (+, 2 C, arom.), 131.4 (+, 2 C, arom.), 136.2 ($C_{quat}$, 1 C), 136.7 ($C_{quat}$, 1 C); **MS** (ESI, $CH_3CN$): m/z (%) = 179.9 [$(M+2H^+ + CH_3CN)^{2+}$] (32), 200.4 [$(M+2H^+ + 2CH_3CN)^{2+}$] (28), 318.0

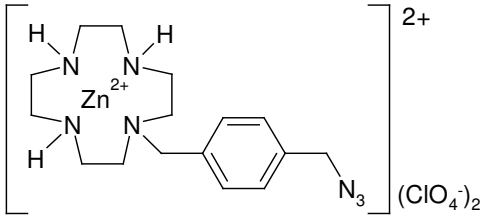
[$MH^+$] (100), 749.5 [$(2M+2H^+ + CF_3COO^-)^+$] (7); **MF**: $[C_{16}H_{29}N_7]^{2+}(CF_3COO^-)_2$ = $C_{20}H_{29}N_7O_4F_6$; **MW** = 545.49 g/mol.




Zn[1-(2-Azido-ethyl)-1,4,7,10tetraaza-cyclododecane] di-perchlorate (**8**):

The TFA-salt **6** (310 mg, 0.66 mmol) was dissolved in little MeOH and treated with 2.9 ml (2.64 mmol, 4 eq) of a strongly basic anion-exchanger ($OH^-$ -form, loading 0.9 mmol/ml). The ion-exchanger resin was washed well with MeOH. The solvent was removed at reduced pressure. The remaining yellow oil was dissolved in 8 ml of a mixture of MeOH/water (9:1) and added dropwise to a solution of $Zn(ClO_4)_2$ x 6 $H_2O$ (492 mg, 1.32 mmol, 2 eq) in 8 ml MeOH. A reddish precipitate occurred. The mixture was stirred at r.t. for 24 h and then heated to reflux for 1 h. To the boiling mixture EtOH (20 ml) was added. After cooling to r.t. the reddish solid was filtered, washed with EtOH and dissolved in very little MeCN. All insoluble compounds were removed with centrifugation. The solvent was removed at reduced pressure and the remaining oil was dried in vacuum. This yielded 327 mg of **8** (0.65 mmol, 98 %) as reddish solid.

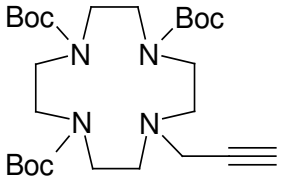
**Mp:** > 220 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.63 – 3.08 (m, 18 H, $CH_2$ + cyclen-$CH_2$), 3.17 – 3.24 (m, 1 H, NH), 3.42 – 3.57 (m, 2 H, NH), 3.68 (t, $^3J$ = 6.0 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 43.6 (–, 2 C, cyclen), 44.8 (–, 2 C, cyclen), 45.9 (–, 2 C, cyclen), 47.3 (–, 1 C), 51.2 (–, 2 C, cyclen), 51.6 (–, 1 C); **MS** (ESI, $H_2O/CH_3CN$): m/z (%) = 152.4 [$K^{2+}$] (75), 172.9 [$(K^{2+}+CH_3CN)^{2+}$] (100), 364.0 [$(K^{2+}+Ac^-)^+$] (80); **MF**: $[C_{10}H_{23}N_7Zn]^{2+}(ClO_4^-)_2$ = $C_{10}H_{23}N_7O_8Cl_2Zn$; **MW** = 505.62 g/mol.



Zn[1-(4-Azidomethyl-benzyl)-1,4,7,10tetraaza-cyclododecane] di-perchlorate (**9**):

The TFA-salt **7** (254 mg, 0.47 mmol) was dissolved in little MeOH and treated with 2.1 ml (1.86 mmol, 4 eq) of a strongly basic anion-exchanger ($OH^-$ - form, loading 0.9 mmol/ml). The ion-exchanger resin was washed well with MeOH. The solvent was removed at reduced pressure. The remaining yellow oil was dissolved in 7 ml of a mixture of MeOH/water (9:1) and added dropwise to a solution of $Zn(ClO_4)_2$ x 6 $H_2O$ (347 mg, 0.93 mmol, 2 eq) in 7 ml MeOH. A reddish precipitate occurred. The mixture was strirred at r.t. for 24 h and then heated to reflux for 1 h. To the boiling mixture EtOH (20 ml) was added. After cooling to r.t. the reddish solid was filtered, washed with EtOH and dissolved in very little MeCN. All insoluble compounds were removed with centrifugation. The solvent was removed at reduced pressure and the remaining oil was dried in vacuum. This yielded 268 mg of **9** (0.46 mmol, 98 %) as reddish solid.

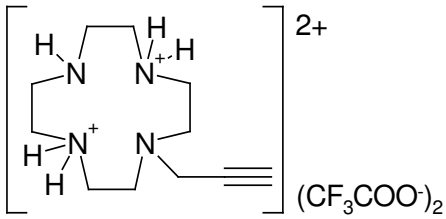
**Mp:** > 220 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.41 – 3.63 (m, 19 H, NH + cyclen-$CH_2$), 4.02 (s, 2 H, Bn-$CH_2$), 4.48 (s, 2 H, Bn-$CH_2$), 7.31 – 7.51 (m, 4 H, arom. CH); **$^{13}$C-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 43.3 (–, 2 C, cyclen), 44.8 (–, 2 C, cyclen), 45.6 (–, 2 C, cyclen), 50.0 (–, 2 C, cyclen), 54.8 (–, 1 C, Bn), 56.2 (–, 1 C, Bn), 129.7 (+, 2 C, arom.), 132.4 ($C_{quat}$, 1 C), 132.8 (+, 2 C, arom.), 137.6 ($C_{quat}$, 1 C); **MS** (ESI, $H_2O/CH_3CN$): m/z (%) = 190.4 [$K^{2+}$] (30), 210.9 [$(K^{2+}+CH_3CN)^{2+}$] (100), 416.0 [$(K^{2+}+Cl^-)^+$] (15), 480.0 [$(K^{2+}+ClO_4^-)^+$] (15), 494.1 [$(K^{2+}+CF_3COO^-)^+$] (15); **MF**: $[C_{16}H_{27}N_7Zn]^{2+}(ClO_4^-)_2 = C_{16}H_{27}N_7O_8Cl_2Zn$; **MW** = 581.72 g/mol.




10-Prop-2-ynyl-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester (**10**):

Compound **1** (473 mg, 1.00 mmol) was dissolved in 10 ml of MeCN and $K_2CO_3$ (182 mg, 1.32 mmol) and catalytic amounts of KI were added. After addition of 195 µl of a 80 % solution of propargyl bromide in toluene (215 mg, 1.80 mmol), the suspension was stirred at 50 °C for 48 h. TLC control of the reaction mixture showed almost complete conversion. The suspension was filtered and the residue was washed with MeCN. The solution was concentrated at reduced pressure and purified with CC (PE:EE = 7:3 to 1:1). This gave alkyne **10** as colourless solid (485 mg, 0.95 mmol, 95 %). $R_f$ (EE:PE = 1:1) = 0.82.

**Mp**: 104 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.37 (s, 18 H, Boc-$CH_3$), 1.39 (s, 9 H, Boc-$CH_3$), 2.14 (s, 1 H, CH), 2.66 – 2.81 (m, 4 H, cyclen-$CH_2$), 3.24 – 3.45 (m, 14 H, 7 $CH_2$); **$^{13}$C-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 6 C, Boc-$CH_3$), 28.7 (+, 3C, Boc-$CH_3$), 39.1 (–), 46.5 (–, cyclen), 47.0 (–, cyclen), 47.6 (–, cyclen), 47.8 (–, cyclen), 49.8 (–, cyclen), 49.9 (–, cyclen), 53.1 (–, cyclen), 54.3 (–, cyclen), 73.6 (+, CH), 77.6 ($C_{quat}$, alkyne), 79.2 ($C_{quat,}$ Boc), 79.4 ($C_{quat,}$ Boc), 79.7 ($C_{quat,}$ Boc), 155.2 ($C_{quat,}$ urethane), 155.7 ($C_{quat,}$ urethane), 156.0 ($C_{quat,}$ urethane); **MS** (ESI, $CH_2Cl_2$/MeOH): m/z (%) = 511.2 [$MH^+$] (100); **MS-HR** (FAB, $CH_2Cl_2$): [$MH^+$] calc.: 510.3417, found: 510.3415 (± = 0.73 ppm); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3252, 2957, 2929, 2841, 2106, 1682, 1456, 1415, 1366, 1251, 1153; **MF**: $C_{26}H_{46}N_4O_6$; **MW** = 510.67 g/mol;




4-Prop-2-ynyl-4,10-diaza-1,7-diazonia-cyclododecane di-trifluoro acetate (**11**):

Synthesis followed **GP 3** using **10** (870 mg, 1.70 mmol), 10 ml of DCM and TFA (3.9 ml, 5.8 g, 51.1 mmol, 30 eq). This gave **11** as yellow crystals in a yield of 747 mg (1.70 mmol, 100 %). In order to obtain **11** in pure form for X-ray structure analysis and EA the solid was recrystallized from MeCN/PE. This yielded colourless crystals (635 mg, 85 %).

**Mp**: 235 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.58 (t, $^4J$ = 2.3 Hz, 1 H, CH), 2.81 – 3.18 (m, 16 H, Cyclen-$CH_2$), 3.53 (d, $^4J$ = 2.3 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 42.6 (–), 43.0 (–, 2 C, cyclen), 43.2 (–, 2 C, cyclen), 45.4 (–, 2 C, cyclen), 48.2 (–, 2 C, cyclen), 75.7 (+, CH), 77.9 ($C_{quat}$); **MS** (ESI, $CH_3CN$): m/z (%) = 105.7 [$(M+2H^+)^{2+}$] (7), 126.3 [$(M+2H^++CH_3CN)^{2+}$] (35), 146.8 [$(M+2H^++2CH_3CN)^{2+}$] (30), 210.7 [$MH^+$] (100); **EA** ($C_{15}H_{24}N_4O_4F_6$) calc.: C 41.10, H 5.52, N 12.78, found: C 40.74, H 5.50, N 12.72; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3323, 3266, 3030, 2856, 2812, 2102, 1681, 1554, 1412, 1199, 1173, 1123; **MF**: $[C_{11}H_{24}N_4]^{2+}$ $(CF_3COO^-)_2$ = $C_{15}H_{24}N_4O_4F_6$; **MW** = 438.37 g/mol;

**X-Ray** structure and crystal data of **11**:

Triclinic; space group: P -1; cell dimensions: a = 9.2944(11) Å, α = 95.692°, b = 10.2766 Å, β = 107.826°, c = 11.2758 Å, γ = 94.101°; V = 1014.3 $Å^3$; Z = 2, $D_x$ = 1.435 $Mg/m^3$; μ = 0.139 $mm^{-1}$; F(000) = 456. Data collection: T = 173 K; graphite monochromator. A translucent colorless crystal with dimensions of 0.500 x 0.360 x 0.240 mm was used to measure 7895 reflections (3591 unique reflections, $R_{int}$ = 0.0170) from 2.32° to 25.94° 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 1.077 for all reflections and 358 parameters.

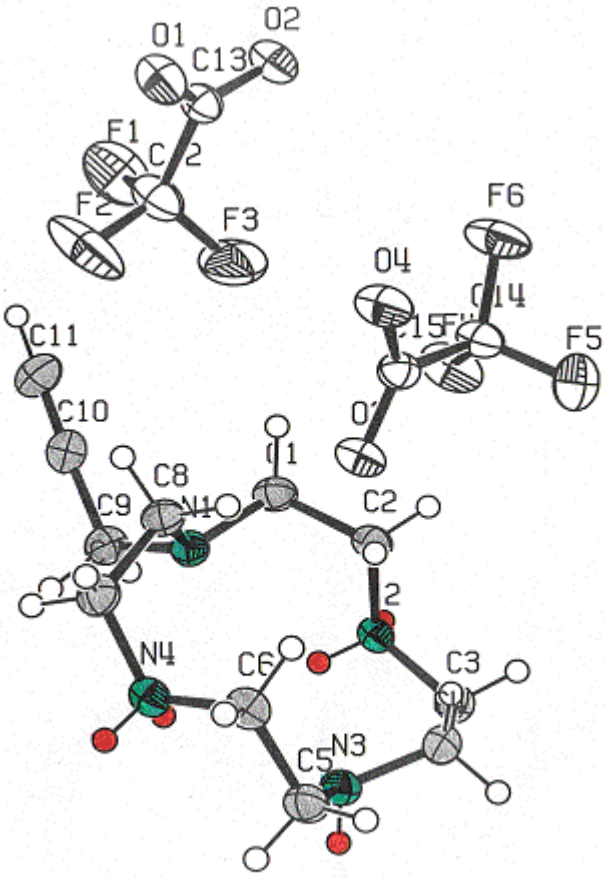


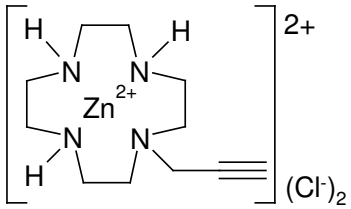
Table of atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters ($A^2$ x $10^3$) for **11**. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor:

| Atom | x | y | z | U(eq) |
|---|---|---|---|---|
| N(1) | 879(1) | 2931(1) | 7497(1) | 26(1) |
| N(2) | 1823(1) | 1518(1) | 5528(1) | 25(1) |
| N(3) | 4336(1) | 857(1) | 7530(1) | 29(1) |
| N(4) | 3544(1) | 2391(1) | 9545(1) | 27(1) |
| C(1) | 575(2) | 3325(2) | 6228(2) | 30(1) |
| C(2) | 1865(2) | 2987(1) | 5722(2) | 27(1) |
| C(3) | 3180(2) | 992(2) | 5281(2) | 30(1) |
| C(4) | 4602(2) | 1309(2) | 6419(2) | 30(1) |
| C(5) | 5464(2) | 1474(2) | 8709(2) | 32(1) |
| C(6) | 4930(2) | 2736(2) | 9180(2) | 30(1) |
| C(7) | 2610(2) | 3494(2) | 9691(2) | 31(1) |
| C(8) | 1814(2) | 3988(1) | 8456(2) | 29(1) |
| C(9) | -526(2) | 2470(2) | 7750(2) | 32(1) |
| C(10) | -1601(2) | 3476(2) | 7704(2) | 33(1) |
| C(11) | -2453(2) | 4292(2) | 7626(2) | 40(1) |
| F(1) | -1124(2) | 7672(1) | 7537(2) | 70(1) |
| F(2) | 891(2) | 7592(1) | 9065(1) | 79(1) |
| F(3) | 816(2) | 6991(1) | 7164(2) | 70(1) |
| O(1) | 1656(1) | 10017(1) | 8724(1) | 39(1) |
| O(2) | 512(1) | 9556(1) | 6636(1) | 34(1) |
| C(12) | 389(2) | 7888(2) | 7907(2) | 41(1) |
| C(13) | 921(2) | 9312(1) | 7754(2) | 29(1) |
| F(4) | 2738(1) | 5830(1) | 5482(1) | 48(1) |
| F(5) | 4995(1) | 6437(1) | 5451(1) | 54(1) |
| F(6) | 3447(1) | 7875(1) | 5545(1) | 50(1) |
| O(3) | 4608(1) | 5784(1) | 7829(1) | 34(1) |
| O(4) | 5004(1) | 7997(1) | 7975(1) | 39(1) |
| C(14) | 3917(2) | 6754(1) | 5969(2) | 32(1) |
| C(15) | 4560(2) | 6858(1) | 7413(2) | 27(1) |

Table of bond legths [Å] and angles [deg] for **11**:

| Bond | Length | Bond | Angle | Bond | Angle |
|---|---|---|---|---|---|
| F(1)-C(12) | 1.334(2) | C(1)-N(1)-C(8) | 110.88(12) | C(3)-C(4)-H(4B) | 110.6(11) |
| F(2)-C(12) | 1.319(2) | C(1)-N(1)-C(9) | 112.40(13) | H(4A)-C(4)-H(4B) | 105.3(16) |
| F(3)-C(12) | 1.347(2) | C(8)-N(1)-C(9) | 112.57(12) | N(3)-C(5)-H(5A) | 112.4(11) |
| F(4)-C(14) | 1.335(2) | C(2)-N(2)-C(3) | 116.21(12) | N(3)-C(5)-H(5B) | 108.6(12) |
| F(5)-C(14) | 1.348(2) | C(4)-N(3)-C(5) | 113.02(13) | C(6)-C(5)-H(5A) | 109.4(11) |
| F(6)-C(14) | 1.3421(19) | C(6)-N(4)-C(7) | 116.42(12) | C(6)-C(5)-H(5B) | 107.8(12) |
| O(1)-C(13) | 1.226(2) | H(2N)-N(2)-H(2O) | 106.5(19) | H(5A)-C(5)-H(5B) | 108.7(16) |
| O(2)-C(13) | 1.257(2) | C(2)-N(2)-H(2O) | 107.2(13) | N(4)-C(6)-H(6A) | 105.3(12) |
| O(3)-C(15) | 1.2403(19) | C(3)-N(2)-H(2N) | 109.9(13) | N(4)-C(6)-H(6B) | 107.7(11) |
| O(4)-C(15) | 1.2515(19) | C(2)-N(2)-H(2N) | 108.1(13) | C(5)-C(6)-H(6A) | 112.2(11) |
| N(1)-C(1) | 1.474(2) | C(3)-N(2)-H(2O) | 108.6(13) | C(5)-C(6)-H(6B) | 110.5(11) |
| N(1)-C(9) | 1.476(2) | C(5)-N(3)-H(3N) | 109.9(13) | H(6A)-C(6)-H(6B) | 111.9(16) |

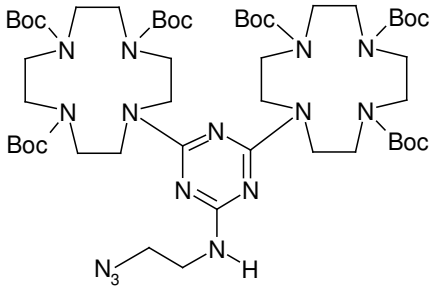
| | | | | | |
|---|---|---|---|---|---|
| N(1)-C(8) | 1.470(2) | C(4)-N(3)-H(3N) | 110.0(13) | N(4)-C(7)-H(7A) | 105.6(11) |
| N(2)-C(2) | 1.4999(19) | C(6)-N(4)-H(4N) | 109.4(13) | N(4)-C(7)-H(7B) | 106.1(12) |
| N(2)-C(3) | 1.497(2) | C(7)-N(4)-H(4N) | 110.6(13) | C(8)-C(7)-H(7A) | 112.8(12) |
| N(3)-C(5) | 1.467(2) | C(7)-N(4)-H(4O) | 107.1(13) | C(8)-C(7)-H(7B) | 110.7(12) |
| N(3)-C(4) | 1.461(2) | C(6)-N(4)-H(4O) | 104.8(13) | H(7A)-C(7)-H(7B) | 108.1(16) |
| N(4)-C(6) | 1.497(2) | H(4N)-N(4)-H(4O) | 108.2(19) | N(1)-C(8)-H(8A) | 107.9(10) |
| N(4)-C(7) | 1.502(2) | N(1)-C(1)-C(2) | 109.36(13) | N(1)-C(8)-H(8B) | 110.3(12) |
| N(2)-H(2O) | 0.90(2) | N(2)-C(2)-C(1) | 108.18(12) | C(7)-C(8)-H(8A) | 108.6(11) |
| N(2)-H(2N) | 0.88(2) | N(2)-C(3)-C(4) | 111.90(13) | C(7)-C(8)-H(8B) | 107.5(12) |
| N(3)-H(3N) | 0.88(2) | N(3)-C(4)-C(3) | 110.74(13) | H(8A)-C(8)-H(8B) | 109.8(15) |
| N(4)-H(4O) | 0.87(2) | N(3)-C(5)-C(6) | 109.89(13) | N(1)-C(9)-H(9A) | 108.9(11) |
| N(4)-H(4N) | 0.91(2) | N(4)-C(6)-C(5) | 109.02(13) | N(1)-C(9)-H(9B) | 107.5(11) |
| C(1)-C(2) | 1.523(2) | N(4)-C(7)-C(8) | 113.15(13) | C(10)-C(9)-H(9A) | 109.1(11) |
| C(3)-C(4) | 1.523(2) | N(1)-C(8)-C(7) | 112.69(12) | C(10)-C(9)-H(9B) | 108.5(11) |
| C(5)-C(6) | 1.525(2) | N(1)-C(9)-C(10) | 114.24(13) | H(9A)-C(9)-H(9B) | 108.4(16) |
| C(7)-C(8) | 1.519(2) | C(9)-C(10)-C(11) | 177.46(19) | C(10)-C(11)-H(11) | 178.6(7) |
| C(9)-C(10) | 1.482(2) | N(1)-C(1)-H(1A) | 110.2(11) | F(1)-C(12)-F(2) | 107.21(17) |
| C(10)-C(11) | 1.185(3) | N(1)-C(1)-H(1B) | 114.0(12) | F(1)-C(12)-F(3) | 104.75(15) |
| C(1)-H(1B) | 1.01(2) | C(2)-C(1)-H(1A) | 108.5(12) | F(1)-C(12)-C(13) | 111.11(14) |
| C(1)-H(1A) | 0.974(19) | C(2)-C(1)-H(1B) | 108.9(11) | F(2)-C(12)-F(3) | 107.51(15) |
| C(2)-H(2B) | 0.95(2) | H(1A)-C(1)-H(1B) | 105.7(16) | F(2)-C(12)-C(13) | 114.33(15) |
| C(2)-H(2A) | 0.97(2) | N(2)-C(2)-H(2A) | 109.9(11) | F(3)-C(12)-C(13) | 111.38(15) |
| C(3)-H(3A) | 0.95(2) | N(2)-C(2)-H(2B) | 109.0(12) | O(1)-C(13)-O(2) | 130.23(14) |
| C(3)-H(3B) | 0.976(19) | C(1)-C(2)-H(2A) | 109.2(11) | O(1)-C(13)-C(12) | 116.05(14) |
| C(4)-H(4B) | 0.970(19) | C(1)-C(2)-H(2B) | 113.2(12) | O(2)-C(13)-C(12) | 113.72(14) |
| C(4)-H(4A) | 0.99(2) | H(2A)-C(2)-H(2B) | 107.3(16) | F(4)-C(14)-F(5) | 106.99(13) |
| C(5)-H(5B) | 0.97(2) | N(2)-C(3)-H(3A) | 107.8(12) | F(4)-C(14)-F(6) | 106.53(14) |
| C(5)-H(5A) | 0.98(2) | N(2)-C(3)-H(3B) | 103.6(11) | F(4)-C(14)-C(15) | 112.72(13) |
| C(6)-H(6B) | 0.975(19) | C(4)-C(3)-H(3A) | 110.7(12) | F(5)-C(14)-F(6) | 106.26(14) |
| C(6)-H(6A) | 0.98(2) | C(4)-C(3)-H(3B) | 110.5(11) | F(5)-C(14)-C(15) | 110.06(14) |
| C(7)-H(7B) | 0.98(2) | H(3A)-C(3)-H(3B) | 112.2(16) | F(6)-C(14)-C(15) | 113.84(13) |
| C(7)-H(7A) | 0.964(19) | N(3)-C(4)-H(4A) | 113.3(12) | O(3)-C(15)-O(4) | 130.18(16) |
| C(8)-H(8A) | 0.997(19) | N(3)-C(4)-H(4B) | 109.2(11) | O(3)-C(15)-C(14) | 114.19(13) |
| C(8)-H(8B) | 0.98(2) | C(3)-C(4)-H(4A) | 107.5(12) | O(4)-C(15)-C(14) | 115.57(13) |
| C(9)-H(9B) | 1.01(2) | | | | |
| C(9)-H(9A) | 1.03(2) | | | | |
| C(11)-H(11) | 0.93(2) | | | | |
| C(12)-C(13) | 1.554(2) | | | | |
| C(14)-C(15) | 1.543(2) | | | | |




Zn[1-Prop-2-ynyl-1,4,7,10tetraaza-cyclododecane] di-chloride (**12**):

The TFA-salt **11** (732 mg, 1.67 mmol) was dissolved in 20 ml of water and eluted over 7.4 ml of a strongly basic anion exchanger ($OH^-$-form, loading: 0.9 mmol/ml, 6.68 mmol, 2 eq). The ion-exchanger resin was washed with water and MeOH. The solution was lyophilized and the obtained frothy solid was dissolved under an atmosphere of $N_2$ in 8 ml of dry MeCN. A solution of 683 mg $ZnCl_2$ (5.01 mmol, 3 eq) in 25 ml of dry MeCN was added and the solution was heated to reflux for 24 h. The solvent was removed at reduced pressure. The residue was suspended in 4 ml dry EtOH, treated with ultra sound for 30 min, filtered and washed with dry EtOH. Drying in vacuum yielded the zinc-complex as colourless hygroscopic solid (434 mg, 1.25 mmol, 75 %).

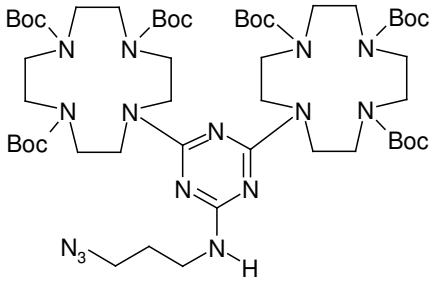
**Mp**: > 250 °C, **$^1$H-NMR** (300 MHz, $CD_3CN$): $\delta$ [ppm] = 2.68 (t, $^4J$ = 2.3 Hz, 1 H, CH), 2.64 – 3.04 (m, 16 H, cyclen-$CH_2$), 3.05 – 3.09 (m, 1 H, NH), 3.14 – 3.20 (m, 2 H, NH), 3.61 (d, $^4J$ = 2.4 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (300 MHz, $CD_3CN$): $\delta$ [ppm] = 43.4 (–, 2 C, cyclen), 44.2 (–, 1 C), 44.9 (–, 2 C, cyclen), 45.6 (–, 2 C, cyclen), 50.9 (–, 2 C, cyclen), 76.7 ($C_{quat}$), 77.1 (+, CH); **MS** (ESI, $H_2O/CH_3CN$): m/z (%) = 308.9 [($K^{2+}$+ $Cl^-$)$^+$] (100); **MS-HR** (PI-LSIMS, $H_2O$/MeCN/glycerine): [($K^{2+}$+$Cl^-$)$^+$] calc.: 309.0824, found: 309.0825 ($\pm$ = 1.12 ppm); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3492, 3269, 3216, 2945, 2886, 2107, 1616, 1468, 1442, 1299, 1087, 974; **MF**: $C_{11}H_{22}N_4Cl_2Zn$; **MW** = 346.61 g/mol;



(2-Azido-ethyl)-[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-amine (**16**):

The chloride **13** (1000 mg, 0.95 mmol) was dissolved in 40 ml of dioxane. Following, potassium carbonate (1962 mg, 14.19 mmol, 15 eq) and 2-Azido-ethylamine hydrochloride (**14**) (580 mg, 4.73 mmol, 5 eq) were added. The suspension was stirred at 90 °C for 3 d. The suspension was filtered and the residue was washed thoroughly with EE. After evaporation of the solvent in vacuum, the obtained brown oil was purified by column chromatography twice. First with PE:EE = 7:3 and then with DCM:MeOH = 98:2. This gave the azide **16** as a colourless solid in a yield of 998 mg (0.90 mmol, 95 %). $R_f$ (EE:PE = 1:1) = 0.73.

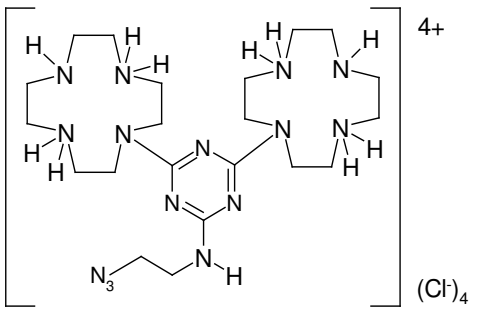
**Mp**: 142 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.37 – 1.39 (m, 54 H, Boc-$CH_3$), 3.24 – 3.58 (m, 36 H, 2 $CH_2$ + cyclen-$CH_2$), 4.93 (bs, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.4 (+, Boc-$CH_3$), 28.5 (+, Boc-$CH_3$), 40.1 (–, 1 C), 50.3 (–, 16 C, cyclen), 51.2 (–, 1 C), 79.7 ($C_{quat,}$ 4 C, Boc), 79.8 ($C_{quat,}$ 2 C, Boc), 156.3 ($C_{quat,}$ 6 C, urethane), 165.3 ($C_{quat,}$ 1 C, triazine), 166.1 ($C_{quat,}$ 2 C, triazine); **MS** (ESI, $CH_2Cl_2$/MeOH): m/z (%) = 1107.0 [$MH^+$] (100), 1129.0 [$M+Na^+$] (8); **EA** ($C_{51}H_{91}N_{15}O_{12}$) calc.: C 55.37, H 8.29, N 18.99, found: C 55.76, H 8.50, N 18.51; **UV/Vis** ($CH_3CN$): λ (lg ε) = 231 nm (4.562); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3402, 3255, 2976, 2932, 2138, 1680, 1539, 1479, 1411, 1367, 1249, 1178, 777; **MF**: $C_{51}H_{91}N_{15}O_{12}$; **MW** = 1106.38 g/mol;



(3-Azido-propyl)-[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-amine (**17**):

The chloride **13** (1000 mg, 0.95 mmol) was dissolved in 40 ml of dioxane. Following, potassium carbonate (1962 mg, 14.19 mmol, 15 eq) and 3-Azido-propylamine hydrochloride (**15**) (646 mg, 4.73 mmol, 5 eq) were added. The suspension was stirred at 90 °C for 3 d. The suspension was filtered and the residue was washed thoroughly with EE. After evaporation of the solvent in vacuum the obtained brown oil was purified by column chromatography twice. First with PE:EE = 7:3 and then with DCM:MeOH = 98:2. This gave the azide **17** as a colourless solid in a yield of 1011 mg (0.90 mmol, 95 %). $R_f$ (EE:PE = 1:1) = 0.74.

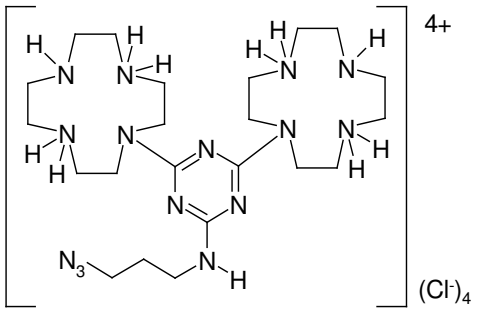
**Mp**: 140 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.37 – 1.39 (m, 54 H, Boc-$CH_3$), 1.76 (tt, $^3J$ = 6.6 Hz, 2 H, $CH_2$), 3.24 – 3.58 (m, 36 H, 2 $CH_2$ + cyclen-$CH_2$), 4.78 (bs, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.4 (+, Boc-$CH_3$), 28.5 (+, Boc-$CH_3$), 29.2 (–, 1 C), 37.9 (–, 1 C), 49.0 (–, 1 C), 50.3 (–, 16 C, cyclen), 79.7 ($C_{quat,}$ 4 C, Boc), 79.8 ($C_{quat,}$ 2 C, Boc), 156.3 ($C_{quat,}$ 6 C, urethane), 165.8 ($C_{quat,}$ 1 C, triazine), 166.5 ($C_{quat,}$ 2 C, triazine); **MS** (ESI, $CH_2Cl_2$/MeOH): m/z (%) = 1121.0 [$MH^+$] (100); **EA** ($C_{52}H_{93}N_{15}O_{12}$) calc.: C 55.75, H 8.37, N 18.75, found: C 56.02, H 8.60, N 18.48; **UV/Vis** ($CH_3CN$): λ (lg ε) = 231 nm (4.559); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3402, 3255, 2976, 2932, 2138, 1680, 1539, 1479, 1411, 1367, 1249, 1178, 777; **MF**: $C_{52}H_{93}N_{15}O_{12}$; **MW** = 1120.40 g/mol;



(2-Azido-ethyl)-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-amine tetra hydrochloride (**18**):

Synthesis followed **GP 4** using **16** (550 mg, 0.50 mmol) and 20 ml of a saturated solution of HCl in $Et_2O$. This yielded 323 mg of **18** as a colourless solid (0.50 mmol, 100 %).

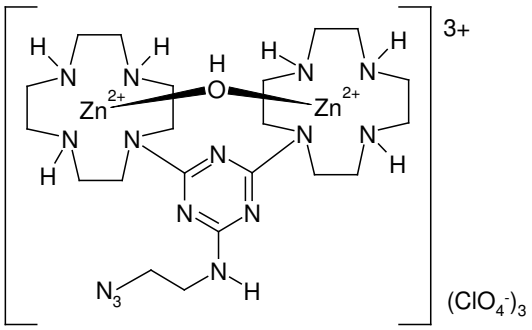
**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 3.08 – 3.26 (m, 24 H, cyclen-$CH_2$), 3.40 (t, $^3J$ = 3.5 Hz, 2 H, $CH_2$), 3.55 (t, $^3J$ = 3.6 Hz, 2 H, $CH_2$), 3.79 (bs, 8 H, cyclen-$CH_2$); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 40.0 (–, 1 C), 44.2 (–, 8 C, cyclen), 46.2 (–, 4 C, cyclen), 47.9 (–, 4 C, cyclen), 49.9 (–, 1 C), 155.4 ($C_{quat,}$ 3 C, triazine); **MS** (ESI, $H_2O$/MeCN + TFA): m/z (%) = 196.7 [$(M+3H^+ +2MeCN)^{3+}$] (26), 253.6 [$(M+2H^+)^{2+}$] (100), 506.4 [$MH^+$] (24), 542.4 [$(M+H^+ +HCl)^+$] (5); **UV/Vis** ($H_2O$): λ (lg ε) = 220 nm (4.637); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3428, 2964, 2940, 2811, 2142, 1678, 1542, 1416, 1352, 1179, 780; **MF**: $C_{21}H_{47}N_{15}Cl_4$; **MW** = 651.52 g/mol;



(3-Azido-propyl)-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-amine tetra hydrochloride (**19**):

Synthesis followed **GP 4** using **17** (560 mg, 0.50 mmol) and 20 ml of a saturated solution of HCl in $Et_2O$. This yielded 333 mg of **19** as colourless solid (0.50 mmol, 100 %).

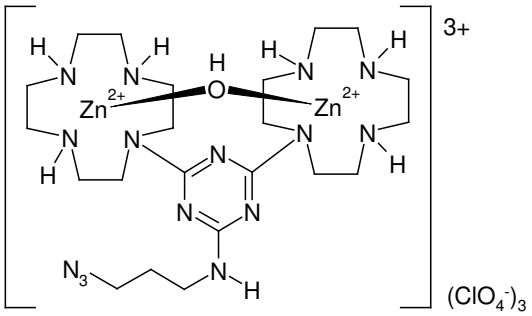
**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.80 (tt, $^3J$ = 3.6 Hz, 2 H, $CH_2$), 3.16 – 3.37 (m, 26 H, $CH_2$ + cyclen-$CH_2$), 3.49 (t, $^3J$ = 3.6 Hz, 2 H, $CH_2$), 3.85 (bs, 8 H, cyclen-$CH_2$); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 27.6 (–, 1 C), 38.3 (–, 1 C), 44.3 (–, 8 C, cyclen), 46.3 (–, 4 C, cyclen), 48.0 (–, 4 C, cyclen), 48.7 (–, 1 C), 155.2 ($C_{quat,}$ 3 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 260.7 $[(M+2H^+)^{2+}]$ (100); **UV/Vis** ($H_2O$): λ (lg ε) = 220 nm (4.637); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3428, 2964, 2940, 2811, 2142, 1678, 1542, 1416, 1352, 1179, 780; **MF**: $C_{22}H_{49}N_{15}Cl_4$; **MW** = 665.53 g/mol;




<u>$Zn_2$[(2-Azido-ethyl)-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-amine] tri-perchlorate hydroxide (**20**):</u>

Synthesis followed **GP 5** using the ammonium-salt **18** (280 mg, 0.43 mmol), 5.7 ml of the anion-exchanger (5.16 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (528 mg, 1.29 mmol, 3 eq). This gave **20** as pale orange solid (382 mg, 0.39 mmol, 90 %).

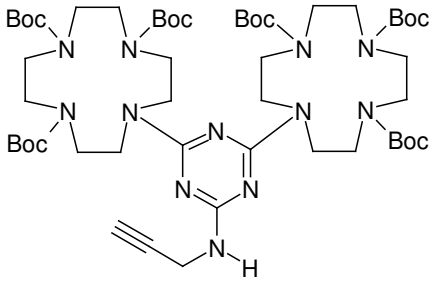
**Mp**: 263 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.71 – 4.40 (m, 42 H), 6.42 (t, $^3J$ = 5.9 Hz, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 41.0 (–, 1 C), 44.5 (–, 4 C, cyclen), 46.5 (–, 2 C, cyclen), 46.7 (–, 2 C, cyclen), 47.5 (–, 2 C, cyclen), 47.5 (–, 2 C, cyclen), 47.7 (–, 2 C, cyclen), 47.9 (–, 2 C, cyclen), 51.6 (–, 1 C), 167.1 ($C_{quat,}$ 1 C, triazine), 170.9 ($C_{quat,}$ 1 C, triazine), 171.2 ($C_{quat,}$ 1 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 360.7 $[(K^{4+}+H_2O+2Cl^-)^{2+}]$ (100); **EA** ($C_{21}H_{48}N_{15}O_{15}Cl_3Zn_2$) calc.: C 25.53, H 4.90, N 21.27, found: C 25.66, H 4.92, N 21.15; **UV/Vis** ($CH_3CN$): λ (lg ε) = 221 nm (4.578); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3405, 3305, 3255, 2942, 2148, 1562, 1343; **MF**: $[C_{21}H_{43}N_{15}Zn_2]$ (OH) $(ClO_4)_3$ x 2 $H_2O$ = $C_{21}H_{48}N_{15}O_{15}Cl_3Zn_2$; **MW** = 987.82 g/mol;



$Zn_2$[(3-Azido-propyl)-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-amine] tri-perchlorate hydroxide (**21**):

Synthesis followed **GP 5** using the ammonium-salt **19** (579 mg, 0.87 mmol), 11.6 ml of the anion-exchanger (10.4 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (972 mg, 2.61 mmol, 3 eq). This gave **21** as pale orange solid (784 mg, 0.78 mmol, 90 %).

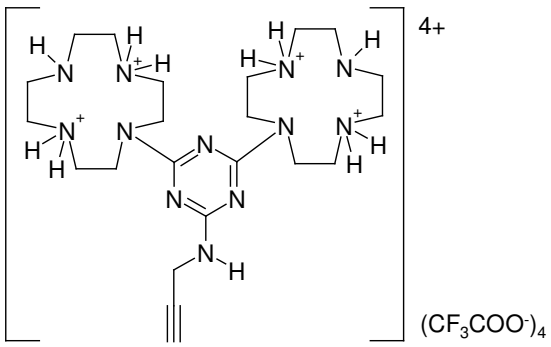
**Mp**: 258 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 1.83 (tt, $^3$J = 6.7 Hz, 2 H, $CH_2$), 2.65 – 4.42 (m, 42 H), 6.32 (t, $^3$J = 5.8 Hz, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 29.6 (–, 1 C), 39.0 (–, 1 C), 44.5 (–, 5 C, cyclen + $CH_2$), 46.3 (–, 2 C, cyclen), 46.5 (–, 2 C, cyclen), 47.4 (–, 2 C, cyclen), 47.4 (–, 2 C, cyclen), 47.8 (–, 2 C, cyclen), 47.9 (–, 2 C, cyclen), 167.0 ($C_{quat,}$ 1 C, triazine), 170.8 ($C_{quat,}$ 1 C, triazine), 171.4 ($C_{quat,}$ 1 C, triazine); **MS** (ESI, MeCN): m/z (%) = 372.6 [$(K^{4+}$-$H^+$+$ClO_4^-)^{2+}$] (65), 381.6 [$(K^{4+}$+$OH^-$+$ClO_4^-)^{2+}$] (100), 862.3 [$(K^{4+}$+$OH^-$+2$ClO_4^-)^+$] (35); **EA** ($C_{22}H_{50}N_{15}O_{15}Cl_3Zn_2$) calc.: C 26.38, H 5.03, N 20.97, found: C 26.71, H 5.16, N 21.03; **UV/Vis** ($CH_3CN$): λ (lg ε) = 221 nm (4.558); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3405, 3305, 3255, 2942, 2148, 1562, 1343; **MF**: [$C_{22}H_{45}N_{15}Zn_2$] (OH) $(ClO_4)_3$ x 2 $H_2O$ = $C_{22}H_{50}N_{15}O_{15}Cl_3Zn_2$; **MW** = 1001.85 g/mol;




[4,6-Bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-prop-2-ynyl-amine (**22**):

The chloride **13** (815 mg, 0.77 mmol) was dissolved in 25 ml of dioxane. Following, potassium carbonate (533 mg, 3.86 mmol, 5 eq) and propargyl amine (247 µl, 212 mg, 3.86 mmol, 5 eq) were added. The suspension was stirred at 90 °C in the dark for 3 d. The suspension was filtered and the residue was washed thoroughly with EE. After evaporation of the solvent in vacuum the obtained brown oil was purified by CC twice. First with PE:EE = 7:3 and then with DCM:MeOH = 98:2. This gave the alkyne **22** as a colourless solid in a yield of 780 mg (0.73 mmol, 94 %). $R_f$ (EE:PE = 1:1) = 0.74.

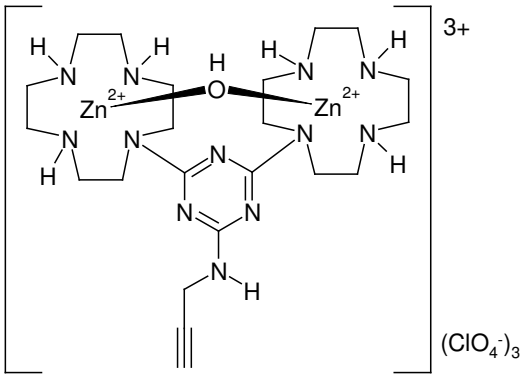
**Mp**: 149 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.37 – 1.39 (m, 54 H, Boc-$CH_3$), 2.09 (t, $^4J$ = 2.3 Hz, 1 H, CH), 3.22 – 3.58 (m, 32 H, cyclen-$CH_2$), 4.07 – 4.10 (m, 2 H, $CH_2$), 4.86 (bs, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.4 (+, Boc-$CH_3$), 28.5 (+, Boc-$CH_3$), 30.4 (–, 1 C), 50.3 (–, 16 C, cyclen), 70.5 (+, 1 C, CH), 79.7 ($C_{quat,}$ 4 C, Boc), 79.8 ($C_{quat,}$ 2 C, Boc), 81.1 ($C_{quat,}$ 1 C, alkyne), 156.3 ($C_{quat,}$ 6 C, urethane), 165.4 ($C_{quat,}$ 1 C, triazine), 166.1 ($C_{quat,}$ 2 C, triazine); **MS** (ESI, $CH_2Cl_2$/MeOH): m/z (%) = 1075.8 [$MH^+$] (100); **EA** ($C_{52}H_{90}N_{12}O_{12}$): calc.: C 58.08, H 8.44, N 15.63, found: C 57.76, H 8.80, N 15.45; **UV/Vis** ($CH_3CN$): λ (lg ε) = 231 nm (4.564); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3402, 3314, 3255, 2976, 2932, 2102, 1680, 1539, 1479, 1411, 1367, 1249, 1178, 777; **MF**: $C_{52}H_{90}N_{12}O_{12}$; **MW** = 1075.36 g/mol;




[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-prop-2-ynyl-amine tetra-trifluoro acetate (**23**):

The Boc-protected compound **22** (327 mg, 0.30 mmol) was dissolved in 20 ml of dry DCM and TFA was added (1.968 ml, 2912 mg, 25.54 mmol, 84 eq). The solution was stirred at r.t. under an atmosphere of $N_2$ for 24 h. The solvent and excess TFA were removed in vacuum. This gave a yellow oil, which was dissolved in MeOH and dried in vacuum several times. This yielded 278 mg of **23** as yellow hygroscopic solid (0.30 mmol, 100 %).

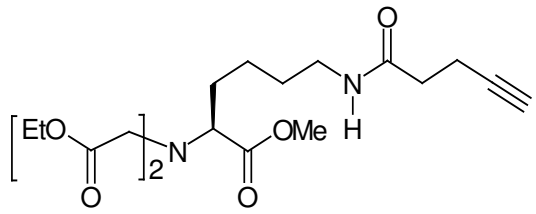
**Mp**: 58 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.47 (t, $^4J$ = 2.5 Hz, 1 H, CH), 3.00 – 3.25 (m, 32 H, cyclen-$CH_2$), 3.78 (d, $^4J$ = 2.5 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 30.9 (–), 43.3 (–, cyclen), 43.8 (–, cyclen), 45.4 (–, cyclen), 46.1 (–, cyclen), 47.0 (–, cyclen), 47.6 (–, cyclen), 72.1 (+, CH), 81.9 ($C_{quat,}$ alkyne), 164.6 ($C_{quat,}$ triazine), 166.6 ($C_{quat,}$ triazine), 168.1 ($C_{quat,}$ triazine); **MS** (ESI, MeCN): m/z (%) = 238.1 [$MH_2^{2+}$] (100), 475.4 [$MH^+$] (27), 589.4 [($MH^+$+$CF_3COOH$)$^+$] (24); **UV/Vis** ($CH_3CN$): λ (lg ε) = 219 nm (4.637); **MF**: $C_{30}H_{46}N_{12}O_8F_{12}$; **MW** = 930.75 g/mol;



$Zn_2$[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl]-prop-2-ynyl-amine tri-perchlorate hydroxide (**24**):

Synthesis followed **GP 5** using the TFA-salt **23** (2317 mg, 2.49 mmol), 33.2 ml of the anion-exchanger (29.9 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (1946 mg, 5.23 mmol, 2.1 eq). This gave **24** as pale orange solid (2192 mg, 2.29 mmol, 92 %).

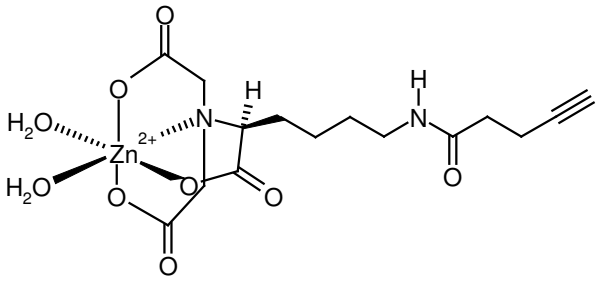
**Mp**: 268 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.54 (t, $^4$J = 2.5 Hz, 1 H, CH), 2.68 – 3.63 (m, 32 H, cyclen-$CH_2$), 3.94 – 4.10 (m, 2 H, NH), 4.17 (dd, $^3$J = 5.8 Hz, $^4$J = 2.5 Hz, 2 H, $CH_2$), 4.30 – 4.39 (m, 2 H, NH), 4.45 – 4.54 (m, 2 H, NH), 6.46 (t, $^3$J = 5.8 Hz, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 31.2 (–, 1 C), 44.4 (–, 4 C, cyclen), 46.8 (–, 2 C, cyclen), 47.1 (–, 2 C, cyclen), 47.5 (–, 4 C, cyclen), 47.7 (–, 4 C, cyclen), 72.2 (+, 1 C, CH), 82.1 ($C_{quat,}$ 1 C, alkyne), 166.7 ($C_{quat,}$ 1 C, triazine), 170.9 ($C_{quat,}$ 1 C, triazine), 170.9 ($C_{quat,}$ 1 C, triazine); **MS** (ESI, MeCN): m/z (%) = 213.9 [$(K^{4+}$-$H^+$+MeCN)$^{3+}$] (32), 227.7 [$(K^{4+}$-$H^+$+2MeCN)$^{3+}$] (44), 233.7 [$(K^{4+}$+$OH^-$ +2MeCN)$^{3+}$] (40), 350.1 [$(K^{4+}$-$H^+$+$OH^-$+2MeCN)$^{2+}$] (40), 359.1 [$(K^{4+}$+$OH^-$+$ClO_4^-$)$^{2+}$] (100), 817.2 [$(K^{4+}$+$OH^-$+2$ClO_4^-$)$^+$] (50); **EA** ($C_{22}H_{47}N_{12}O_{15}Cl_3Zn_2$) calc.: C 27.62, H 4.95, N 17.57, found: C 27.77, H 5.19, N 17.88; **UV/Vis** ($CH_3CN$): λ (lg ε) = 221 nm (4.565); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3405, 3305, 3255, 2942, 2118, 1562, 1343; **MF**: [$C_{22}H_{42}N_{12}Zn_2$] (OH) $(ClO_4)_3$ x 2 $H_2O$ = $C_{22}H_{47}N_{12}O_{15}Cl_3Zn_2$; **MW** = 956.81 g/mol;



2-(Bis-ethoxycarbonylmethyl-amino)-6-pent-4-ynoylamino-hexanoic acid methyl ester (**26**):

The 4-pentynoic acid (338 mg, 2.65 mmol, 1.3 eq) was dissolved in 5 ml of dry DCM and cooled to 0 °C in an icebath. Following, HOBt (512 mg, 3.79 mmol, 1.43 eq), EDC (670 µl, 588 mg, 3.79 mmol, 1.43 eq) and DIEA (346 µl, 489 mg, 3.79 mmol, 1.43 eq) were added in this sequence (important). The solution was stirred at 0 °C for 30 min. Then, the amine **25** (880 mg, 2.65 mmol, 1 eq), dissolved in 10 ml of dry DCM, was added dropwise. The solution was stirred at r.t. under an atmosphere of $N_2$ for 24 h. After that time 50 ml of DCM were added and the solution was washed with 30 ml of a 0.1 N aqueous solution of citric acid and 30 ml water. The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuum. The crude product was purified by CC with EE:PE = 3:2 to 4:1. This gave **26** as a colourless oil in a yield of 656 mg (1.59 mmol, 60 %). $R_f$ (EE:PE = 1:1) = 0.21.

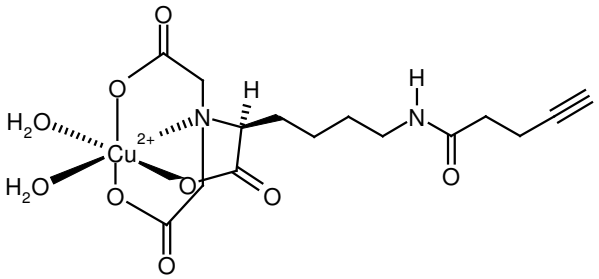
**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.06 (t, $^3J$ = 7.1 Hz, 6 H, $CH_3$), 1.19 – 1.41 (m, 4 H, $CH_2$), 1.46 – 1.53 (m, 2 H, $CH_2$), 1.84 (t, $^4J$ = 2.5 Hz, 1 H, alkyne-CH), 2.19 – 2.24 (m, 2 H, $CH_2$), 2.27 – 2.33 (m, 2 H, $CH_2$), 2.97 – 3.10 (m, 2 H, $CH_2$), 3.22 (t, $^3J$ = 7.6 Hz, 1 H, C*H), 3.41 (s, 4 H, N-$CH_2$), 3.47 (s, 3 H, $CH_3$), 3.95 (q, $^3J$ = 7.1 Hz, 4 H, $CH_2$), 6.58 (t, $^3J$ = 5.2 Hz, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 2 C, $CH_3$), 15.0 (–, 1 C), 22.7 (–, 1 C), 28.3 (–, 1 C), 29.6 (–, 1 C), 35.3 (–, 1 C), 39.2 (–, 1 C), 51.5 (+, 1 C, C*H), 52.8 (–, 2 C), 60.7 (–, 2 C), 64.2 (+, 1 C, $CH_3$), 69.1 (+, CH, alkyne), 83.3 ($C_{quat}$, 1 C, alkyne), 171.1 ($C_{quat}$, 1 C), 171.5 ($C_{quat}$, 2 C, Et-ester), 173.24 ($C_{quat}$, 1 C, amide); **MS** (ESI, MeOH): m/z (%) = 413.2 [$MH^+$] (100), 435.2 [$MNa^+$] (7); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3311, 3264, 2971, 2107, 1735, 1689, 1646, 1534, 1450, 1282, 1166; **MF**: $C_{20}H_{32}N_2O_7$; **MW** = 412.48 g/mol;



Li[Zn(2-(Bis-carboxylate-methyl-amino)-6-pent-4-ynoylamino-hexanoate)] di-hydrate (**27**):

Compound **26** (429 mg, 1.04 mmol) was dissolved in a mixture of 10 ml of acetone and 7 ml water. Then, LiOH (74.8 mg, 3.12 mmol, 3 eq) was added and the solution was stirred at r.t. for 24 h. The acetone was evaporated and the water was removed by lyophilisation. This yielded 375 mg of the carboxylate as colourless solid (1.04 mmol, 100 %, MF: $C_{15}H_{19}N_2O_7Li_3$; MW = 360.15 g/mol). NMR- and MS-spectroscopy confirmed the complete cleavage of all ester-groups. The lithium-salt was dissolved in 15 ml water and $ZnCl_2$ (142 mg, 1.04 mmol, 1 eq) and $5ZnO{\cdot}2CO_3$ x 4 $H_2O$ (623 mg, 1.04 mmol, 1 eq) were added. The solution was stirred at r.t. for 16 h and then heated to 60 °C for 2 h. All inorganic compounds were removed by filtration and the aqueous solution was dried by lyophilisation. The crude product was purified by recrystallisation in EtOH/water. Diethyl ether was added to complete precipitation. This yielded 421 mg of **27** as colourless solid (0.94 mmol, 90 %).

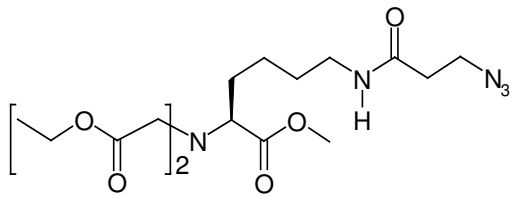
**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.29 – 1.65 (m, 6 H, 3 $CH_2$), 2.27 – 2.43 (m, 5 H, 2 $CH_2$ + alkyne-CH), 3.02 – 3.44 (m, 7 H, 3 $CH_2$ + C*H); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 14.7 (–, 1 C), 24.7 (–, 1 C), 27.0 (–, 1 C), 28.4 (–, 1 C), 34.6 (–, 1 C), 39.0 (–, 1 C), 55.3 (–, 1 C), 60.7 (–, 1 C), 69.2 (+, 1 C, C*H), 70.2 (+, alkyne-CH), 82.9 ($C_{quat}$, 1 C, alkyne), 174.5 ($C_{quat}$, 1 C), 177.9 ($C_{quat}$, 1 C), 178.2 ($C_{quat}$, 1 C), 179.5 ($C_{quat}$, 1 C); **MS** (neg. ESI, $H_2O$/MeOH/MeCN + $NH_4Ac$): m/z (%) = 403.1 [$A^-$] (100), 469.2 [($A^-$+LiOAc)$^-$] (55), 535.3 [($A^-$+2LiOAc)$^-$] (50), 601.3 [($A^-$+3LiOAc)$^-$] (28); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3443, 3253, 2940, 2870, 1640, 1412, 1138; **MF**: $C_{15}H_{19}N_2O_7ZnLi$ x 2 $H_2O$; **MW** = 447.68 g/mol;



Li[Cu(2-(Bis-carboxylate-methyl-amino)-6-pent-4-ynoylamino-hexanoate)] di-hydrate (**28**):

Compound **26** (429 mg, 1.04 mmol) was dissolved in a mixture of 10 ml acetone and 7 ml water. Then, LiOH (74.8 mg, 3.12 mmol, 3 eq) was added and the solution was stirred at r.t. for 24 h. The acetone was evaporated and the water is removed by lyophilisation. This yielded 375 mg of the carboxylate as a colourless solid (1.04 mmol, 100 %, MF: $C_{15}H_{19}N_2O_7Li_3$; MW = 360.15 g/mol). NMR- and MS-spectroscopy confirmed the complete cleavage of all ester-groups. The lithium-salt was dissolved in 15 ml water and $CuSO_4$ x 5 $H_2O$ (260 mg, 1.04 mmol, 1 eq) was added. The solution was stirred at r.t. for 16 h and then heated to 60 °C for 2 h. The aqueous solution was dried by lyophilisation. The bluish-green crude product was purified by recrystallisation in EtOH/water. Diethyl ether was added to complete precipitation. This yielded 419 mg of **28** as a bluish-green solid (0.94 mmol, 90 %).

**Mp**: > 250 °C; **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 402.0 [$A^-$] (100); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3443, 3253, 2940, 2870, 1640, 1412, 1138; **MF**: $C_{15}H_{19}N_2O_7CuLi$ x 2 $H_2O$; **MW** = 445.84 g/mol;

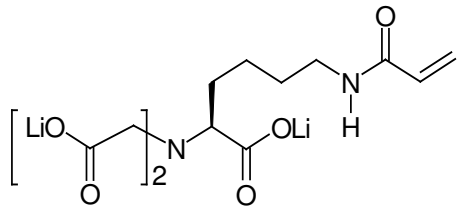


6-(3-Azido-propionylamino)-2-(bis-ethoxycarbonylmethyl-amino)-hexanoic acid methyl ester (**29**):

The β-azido-propionic acid (336 mg, 2.93 mmol, 1.3 eq) was dissolved in 5 ml of dry DCM under an atmosphere of $N_2$ and cooled to 0 °C in an icebath. Following, HOBt (434 mg, 3.21 mmol, 1.43 eq), EDC (569 µl, 499 mg, 3.21 mmol, 1.43 eq) and DIEA (294 µl, 415 mg, 3.21 mmol, 1.43 eq) were added in this sequence. The solution was

stirred at 0 °C for 30 min. Then, the amine **25** (747 mg, 2.25 mmol, 1 eq), dissolved in 10 ml dry DCM, was added dropwise. The solution was stirred at r.t. for 24 h. After that time 50 ml DCM were added and the solution was washed with 30 ml of a 0.1 N aqueous solution of citric acid and 30 ml water. The organic layer was dried with $MgSO_4$, filtered and concentrated at reduced pressure. The crude product was purified by CC. EE:PE in a ratio of 3:2 to 4:1. This gave **29** as colourless oil in a yield of 531 mg (1.24 mmol, 55 %). $R_f$ (EE:PE = 1:1) = 0.21.

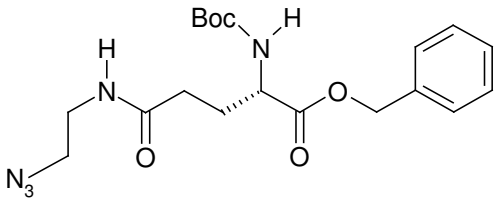
**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.14 (t, $^3J$ = 7.2 Hz, 6 H, $CH_3$), 1.32 – 1.47 (m, 4 H, $CH_2$), 1.54 – 1.62 (m, 2 H, $CH_2$), 2.35 (t, $^3J$ = 6.6 Hz, 2 H, $CH_2$), 3.07 – 3.20 (m, 2 H, $CH_2$), 3.31 (t, $^3J$ = 7.6 Hz, 1 H, C*H), 3.46 – 3.50 (m, 6 H, $CH_2$ + 2 N-$CH_2$), 3.56 (s, 3 H, $CH_3$), 4.03 (q, $^3J$ = 7.2 Hz, 4 H, $CH_2$), 6.54 – 6.57 (m, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, $CH_3$), 22.6 (–, 1 C), 28.0 (–, 1 C), 29.4 (–, 1 C), 35.5 (–, 1 C), 39.2 (–, 1 C), 47.4 (–, 1 C), 51.3 (+, 1 C, C*H), 52.6 (–, 2 C), 60.6 (–, 2 C), 64.0 (+, 1 C, O-$CH_3$), 170.0 ($C_{quat}$, 1 C, ester), 171.4 ($C_{quat}$, 2 C, ester), 173.13 ($C_{quat}$, 1 C, amide); **MS** (ESI, MeOH): m/z (%) = 430.3 [$MH^+$] (100), 452.3 [$M+Na^+$] (15); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3311, 3264, 2971, 2146, 1735, 1689, 1646, 1534, 1450, 1282, 1166; **MF**: $C_{18}H_{31}N_5O_7$; **MW** = 429.47 g/mol;




<u>$Li_3$[6-Acryloylamino-2-(bis-carboxylate-methyl-amino)-hexanoate] (**30**):</u>

The azide **29** (429 mg, 1.00 mmol) was dissolved in aqueous acetone (40 Vol% $H_2O$) and 71.9 mg LiOH (3.00 mmol, 3 eq) were added. The solution was stirred at r.t. for 24 h. After that time the acetone was evaporated and the water was removed by lyophilisation. This yielded 334 mg of **30** as colourless solid (1.00 mmol, 100 %).

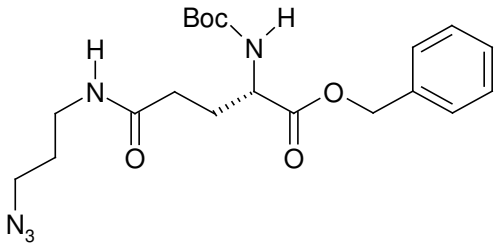
**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.13 – 1.64 (m, 6 H, 3 $CH_2$), 2.86 – 3.14 (m, 7 H, C*H + 3 $CH_2$), 5.59 (dd, $^3J$ = 1.8 Hz, $^3J$ = 9.7 Hz, 1 H, CH), 6.00 (dd, $^3J$ = 1.8 Hz, $^2J$ = 17.2 Hz, 1 H, $CH_2$), 6.11 (dd, $^2J$ = 17.2 Hz, $^3J$ = 9.7 Hz, 1 H, $CH_2$); **MS** (pos. ESI, $H_2O$/MeOH + $NH_4Cl$): m/z (%) = 317.0 [$(A^{3-}+4H^+)^+$] (70), 323.1 [$(A^{3-}+Li^+ +3H^+)^+$] (100), 329.1 [$(A^{3-}+2Li^+ +2H^+)^+$]; **MF**: $C_{13}H_{17}N_2O_7Li_3$; **MW** = 334.11 g/mol;



4-(2-Azido-ethylcarbamoyl)-2-tert-butoxycarbonyl-amino-butyric acid benzyl ester (**32**):

The protected glutamic acid **31** (500 mg, 1.48 mmol) was dissolved in 2 ml of dry DMF and cooled in an ice bath. Following, EDC (317 µl, 278 mg, 1.79 mmol, 1.21 eq), HOBt (242 mg, 1.79 mmol, 1.21 eq), DIEA (298 µl, 421 mg, 3.26 mmol, 2.2 eq) and **14** (200 mg, 1.63 mmol, 1.1 eq) were added. The ice bath was removed and the solution was stirred at r.t. for 24 h. After that time the solution was cooled to 0 °C and water at 0 °C was added. A pale yellow oil separated. The aqueous solution was discarded. The oil was dissolved in 50 ml DCM. The solution was washed with 30 ml water, 30 ml 1 M aqueous citric acid, 30 ml water and 30 ml brine. The organic layer was dried with $Na_2SO_4$ and the solvent was removed in vacuum. This yielded 588 mg of **32** (1.45 mmol, 98 %) as a pale yellow crystalline solid in sufficient purity for the following reaction steps. For analytical data the crude product was purified by column chromatography with EE:PE in a ratio of 2:3. This gave pure **32** in a yield of 576 mg (1.42 mmol, 96 %). $R_f$ (EE:PE = 1:1) = 0.36.

**Mp**: 118 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.33 (s, 9 H, Boc-$CH_3$), 1.82 – 1.94 (m, 1 H, $CH_2$), 2.02 – 2.13 (m, 1 H, $CH_2$), 2.19 (t, $^3$J = 7.0 Hz, 2 H, $CH_2$), 3.27 – 3.28 (m, 4 H, 2 $CH_2$), 4.19 – 4.25 (m, 1 H, C*H), 5.02 (d, $^2$J = 12.4 Hz, 1 H, Bn-$CH_2$), 5.09 (d, $^2$J = 12.4 Hz, 1 H, Bn-$CH_2$), 5.66 (d, $^3$J = 8.0 Hz, 1 H, NH), 6.85 – 6.88 (m, 1 H, NH), 7.22 – 7.25 (m, 5 H, arom. CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.1 (–, 1 C), 28.3 (+, 3 C, Boc-$CH_3$), 32.3 (–, 1 C), 38.9 (–, 1 C), 50.5 (–, 1 C), 53.3 (+, C*H), 67.1 (–, 1 C, Bn), 79.9 ($C_{quat,}$ Boc), 128.2 (+, 2 C, arom.), 128.4 (+, 1 C, arom.), 128.6 (+, 2 C, arom.), 135.3 ($C_{quat,}$ 1 C, arom.), 155.9 ($C_{quat,}$ urethane), 172.2 ($C_{quat}$), 172.6 ($C_{quat}$); **MS** (ESI, DCM/MeOH): m/z (%) = 306.1 [(M-$C_5H_9O_2^-$)$^+$] (15), 350.1 [($MH^+$-$C_4H_8$)$^+$] (20), 406.2 [$MH^+$] (100); **EA** ($C_{19}H_{27}N_5O_5$) calc.: C 56.28, H 6.71, N 17.27, found: C 56.40, H 6.81, N 17.33; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3344, 3028, 2970, 2148, 1734, 1688, 1647, 1535, 1455, 1283; **MF**: $C_{19}H_{27}N_5O_5$; **MW** = 405.45 g/mol;

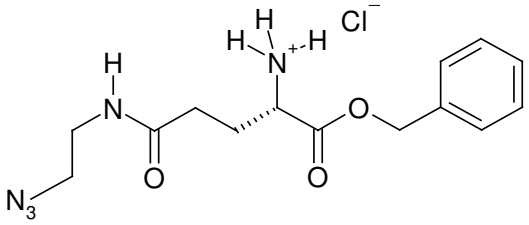


4-(3-Azido-propylcarbamoyl)-2-tert-butoxycarbonylamino-butyric acid benzyl ester (**33**):

The protected glutamic acid **31** (900 mg, 2.67 mmol) was dissolved in 2 ml of dry DMF and cooled in an ice bath. Following, EDC (571 µl, 501 mg, 2.93 mmol, 1.21 eq), HOBt (436 mg, 3.23 mmol, 1.21 eq), DIEA (537 µl, 759 mg, 5.87 mmol, 2.2 eq) and **15** (401 mg, 2.93 mmol, 1.1 eq) were added. The ice bath was removed and the solution was stirred at r.t. for 24 h. Then the solution was cooled to 0 °C and water at 0 °C was added. A pale yellow oil separated. The aqueous solution was discarded. The oil was dissolved in 50 ml DCM. The solution was washed with 30 ml water, 30 ml 1 M aqueous citric acid, 30 ml water and 30 ml brine. The organic layer was dried with $Na_2SO_4$ and the solvent was removed in vacuum. This yielded 1098 mg of **33** (2.62 mmol, 98 %) as a pale yellow crystalline solid in sufficient purity for the following reaction steps. For analytical data the crude product was purified by column chromatography with EE:PE in a ratio of 2:3. This gave pure **33** as white crystalline solid in a yield of 1074 mg (2.56 mmol, 96 %). $R_f$ (EE:PE = 1:1) = 0.37.

**Mp**: 116 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9 H, Boc-$CH_3$), 1.72 – 1.81 (m, 2 H, $CH_2$), 1.85 – 1.98 (m, 1 H, $CH_2$), 2.11 – 2.24 (m, 3 H, $CH_2$), 3.27 – 3.36 (m, 4 H, $CH_2$), 4.26 – 4.33 (m, 1 H, C*H), 5.11 (d, $^2$J = 12.1 Hz, 1 H, Bn-$CH_2$), 5.20 (d, $^2$J = 12.1 Hz, 1 H, Bn-$CH_2$), 5.35 (d, $^3$J = 7.7 Hz, 1 H, NH), 6.28 – 6.33 (m, 1 H, NH), 7.32 – 7.37 (m, 5 H, arom. CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.3 (+, 3 C, Boc-$CH_3$), 28.8 (–, 1 C), 29.1 (–, 1 C), 32.6 (–, 1 C), 37.1 (–, 1 C), 49.3 (–, 1 C), 53.1 (+, C*H), 67.3 (–, 1 C, Bn), 80.3 ($C_{quat,}$ Boc), 128.4 (+, 2 C, arom.), 128.6 (+, 1 C, arom.), 128.7 (+, 2 C, arom.), 135.2 ($C_{quat,}$ 1 C, arom.), 155.9 ($C_{quat,}$ 1 C, urethane), 172.1 ($C_{quat}$, 2 C, amide + ester); **MS** (pos. ESI, DCM/MeOH): m/z (%) = 320.1 [(M-$C_5H_9O_2^-$)$^+$] (30), 364.1 [($MH^+$-$C_4H_8$)$^+$] (40), 420.2 [$MH^+$] (100); **MS** (neg. ESI, DCM/MeOH): m/z (%) = 236.0 [(M-$H^+$-BnOH-$^t$BuOH)$^-$] (35), 310.1 [(M-$H^+$-BnOH)$^-$] (75), 454.3 [M+$Cl^-$] (20), 478.3 [M+$Ac^-$] (100); **EA** ($C_{20}H_{29}N_5O_5$) calc.: C 57.27, H 6.97, N 16.70, found: C 57.29, H 7.08, N 16.65; **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3344, 3028,

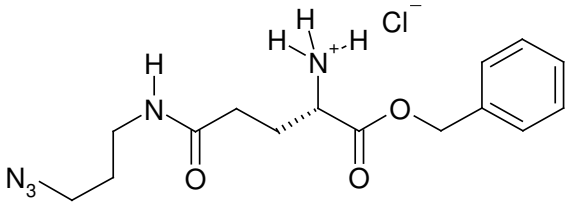
2970, 2148, 1734, 1688, 1647, 1535, 1455, 1283; **MF**: $C_{20}H_{29}N_5O_5$; **MW** = 419.48 g/mol;



3-(2-Azido-ethylcarbamoyl)-1-benzyloxycarbonyl-propyl-ammonium chloride (**34**):

The amide **32** (1220 mg, 3.01 mmol) was dissolved in little dry EE and 20 ml of a saturated solution of HCl in dry diethyl ether were added (1 ml per 0.15 mmol Boc). The mixture was stirred at r.t. under an atmosphere of $N_2$ for 15 h. After 30 min the product began to separate as a colourless oil. The solvent was removed in vacuum. This gave **34** as a pale yellow hygroscopic solid in quantitative yield (1028 mg, 3.01 mmol).

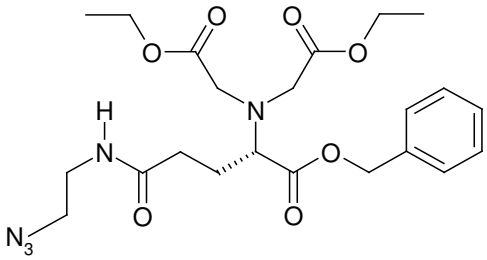
**$^1$H-NMR** (300 MHz, $CD_3CN$): $\delta$ [ppm] = 2.29 – 2.35 (m, 2 H, $CH_2$), 2.46 – 2.63 (m, 2 H, $CH_2$), 3.25 – 3.35 (m, 4 H, $CH_2$), 4.28 – 4.34 (m, 1 H, C*H), 5.22 (s, 2 H, Bn-$CH_2$), 7.29 – 7.42 (m, 5 H, arom. CH), 7.76 – 7.80 (m, 1 H, NH), 8.82 (bs, 3 H, $NH_3^+$); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): $\delta$ [ppm] = 27.0 (–, 1 C), 32.5 (–, 1 C), 39.8 (–, 1 C), 51.2 (–, 1 C), 53.8 (+, C*H), 69.0 (–, 1 C, Bn), 129.5 (+, 2 C, arom.), 129.6 (+, 1 C, arom.), 129.7 (+, 2 C, arom.), 136.3 ($C_{quat,}$ 1 C, arom.), 170.4 ($C_{quat}$), 173.9 ($C_{quat}$); **MS** (ESI, MeCN): m/z (%) = 306.1 [$MH^+$] (100), 611.4 [(2 $MH^+$-$H^+$)$^+$] (7); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3324, 3069, 3028, 2970, 2142, 2118, 1731, 1681, 1643, 1533, 1449; **MF**: $C_{14}H_{20}N_5O_3Cl$; **MW** = 341.80 g/mol;



3-(3-Azido-propylcarbamoyl)-1-benzyloxycarbonyl-propyl-ammonium chloride (**35**):

The amide **33** (1401 mg, 3.34 mmol) was dissolved in little dry EE and 22 ml of a saturated solution of HCl in dry diethyl ether were added (1 ml per 0.15 mmol Boc). The mixture was stirred at r.t. under an atmosphere of $N_2$ for 15 h. After 30 min the product began to separate as a colourless oil. The solvent was removed in vacuum. This gave **35** as a pale yellow hygroscopic solid in quantitative yield (1188 mg, 3.34 mmol).

**$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 1.69 (tt, $^3J$ = 6.7 Hz, 2 H, $CH_2$), 2.33 – 2.42 (m, 2 H, $CH_2$), 2.61 – 2.67 (m, 2 H, $CH_2$), 3.16 – 3.21 (m, 2 H, $CH_2$), 3.30 (t, $^3J$ = 6.7 Hz, 2 H, $CH_2$), 4.31 – 4.37 (m, 1 H, C*H), 5.24 (s, 2 H, Bn-$CH_2$), 7.29 – 7.44 (m, 5 H, arom. CH), 8.21 – 8.27 (m, 1 H, NH), 8.89 (bs, 3 H, $NH_3^+$); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 27.2 (–, 1 C), 29.2 (–, 1 C), 32.2 (–, 1 C), 38.1 (–, 1 C), 49.9 (–, 1 C), 53.8 (+, C*H), 69.1 (–, 1 C, Bn), 129.5 (+, 2 C, arom.), 129.6 (+, 1 C, arom.), 129.7 (+, 2 C, arom.), 136.2 ($C_{quat,}$ 1 C, arom.), 170.4 ($C_{quat}$), 174.3 ($C_{quat}$); **MS** (ESI, MeCN): m/z (%) = 320.1 [$MH^+$] (100), 639.4 [(2 $MH^+$-$H^+$)$^+$] (7); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3334, 3069, 3028, 2967, 2141, 2118, 1732, 1681, 1642, 1533, 1450; **MF**: $C_{15}H_{22}N_5O_3Cl$; **MW** = 355.82 g/mol.

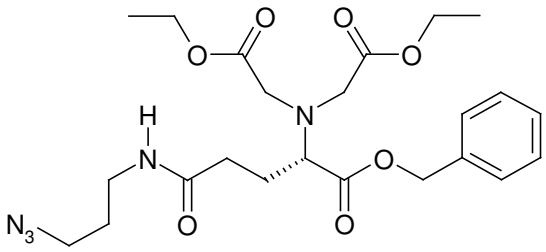


4-(2-Azido-ethylcarbamoyl)-2-(bis-ethoxycarbonylmethyl-amino)-butyric acid benzyl ester (**36**):

Synthesis followed **GP 6** using **34** (1136 mg, 3.32 mmol), bromo-ethylacetate (22.1 ml, 33.3 g, 0.2 mol), NaF (1256 mg, 29.9 mmol), $SiO_2$ (999 mg, 16.62 mmol) and potassium carbonate (459 mg, 3.32 mmol). This yielded 1458 mg of **36**

(3.05 mmol, 92 %) as colourless oil. Compound **36** was not stable at r.t. and decomposed within a few days, turning yellow.

**$^1$H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = 1.10 (t, $^3J$ = 7.2 Hz, 6 H, $CH_3$), 1.80 – 1.88 (m, 1 H, Glu-$CH_2$), 1.95 – 2.01 (m, 1 H, Glu-$CH_2$), 2.27 (ddd, $^3J$ = 6.3 Hz, $^2J$ = 14.1 Hz, 1 H, Glu-$CH_2$), 2.35 (ddd, $^3J$ = 6.0 Hz, $^3J$ = 8.8 Hz, $^2J$ = 14.1 Hz, 1 H, Glu-$CH_2$), 3.20 – 3.28 (m, 4 H, 2 $CH_2$), 3.39 (dd, $^3J$ = 11.2 Hz, $^3J$ = 4.2 Hz, 1 H, C*H), 3.45 (d, $^2J$ = 17.5 Hz, 2 H, N-$CH_2$), 3.48 (d, $^2J$ = 17.5 Hz, 2 H, $CH_2$), 3.98 (q, $^3J$ = 7.2 Hz, 4 H, $CH_2$), 4.96 (d, $^2J$ = 12.3 Hz, 1 H, $CH_2$), 4.99 (d, $^2J$ = 12.3 Hz, 1 H, $CH_2$), 6.97 – 6.99 (m, 1 H, NH), 7.15 – 7.22 (m, 5 H, arom. CH); **$^{13}$C-NMR** (150.9 MHz, $CDCl_3$): δ [ppm] = 14.0 (+, 2 C, $CH_3$), 25.8 (–, 1 C, Glu), 32.2 (–, 1 C, Glu), 38.7 (–, 1 C), 50.5 (–, 1 C, $N_3$-$CH_2$), 52.8 (–, 2 C, N-$CH_2$), 60.5 (–, 2 C, Et-ester-$CH_2$), 64.0 (+, 1 C, C*H), 66.3 (–, 1 C, Bn-$CH_2$), 128.1 (+, 2 C, arom.), 128.1 (+, 1 C, arom.), 128.4 (+, 2 C, arom.), 135.6 ($C_{quat}$, 1 C, arom.), 171.3 ($C_{quat}$, 2 C, Et-ester), 171.8 ($C_{quat}$, 1 C, Bn-ester), 173.0 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 478.1 [$MH^+$] (100); **MS-HR** (EI-MS): [$M^{+\cdot}$] calc.: 477.2223, found: 477.2223 (± 0.69 ppm); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3342, 3311, 3267, 3026, 2968, 2139, 1741, 1737, 1642, 1531, 1449; **MF**: $C_{22}H_{31}N_5O_7$; **MW** = 477.52 g/mol.

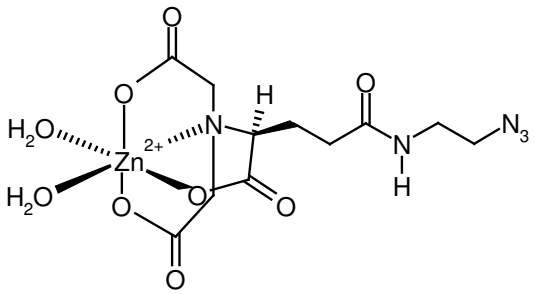


4-(3-Azido-propylcarbamoyl)-2-(bis-ethoxycarbonylmethyl-amino)-butyric acid benzyl ester (**37**):

Synthesis followed **GP 6** using **35** (631 mg, 1.77 mmol), bromo-ethylacetate (11.8 ml, 17.8 g, 0.1 mol), NaF (671 mg, 16.0 mmol), $SiO_2$ (533 mg, 8.87 mmol) and potassium carbonate (245 mg, 1.77 mmol, 1 eq). This yielded 800 mg of **37** (1.63 mmol, 92 %) as a colourless oil. Compound **37** was not stable at r.t. and decompose slowly within a few days turning yellow.

**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.16 (t, $^3J$ = 7.2 Hz, 6 H, $CH_3$), 1.68 (tt, $^3J$ = 6.9 Hz, 2 H, $CH_2$), 1.79 – 1.93 (m, 1 H, Glu-$CH_2$), 1.99 – 2.10 (m, 1 H, Glu-$CH_2$), 2.24

– 4.42 (m, 2 H, Glu-$CH_2$), 3.13 – 3.27 (m, 4 H, 2 $CH_2$), 3.40 (dd, $^3J$ = 11.1 Hz, $^3J$ = 4.5 Hz, 1 H, C*H), 3.47 (d, $^2J$ = 18.1 Hz, 2 H, N-$CH_2$), 3.53 (d, $^2J$ = 18.1 Hz, 2 H, $CH_2$), 4.05 (q, $^3J$ = 7.2 Hz, 4 H, $CH_2$), 5.00 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 5.05 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 6.75 (t, $^3J$ = 5.9 Hz, 1 H, NH), 7.22 – 7.28 (m, 5 H, arom. CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, $CH_3$), 25.9 (–, 1 C, Glu), 28.8 (–, 1 C), 32.4 (–, 1 C, Glu), 36.8 (–, 1 C), 49.1 (–, 1 C, $N_3$-$CH_2$), 52.9 (–, 2 C, N-$CH_2$), 60.7 (–, 2 C, Et-ester-$CH_2$), 64.1 (+, 1 C, C*H), 66.4 (–, 1 C, Bn-$CH_2$), 128.2 (+, 2 C, arom.), 128.3 (+, 1 C, arom.), 128.5 (+, 2 C, arom.), 135.6 ($C_{quat}$, 1 C, arom.), 171.5 ($C_{quat}$, 2 C, Et-ester), 172.0 ($C_{quat}$, 1 C, Bn-ester), 172.9 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 492.4 [$MH^+$] (100), 983.8 [$(2M+H^+)^+$] (5); **EA** ($C_{23}H_{33}N_5O_7$) calc.: C 56.20, H 6.77, N 14.25, found: C 55.86, H 6.61, N 14.07; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3344, 3310, 3268, 3025, 2968, 2139, 1742, 1737, 1642, 1532, 1449; **MF**: $C_{23}H_{33}N_5O_7$; **MW** = 491.54 g/mol;



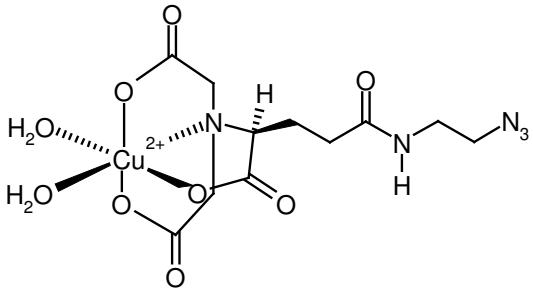

Li[Zn(4-(2-Azido-ethylcarbamoyl)-2-(bis-carboxylate-methyl-amino)-butanoate)] di-hydrate (**40**):

*This procedure can be used as a general procedure for the synthesis of NTA-complexes*:

The ester-protected compound **36** (1357 mg, 2.84 mmol) was dissolved in 50 ml of a 3:2 mixture of THF/water. After addition of LiOH (204 mg, 8.53 mmol, 3 eq) the solution was stirred at r.t. for 24 h. The THF was evaporated and the water was removed by lyophilisation. This yielded 1000 mg of the carboxylate **38** as a colourless solid (2.84 mmol, 100 %, MF: $C_{11}H_{14}N_5O_7Li_3$; MW = 349.08 g/mol). NMR- and MS-spectroscopy confirmed the complete cleavage of all ester-groups. The lithium-salt was dissolved in 25 ml water and $ZnCl_2$ (387 mg, 2.84 mmol, 1 eq) and $5ZnO{\cdot}2CO_3$ x 4 $H_2O$ (1701 mg, 2.84 mmol, 5 eq) were added. The solution was stirred at r.t. for 16 h and then heated to 60 °C for 3 h. All inorganic compounds were removed by

filtration and the aqueous solution was dried by lyophilisation. The white crude product was purified by recrystallisation from EtOH/water. Diethyl ether was added in order to complete precipitation. This yielded 1118 mg of **40** as colourless hygroscopic solid (2.56 mmol, 90 %).

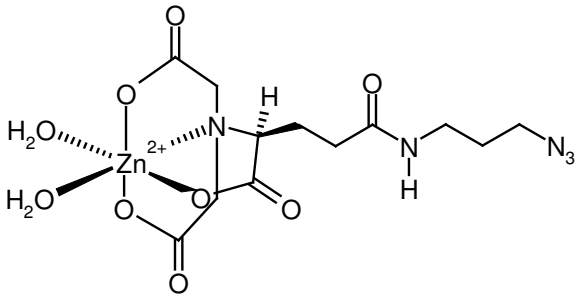
**MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 392.0 [$A^-$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3448, 3250, 2940, 2870, 2143, 1641, 1413; **MF**: $C_{11}H_{14}N_5O_7ZnLi$ x 2 $H_2O$; **MW** = 436.61 g/mol;



Li[Cu(4-(2-Azido-ethylcarbamoyl)-2-(bis-carboxylate-methyl-amino)-butanoate)] di-hydrate (**41**):

Procedure was the same as for the synthesis of **40** using **36** (1357 mg, 2.84 mmol), LiOH (204 mg, 8.53 mmol, 3 eq) and $Cu_2(OH)_2CO_3$ (628 mg, 2.84 mmol, 2 eq). The blue crude product was purified by recrystallisation from EtOH/water. Diethylether was added in order to complete precipitation. This yielded 1113 mg of **41** as blue hygroscopic solid (2.56 mmol, 90 %).

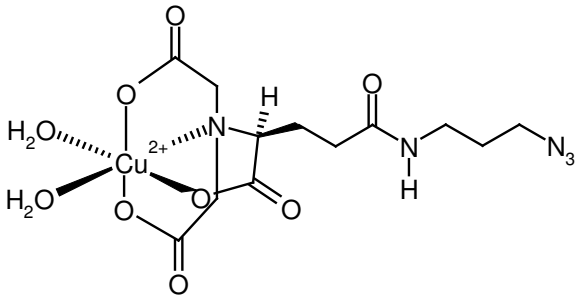
**MS** (neg. ESI, $H_2O$/MeOH): m/z (%) = 390.9 [$A^-$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3448, 3252, 2939, 2869, 2142, 1641, 1413; **MF**: $C_{11}H_{14}N_5O_7CuLi$ x 2 $H_2O$; **MW** = 434.78 g/mol;



Li[Zn(4-(3-Azido-propylcarbamoyl)-2-(bis-carboxylate-methyl-amino)-butanoate)] di-hydrate (**42**):

Procedure was the same as for the synthesis of **40** using **37** (1396 mg, 2.84 mmol), LiOH (204 mg, 8.53 mmol, 3 eq), $ZnCl_2$ (387 mg, 2.84 mmol, 1 eq) and $5ZnO{\cdot}2CO_3$ x 4 $H_2O$ (1701 mg, 2.84 mmol, 5 eq). This yielded 1031 mg of the carboxylate **39** as a colourless solid (2.84 mmol, 100 %, MF: $C_{12}H_{16}N_5O_7Li_3$; MW = 363.10 g/mol) and then 1153 mg of **42** as colourless hygroscopic solid (2.56 mmol, 90 %).

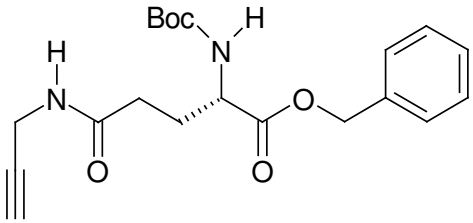
**MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 406.0 [$A^-$] (100); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3448, 3250, 2940, 2870, 2143, 1641, 1413; **MF**: $C_{12}H_{16}N_5O_7ZnLi$ x 2 $H_2O$; **MW** = 450.63 g/mol;



Li[Cu(4-(3-Azido-propylcarbamoyl)-2-(bis-carboxylate-methyl-amino)-butanoate)] di-hydrate (**43**):

Procedure was the same as for the synthesis of **40** using **37** (1396 mg, 2.84 mmol), LiOH (204 mg, 8.53 mmol, 3 eq), and $Cu_2(OH)_2CO_3$ (628 mg, 2.84 mmol, 2 eq). This yielded 1031 mg of the carboxylate **39** as a colourless solid (2.84 mmol, 100 %, MF: $C_{12}H_{16}N_5O_7Li_3$; MW = 363.10 g/mol) and then 1149 mg of **43** as blue hygroscopic solid (2.56 mmol, 90 %).

**MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 405.0 [$A^-$] (100); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3448, 3252, 2939, 2869, 2142, 1641, 1413; **MF**: $C_{12}H_{16}N_5O_7CuLi$ x 2 $H_2O$; **MW** = 448.80 g/mol;

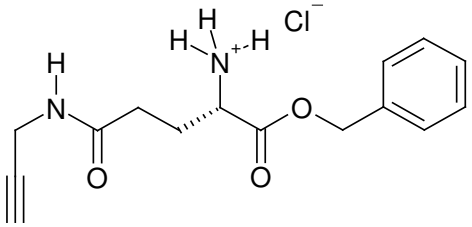



2-tert-Butoxycarbonylamino-4-prop-2-ynylcarbamoyl-butyric acid benzyl ester (**44**):

The protected glutamic acid **31** (1000 mg, 2.96 mmol) was dissolved in 2 ml of dry DMF and cooled in an ice-bath. Following, EDC (634 µl, 551 mg, 3.58 mmol, 1.21 eq), HOBt (484 mg, 3.58 mmol, 1.21 eq), DIEA (327 µl, 463 mg, 3.58 mmol, 1.21 eq) and propargylamine (209 µl, 179 mg, 3.26 mmol, 1.1 eq) were added. The ice bath was removed and the solution was stirred in the dark at r.t. for 24 h. The solution was cooled to 0 °C and water at 0 °C was added in order to precipitate the product. After filtration, the white solid was dissolved in 50 ml DCM. The solution was washed with 30 ml water, 30 ml 1 M aqueous citric acid, 30 ml water and 30 ml brine. The organic layer was dried with $Na_2SO_4$ and the solvent was removed in vacuum. This yielded 1086 mg of **44** (2.90 mmol, 98 %) as a white crystalline solid in sufficient purity for the following reaction steps. For analytical data the crude product was purified by column chromatography with EE:PE in a ratio of 2:3. This gave pure **44** in a yield of 1066 mg (2.85 mmol, 96 %). $R_f$ (EE:PE = 1:1) = 0.37.

**Mp**: 122 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9 H, Boc-$CH_3$), 1.91 – 2.03 (m, 1 H, $CH_2$), 2.11 – 2.19 (m, 1 H, $CH_2$), 2.22 (t, $^4J$ = 2.5 Hz, 1 H, CH), 2.25 – 2.30 (m, 2 H, $CH_2$), 3.98 (dd, $^4J$ = 2.5 Hz, $^3J$ = 5.2 Hz, 2 H, propargyl-$CH_2$), 4.27 – 4.34 (m, 1 H, C*H), 5.12 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 5.18 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 5.63 (d, $^3J$ = 8.2 Hz, 1 H, NH), 6.85 (t, $^3J$ = 5.2 Hz, 1 H, NH), 7.31 – 7.36 (m, 5 H, arom. CH); **$^{13}$C-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 28.3 (+, 3 C, Boc-$CH_3$), 29.0 (–, 1 C), 29.2 (–, 1 C), 32.3 (–, 1 C), 53.0 (+, C*H), 67.3 (–, 1 C, Bn), 71.5 (+, alkyne-CH), 79.5 ($C_{quat}$, alkyne), 80.3 ($C_{quat,}$ Boc), 128.4 (+, 2 C, arom.), 128.6 (+, 1 C, arom.), 128.7 (+, 2 C, arom.), 135.2 ($C_{quat,}$ 1 C, arom.), 155.8 ($C_{quat,}$ urethane), 171.5 ($C_{quat}$), 172.1 ($C_{quat}$); **MS** (CI-MS, $NH_3$): m/z (%) = 275.2 [$(M+H^+-C_4H_8+CO_2)^+$]

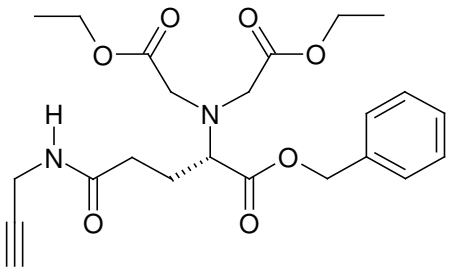
(100), 319.2 [$(M+H^+-C_4H_8)^+$] (65), 336.1 [$(M+NH_4^+-C_4H_8)^+$] (20), 375.2 [$MH^+$] (40); **EA** ($C_{20}H_{26}N_2O_5$) calc.: C 64.16, H 7.00, N 7.48, found: C 63.85, H 7.03, N 7.40; **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3344, 3313, 3266, 2970, 1736, 1690, 1645, 1535, 1451, 1283, 1165, 727; **MF**: $C_{20}H_{26}N_2O_5$; **MW** = 374.44 g/mol;



1-Benzyloxycarbonyl-3-prop-2-ynylcarbamoyl-propyl-ammonium chloride (**45**):

The amide **44** (1000 mg, 2.67 mmol) was dissolved in little dry EE and 18 ml of a saturated solution of HCl in dry diethyl ether were added (1 ml per 0.15 mmol Boc). The mixture is stirred at r.t. under an atmosphere of $N_2$ for 15 h. After 30 min the product began to separate as a colourless oil. The solvent was removed in vacuum. This gave **45** as a colourless hygroscopic solid in quantitative yield (830 mg, 2.67 mmol).

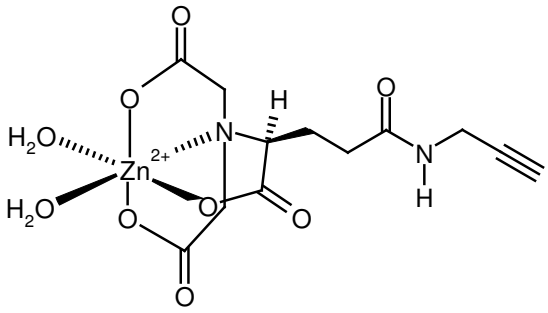
**$^1$H-NMR** (300 MHz, $CD_3CN$): $\delta$ [ppm] = 2.27 – 2.34 (m, 2 H, $CH_2$), 2.47 (t, $^4J$ = 2.5 Hz, 1 H, alkyne), 2.47 – 2.56 (m, 2 H, $CH_2$), 3.86 – 3.88 (m, $^4J$ = 2.5 Hz, 2 H, propargyl-$CH_2$), 4.26 – 4.31 (m, 1 H, C*H), 5.19 (d, $^2J$ = 12.5 Hz, 1 H, Bn-$CH_2$), 5.23 (d, $^2J$ = 12.5 Hz, 1 H, Bn-$CH_2$), 7.31 – 7.42 (m, 5 H, arom. CH), 7.87 (t, $^3J$ = 5.5 Hz, 1 H, NH), 8.82 (bs, 3 H, $NH_3^+$); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): $\delta$ [ppm] = 26.8 (–, 1 C), 29.5 (–, 1 C), 32.2 (–, 1 C), 53.7 (+, C*H), 69.1 (–, 1 C, Bn), 72.5 (+, alkyne), 81.3 ($C_{quat}$, alkyne), 129.5 (+, 2 C, arom.), 129.6 (+, 1 C, arom.), 129.7 (+, 2 C, arom.), 136.2 ($C_{quat,}$ 1 C, arom.), 170.3 ($C_{quat}$), 173.2 ($C_{quat}$); **MS** (ESI, $H_2O$): m/z (%) = 275.0 [$MH^+$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3324, 3069, 3028, 2970, 2121, 1731, 1681, 1643, 1533, 1449; **MF**: $C_{15}H_{19}N_2O_3Cl$; **MW** = 310.78 g/mol;



2-(Bis-ethoxycarbonylmethyl-amino)-4-prop-2-ynylcarbamoyl-butyric acid benzyl ester (**46**):

Synthesis followed **GP 6** using **45** (1602 mg, 5.15 mmol), bromo-ethylacetate (34.2 ml, 51.7 g, 0.31 mol), NaF (1949 mg, 46.4 mmol), $SiO_2$ (1549 mg, 25.8 mmol) and potassium carbonate (712 mg, 5.15 mmol). This yielded 2116 mg of **46** (4.74 mmol, 92 %) as colourless oil.

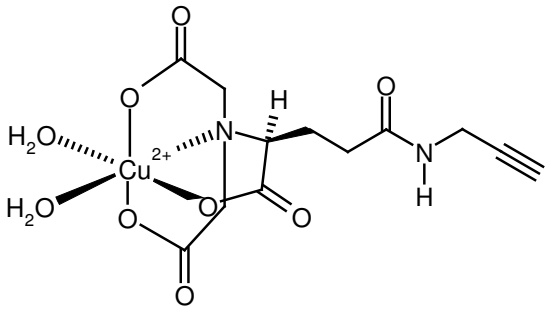
**$^1$H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = 1.17 (t, $^3J$ = 7.2 Hz, 6 H, $CH_3$), 1.89 (dddd, $^2J$ = 14.9 Hz , $^3J$ = 5.5 Hz, $^3J$ = 5.8 Hz, $^3J$ = 11.6 Hz, 1 H, $CH_2$), 2.06 (dddd, $^2J$ = 14.9 Hz, $^3J$ = 4.5 Hz, $^3J$ = 5.7 Hz, $^3J$ = 10.1 Hz, 1 H, $CH_2$), 2.09 (dd, $^4J$ = 2.6 Hz, $^4J$ = 2.6 Hz, 1 H, CH), 2.30 (ddd, $^3J$ = 5.5 Hz, $^3J$ = 5.8 Hz, $^2J$ = 13.9 Hz, 1 H, $CH_2$), 2.41 (ddd, $^3J$ = 5.8 Hz, $^3J$ = 10.1 Hz, $^2J$ = 13.9 Hz, 1 H, $CH_2$), 3.40 (dd, $^3J$ = 11.6 Hz, $^3J$ = 4.5 Hz, 1 H, C*H), 3.52 (s, 4 H, $CH_2$), 3.83 (ddd, $^2J$ = 17.5 Hz, $^3J$ = 5.0 Hz, $^4J$ = 2.6 Hz, 1 H, $CH_2$), 4.00 (ddd, $^2J$ = 17.5 Hz, $^3J$ = 5.9 Hz, $^4J$ = 2.6 Hz, 1 H, $CH_2$), 4.05 (q, $^3J$ = 7.2 Hz, 4 H, $CH_2$), 5.01 (d, $^2J$ = 12.3 Hz, 1 H, $CH_2$), 5.05 (d, $^2J$ = 12.3 Hz, 1 H, $CH_2$), 6.88 (dd, $^3J$ = 5.0 Hz, $^3J$ = 6.0 Hz, 1 H, NH), 7.22 – 7.30 (m, 5 H, arom. CH); **$^{13}$C-NMR** (150.9 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, $CH_3$), 25.8 (–, 1 C, Glu), 28.8 (–, 1 C), 32.3 (–, 1 C, Glu), 53.0 (–, 2 C, N-$CH_2$), 60.8 (–, 2 C, Et-ester-$CH_2$), 64.1 (+, 1 C, C*H), 66.5 (–, 1 C, Bn-$CH_2$), 70.8 (+, 1 C, alkyne), 80.2 ($C_{quat}$, 1 C, alkyne), 128.2 (+, 2 C, arom.), 128.3 (+, 1 C, arom.), 128.6 (+, 2 C, arom.), 135.4 ($C_{quat}$, 1 C, arom.), 171.5 ($C_{quat}$, 2 C, Et-ester), 171.9 ($C_{quat}$, 1 C, Bn-ester), 172.5 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 447.1 [$MH^+$] (100); **MS-HR** (EI-MS): [$M^{+\cdot}$] calc.: 446.2053, found: 446.2053 (± 0.43 ppm); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3344, 3313, 3266, 3030, 2970, 2119, 1742, 1736, 1643, 1533, 1450; **MF**: $C_{23}H_{30}N_2O_7$; **MW** = 446.50 g/mol;



Li[Zn(2-(Bis-carboxylate-methyl-amino)-4-prop-2-ynylcarbamoyl-butanoate)] di-hydrate (**48**):

Procedure was the same as for the synthesis of **40** using **46** (1000 mg, 2.24 mmol), LiOH (161 mg, 6.72 mmol, 3 eq), $ZnCl_2$ (305 mg, 2.24 mmol, 1 eq) and $5ZnO{\cdot}2CO_3$ x 4 $H_2O$ (1341 mg, 2.24 mmol, 5 eq). This yielded 712 mg of the carboxylate **47** as a colourless solid (2.24 mmol, 100 %, MF: $C_{12}H_{13}N_2O_7Li_3$; MW = 318.07 g/mol) and then 819 mg of **48** as colourless hygroscopic solid (2.02 mmol, 90 %).

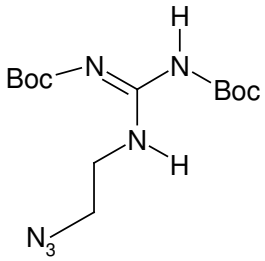
**MS** (neg. ESI, $H_2O$/MeOH + $NH_4Ac$): m/z (%) = 361.0 [$A^-$] (100), 427.0 [($A^-$+LiOAc)$^-$] (25); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3448, 3250, 2940, 2870, 2112, 1641, 1413; **MF**: $C_{12}H_{13}N_2O_7ZnLi$ x 2 $H_2O$; **MW** = 405.60 g/mol;



Li[Cu(2-(Bis-carboxylate-methyl-amino)-4-prop-2-ynylcarbamoyl-butanoate)] di-hydrate (**49**):

Procedure was the same as for the synthesis of **40** using **46** (1000 mg, 2.24 mmol), LiOH (161 mg, 6.72 mmol, 3 eq) and $Cu_2(OH)_2CO_3$ (495 mg, 2.24 mmol, 2 eq). This yielded 712 mg of the carboxylate **47** as a colourless solid (2.24 mmol, 100 %, MF: $C_{12}H_{13}N_2O_7Li_3$; MW = 318.07 g/mol) and then 815 mg of **49** as greenish brown hygroscopic solid (2.02 mmol, 90 %).

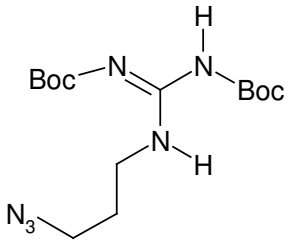
**MS** (neg. ESI, $H_2O$/MeOH + $NH_4Ac$): m/z (%) = 360.0 [$A^-$] (75), 426.0 [($A^-$+LiOAc)$^-$] (100), 492.2 [($A^-$+2LiOAc)$^-$] (55); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3446, 3251, 2937, 2867, 2111, 1641, 1413; **MF**: $C_{12}H_{13}N_2O_7CuLi$ x 2 $H_2O$; **MW** = 403.76 g/mol;




*N*,*N*'-Di-Boc-*N*-(2-azido-ethyl)-guanidine (**50**):

The azide **14** (775 mg, 6.32 mmol, 2.5 eq) and $NEt_3$ (1052 µl, 768 mg, 7.59 mmol, 3 eq) were suspended in 10 ml of dry THF. After addition of N,N'-Di-Boc-S-methyl-isothiourea (735 mg, 2.53 mmol) the suspension was stirred at 40 °C under an atmosphere of $N_2$ for 7 h. TLC control showed incomplete conversion and formation of a side product. The solvent and the formed methyl thiole were removed in vacuum. The remaining solid was treated with EE in order to dissolve **50**. After filtration the solvent was evaporated and the crude product was purified by CC. PE:EE in a ratio of 9:1 ($R_f$ = 0.25). This yielded 689 mg (2.10 mmol, 83 %) of **50** as colourless crystalline solid.

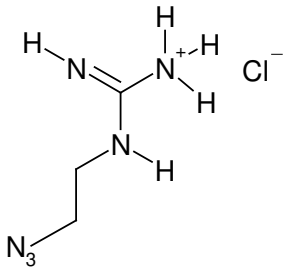
**Mp**: 63 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.49 (s, 18 H, Boc-$CH_3$), 3.50 (t, $^3$J = 5.8 Hz, 2 H, $CH_2$), 3.60 (dt, $^3$J = 6.0 Hz, $^3$J = 5.8 Hz, 2 H, $CH_2$), 8.56 – 8.60 (m, 1 H, NH), 11.47 (s, 1 H, NH) **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C, Boc-$CH_3$), 28.3 (+, 3 C, Boc-$CH_3$), 40.0 (–, 1 C), 50.5 (–, 1 C), 79.5 ($C_{quat}$, 1 C, Boc), 83.4 ($C_{quat}$, 1 C, Boc), 153.2 ($C_{quat}$, 1 C, urethane), 156.3 ($C_{quat}$, 1 C, urethane), 163.4 ($C_{quat}$, 1 C); **MS** (ESI, DCM/MeOH + $NH_4Ac$): m/z (%) = 329.2 [$MH^+$] (100), 273.0 [($MH^+$ - $C_4H_8$)$^+$] (40), 216.9 [($MH^+$ - 2 $C_4H_8$)$^+$]; **EA** ($C_{13}H_{24}N_6O_4$) calc.: C 47.55, H 7.37, N 25.59, found: C 63.85, H 7.03, N 7.40; **UV/Vis** ($CHCl_3$): λ (lg ε) = 242 nm (4.258); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3437, 3326, 3136, 3006, 2977, 2932, 2096, 1737, 1650, 1624, 1573, 1415, 1368, 1143, 1054; **MF**: $C_{13}H_{24}N_6O_4$; **MW** = 328.37 g/mol;



*N*,*N*'-Di-Boc-*N*-(3-azido-propyl)-guanidine (**51**):

The azide **15** (775 mg, 5.67 mmol, 2.5 eq) and $NEt_3$ (944 µl, 689 mg, 6.81 mmol, 3 eq) were suspended in 10 ml of dry THF. After addition of N,N'-Di-Boc-S-methyl-isothiourea (659 mg, 2.27 mmol) the suspension was stirred at 40 °C under an atmosphere of $N_2$ for 7 h. TLC control showed incomplete conversion and formation of a side product. The solvent and the formed methyl thiole were removed in vacuum. The remaining solid was treated with EE in order to dissolve **51**. After filtration the solvent was evaporated and the crude product was purified by CC. PE:EE in a ratio of 9:1 ($R_f$ = 0.25). This yielded 630 mg (1.84 mmol, 81 %) of **51** as colourless crystalline solid.

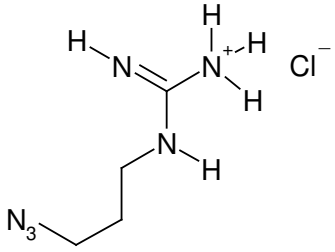
**Mp**: 65 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 18 H, Boc-$CH_3$), 1.79 (tt, $^3$J = 6.7 Hz, 2 H, $CH_2$), 3.32 (t, $^3$J = 6.7 Hz, 2 H, $CH_2$), 3.45 (dt, $^3$J = 6.7 Hz, $^3$J = 5.8 Hz, 2 H, $CH_2$), 8.36 (t, $^3$J = 5.8 Hz, 1 H, NH), 11.44 (s, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C, Boc-$CH_3$), 28.2 (+, 3 C, Boc-$CH_3$), 28.3 (–, 1 C), 38.2 (–, 1 C), 49.0 (–, 1 C), 79.2 ($C_{quat}$, 1 C, Boc), 83.1 ($C_{quat}$, 1 C, Boc), 153.2 ($C_{quat}$, 1 C, urethane), 156.2 ($C_{quat}$, 1 C, urethane), 163.5 ($C_{quat}$, 1 C); **MS** (ESI, DCM/MeOH + $NH_4Ac$): m/z (%) = 343.1 [$MH^+$] (100); **EA** ($C_{14}H_{26}N_6O_4$) calc.: C 49.11, H 7.65, N 24.54, found: C 63.85, H 7.03, N 7.40; **UV/Vis** ($CHCl_3$): λ (lg ε) = 242 nm (4.273); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3437, 3326, 3136, 3006, 2977, 2932, 2096, 1737, 1650, 1624, 1573, 1415, 1368, 1143, 1054; **MF**: $C_{14}H_{26}N_6O_4$; **MW** = 342.40 g/mol;



*N*-(2-azido-ethyl)-guanidinium chloride (**52**):

The Boc-protected compound **50** (356 mg, 1.08 mmol) was dissolved in 10 ml of dry DCM and 1.6 ml of TFA (2.46 g, 21.6 mmol, 20 eq) were added. The solution was stirred at r.t. under an atmosphere of $N_2$ for 16 h. The solvent was removed in vacuum. This gave a pale yellow oil. In order to exchange the anion and to remove remaining excess TFA the oil is dissolved in 4 ml of 36 % aqueous HCl and 2 ml MeOH. The solution was stirred at r.t. for 1 h. The solvents were removed in vacuum. This yielded 178 mg (1.08 mmol, 100 %) of **52** as colourless oil.

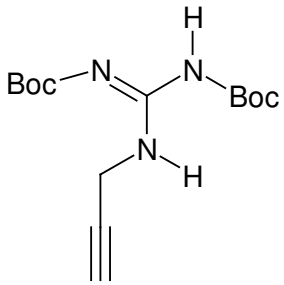
**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 3.35 (t, $^3J$ = 5.8 Hz, 2 H, $CH_2$), 3.48 (t, $^3J$ = 5.8 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 40.8 (–, 1 C), 50.0 (–, 1 C), 157.2 ($C_{quat}$, 1 C); **MS** (ESI, $H_2O$/MeCN/TFA): m/z (%) = 129.2 [$MH^+$] (100); **MF**: $C_3H_9N_6Cl$; **MW** = 164.60 g/mol;



*N*-(3-azido-propyl)-guanidinium chloride (**53**):

The Boc-protected compound **51** (630 mg, 1.84 mmol) was dissolved in 10 ml of dry DCM and 2.84 ml of TFA (4.21 g, 36.9 mmol, 20 eq) were added. The solution was stirred at r.t. under an atmosphere of $N_2$ for 16 h. The solvent was removed in vacuum. This gave a pale yellow oil. In order to exchange the anion and to remove remaining excess TFA the oil was dissolved in 4 ml of 36 % aqueous HCl and 2 ml MeOH. The solution was stirred at r.t. for 1 h. The solvents were removed in vacuum. This yielded 329 mg (1.84 mmol, 100 %) of **53** as colourless oil.

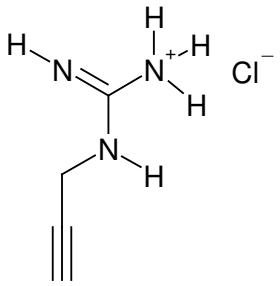
**$^1$H-NMR** (300 MHz, $CD_3OD$): δ [ppm] = 1.84 (tt, $^3J$ = 7.0 Hz, $^3J$ = 6.6 Hz, 2 H, $CH_2$), 3.30 (t, $^3J$ = 7.0 Hz, 2 H, $CH_2$), 3.45 (t, $^3J$ = 6.6 Hz, 2 H, $CH_2$); **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.81 (psq, $^3J$ = 6.6 Hz, 2 H, $CH_2$), 3.23 (t, $^3J$ = 6.7 Hz, 2 H, $CH_2$), 3.39 (t, $^3J$ = 6.6 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (75.5 MHz, $CD_3OD$): δ [ppm] = 29.3 (–, 1 C), 39.9 (–, 1 C), 49.8 (–, 1 C), 158.8 ($C_{quat}$, 1 C); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 27.3 (–, 1 C), 38.7 (–, 1 C), 48.5 (–, 1 C), 157.0 ($C_{quat}$, 1 C); **MS** (ESI, $H_2O$/MeCN/TFA): m/z (%) = 143.2 [$MH^+$] (100); **MF**: $C_4H_{11}N_6Cl$; **MW** = 178.62 g/mol;




*N,N'*-Di-Boc-*N*-(prop-2-ynyl)-guanidine (**54**):

In a round bottom flask N,N'-Di-Boc-S-methyl-isothiourea (738 mg, 2.54 mmol) was dissolved in 10 ml THF and propargyl amine (436 µl, 350 mg, 6.35 mmol, 2.5 eq) was added. The solution was stirred at 40 °C under an atmosphere of $N_2$ for 7 h. The solvent and surplus amine were removed in vacuum and the remaining solid was purified by CC with PE:EE in a ratio of 9:1 ($R_f$ = 0.25). This yielded 640 mg (2.14 mmol, 84 %) of **54** as colourless crystalline solid.

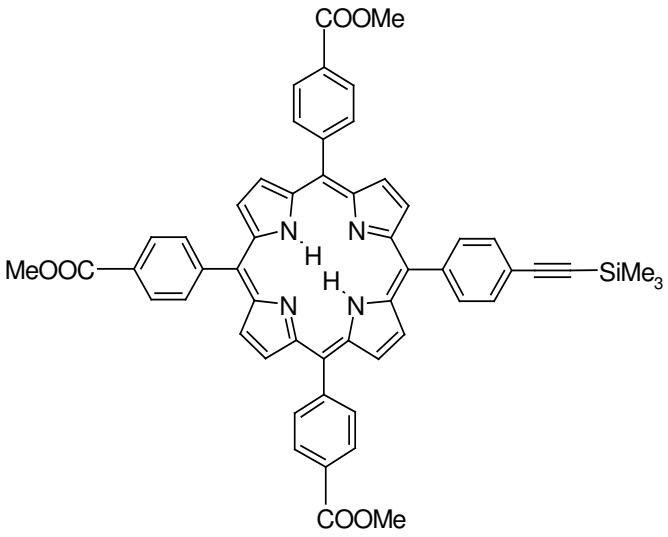
**Mp**: 69 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.39 (s, 9 H, Boc-$CH_3$), 1.40 (s, 18 H, Boc-$CH_3$), 2.22 (t, $^4J$ = 2.6 Hz, 1 H, CH), 4.13 (dd, $^3J$ = 4.9 Hz, $^4J$ = 2.7 Hz, 2 H, $CH_2$), 8.36 (t, $^3J$ = 4.9 Hz, 1 H, NH), 11.37 (s, 1 H, NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C, Boc-$CH_3$), 28.2 (+, 3 C, Boc-$CH_3$), 30.6 (–, 1 C), 72.3 (+, 1 C, CH), 78.8 ($C_{quat}$, 1 C, alkyne), 79.4 ($C_{quat}$, 1 C, Boc), 83.2 ($C_{quat}$, 1 C, Boc), 152.9 ($C_{quat}$, 1 C, urethane), 155.5 ($C_{quat}$, 1 C, urethane), 163.2 ($C_{quat}$, 1 C); **MS** (CI): m/z (%) = 298.3 [$MH^+$] (100), 242.2 [$(M + H^+ - C_4H_8)^+$] (10); **EA** ($C_{14}H_{23}N_3O_4$) calc.: C 56.55, H 7.80, N 14.13, found: C 56.28, H 7.58, N 13.97; **UV/Vis** ($CHCl_3$): λ (lg ε) = 242 nm (4.265); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3434, 3324, 3135, 3003, 2978, 2935, 2110, 1737, 1650, 1623, 1573, 1414, 1367, 1143, 1052; **MF**: $C_{14}H_{23}N_3O_4$; **MW** = 297.35 g/mol;



*N*-(prop-2-ynyl)-guanidinium chloride (**55**):

The Boc-protected compound **54** (467 mg, 1.57 mmol) was dissolved in 10 ml of dry DCM and 2.42 ml of TFA (3.58 g, 31.4 mmol, 20 eq) were added. The solution was stirred at r.t. under an atmosphere of $N_2$ for 16 h. The solvent was removed in vacuum. This gave a pale yellow oil. In order to exchange the anion and to remove remaining excess TFA the oil is dissolved in 4 ml of 36 % aqueous HCl and 2 ml MeOH. The solution was stirred at r.t. for 1 h. The solvents were removed in vacuum. This yielded 210 mg (1.57 mmol, 100 %) of **55** as colourless oil.

**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 2.72 (t, $^4J$ = 2.5 Hz, 1 H, CH), 4.01 (d, $^4J$ = 2.5 Hz, 2 H, $CH_2$); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 30.8 (–, 1 C), 73.8 (+, 1 C), 77.9 ($C_{quat}$, 1 C, alkyne), 156.9 ($C_{quat}$, 1 C); **MS** (CI-MS, $NH_3$): m/z (%) = 98.2 [$MH^+$] (100); **MF**: $C_4H_8N_3Cl$; **MW** = 133.58 g/mol;



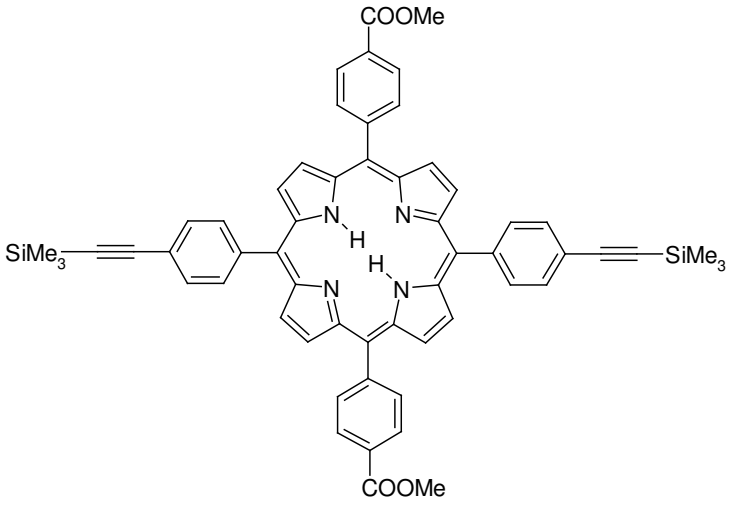

Formic acid 4-[15,20-bis-(4-formyloxymethyl-phenyl)-10-(4-trimethylsilanylethynyl-phenyl)-porphyrin-5-yl]-benzyl ester (**56**):

Synthesis followed **GP 7** using pyrrole (280 µl, 268 mg, 4.00 mmol, 8 eq), 4-formyl-bezoicacid-methylester (575 mg, 3.50 mmol, 7 eq), 4-((trimethylsilyl)-ethinyl)-benzaldehyde (101 mg, 0.50 mmol), 37 µl of $BF_3 \cdot OEt_2$ and p-chloranil (738 mg, 3.00 mmol, 6 eq). Identification of the product in the crude mixture was possible using TLC which showed 6 red fluorescent spots (ex: 336 nm): spot 1 = 4 TMS groups, spot 2 = 3 TMS groups, spots 3 and 4 = 2 TMS groups (isomers), *spot 5 = product*, spot 6 = 4 methoxy groups. Purification by CC had to be done twice. First with PE/DCM/EE in a ratio of 80:20:5 to 80:20:12 and then in a ratio of 75:25:5 to 75:25:10. This gave porphyrin **56** as purple crystals in a yield of 93 mg (0.11 mmol, 26 %). $R_f$ (PE:DCM:EE = 8:2:1) = 0.28.

**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = - 2.83 (s, 2 H, NH), 0.38 (s, 9 H, TMS-$CH_3$), 4.12 (s, 9 H, 3 $CH_3$), 7.88 (d, $^3$J = 8.1 Hz, 2 H, arom. CH), 8.15 (d, $^3$J = 8.1 Hz, 2 H, arom. CH), 8.29 (d, $^3$J = 8.1 Hz, 6 H, arom. CH), 8.45 (d, $^3$J = 8.1 Hz, 6 H, arom. CH), 8.80 – 8.85 (m, 8 H, porphyrin-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 0.0 (+, 3 C, TMS-$CH_3$), 52.4 (+, 3 C, $CH_3$), 95.7 ($C_{quat}$, 1 C, alkyne), 104.8 ($C_{quat}$, 1 C, alkyne), 119.1 ($C_{quat}$, 1 C, porphyrin), 119.2 ($C_{quat}$, 2 C, porphyrin), 119.9 ($C_{quat}$, 1 C, porphyrin), 122.8 ($C_{quat}$, 1 C), 127.9 (+, 6 C), 129.7 ($C_{quat}$, 3 C), 130.4 (+, 2 C), 134.3 (+, 2 C), 134.5 (+, 6 C), 142.0 ($C_{quat}$, 1 C), 146.6 ($C_{quat}$, 3 C), 167.3 ($C_{quat}$, 3 C, ester); **MS** (PI-EIMS, 70 eV): m/z (%) = 884.0 [$M^{+\cdot}$] (100); **UV/Vis** ($CH_3CN$): λ (lg ε) = 646 nm (3.599), 591 nm (3.764), 551 nm (3.959), 516 nm (4.272), 420 nm (5.680), 298 nm (4.271), 260 nm (4.487); **FI** ($CH_3CN$, $\lambda_{ex}$=

420 nm): $\lambda_{em}$ (a.u.) = 655 nm (90), 720 nm (40); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3433, 3319, 3118, 3036, 2998, 2946, 2708, 2113, 1934, 1812, 1723, 1608, 1436, 965, 802, 760; **MF**: $C_{55}H_{44}N_4O_6Si$; **MW** = 885.07 g/mol;

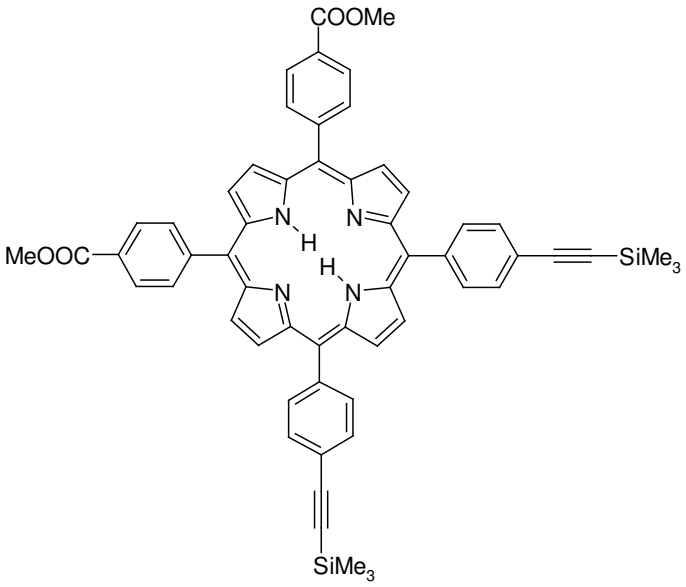
**Note:** due to broadening of signals of pyrrole-CH and $-C_{quat}$ in the $^{13}C$-NMR spectrum, these signals are not given for all porphyrin-compounds!




5,15-bis-(4-trimethylsilanylethynyl-phenyl)-10,20-bis-(4-formyloxymethyl-phenyl)-porphyrin (**57**):

Porphyrin **57** was a side-product of the synthesis of **56** and was obtained after CC as purple crystals in a yield of 24 mg (0.03 mmol, 5 %).

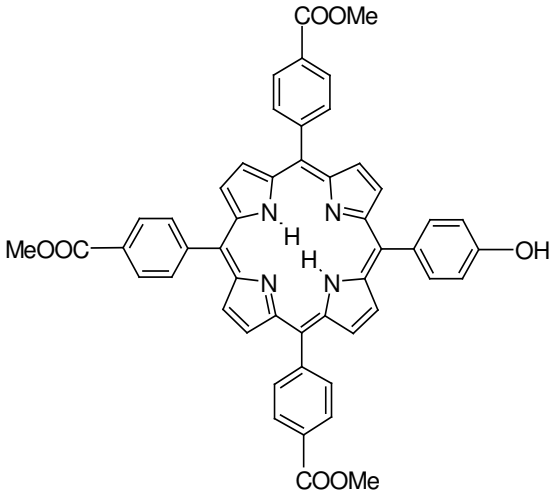
**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (300 MHz, $CDCl_3$): $\delta$ [ppm] = - 2.84 (s, 2 H, NH), 0.38 (s, 18 H, TMS-$CH_3$), 4.12 (s, 6 H, 3 $CH_3$), 7.87 (d, $^3J$ = 8.3 Hz, 4 H, arom. CH), 8.15 (d, $^3J$ = 8.3 Hz, 4 H, arom. CH), 8.29 (d, $^3J$ = 8.3 Hz, 4 H, arom. CH), 8.45 (d, $^3J$ = 8.3 Hz, 4 H, arom. CH), 8.80 (d, $^3J$ = 4.8 Hz, 4 H, porphyrin-CH), 8.85 (d, $^3J$ = 4.8 Hz, 4 H, porphyrin-CH); **$^{13}$C-NMR** (150.9 MHz, $CDCl_3$): $\delta$ [ppm] = 0.1 (+, 6 C, TMS-$CH_3$), 52.4 (+, 2 C, $CH_3$), 95.8 ($C_{quat}$, 2 C, alkyne), 104.9 ($C_{quat}$, 2 C, alkyne), 119.2 ($C_{quat}$, 2 C, porphyrin), 119.8 ($C_{quat}$, 2 C, porphyrin), 122.8 ($C_{quat}$, 2 C), 128.0 (+, 4 C), 129.7 ($C_{quat}$, 2 C), 130.4 (+, 4 C), 134.4 (+, 4 C), 134.5 (+, 4 C), 142.1 ($C_{quat}$, 2 C), 146.8 ($C_{quat}$, 2 C), 167.3 ($C_{quat}$, 2 C, ester); **MS** (PI-EIMS, 70 eV): m/z (%) = 922.0 [$M^{+\cdot}$] (100); **UV/Vis** ($CH_3CN$): $\lambda$ (lg $\varepsilon$) = 646 nm (3.599), 591 nm (3.764), 551 nm (3.959), 516 nm (4.272), 420 nm (5.680), 298 nm (4.271), 260 nm (4.487); **Fl** ($CH_3CN$, $\lambda_{ex}$= 420 nm): $\lambda_{em}$ (a.u.) = 655 nm (90), 720 nm (40); **MF**: $C_{58}H_{50}N_4O_4Si_2$; **MW** = 923.23 g/mol;




5,10-bis-(4-trimethylsilanylethynyl-phenyl)-15,20-bis-(4-formyloxymethyl-phenyl)-porphyrin (**58**):

Porphyrin **58** was a side-product of the synthesis of **56** and was obtained after CC as purple crystals in a yield of 23 mg (0.03 mmol, 5 %).

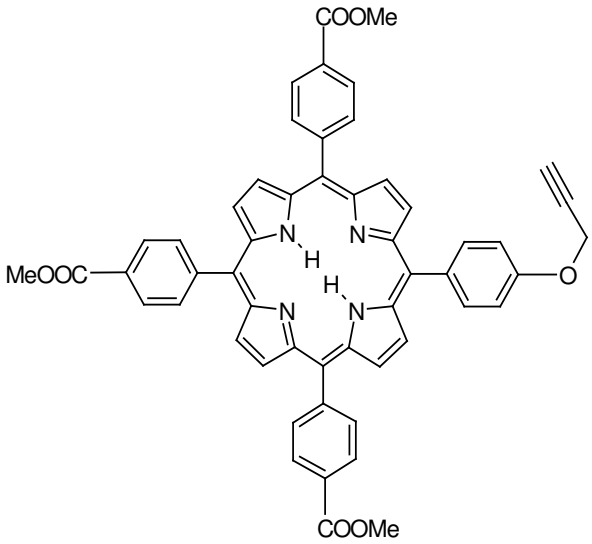
**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (600 MHz, $CDCl_3$): δ [ppm] = - 2.82 (s, 2 H, NH), 0.38 (s, 18 H, TMS-$CH_3$), 4.11 (s, 6 H, 3 $CH_3$), 7.87 (d, $^3$J = 8.7 Hz, 4 H, arom. CH), 8.15 (d, $^3$J = 8.7 Hz, 4 H, arom. CH), 8.28 (d, $^3$J = 8.3 Hz, 4 H, arom. CH), 8.44 (d, $^3$J = 8.3 Hz, 4 H, arom. CH), 8.80 (d, $^3$J = 4.8 Hz, 2 H, porphyrin-CH), 8.81 (s, 2 H, porphyrin-CH), 8.83 (s, 2 H, porphyrin-CH), 8.84 (d, $^3$J = 4.8 Hz, 2 H, porphyrin-CH); **$^{13}$C-NMR** (150.9 MHz, $CDCl_3$): δ [ppm] = 0.1 (+, 6 C, TMS-$CH_3$), 52.5 (+, 2 C, $CH_3$), 95.8 ($C_{quat}$, 2 C, alkyne), 104.9 ($C_{quat}$, 2 C, alkyne), 119.1 ($C_{quat}$, 2 C, porphyrin), 119.9 ($C_{quat}$, 2 C, porphyrin), 122.8 ($C_{quat}$, 2 C), 128.0 (+, 4 C), 129.8 ($C_{quat}$, 2 C), 130.4 (+, 4 C), 134.4 (+, 4 C), 134.5 (+, 4 C), 142.2 ($C_{quat}$, 2 C), 146.8 ($C_{quat}$, 2 C), 167.3 ($C_{quat}$, 2 C, ester); **MS** (PI-EIMS, 70 eV): m/z (%) = 922.0 [$M^{+\cdot}$] (100); **UV/Vis** ($CH_3CN$): λ (lg ε) = 646 nm (3.599), 591 nm (3.764), 551 nm (3.959), 516 nm (4.272), 420 nm (5.680), 298 nm (4.271), 260 nm (4.487); **Fl** ($CH_3CN$, $\lambda_{ex}$= 420 nm): $\lambda_{em}$ (a.u.) = 655 nm (90), 720 nm (40); **MF**: $C_{58}H_{50}N_4O_4Si_2$; **MW** = 923.23 g/mol;




Formic acid 4-[10,15-bis-(4-formyloxymethyl-phenyl)-20-(4-hydroxy-phenyl)-porphyrin-5-yl]-benzyl ester (**59**):

Synthesis followed **GP 7** using pyrrole (280 µl, 268 mg, 4.00 mmol, 8 eq), 4-formyl-bezoicacid-methylester (575 mg, 3.50 mmol, 7 eq), 4-hydroxy-benzaldehyde (61 mg, 0.50 mmol), 37 µl of $BF_3{\cdot}OEt_2$ and p-chloranil (738 mg, 3.00 mmol, 6 eq). Identification of the product in the crude mixture was possible using TLC which showed 6 red fluorescent spots (ex: 336 nm) of which the second one revealed to be the product. Purification by CC had to be done twice. First with PE/DCM/EE in a ratio of 6:3:3 to 6:3:4 and then in a ratio of 60:30:15 to 60:30:25. This gave porphyrin **59** as purple crystals in a yield of 90 mg (0.11 mmol, 22 %). $R_f$ (PE:DCM:EE = 65:30:40) = 0.48.

**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = - 2.80 (s, 2 H, NH), 4.12 (s, 9 H, 3 $CH_3$), 7.21 (d, $^3$J = 8.8 Hz, 2 H, arom. CH), 8.06 (d, $^3$J = 8.8 Hz, 2 H, arom. CH), 8.30 (d, $^3$J = 8.3 Hz, 6 H, arom. CH), 8.45 (d, $^3$J = 8.3 Hz, 6 H, arom. CH), 8.79 – 8.81 (m, 8 H, porphyrin-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 52.5 (+, 3 C, $CH_3$), 113.8 (+, 2 C, phenol), 119.2 ($C_{quat}$, 4 C, porphyrin), 128.0 (+, 6 C), 129.7 ($C_{quat}$, 3 C), 134.3 ($C_{quat}$, 1 C, phenol), 134.6 (+, 6 C), 135.8 (+, 2 C, phenol), 146.8 ($C_{quat}$, 3 C), 155.7 ($C_{quat}$, 1 C, phenol), 167.3 ($C_{quat}$, 3 C, ester); **MS** (PI-EIMS, 70 eV): m/z (%) = 803.9 [$M^{+\cdot}$] (100); **EA** ($C_{50}H_{38}N_4O_8$) calc.: C 72.98 H 4.65 N 6.81, found: C 73.48 H 4.72 N 6.58; **UV/Vis** ($CH_3CN$): λ (lg ε) = 647 nm (3.602), 592 nm (3.774), 553 nm (3.965), 517 nm (4.283), 421 nm (5.697), 299 nm (4.285), 261 nm (4.502); **Fl** ($CH_3CN$, $\lambda_{ex}$= 421 nm): $\lambda_{em}$ (a.u.) = 655 nm (95), 720 nm (43); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3448, 3319, 3118, 3036, 2998, 2946, 2708, 1934, 1812, 1723, 1608, 1436, 1380, 1140, 965, 802, 760; **MF**: $C_{50}H_{36}N_4O_7$; **MW** = 804.86 g/mol;

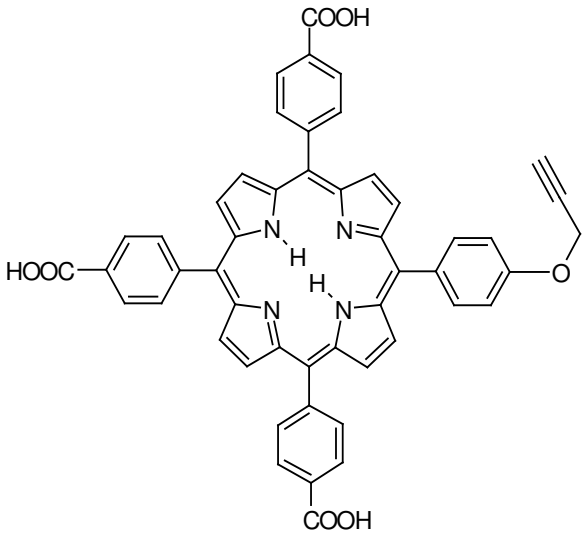



Formic acid 4-[10,20-bis-(4-formyloxymethyl-phenyl)-15-(4-prop-2-ynyloxy-phenyl)-porphyrin-5-yl]-benzyl ester (**60**):

The phenol **59** (405 mg, 0.50 mmol) was dissolved in 20 ml of dry THF under an atmosphere of $N_2$. After addition of $K_2CO_3$ (417 mg, 3.02 mmol, 6 eq), catalytic amounts of 18-C-6 and KI (each approx. 25 mg), a solution of propargyl bromide in toluene (80 %, 163 µl, 180 mg, 1.51 mmol, 3 eq) was added. The mixture was stirred at r.t. for 48 h. TLC control showed almost complete conversion and no side-products. The suspension was filtered and the filtrate was concentrated at reduced pressure. The crude product was purified by CC. PE/DCM/EE in a ratio of 65:25:20. This yielded 390 mg of **60** as purple crystals (0.46 mmol, 92 %). $R_f$ (PE:DCM:EE = 65:25:20) = 0.51.

**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = - 2.80 (s, 2 H, NH), 2.69 (t, $^4J$ = 2.2 Hz, 1 H, CH), 4.11 (s, 9 H, 3 $CH_3$), 4.97 (d, $^4J$ = 2.2 Hz, 2 H, $CH_2$), 7.36 (d, $^3J$ = 8.6 Hz, 2 H, arom. CH), 8.12 (d, $^3J$ = 8.6 Hz, 2 H, arom. CH), 8.29 (d, $^3J$ = 8.3 Hz, 6 H, arom. CH), 8.44 (d, $^3J$ = 8.3 Hz, 6 H, arom. CH), 8.79 – 8.81 (m, 8 H, porphyrin-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 51.3 (+, 3 C, $CH_3$), 54.9 (–, 1 C), 74.8 (+, 1 C, CH), 77.5 ($C_{quat}$, 1 C, alkyne), 112.0 (+, 2 C, phenol), 117.8 ($C_{quat}$, 1 C, porphyrin), 118.0 ($C_{quat}$, 2 C, porphyrin), 119.5 ($C_{quat}$, 1 C, porphyrin), 126.8 (+, 6 C), 128.5 ($C_{quat}$, 3 C), 133.4 (+, 6 C), 133.8 ($C_{quat}$, 1 C, phenol), 134.4 (+, 2 C, phenol), 145.6 ($C_{quat}$, 3 C), 156.4 ($C_{quat}$, 1 C, phenol), 166.1 ($C_{quat}$, 3 C, ester); **MS** (ESI, DCM/MeOH): m/z (%) = 843.4 [$MH^+$] (100); **EA** ($C_{53}H_{38}N_4O_7$) calc.: C 75.52 H 4.54 N 6.65, found: C 75.00 H 4.86 N 6.48; **UV/Vis** ($CH_2Cl_2$): λ (lg ε) = 647 nm (3.598), 591 nm (3.772), 552 nm (3.960), 516 nm (4.278), 420 nm (5.690), 305 nm

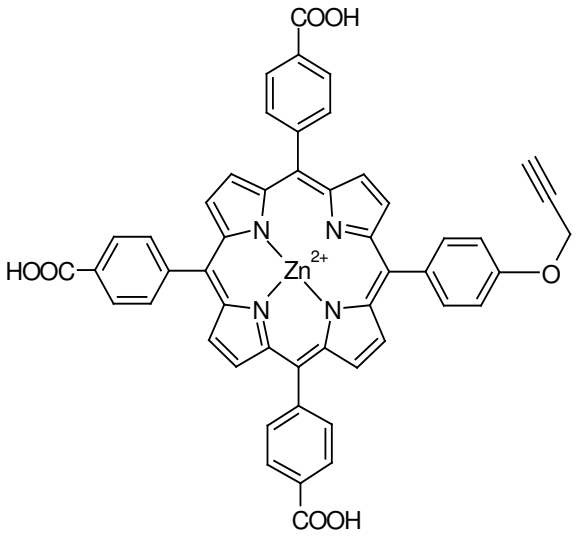
(4.281); **Fl** ($CH_3CN$, $\lambda_{ex}$= 420 nm): $\lambda_{em}$ (a.u.) = 655 nm (95), 720 nm (43); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3431, 3320, 3120, 3036, 2996, 2948, 2709, 2113, 1934, 1813, 1722, 1607, 1435, 1275, 1107, 964, 802, 760; **MF**: $C_{53}H_{38}N_4O_7$; **MW** = 842.91 g/mol;




5,10,15-tris-(4-carboxy-phenyl)-20-(4-prop-2-ynyloxy-phenyl)-porphyrin (**61**):

The methyl ester **60** (200 mg, 0.24 mmol) was dissolved in 20 ml of THF. After addition of LiOH (170 mg, 7.11 mmol, 30 eq), dissolved in 7 ml $H_2O$, the mixture was heated to reflux for 24 h. The solution was acidified with 6 N aq. HCl to pH 2, which caused the product to precipitate. The solution was separated by centrifugation and discarded. The precipitate was washed with water and dried in vacuum. The crude product was purified by CC with $CHCl_3$/MeOH/AcOH in a ratio of 84:15:1. This yielded 180 mg of **61** as purple crystals (0.23 mmol, 95 %). $R_f$ ($CHCl_3$:MeOH:AcOH = 84:15:1) = 0.31.

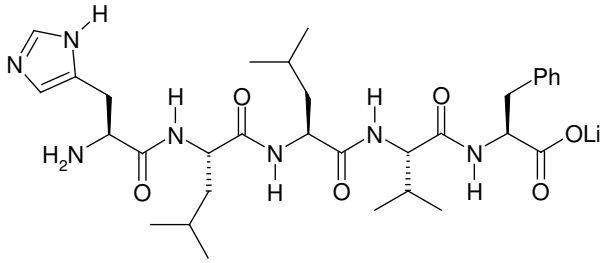
**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (300 MHz, acetone-$d_6$): $\delta$ [ppm] = - 2.74 (s, 2 H, NH), 5.13 (d, $^4J$ = 2.2 Hz, 2 H, $CH_2$), 7.49 (d, $^3J$ = 8.5 Hz, 2 H, arom. CH), 8.22 (d, $^3J$ = 8.5 Hz, 2 H, arom. CH), 8.41 (d, $^3J$ = 8.3 Hz, 6 H, arom. CH), 8.51 (d, $^3J$ = 8.3 Hz, 6 H, arom. CH), 8.91 – 8.98 (m, 8 H, porphyrin-CH); Assignment of the signal of the alkyne-CH was not possible as it was covered by the $H_2O$-signal. **$^{13}$C-NMR** cannot be given as **61** was too poorly soluble in all common deuterated solvents. **MS** (ESI, DCM/MeOH): m/z (%) = 801.3 [$MH^+$] (100); **UV/Vis** (Hepes-buffer pH 7.5): $\lambda$ (lg $\varepsilon$) = 647 nm (3.586), 591 nm (3.762), 552 nm (3.948), 516 nm (4.270), 420 nm (5.678), 305 nm (4.268); **MF**: $C_{50}H_{32}N_4O_7$; **MW** = 800.83 g/mol;




Zn[5,10,15-tris-(4-carboxy-phenyl)-20-(4-prop-2-ynyloxy-phenyl)-porphyrin] (**62**):

The porphyrin **61** (285 mg, 0.36 mmol) was suspended in 10 ml water. A saturated aq. solution of $NaHCO_3$ was added dropwise until a clear solution was formed. A solution of $Zn(OAc)_2$ x 2 $H_2O$ (781 mg, 3.56 mmol, 10 eq) in 5 ml water was added dropwise. A reddish brown precipitate appeared immediately. The mixture was heated to 80 °C for 5 minutes. After cooling to r.t. the product was separated by centrifugation and the colourless solution was discarded. The product was washed with water and dried in vacuum. This yielded 307 mg of **62** as purple crystalline solid (0.36 mmol, 100 %).

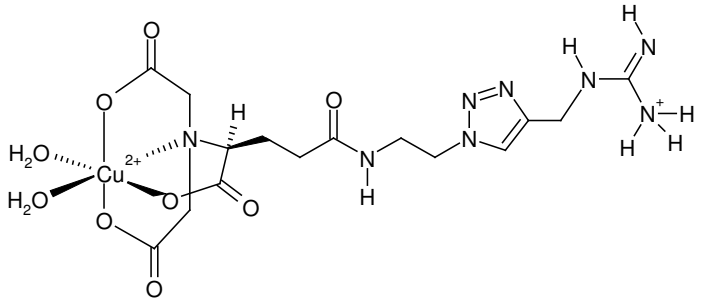
**Mp**: > 320 °C (decomp.); **$^1$H-NMR** (600 MHz, $CD_3COOD$:$CDCl_3$ = 1:1): $\delta$ [ppm] = 2.77 (t, $^4J$ = 2.3 Hz, 1 H, CH), 4.98 (d, $^4J$ = 2.3 Hz, 2 H, $CH_2$), 7.36 (d, $^3J$ = 8.7 Hz, 2 H, arom. CH), 8.13 (d, $^3J$ = 8.7 Hz, 2 H, arom. CH), 8.33 (d, $^3J$ = 8.0 Hz, 6 H, arom. CH), 8.47 (d, $^3J$ = 8.0 Hz, 6 H, arom. CH), 8.67 (d, $^3J$ = 4.6 Hz, 6 H, porphyrin-CH), 8.87 (s, 4 H, porphyrin-CH), 8.97 (d, $^3J$ = 4.6 Hz, 6 H, porphyrin-CH); **$^{13}$C-NMR** cannot be given as **62** was too poorly soluble in all common deuterated solvents. **MS** (ESI, $H_2O$/MeOH): m/z (%) = 865.3 [$MH^+$] (100); **UV/Vis** (Hepes-buffer, pH 7.5): $\lambda$ (lg $\varepsilon$) = 599 nm (3.893), 558 nm (4.151), 423 nm (5.642), 310 nm (4.295); **Fl** (Hepes-buffer, pH 7.5, $\lambda_{ex}$= 423 nm): $\lambda_{em}$ (a.u.) = 610 nm (89), 660 nm (39); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3433, 2925, 2854, 2122, 1697, 1605, 1400, 998; **MF**: $C_{50}H_{30}N_4O_7Zn$; **MW** = 864.19 g/mol;



Li[2-[2-(2-{2-[2-Amino-3-(3H-imidazol-4-yl)-propionylamino]-4-methyl-pentanoyl-amino}-4-methyl-pentanoylamino)-3-methyl-butyrylamino]-3-phenyl-propionate] (**63**):

The commercially available peptide H-His-Leu-Leu-Val-Phe-OMe x 2 HCl (71 mg, 0.1 mmol) was dissolved in 5 ml of a 1:1 mixture of water:acetonitrile. LiOH was added (7.2 mg, 0.3 mmol, 3 eq). The solution was stirred at r.t. for 20 h and lyophilized. This gave **63** as colourless frothy solid in combination with 2 eq LiCl (72 mg, 0.1 mmol, 100 %).

**$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 0.68 – 0.79 (m, 18 H), 1.19 – 1.50 (m, 6 H), 1.76 – 1.83 (m, 1 H), 2.74 – 2.81 (m, 3 H, Phe-$CH_2$ + His-$CH_2$), 3.00 (dd, $^2J$ = 13.9 Hz, $^3J$ = 4.8 Hz, 1 H, Phe-$CH_2$), 3.53 (t, $^3J$ = 6.2 Hz, 1 H, His-C*H), 3.91 (d, $^3J$ = 8.2 Hz, 1 H, Val-C*H), 4.12 – 4.23 (m, 2 H), 4.28 – 4.32 (m, 1 H), 6.74 (s, 1 H, His), 7.05 – 7.20 (m, 5 H, Phe), 7.51 (s, 1 H, His); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 628.5 [$MH^+$] (100), 634.5 [$(M+Li^+)^+$] (65); **MF**: $C_{32}H_{48}N_7O_6Li$; **MW** = 633.72 g/mol;

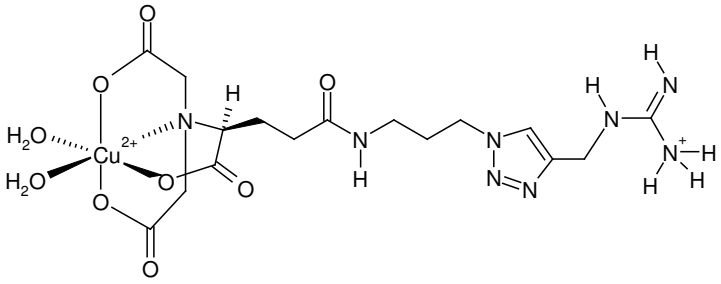


Cu[2-(Bis-carboxylate-methyl-amino)-4-[2-(4-guanidinium-methyl-[1,2,3]triazol-1-yl)-ethylcarbamoyl]-butanoate] (**64**):

Synthesis followed **GP 8** using **41** (87 mg, 0.20 mmol), **55** (27 mg, 0.20 mmol), sodium ascorbate (8 mg, 0.04 mmol, 20 mol%) and $CuSO_4$ x 5 $H_2O$ (2.5 mg, 0.01 mmol, 5 mol%). This yielded 79 mg (0.15 mmol, 75 %) of **64** as green solid.

**MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 490.0 [MH+] (80), 496.1 [$(M+Li^+)^+$] (100), 512.1 [$(M+Na^+)^+$] (60); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 488.3 [$(M-H^+)^-$] (100);

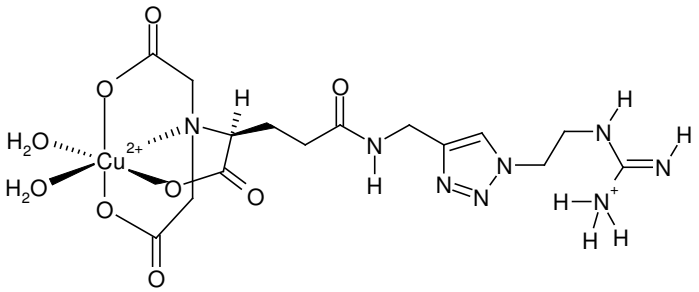
**IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3387, 2936, 2873, 2103, 1624, 1388, 1126, 915; **MF**: $C_{15}H_{22}N_8O_7Cu$ x 2 $H_2O$; **MW** = 525.97 g/mol;



Cu[2-(Bis-carboxylate-methyl-amino)-4-[3-(4-guanidinium-methyl-[1,2,3]triazol-1-yl)-propylcarbamoyl]-butanoate] (**65**):

Synthesis followed **GP 8** using **43** (90 mg, 0.20 mmol), **55** (27 mg, 0.20 mmol), sodium ascorbate (8 mg, 0.04 mmol, 20 mol%) and $CuSO_4$ x 5 $H_2O$ (2.5 mg, 0.01 mmol, 5 mol%). This yielded 79 mg (0.15 mmol, 73 %) of **65** as green solid.

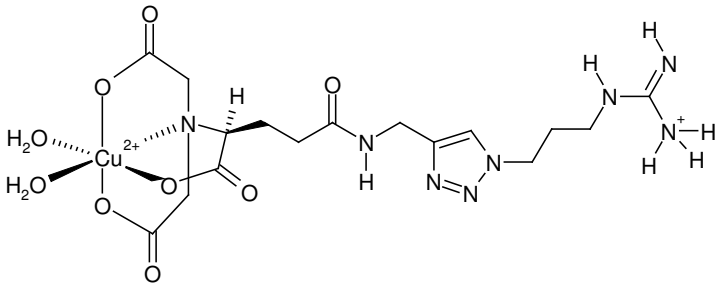
**MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 504.0 [MH+] (70), 510.1 [$(M+Li^+)^+$] (100), 526.1 [$(M+Na^+)^+$] (60); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 502.2 [$(M-H^+)^-$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3392, 2932, 2874, 2105, 1622, 1387, 1126, 915; **MF**: $C_{16}H_{24}N_8O_7Cu$ x 2 $H_2O$; **MW** = 539.99 g/mol;



Cu[2-(Bis-carboxylate-methyl-amino)-4-{[1-(2-guanidinium-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-carbamoyl}-butanoate] (**66**):

Synthesis followed **GP 8** using **49** (84 mg, 0.21 mmol), **52** (34 mg, 0.21 mmol), sodium ascorbate (8 mg, 0.04 mmol, 20 mol%) and $CuSO_4$ x 5 $H_2O$ (2.6 mg, 0.01 mmol, 5 mol%). This yielded 78 mg (0.15 mmol, 71 %) of **66** as green solid.

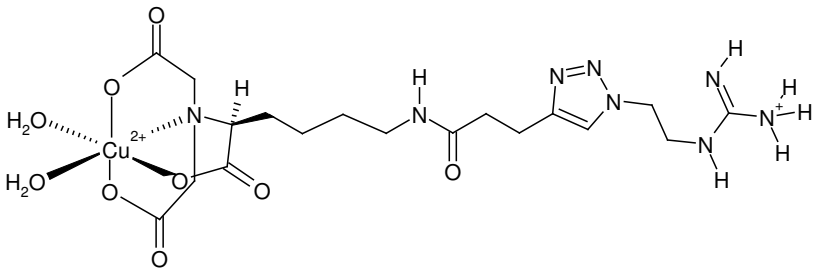
**MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 488.3 [$(M-H^+)^-$] (100); **MF**: $C_{15}H_{22}N_8O_7Cu$ x 2 $H_2O$; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3389, 2936, 2872, 2103, 1626, 1388, 1127, 915; **MW** = 525.97 g/mol;



Cu[2-(Bis-carboxylate-methyl-amino)-4-{[1-(3-guanidinium-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-carbamoyl}-butanoate] (**67**):

Synthesis followed **GP 8** using **49** (84 mg, 0.21 mmol), **53** (37 mg, 0.21 mmol), sodium ascorbate (8 mg, 0.04 mmol, 20 mol%) and $CuSO_4$ x 5 $H_2O$ (2.6 mg, 0.01 mmol, 5 mol%). This yielded 85 mg (0.15 mmol, 76 %) of **67** as green solid.

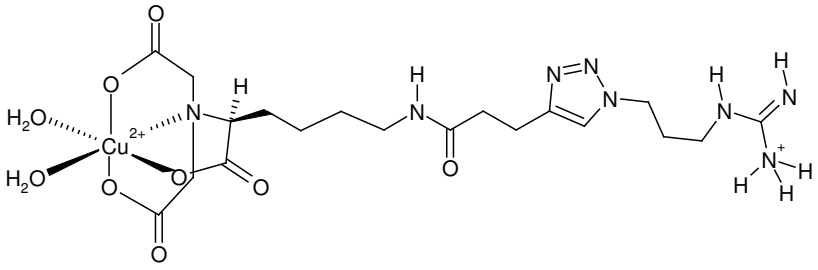
**MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 504.0 [MH+] (60), 510.1 [$(M+Li^+)^+$] (100), 526.1 [$(M+Na^+)^+$] (50); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 502.2 [$(M-H^+)^-$] (100); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3387, 2936, 2873, 2103, 1624, 1388, 1126, 915; **MF**: $C_{16}H_{24}N_8O_7Cu$ x 2 $H_2O$; **MW** = 539.99 g/mol;



Cu[2-(Bis-carboxylate-methyl-amino)-6-{3-[1-(2-guanidinium-ethyl)-1H-[1,2,3]triazol-4-yl]-propionylamino}-hexanoate] (**68**):

Synthesis followed **GP 8** using **28** (75 mg, 0.15 mmol), **52** (25 mg, 0.15 mmol), sodium ascorbate (6 mg, 0.03 mmol, 20 mol%) and $CuSO_4$ x 5 $H_2O$ (2 mg, 0.01 mmol, 5 mol%). This yielded 61 mg (0.11 mmol, 72 %) of **68** as green solid.

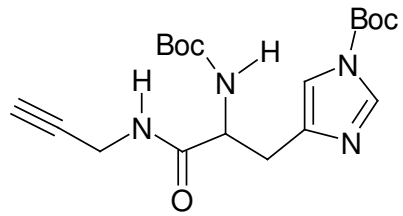
**MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 532.3 [$MH^+$] (100); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 530.4 [$(M-H^+)^-$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3390, 2938, 2879, 2107, 1625, 1389, 1128, 916; **MF**: $C_{18}H_{28}N_8O_7Cu$ x 2 $H_2O$; **MW** = 568.05 g/mol;



Cu[2-(Bis-carboxylate-methyl-amino)-6-{3-[1-(3-guanidinium-propyl)-1H-[1,2,3]triazol-4-yl]-propionylamino}-hexanoate] (**69**):

Synthesis followed **GP 8** using **28** (75 mg, 0.15 mmol), **53** (27 mg, 0.15 mmol), sodium ascorbate (6 mg, 0.03 mmol, 20 mol%) and $CuSO_4$ x 5 $H_2O$ (2 mg, 0.01 mmol, 5 mol%). This yielded 61 mg (0.11 mmol, 70 %) of **69** as green solid.

**MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 546.1 [MH+] (90), 552.2 [$(M+Li^+)^+$] (100), 568.2 [$(M+Na^+)^+$] (70); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 544.2 [$(M-H^+)^-$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3391, 2939, 2881, 2108, 1626, 1389, 1129, 916; **MF**: $C_{19}H_{30}N_8O_7Cu$ x 2 $H_2O$; **MW** = 582.07 g/mol;

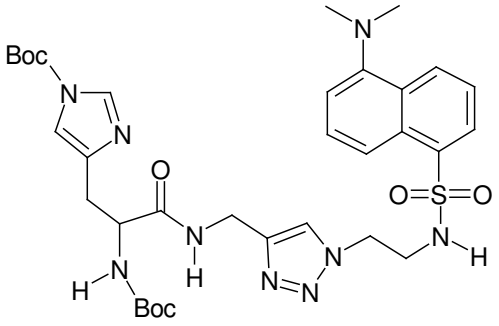


4-(2-tert-Butoxycarbonylamino-2-prop-2-ynylcarbamoyl-ethyl)-imidazole-1-carboxylic acid tert-butyl ester (**70**):

The twofold Boc-protected histidine (700 mg, 1.3 mmol, di-cyclohexylamine salt) was suspended in 5 ml of dry DMF under an atmosphere of $N_2$. The addition of TBTU (503 mg, 1.57 mmol, 1.2 eq), HOBt (274 mg, 1.72 mmol, 1.32 eq) and DIEA (290 µl, 219 mg, 1.70 mmol, 1.3 eq) yielded a clear solution. After addition of propargyl amine (358 µl, 287 mg, 5.2 mmol, 4 eq) the solution was stirred at r.t. for 24 h. Water (50 ml) was added and the mixture was extracted twice with DCM (100 ml each

time). The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated at reduced pressure to give a yellow oil. Purification of the crude product by CC with EE:PE = 1:1 yielded 383 mg (0.98 mmol, 75 %) of **70** as colourless oil. $R_f$ (EE:PE = 1:1) = 0.35.

**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.15 (s, 9 H, $CH_3$), 1.33 (s, 9 H, $CH_3$), 2.97 (t, $^4J$ = 2.5 Hz, 1 H, alkyne-CH), 2.72 – 2.77 (m, 2 H, His-$CH_2$), 3.72 (dd, $^3J$ = 5.5 Hz, $^4J$ = 2.5 Hz, 2 H, propargyl $CH_2$), 4.19 – 4.25 (m, 1 H, C*H), 6.01 (d, $^3J$ = 8.0 Hz, 1 H, NH), 6.93 (s, 1 H, His-CH), 7.45 (t, $^3J$ = 5.5 Hz, 1 H, NH), 7.74 (s, 1 H, His-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 27.6 (+, 3 C, $CH_3$), 28.1 (+, 3 C, $CH_3$), 28.6 (–, 1 C, His), 30.6 (–, 1 C, propargyl), 52.7 (+, 1 C, C*H), 70.9 (+, alkyne-CH), 79.3 ($C_{quat}$, 1 C, Boc), 79.6 ($C_{quat}$, 1 C, alkyne), 85.2 ($C_{quat}$, 1 C, Boc), 114.5 (+, 1 C, imidazole), 136.5 (+, 1 C, imidazole), 138.9 ($C_{quat}$, 1 C, imidazole), 146.7 ($C_{quat}$, 1 C, urethane), 155.4 ($C_{quat}$, 1 C, urethane), 171.2 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 393.1 [$MH^+$] (100); **MF**: $C_{19}H_{28}N_4O_5$; **MW** = 392.46 g/mol;

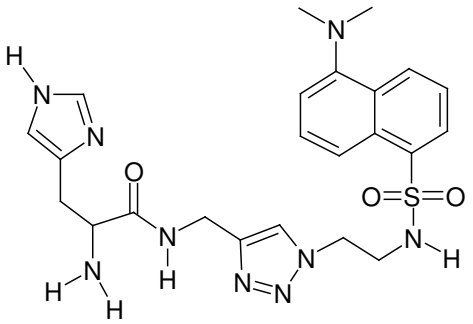



4-[2-tert-Butoxycarbonylamino-2-({1-[2-(5-dimethylamino-naphthalene-1-sulfonyl-amino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-carbamoyl)-ethyl]-imidazole-1-carboxylic acid tert-butyl ester (**72**):

The alkyne **70** (510 mg, 1.30 mmol) and the azide **71** (415 mg, 1.30 mmol) were dissolved in 10 ml of MeCN. After addition of CuI (50 mg, 0.26 mmol, 20 mol%) and 2,4-lutidine (176 µl, 164 mg, 1.30 mmol, 1 eq) the solution was stirred at r.t. for 16 h. An aq. hydrogen peroxide solution (3 %, 20 ml) and a sat. aq. solution of EDTA (20 ml) were added and the mixture was stirred strongly for 30 min. After phase separation, the aq. phase was extracted with DCM (50 ml). The organic layers were combined, dried over $Na_2SO_4$ and concentrated at reduced pressure. This yielded a green crude product which was purified by CC with DCM:MeOH 96:4. Note: TLC with

this eluent shows only a baseline spot. Behaviour of **72** on the column is different from that one on TLC plates. Compound **72** was obtained as greenish solid in a yield of 85 % (787 mg, 1.11 mmol).

**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.36 (s, 9 H, $CH_3$), 1.56 (s, 9 H, $CH_3$), 2.85 (s, 6 H, 2 $CH_3$), 2.96 (t, $^3J$ = 5.5 Hz, 2 H, $CH_2$), 3.37 – 3.43 (m, 2 H, $CH_2$), 4.27 – 4.44 (m, 5 H, 2 $CH_2$ + C*H), 6.09 (d, $^3J$ = 6.9 Hz, 1 H, NH), 6.94 (t, $^3J$ = 6.1 Hz, 1 H, NH), 7.10 (d, $^4J$ = 1.1 Hz, 1 H, His-CH), 7.13 (d, $^3J$ = 7.4 Hz, 1 H, dansyl-CH), 7.41 – 7.51 (m, 4 H, triazole-CH + NH + 2 dansyl-CH), 7.92 (d, $^4J$ = 1.1 Hz, 1 H, His-CH), 8.18 – 8.21 (m, 2 H, dansyl-CH), 8.51 (d, $^3J$ = 8.5 Hz, 1 H, dansyl-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 27.9 (+, 3 C, $CH_3$), 28.3 (+, 3 C, $CH_3$), 30.6 (–, 1 C), 34.8 (–, 1 C), 42.6 (–, 1 C), 45.4 (+, 2 C), 50.3 (–, 1 C), 54.4 (+, 1 C, C*H), 79.9 ($C_{quat}$, 1 C, Boc), 85.7 ($C_{quat}$, 1 C, Boc), 114.7 (+, 1 C, dansyl), 115.3 (+, 1 C, imidazole), 118.9 (+, 1 C, dansyl), 123.1 (+, 1 C, dansyl), 123.7 (+, 1 C, triazole), 128.4 (+, 1 C, dansyl), 129.3 (+, 1 C, dansyl), 129.5 ($C_{quat}$, 1 C, dansyl), 129.9 ($C_{quat}$, 1 C, dansyl), 130.6 (+, 1 C, dansyl), 134.7 ($C_{quat}$, 1 C, dansyl), 136.8 (+, 1 C, imidazole), 138.9 ($C_{quat}$, 1 C, imidazole), 144.6 ($C_{quat}$, 1 C, triazole), 146.9 ($C_{quat}$, 1 C, urethane), 151.9 ($C_{quat}$, 1 C, dansyl), 155.6 ($C_{quat}$, 1 C, urethane), 171.8 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 712.3 [$MH^+$] (100); **UV/Vis** ($CH_3CN$): λ (lg ε) = 330 nm (3.405); **MF**: $C_{33}H_{45}N_9SO_7$; **MW** = 711.84 g/mol;



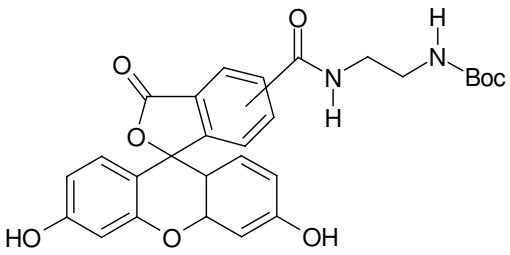
2-Amino-N-{1-[2-(5-dimethylamino-naphthalene-1-sulfonylamino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-3-(1H-imidazol-4-yl)-propionamide (**73**):

Synthesis followed **GP 4** using **72** (270 mg, 0.38 mmol) and 5 ml of HCl saturated $Et_2O$. The pale green solid, which was obtained after drying in vacuum was dissolved in 10 ml of water. A sat. aq. solution of $NaHCO_3$ was added to adjust the pH value to 9. The solution was extracted 8 times with DCM (40 ml each time). The organic

phases were combined, dried over $Na_2SO_4$ and concentrated at reduced pressure. Drying in vacuum yielded 185 mg of **73** (0.36 mmol, 95 %) as green solid.

**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 2.75 (bs, 2 H, $NH_2$), 2.79 (s, 6 H, 2 $CH_3$), 2.84 – 2.90 (m, 2 H, $CH_2$), 3.25 – 3.32 (m, 2 H, $CH_2$), 3.56 – 3.62 (m, 1 H, C*H), 4.25 – 4.36 (m, 4 H, 2 $CH_2$), 6.66 (bs, 1 H, His-CH), 7.04 (d, $^3$J = 7.4 Hz, 1 H, dansyl-CH), 7.32 – 7.45 (m, 5 H, triazole-CH + 2 NH + 2 dansyl-CH), 8.12 – 8.25 (m, 3 H, dansyl-CH + His-CH), 8.45 (d, $^3$J = 8.8 Hz, 1 H, dansyl-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 31.0 (–, 1 C), 33.4 (–, 1 C), 41.6 (–, 1 C), 44.3 (+, 2 C), 49.6 (–, 1 C), 54.0 (+, 1 C, C*H), 114.3 (+, 1 C, dansyl), 117.9 (+, 1 C, dansyl), 122.1 (+, 1 C, dansyl), 122.5 (+, 1 C, triazole), 127.3 (+, 1 C, dansyl), 128.1 (+, 1 C, dansyl), 128.4 ($C_{quat}$, 1 C, dansyl), 128.8 ($C_{quat}$, 1 C, dansyl), 129.4 (+, 1 C, dansyl), 133.7 ($C_{quat}$, 1 C, dansyl), 134.3 (+, 1 C, imidazole), 143.7 ($C_{quat}$, 1 C, triazole), 150.8 ($C_{quat}$, 1 C, dansyl), 173.9 ($C_{quat}$, 1 C, amide); **Note:** Due to broadening, the signals of the imidazole-$C_{quat}$ and –CH cannot be given. **MS** (ESI, $H_2O$/MeCN): m/z (%) = 512.2 [$MH^+$] (100); **UV/Vis** (Hepes-buffer pH 7.5): λ (lg ε) = 329 nm (3.296), 247 nm (3.832); **Fl** (Hepes-buffer, pH 7.5, $\lambda_{ex}$= 330 nm): $\lambda_{em}$ = 561 nm; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3354, 3140, 3086, 2955, 2858, 2790, 1662, 1573, 1451, 1316, 1258, 1142, 1096, 796; **MF**: $C_{23}H_{29}N_9SO_3$; **MW** = 511.60 g/mol;

**Note:** As compounds **74** – **77** were synthesized from an isomere mixture of 5/6-carboxy fluorescein (with unknown ratio), assignment of signals in $^1$H and $^{13}$C NMR spectra was not possible.

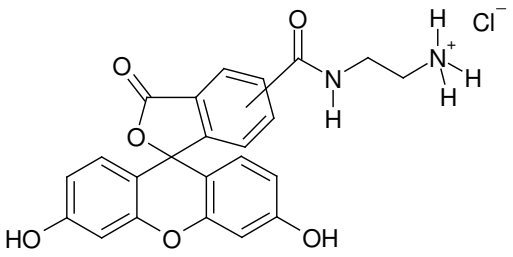



5/6-[(2-Amino-ethyl)-carbamic acid tert-butyl ester]-carboxy-fluorescein (**74**):

The isomere mixture of 5/6-carboxy fluorescein (1000 mg, 2.66 mmol) was dissolved in 15 ml of dry DMF under an atmosphere of $N_2$. Following, TBTU (1033 mg, 3.22 mmol, 1.21 eq), HOBt (511 mg, 3.22 mmol, 1.21 eq) and DIEA (1.0 ml, 756 mg,

5.85 mmol, 2.2 eq) were added. The solution was stirred at r.t. for 15 min. After addition of Boc-protected ethylene diamine (468 mg, 2.92 mmol, 1.1 eq) the solution was stirred at r.t. for 18 h. An aq. solution of citric acid (100 mg in 40 ml of water) was added in order to precipitate the product. After filtration, the residue was washed with little water and dried in vacuum. The crude product was purified by CC with EE. This yielded 1100 mg (2.12 mmol, 80 %) of **74** as orange solid. $R_f$ (EE:PE = 4:1) = 0.2.

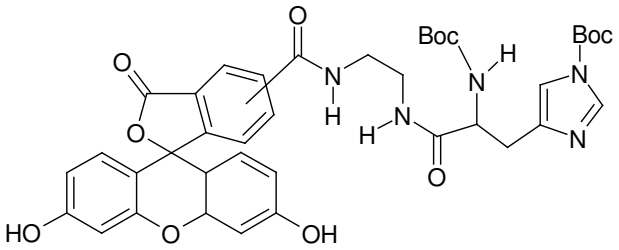
**MS** (ESI, MeOH): m/z (%) = 519.2 [$MH^+$] (100); **MF**: $C_{28}H_{26}N_2O_8$; **MW** = 518.52 g/mol;



5/6-(2-Amino-ethylamine)-carboxy-fluorescein hydrochloride (**75**):

Synthesis followed **GP 4** using **74** (1100 mg, 2.12 mmol) and 14 ml of HCl saturated diethyl ether. This yielded 964 mg of **75** (2.12 mmol, quant.) as pale yellow solid.

**MS** (ESI, $H_2O$/MeCN/TFA): m/z (%) = 419.1 [$MH^+$] (100); **MF**: $C_{23}H_{19}N_2O_6Cl$; **MW** = 454.87 g/mol;



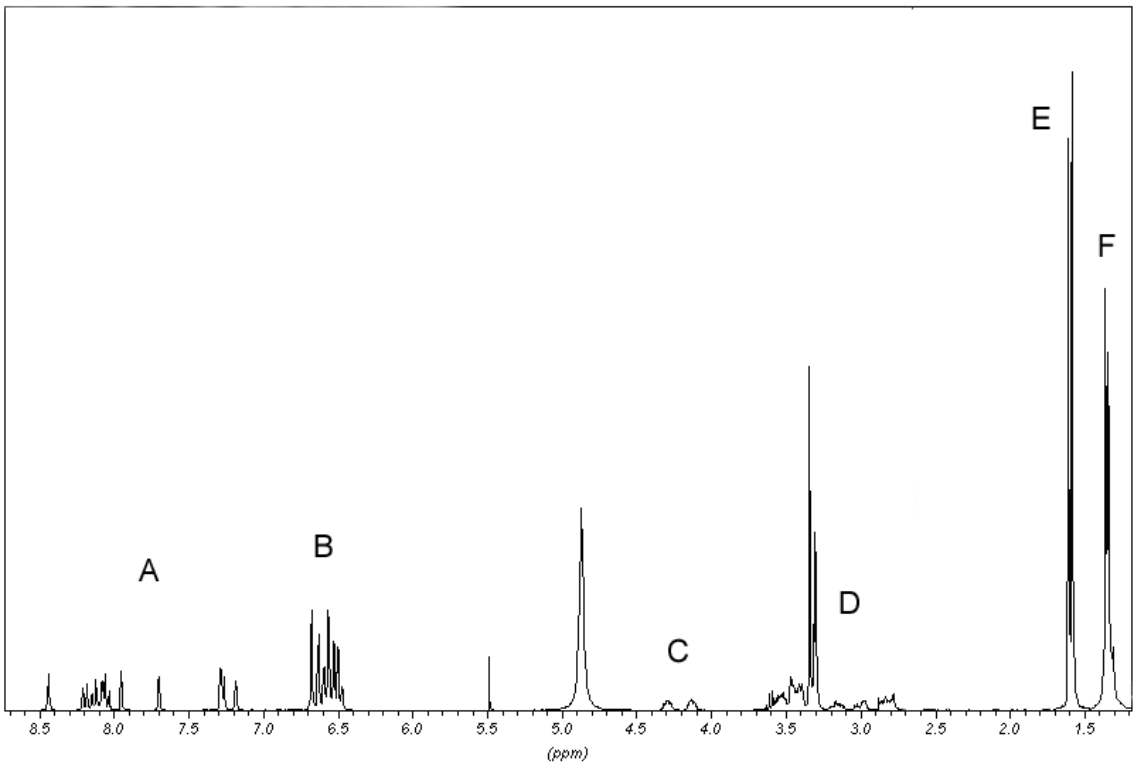
5/6-[4-[2-(2-Amino-ethylcarbamoyl)-2-tert-butoxycarbonylamino-ethyl]-imidazole-1-carboxylic acid tert-butyl ester]-carboxy-fluorescein (**76**):

The twofold Boc-protected histidine (1015 mg, 1.89 mmol, di-cyclohexyl-amine salt) was suspended in 20 ml of dry DMF under an atmosphere of $N_2$. The addition of TBTU (668 mg, 2.08 mmol, 1.21 eq), HOBt (331 mg, 2.08 mmol, 1.21 eq) and DIEA (1236 µl, 933 mg, 7.22 mmol, 4.2 eq) yielded a clear solution. After addition of **75** (782 mg, 1.72 mmol), the solution was stirred at r.t. for 3 d. An aq. solution of citric acid (150 mg in 80 ml of water) was added in order to precipitate the product. After

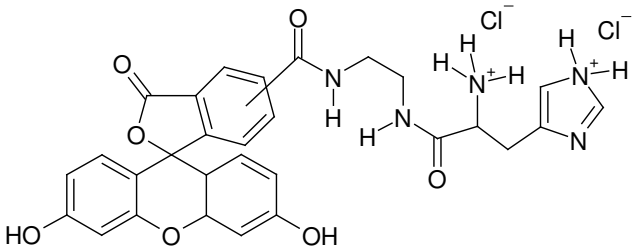
filtration, the residue was washed with water and dried in vacuum. The aqueous phases were combined and extracted with 100 ml EE. The organic phase was dried with $Na_2SO_4$ and concentrated at reduced pressure. Both crude products were purified separately by CC with EE:EtOH (from 9:1 to 2:1). Fractions containing the product were combined, concentrated and purified a second time with DCM:MeOH = 4:1. In order to separate silica gel that was dissolved during CC, the product was taken up in EE and centrifugated. This yielded 615 mg (0.81 mmol, 47 %) of **76** as orange solid. $R_f$ (EE:EtOH = 9:1) = 0.4. **Note:** As it was very difficult to separate the dicyclohexylamine it is recommended to use the twofold Boc-protected histidine *benzene adduct*!

**MS** (ESI, MeOH): m/z (%) = 756.3 [$MH^+$] (100); **MF**: $C_{39}H_{41}N_5O_{11}$; **MW** = 755.78 g/mol;

**$^1$H-NMR** spectrum of **76** (300 MHz, methanol-d4):




The spectrum shows a set of signals for each isomer. δ [ppm] = 1.3 – 1.4 (**F**, 9 H, Boc-$CH_3$), 1.5 – 1.7 (**E**, 9 H, Boc-$CH_3$), 2.8 – 3.6 (**D**, 6 H, 3 $CH_2$), 4.1 – 4.3 (**C**, 1 H, C*H), 6.5 – 6.7 (**B**, 6 H), 7.2 – 8.5 (**A**, 5 H).

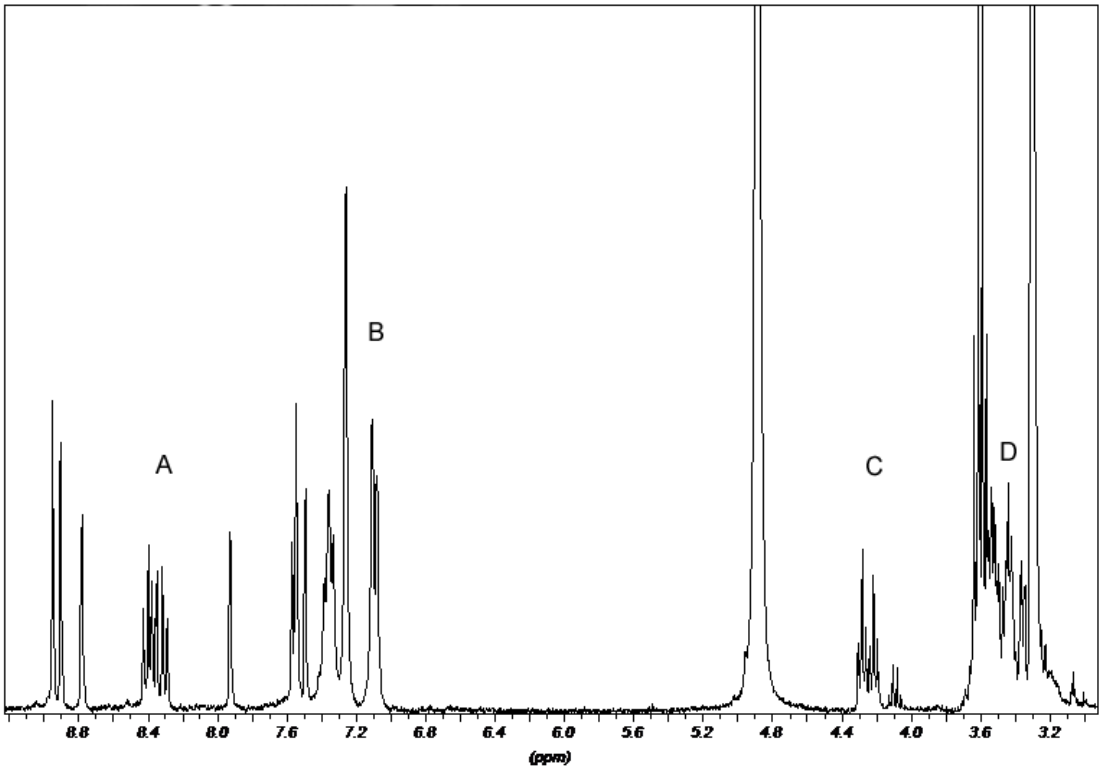


5/6-[2-Amino-N-(2-amino-ethyl)-3-(1H-imidazol-4-yl)-propionamide]-carboxy-fluorescein di-hydrochloride (**77**):

Synthesis followed **GP 4** using **76** (100 mg, 0.13 mmol) and 2 ml of HCl saturated diethyl ether. This yielded 83 mg of **77** (0.13 mmol, quant.) as pale yellow solid.

**MS** (ESI, MeOH/$H_2O$): m/z (%) = 556.2 [$MH^+$] (100); **MF**: $C_{29}H_{27}N_5O_7Cl_2$; **MW** = 628.47 g/mol;

**$^1$H-NMR** spectrum of **77** (300 MHz, methanol-d4):




The spectrum shows a set of signals for each isomer. δ [ppm] = 3.2 – 3.6 (**D**, 6 H, 3 $CH_2$), 4.2 – 4.3 (**C**, 1 H, C*H), 7.1 – 7.6 (**B**, 6 H), 7.9 – 9.0 (**A**, 5 H).

## 2.5.3. Synthesis of Receptors for Stepwise TGS

### *2.5.3.1. General Procedures*

**GP 9** – Click-reaction:

Equimolar amounts of the alkyne and the azide compound were dissolved in a 1:2 mixture of $^{t}$BuOH:EtOH (10 ml per mmol alkyne). A solution of sodium ascorbate (2 eq) in aqueous acetate buffer (pH 5, c = 0.5 mol/L) was added (4 ml buffer per mmol alkyne). Finally an aqueous solution of $CuSO_4$ x 5 $H_2O$ (c = 1 mol/L, 1 eq) was added. The colour of the mixture turned to brown immediately and then slowly to yellow. The mixture was stirred at r.t. for 5 h and diluted with 50 ml of water (an orange precipitate occured). The suspension was extracted with EE (3 x 50 ml). The organic layers were combined, together with the orange precipitate and washed thoroughly with a sat. aq. solution of EDTA until the orange solid dissolved completely. The blue aqueous layer was discarded and the organic layer was dried with $Na_2SO_4$. Filtration and removal of the solvent in vacuum yielded the crude product as a white solid, which was purified by column chromatography (CC). Chromatography had to be done twice. First with DCM:MeOH and then with EE:EtOH (gradient of 98:2 to 96:4 for both mixtures).

**GP 10** – Cleavage of Boc protecting-groups with HCl-saturated diethyl ether:
The Boc-protected compound was dissolved in diethyl ether (20 ml per mmol). A saturated solution of HCl in diethyl ether was added (1 ml per 0.15 mmol Boc). After a few minutes a white precipitate appeared. The mixture was stirred at r.t. under an atmosphere of $N_2$ for 48 h. The solvent was removed at reduced pressure and the remaining colourless solid was dried in vacuum.

**GP 11** – Deprotonation and cleavage of ester-groups:
The ammonium salt was dissolved in water (10 ml per mmol) and eluted over a strongly basic anion exchanger ($OH^-$-form, loading 0.9 mmol/ml, 16 eq). The resin was washed with a little MeOH. Both solutions were combined and LiOH was added (3 eq). The solution was stirred at r.t. for 24 h. Lyophilisation yielded the completely deprotected compounds. $^{1}$H-NMR as well as $^{13}$C-NMR spectra of compounds **37** – **41**

could not be analyzed in detail because of too slow movement of cyclen rings in NMR timescale. However, spectra showed no signals that could be assigned to ester functions. MS spectroscopy also confirmed the complete cleavage of all ester groups.

**GP 12** – complexation of $Zn^{2+}$:

The polydentate ligand was dissolved in MeOH (10 ml per mmol). A slight excess of $Zn(ClO_4)_2$ x 6 $H_2O$ (3.3 eq) was dissolved in water (10 ml per mmol). Both solutions were added simultaneously drop wise to water (5 ml per mmol) at 80 °C. In the case that a precipitate appeared, water was added until the precipitate dissolved. After complete addition of both solutions the pH value was checked. If it was lower than 8, an aqueous solution of LiOH (0.1 mol/l) was added. The mixture was heated to reflux for 6 h. Following, EtOH was added to the boiling solution until the product began to precipitate. After cooling to r.t. the suspension was stored at -20 °C over night in order to complete precipitation. The product was separated by centrifugation and washed with MeOH and EtOH. The solution was concentrated at reduced pressure and dried in vacuum. If necessary, the remaining solid was recrystallized from water/MeOH/EtOH to raise the yield.

**GP 13** – Click-reaction:

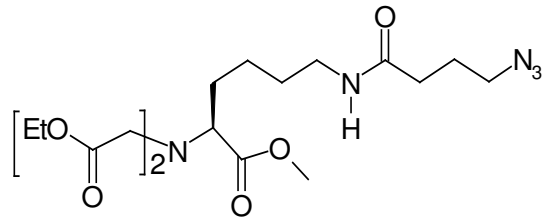
Synthetic procedure was the same like **GP 9**. Only CC was different: mixtures of PE:EE (gradient 1:1 to 1:9) were used to purify the crude product.

**GP 14** – complexation of $Zn^{2+}$:

The ammonium salt was dissolved in water (10 ml per mmol) and eluted over a strongly basic anion exchanger ($OH^-$-form, loading 0.9 mmol/ml, 16 eq). The resin was washed with a little MeOH. Both solutions were combined and lyophilized. This yielded the amine, which was dissolved in MeOH (10 ml per mmol). A slight excess of $Zn(ClO_4)_2$ x 6 $H_2O$ (3.3 eq) was dissolved in water (10 ml per mmol). Both solutions were added simultaneously drop wise to water (5 ml per mmol) at 80 °C. In the case that a precipitate appeared, water was added until the precipitate dissolved. After complete addition of both solutions an aqueous solution of LiOH (0.1 mol/l) was added until $Zn(OH)_2$ began to precipitate. This was done in order to prevent the

amine ligand from protonation. The mixture was heated to reflux for 8 h. Following, EtOH was added to the boiling solution until the product began to precipitate. After cooling to r.t. the suspension was stored at -20 °C over night in order to complete precipitation. The product was separated by centrifugation and washed with MeOH and EtOH. The solution was concentrated at reduced pressure and dried in vacuum. If necessary, the remaining solid was recrystallized from water/MeOH/EtOH to raise the yield.

*2.5.3.2. Synthesis of New Compounds*

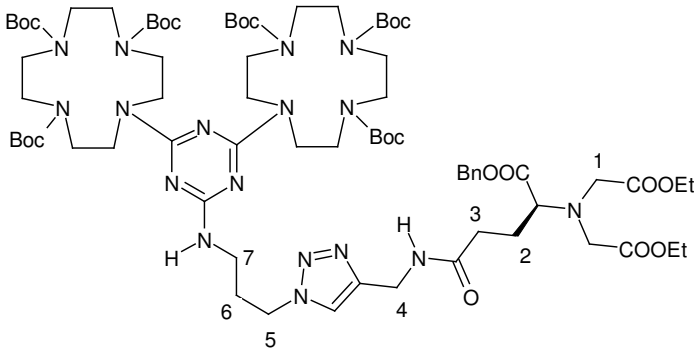


6-(4-Azido-butyrylamino)-2-(bis-ethoxycarbonylmethyl-amino)-hexanoic acid methyl ester (**79**):

The γ-azido-butyric acid (321 mg, 2.49 mmol, 1.2 eq) was dissolved in 5 ml of dry DCM and cooled to 0 °C in an ice bath. Following, HOBt (370 mg, 2.74 mmol, 1.32 eq), EDC (484 µl, 425 mg, 2.74 mmol, 1.32 eq) and DIEA (468 µl, 354 mg, 2.74 mmol, 1.32 eq) were added in this sequence. The solution was stirred at 0 °C for 30 min. Then, the amine **25** (689 mg, 2.07 mmol) dissolved in 10 ml dry DCM was added drop wise. The solution was stirred at r.t. under an atmosphere of $N_2$ for 24 h. After that time 50 ml DCM were added and the solution was washed with 30 ml of a 0.1 N aqueous solution of citric acid and 30 ml water. The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuum. The crude product was purified by column chromatography with EE:PE in a ratio of 3:2 to 4:1. This gave **79** as a colourless oil in a yield of 550 mg (1.24 mmol, 60 %). $R_f$ (EE:PE = 1:1) = 0.21.

**$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.14 (t, $^3$J = 7.1 Hz, 6 H, $CH_3$), 1.29 – 1.49 (m, 4 H, $CH_2$), 1.55 – 1.62 (m, 2 H, $CH_2$), 1.80 (tt, $^3$J = 6.7 Hz, $^3$J = 7.3 Hz, 2 H, $CH_2$), 2.17 (t, $^3$J = 7.3 Hz, 2 H, $CH_2$), 3.03 – 3.19 (m, 2 H, $CH_2$), 3.23 (t, $^3$J = 6.7 Hz, 2 H, $CH_2$), 3.32 (t, $^3$J = 7.6 Hz, 1 H, C*H), 3.49 (s, 2 H, N-$CH_2$), 3.50 (s, 2 H, N-$CH_2$), 3.56 (s, 3 H, Me-ester-$CH_3$), 4.03 (q, $^3$J = 7.1 Hz, 4 H, $CH_2$), 6.37 (t, $^3$J = 5.0 Hz, 1 H, NH);

**$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, $CH_3$), 22.7 (–, 1 C), 24.9 (–, 1 C), 28.2 (–, 1 C), 29.5 (–, 1 C), 32.9 (–, 1 C), 39.1 (–, 1 C), 50.8 (–, 1 C), 51.3 (+, 1 C, C*H), 52.6 (–, 2 C), 60.5 (–, 2 C), 64.0 (+, 1 C, $CH_3$), 171.4 ($C_{quat}$, 2 C, Et-ester), 171.9 ($C_{quat}$, 1 C, Me-ester), 173.1 ($C_{quat}$, 1 C, amide); **MS** (ESI, MeOH): m/z (%) = 444.2 [$MH^+$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3309, 3265, 2948, 2888, 2148, 1734, 1688, 1647, 1535, 1455, 1283; **MF**: $C_{19}H_{33}N_5O_7$; **MW** = 443.48 g/mol;

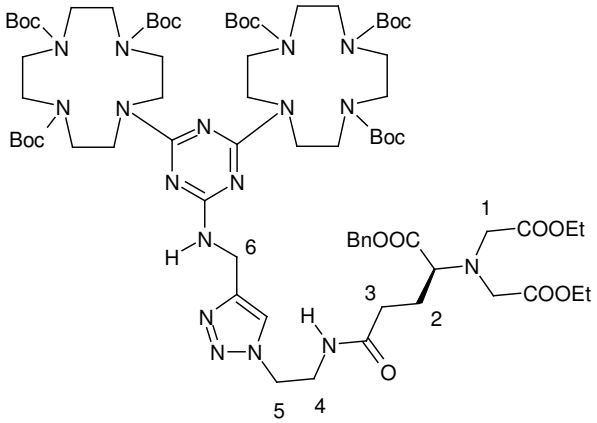



2-(Bis-ethoxycarbonylmethyl-amino)-4-[(1-{3-[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-carbamoyl]-butyric acid benzyl ester (**80**):

Synthesis followed **GP 9** using **17** (1090 mg, 0.97 mmol), **46** (434 mg, 0.97 mmol), sodium ascorbate (386 mg, 1.95 mmol) and $CuSO_4$ x 5 $H_2O$ (243 mg, 0.97 mmol). This yielded 1432 mg of **80** (0.91 mmol, 94 %) as colourless solid. $R_f$ (DCM:MeOH = 95:5) = 0.30.

**Mp**: 110 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.16 (t, $^3J$ = 7.2 Hz, 6 H, Et-ester-$CH_3$), 1.37 (bs, 36 H, Boc-$CH_3$), 1.39 (bs, 18 H, Boc-$CH_3$), 1.83 – 1.95 (m, 1 H, $C^2H_2$), 2.00 – 2.10 (m, 3 H, $C^6H_2$ + $C^2H_2$), 2.28 – 2.47 (m, 2 H, $C^3H_2$), 3.22 – 3.62 (m, 39 H, C*H + $C^7H_2$ + $C^1H_2$ + cyclen-$CH_2$), 4.04 (q, $^3J$ = 7.2 Hz, 4 H, Et-ester-$CH_2$), 4.31 – 4.41 (m, 4 H, $C^4H_2$ + $C^5H_2$), 4.91 – 5.00 (m, 1 H, NH), 5.01 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 5.06 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 7.10 (t, $^3J$ = 5.5 Hz, 1 H, amide-NH), 7.25 – 7.30 (m, 5 H, arom. CH), 7.53 (bs, 1 H, triazole-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, Et-ester), 25.9 (–, 1 C, $C^2$), 28.4 (+, Boc-$CH_3$), 28.5 (+, Boc-$CH_3$), 30.7 (–, 1 C, $C^6$), 32.3 (–, 1 C, $C^3$), 35.0 (–, 1 C, $C^4$), 37.5 (–, 1 C, $C^7$), 47.6 (–, 1 C, $C^5$), 50.3 (–, broad signal, 16 C, cyclen), 52.9 (–, 2 C, $C^1$), 60.7 (–, 2 C, Et-ester), 64.1 (+, 1 C, C*H), 66.4 (–, 1 C, Bn-$CH_2$), 79.7 ($C_{quat}$, 4 C, Boc), 79.9 ($C_{quat}$, 2 C, Boc), 122.3 (+, 1 C, triazole), 128.2 (+, 2 C, arom.), 128.3 (+,

1 C, arom.), 128.6 (+, 2 C, arom.), 135.6 ($C_{quat}$, 1 C, arom.), 145.1 ($C_{quat}$, 1 C, triazole), 156.3 ($C_{quat}$, 6 C, urethane), 165.3 ($C_{quat}$, 1 C, triazine), 166.1 (very broad signal, 2 C, triazine), 171.5 ($C_{quat}$, 2 C, Et-ester), 172.0 ($C_{quat}$, 1 C, Bn-ester), 172.9 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 1589.6 [$(M+Na^+)^+$] (7), 1567.6 [$MH^+$] (37), 784.2 [$(M+2H^+)^{2+}$] (100); **EA** ($C_{75}H_{123}N_{17}O_{19}$) calc.: C 57.49, H 7.91, N 15.20, found: C 57.11, H 8.08, N 15.05; **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3387, 2974, 2933, 1740, 1691, 1539, 1410, 1366, 1165, 1028, 777; **MF**: $C_{75}H_{123}N_{17}O_{19}$; **MW** = 1566.90 g/mol;

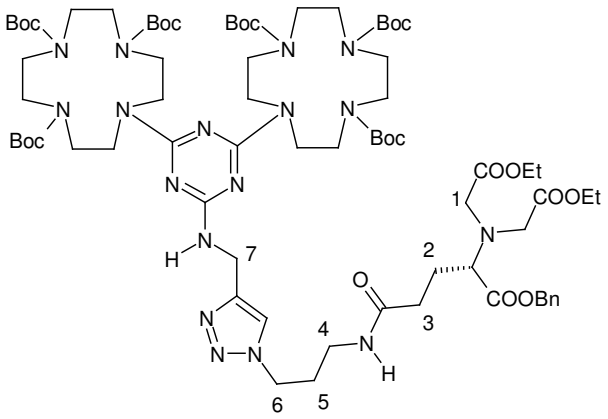



2-(Bis-ethoxycarbonylmethyl-amino)-4-[2-(4-{[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethylcarbamoyl]-butyric acid benzyl ester (**81**):

Synthesis followed **GP 9** using **22** (901 mg, 0.84 mmol), **36** (400 mg, 0.84 mmol), sodium ascorbate (332 mg, 1.68 mmol) and $CuSO_4$ x 5 $H_2O$ (209 mg, 0.84 mmol). This yielded 1249 mg of **81** (0.81 mmol, 96 %) as colourless solid. $R_f$ (DCM:MeOH = 95:5) = 0.30.

**Mp**: 110 °C; **$^1$H-NMR** (400 MHz, $CDCl_3$): δ [ppm] = 1.16 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.36 (bs, 36 H, Boc-$CH_3$), 1.39 (bs, 18 H, Boc-$CH_3$), 1.82 – 1.91 (m, 1 H, $C^2H_2$), 1.99 – 2.08 (m, 1 H, $C^2H_2$), 2.30 (ddd, $^2J$ = 14.0 Hz, $^3J$ = 5.9 Hz, $^3J$ = 5.9 Hz, 1 H, $C^3H_2$), 2.40 (ddd, $^2J$ = 14.0 Hz, $^3J$ = 9.0 Hz, $^3J$ = 5.9 Hz, 1 H, $C^3H_2$), 3.20 – 3.70 (m, 39 H, C*H + $C^4H_2$ + $C^1H_2$ + cyclen-$CH_2$), 4.03 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.37 – 4.41 (m, 2 H, $C^5H_2$), 4.52 – 4.55 (m, 2 H, $C^6H_2$), 5.03 (d, $^2J$ = 12.3 Hz, 1 H, Bn-$CH_2$), 5.07 (d, $^2J$ = 12.3 Hz, 1 H, Bn-$CH_2$), 5.23 – 5.29 (m, 1 H, NH), 6.95 (t, $^3J$ = 5.5 Hz, 1 H, amide-NH), 7.23 – 7.31 (m, 5 H, arom. CH), 7.63 (bs, 1 H, triazole-CH); **$^{13}$C-NMR** (100.6 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, Et-ester), 25.7 (–, 1 C, $C^2$), 28.4 (+, Boc-$CH_3$), 28.5 (+, Boc-$CH_3$), 32.3 (–, 1 C, $C^3$), 36.3 (–, 1 C, $C^6$), 39.4 (–,

1 C, $C^4$), 49.4 (–, 1 C, $C^5$), 50.2 (–, broad signal, 16 C, cyclen), 53.0 (–, 2 C, $C^1$), 60.7 (–, 2 C, Et-ester), 64.0 (+, 1 C, C*H), 66.5 (–, 1 C, Bn-$CH_2$), 79.7 ($C_{quat}$, 4 C, Boc), 79.8 ($C_{quat}$, 2 C, Boc), 122.4 (+, 1 C, triazole), 128.2 (+, 2 C, arom.), 128.3 (+, 1 C, arom.), 128.6 (+, 2 C, arom.), 135.6 ($C_{quat}$, 1 C, arom.), 146.0 ($C_{quat}$, 1 C, triazole), 156.3 ($C_{quat}$, 6 C, urethane), 165.6 ($C_{quat}$, 1 C, triazine), 166.1 ($C_{quat}$, very broad signal, 2 C, triazine), 171.5 ($C_{quat}$, 2 C, Et-ester), 172.0 ($C_{quat}$, 1 C, Bn-ester), 173.3 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 1553.5 [$MH^+$] (40), 777.3 [$(M+2H^+)^{2+}$] (100); **EA** ($C_{74}H_{121}N_{17}O_{19}$) calc.: C 57.24, H 7.85, N 15.33, found: C 56.94, H 8.09, N 15.07; **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3389, 2973, 2933, 1741, 1691, 1538, 1410, 1366, 1166, 1029, 777; **MF**: $C_{74}H_{121}N_{17}O_{19}$; **MW** = 1552.88 g/mol;

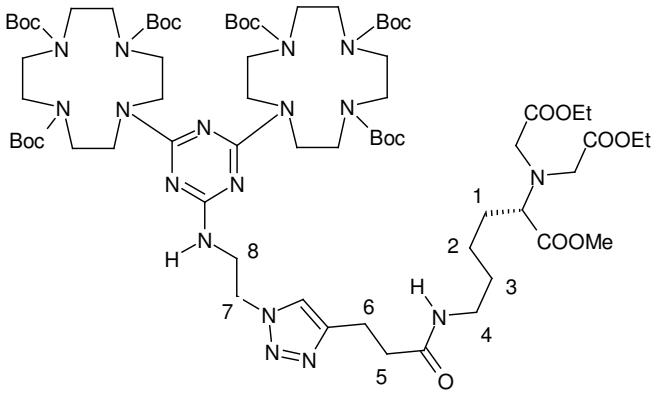



2-(Bis-ethoxycarbonylmethyl-amino)-4-[3-(4-{[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propylcarbamoyl]-butyric acid benzyl ester (**82**):

Synthesis followed **GP 9** using **22** (1009 mg, 0.94 mmol), **37** (452 mg, 0.94 mmol), sodium ascorbate (372 mg, 1.88 mmol) and $CuSO_4$ x 5 $H_2O$ (234 mg, 0.94 mmol). This yielded 1371 mg of **82** (0.87 mmol, 93 %) as colourless solid. $R_f$ (DCM:MeOH = 95:5) = 0.31.

**Mp**: 110 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.16 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.36 (bs, 36 H, Boc-$CH_3$), 1.38 (bs, 18 H, Boc-$CH_3$), 1.82 – 1.94 (m, 1 H, $C^2H_2$), 1.96 – 2.11 (m, 3 H, $C^2H_2$ + $C^5H_2$), 2.28 – 2.45 (m, 2 H, $C^3H_2$), 3.06 – 3.64 (m, 39 H, C*H + $C^4H_2$ + $C^1H_2$ + cyclen-$CH_2$), 4.04 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.30 (t, $^3J$ = 6.9 Hz, 2 H, $C^6H_2$), 4.52 – 4.56 (m, 2 H, $C^7H_2$), 5.01 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 5.06 (d, $^2J$ = 12.4 Hz, 1 H, Bn-$CH_2$), 5.16 – 5.24 (m, 1 H, NH), 6.83 (t, $^3J$ = 5.9 Hz, 1 H, amide-NH), 7.21 – 7.31 (m, 5 H, arom. CH), 7.66 (bs, 1 H, triazole-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 2 C, Et-ester), 25.9 (–, 1 C, $C^2$), 28.4

(+, Boc-$CH_3$), 28.5 (+, Boc-$CH_3$), 30.3 (–, 1 C, $C^5$), 32.4 (–, 1 C, $C^3$), 36.2 (–, 2 C, $C^4$ + $C^7$), 47.6 (–, 1 C, $C^6$), 50.2 (–, broad signal, 16 C, cyclen), 52.9 (–, 2 C, $C^1$), 60.7 (–, 2 C, Et-ester), 64.1 (+, 1 C, C*H), 66.5 (–, 1 C, Bn-$CH_2$), 79.7 ($C_{quat}$, 4 C, Boc), 79.8 ($C_{quat}$, 2 C, Boc), 122.3 (+, 1 C, triazole), 128.3 (+, 2 C, arom.), 128.3 (+, 1 C, arom.), 128.6 (+, 2 C, arom.), 135.5 ($C_{quat}$, 1 C, arom.), 145.8 ($C_{quat}$, 1 C, triazole), 156.3 ($C_{quat}$, 6 C, urethane), 165.5 ($C_{quat}$, 1 C, triazine), 166.6 ($C_{quat}$, broad signal, 2 C, triazine), 171.5 ($C_{quat}$, 2 C, Et-ester), 172.0 ($C_{quat}$, 1 C, Bn-ester), 173.2 ($C_{quat}$, 1 C, amide); **MS** (ESI, DCM/MeOH): m/z (%) = 1576.6 [$MH^+$] (45), 784.2 [$(M+2H^+)^{2+}$] (100); **EA** ($C_{75}H_{123}N_{17}O_{19}$) calc.: C 57.49, H 7.91, N 15.20, found: C 57.15, H 8.07, N 15.01; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3387, 2974, 2933, 1740, 1691, 1539, 1410, 1366, 1165, 1028, 777; **MF**: $C_{75}H_{123}N_{17}O_{19}$; **MW** = 1566.90 g/mol;

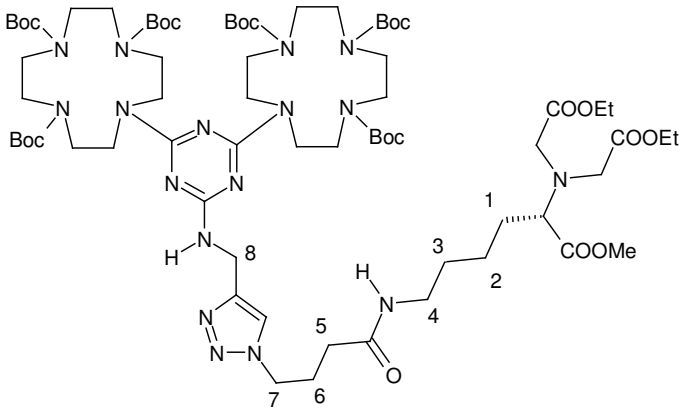



2-(Bis-ethoxycarbonylmethyl-amino)-6-[3-(1-{2-[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1H-[1,2,3]triazol-4-yl)-propionylamino]-hexanoic acid methyl ester (**83**):

Synthesis followed **GP 9** using **16** (1218 mg, 1.10 mmol), **26** (454 mg, 1.10 mmol), sodium ascorbate (463 mg, 2.20 mmol) and $CuSO_4$ x 5 $H_2O$ (275 mg, 1.10 mmol). This yielded 1519 mg of **83** (1.00 mmol, 91 %) as colourless solid. $R_f$ (DCM:MeOH = 95:5) = 0.31.

**Mp**: 108 °C; **$^1$H-NMR** (400 MHz, $CDCl_3$): δ [ppm] = 1.22 (t, $^3$J = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.37 – 1.50 (m, 58 H, $C^2H_2$, $C^3H_2$, Boc-$CH_3$), 1.62 – 1.68 (m, 2 H, $C^1H_2$), 2.57 (t, $^3$J = 7.3 Hz, 2 H, $C^5H_2$), 3.00 (t, $^3$J = 7.3 Hz, 2 H, $C^6H_2$), 3.12 – 3.23 (m, 2 H, $C^4H_2$), 3.23 – 3.74 (m, 33 H, C*H + Cyclen-$CH_2$), 3.576 (s, 2 H, N-$CH_2$), 3.583 (s, 2 H, N-$CH_2$), 3.64 (s, 3 H, Me-ester-$CH_3$), 3.76 – 3.81 (m, 2 H, $C^8H_2$), 4.10 (q, $^3$J = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.46 – 4.49 (m, 2 H, $C^7H_2$), 4.88 – 4.93 (m, 1 H, NH), 6.29 – 6.31 (m, 1 H, amide-NH), 7.50 (bs, 1 H, triazole-CH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$):

δ [ppm] = 14.2 (+, 2 C, Et-ester-$CH_3$), 21.6 (–, 1 C, $C^6$), 23.0 (–, 1 C, $C^2$), 28.5 (+, 18 C, Boc-$CH_3$), 28.7 (–, 1 C, $C^3$), 29.8 (–, 1 C, $C^1$), 35.6 (–, 1 C, $C^5$), 39.2 (–, 1 C, $C^4$), 41.0 (–, 1 C, $C^8$), 49.8 (–, 1 C, $C^7$), 50.3 (–, 16 C, cyclen), 51.4 (+, 1 C, Me-ester), 52.7 (–, 2 C, N-$CH_2$), 60.6 (–, 2 C, Et-ester), 64.4 (+, 1 C, C*H), 79.8 ($C_{quat}$, 4 C, Boc), 80.0 ($C_{quat}$, 2 C, Boc), 122.3 (+, 1 C, triazole), 146.7 ($C_{quat}$, 1 C, triazole), 156.5 ($C_{quat}$, 6 C, urethane), 165.7 ($C_{quat}$, 1 C, triazine), 166.6 (very broad signal, $C_{quat}$, 2 C, triazine), 171.4 ($C_{quat}$, 2 C, Et-ester), 172.0 ($C_{quat}$, 1 C, amide), 173.1 ($C_{quat}$, 1 C, Me-ester); **MS** (ESI, DCM/MeOH): m/z (%) = 1541.3 [$(M+Na^+)^+$] (20), 1519.3 [$MH^+$] (60), 760.2 [$(M+2H^+)^{2+}$] (100), 710.2 [$(M+3H^+-CO_2-(C_4H_8)^+)^{2+}$] (60), 660.2 [$(M+4H^+-2CO_2-2(C_4H_8)^+)^{2+}$] (40); **EA** ($C_{71}H_{123}N_{17}O_{19}$) calc.: C 56.15, H 8.16, N 15.68, found: C 56.05, H 8.21, N 15.60; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3391, 2978, 2935, 1739, 1690, 1538, 1411, 1366, 1166, 1028; **MF**: $C_{71}H_{123}N_{17}O_{19}$; **MW** = 1518.86 g/mol;

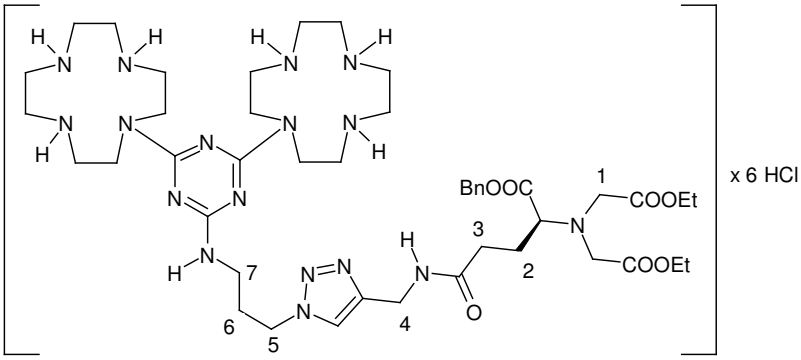



2-(Bis-ethoxycarbonylmethyl-amino)-6-[4-(4-{[4,6-bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-butyrylamino]-hexanoic acid methyl ester (**84**):

Synthesis followed **GP 9** using **22** (749 mg, 0.70 mmol), **79** (309 mg, 0.70 mmol), sodium ascorbate (276 mg, 1.39 mmol) and $CuSO_4$ x 5 $H_2O$ (174 mg, 0.70 mmol). This yielded 953 mg of **84** (0.63 mmol, 90 %) as colourless solid. $R_f$ (DCM:MeOH = 95:5) = 0.31.

**Mp**: 108 °C; **$^1$H-NMR** (400 MHz, $CDCl_3$): δ [ppm] = 1.19 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.37 – 1.52 (m, 58 H, $C^2H_2$, $C^3H_2$, Boc-$CH_3$), 1.61 – 1.67 (m, 2 H, $C^1H_2$), 2.12 – 2.18 (m, 4 H, $C^5H_2$, $C^6H_2$), 3.11 – 3.73 (m, 35 H, C*H, $C^4H_2$, cyclen-$CH_2$), 3.546 (s, 2 H, N-$CH_2$), 3.552 (s, 2 H, N-$CH_2$), 3.62 (s, 3 H, Me-ester-$CH_3$), 4.07 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.34 (t, $^3J$ = 6.1 Hz, 2 H, $C^7H_2$), 4.56 – 4.58 (m, 2 H, $C^8H_2$), 5.20 – 5.26 (m, 1 H, NH), 6.35 – 6.38 (m, 1 H, amide-NH), 7.63 (bs, 1 H, triazole); **$^{13}$C-NMR**

(100.6 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 2 C, Et-ester-$CH_3$), 22.9 (–, 1 C, $C^2$), 26.3 (–, 1 C, $C^6$), 28.4 – 28.5 (19 C, $C^3$ and Boc-$CH_3$), 29.7 (–, 1 C, $C^1$), 32.6 (–, 1 C, $C^5$), 36.3 (–, 1 C, $C^8$), 39.2 (–, 1 C, $C^4$), 49.3 (–, 1 C, $C^7$), 50.2 (–, 16 C, cyclen), 51.4 (+, 1 C, Me-ester), 52.7 (–, 2 C, N-$CH_2$), 60.6 (–, 2 C, Et-ester), 64.3 (+, 1 C, C*H), 79.8 ($C_{quat}$, 4 C, Boc), 79.8 ($C_{quat}$, 2 C, Boc), 122.0 (+, 1 C, triazole), 146.1 ($C_{quat}$, 1 C, triazole), 156.3 ($C_{quat}$, 6 C, urethane), 165.6 ($C_{quat}$, 1 C, triazine), 166.4 (broad signal, $C_{quat}$, 2 C, triazine), 171.4 ($C_{quat}$, 2 C, Et-ester), 171.5 ($C_{quat}$, 1 C, amide), 173.1 ($C_{quat}$, 1 C, Me-ester); **MS** (ESI, DCM/MeOH): m/z (%) = 1519.4 [$MH^+$] (22), 760.1 [$(M+2H^+)^{2+}$] (100), 710.2 [$(M+3H^+-CO_2-(C_4H_8)^+)^{2+}$] (20); **EA** ($C_{71}H_{123}N_{17}O_{19}$) calc.: C 56.15, H 8.16, N 15.68, found: C 56.01, H 8.23, N 15.57; **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3390, 2978, 2934, 1739, 1689, 1538, 1411, 1366, 1166, 1028; **MF**: $C_{71}H_{123}N_{17}O_{19}$; **MW** = 1518.86 g/mol;

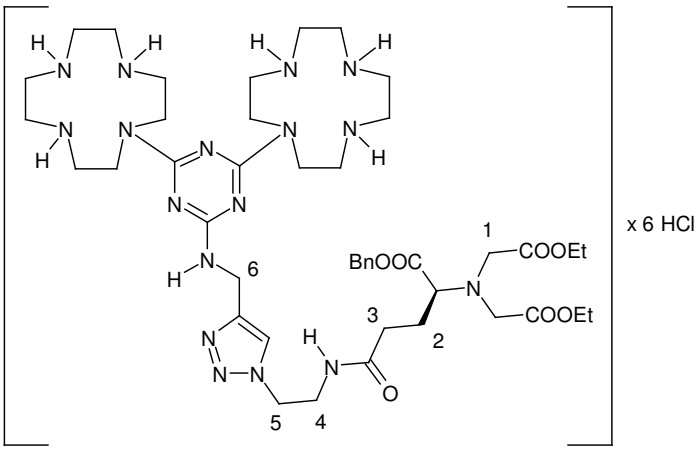



2-(Bis-ethoxycarbonylmethyl-amino)-4-[(1-{3-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-carbamoyl]-butyric acid benzyl ester hexa-hydrochloride (**85**):

Synthesis followed **GP 10** using **80** (625 mg, 0.40 mmol) and 16 ml of HCl/$Et_2O$. This yielded 474 mg of **85** (0.40 mmol, quant.) as colourless hygroscopic solid.

**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.01 (t, $^3$J = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.79 – 1.92 (m, 2 H, $C^2H_2$), 2.04 (tt, $^3$J = 6.7 Hz, 2 H, $C^6H_2$), 2.26 (t, $^3$J = 6.9 Hz, 2 H, $C^3H_2$), 3.05 – 3.35 (m, 26 H, $C^7H_2$ + cyclen-$CH_2$), 3.51 (t, $^3$J = 7.6 Hz, 1 H, C*H), 3.57 (s, 4 H, $C^1H_2$), 3.67 – 3.79 (m, 8 H, cyclen-$CH_2$), 3.93 (q, $^3$J = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.25 (s, 2 H, $C^4H_2$), 4.32 (t, $^3$J = 6.7 Hz, 2 H, $C^5H_2$), 4.90 – 4.98 (m, 2 H, Bn-$CH_2$), 7.16 – 7.21 (m, 5 H, arom. CH), 7.83 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 13.3 (+, 2 C, Et-ester), 24.7 (–, 1 C, $C^2$), 28.7 (–, 1 C, $C^6$), 31.6 (–, 1 C, $C^3$), 34.0 (–, 1 C, $C^4$), 37.8 (–, 1 C, $C^7$), 44.3 (–, broad signal, 8 C, cyclen), 46.4

(–, 4 C, cyclen), 48.1 (–, broad signal, 4 C, cyclen), 48.4 (–, 1 C, $C^5$), 53.2 (–, 2 C, $C^1$), 62.3 (–, 2 C, Et-ester), 64.6 (+, 1 C, C*H), 67.4 (–, 1 C, Bn-$CH_2$), 124.5 (+, 1 C, triazole), 128.3 (+, 2 C, arom.), 128.4 (+, 1 C, arom.), 128.8 (+, 2 C, arom.), 135.0 ($C_{quat}$, 1 C, arom.), 144.0 ($C_{quat}$, 1 C, triazole), 155.2 ($C_{quat}$, 2 C, triazine), 163.4 (broad signal, 1 C, triazine), 171.9 ($C_{quat}$, 2 C, Et-ester), 172.1 ($C_{quat}$, 1 C, Bn-ester), 174.9 ($C_{quat}$, 1 C, amide); **MS** (ESI, MeCN/$H_2O$): m/z (%) = 966.7 [$MH^+$] (4), 483.9 [$(M+2H^+)^{2+}$] (100), 323.0 [$(M+3H^+)^{3+}$] (16); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3431, 2961, 2938, 2810, 1736, 1651, 1610, 1531, 1426, 1340, 751; **MF**: $C_{45}H_{81}N_{17}O_7Cl_6$; **MW** = 1184.96 g/mol;

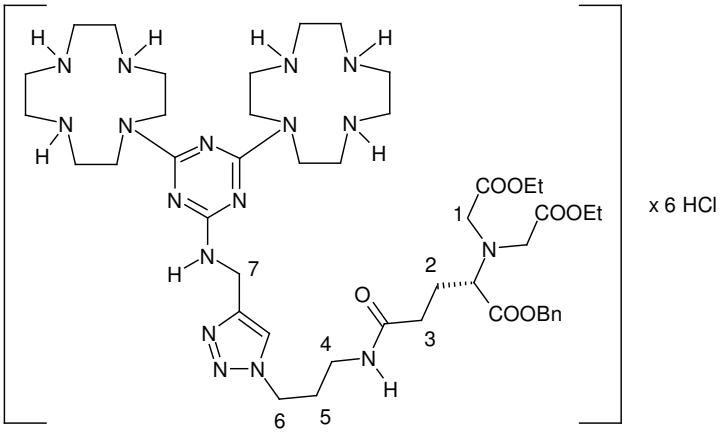



2-(Bis-ethoxycarbonylmethyl-amino)-4-[2-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethylcarbamoyl]-butyric acid benzyl ester hexa-hydrochloride (**86**):

Synthesis followed **GP 10** using **81** (625 mg, 0.40 mmol) and 16 ml of HCl/$Et_2O$. This yielded 471 mg of **86** (0.40 mmol, quant.) as colourless hygroscopic solid.

**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.04 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.70 – 1.82 (m, 2 H, $C^2H_2$), 2.14 (t, $^3J$ = 7.1 Hz, 1 H, $C^3H_2$), 3.04 – 3.31 (m, 24 H, cyclen-$CH_2$), 3.43 – 3.49 (m, 3 H, C*H + $C^4H_2$), 3.59 (s, 4 H, $C^1H_2$), 3.72 – 3.81 (m, 8 H, cyclen-$CH_2$), 3.96 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.32 – 4.36 (m, 2 H, $C^5H_2$), 4.55 (s, 2 H, $C^6H_2$), 4.95 – 5.01 (m, 2 H, Bn-$CH_2$), 7.21 – 7.24 (m, 5 H, arom. CH), 7.86 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 13.3 (+, 2 C, Et-ester), 24.7 (–, 1 C, $C^2$), 31.7 (–, 1 C, $C^3$), 35.8 (–, 1 C, $C^6$), 39.0 (–, 1 C, $C^4$), 43.9 – 44.3 (–, broad signal, 8 C, cyclen), 46.1 (–, 4 C, cyclen), 47.7 (–, 2 C, cyclen), 48.2 (–, 2 C, cyclen), 49.8 (–, 1 C, $C^5$), 53.2 (–, 2 C, $C^1$), 62.3 (–, 2 C, Et-ester), 64.6 (+, 1 C, C*H), 67.5 (–, 1 C, Bn-$CH_2$), 124.3 (+, 1 C, triazole), 128.4 (+, 2 C, arom.), 128.8

(+, 1 C, arom.), 128.8 (+, 2 C, arom.), 135.1 ($C_{quat}$, 1 C, arom.), 144.2 ($C_{quat}$, 1 C, triazole), 155.5 ($C_{quat}$, 2 C, triazine), 163.6 (broad signal, $C_{quat}$, 1 C, triazine), 172.0 ($C_{quat}$, 2 C, Et-ester), 172.1 ($C_{quat}$, 1 C, Bn-ester), 175.1 ($C_{quat}$, 1 C, amide); **MS** (ESI, MeCN/$H_2O$): m/z (%) = 952.6 [$MH^+$] (7), 476.9 [$(M+2H^+)^{2+}$] (100); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3433, 2961, 2939, 2810, 1736, 1651, 1611, 1531, 1426, 1340, 752; **MF**: $C_{44}H_{79}N_{17}O_7Cl_6$; **MW** = 1170.94 g/mol;

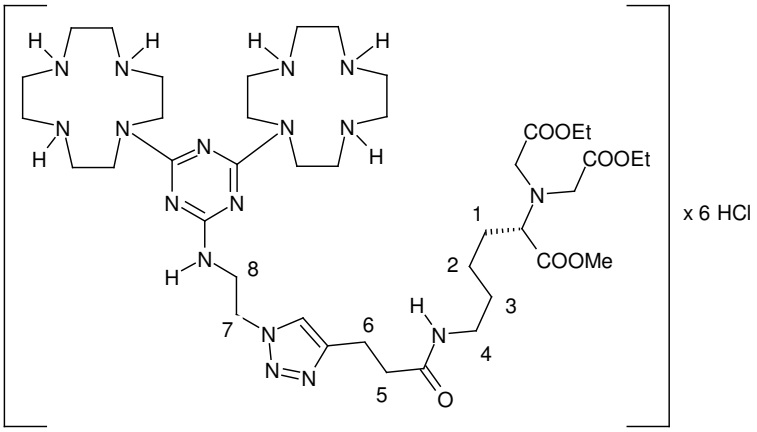



2-(Bis-ethoxycarbonylmethyl-amino)-4-[3-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propylcarbamoyl]-butyric acid benzyl ester hexa-hydrochloride (**87**):

Synthesis followed **GP 10** using **82** (675 mg, 0.43 mmol) and 17 ml of HCl/$Et_2O$. This yielded 510 mg of **87** (0.43 mmol, quant.) as colourless hygroscopic solid.

**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.05 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.75 – 1.87 (m, 2 H, $C^2H_2$), 1.88 – 2.04 (m, 2 H, $C^5H_2$), 2.17 (t, $^3J$ = 7.0 Hz, 2 H, $C^3H_2$), 3.03 – 3.50 (m, 27 H, C*H + $C^4H_2$ + cyclen-$CH_2$), 3.58 (s, 4 H, $C^1H_2$), 3.70 – 3.82 (m, 8 H, cyclen-$CH_2$), 3.95 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.25 – 4.30 (m, 2 H, $C^6H_2$), 4.55 (s, 2 H, $C^7H_2$), 4.92 – 4.99 (m, 2 H, Bn-$CH_2$), 7.18 – 7.22 (m, 5 H, arom. CH), 7.85 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 13.3 (+, 2 C, Et-ester), 25.0 (–, 1 C, $C^2$), 29.4 (–, 1 C, $C^5$), 31.7 (–, 1 C, $C^3$), 35.5 (–, 2 C, $C^4$), 35.7 (–, 2 C, $C^7$), 43.9 – 44.4 (–, broad signal, 8 C, cyclen), 46.2 (–, 4 C, cyclen), 47.6 (–, 2 C, cyclen), 48.1 (–, 2 C, cyclen), 48.4 (–, 1 C, $C^6$), 53.2 (–, 2 C, $C^1$), 62.3 (–, 2 C, Et-ester), 64.6 (+, 1 C, C*H), 67.4 (–, 1 C, Bn-$CH_2$), 124.5 (+, 1 C, triazole), 128.3 (+, 2 C, arom.), 128.4 (+, 1 C, arom.), 128.7 (+, 2 C, arom.), 135.0 ($C_{quat}$, 1 C, arom.), 144.1 ($C_{quat}$, 1 C, triazole), 155.3 ($C_{quat}$, 2 C, triazine), 163.5 (broad signal, $C_{quat}$, 1 C, triazine), 171.7 ($C_{quat}$, 2 C, Et-ester), 172.0 ($C_{quat}$, 1 C, Bn-ester), 174.9 ($C_{quat}$, 1 C,

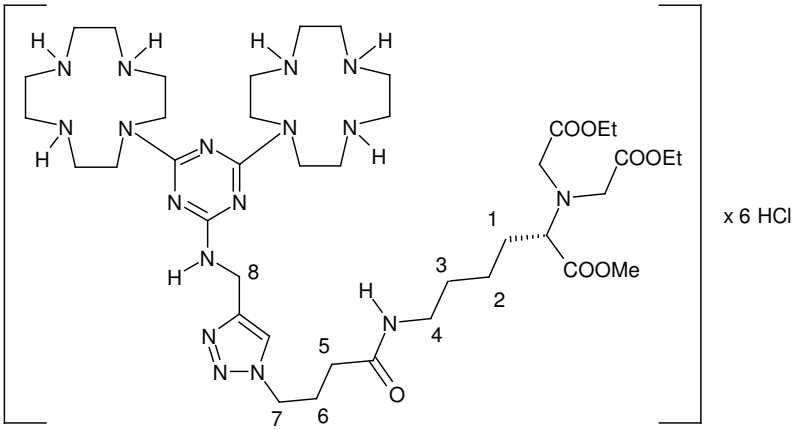
amide); **MS** (ESI, MeCN/$H_2O$): m/z (%) = 966.7 [$MH^+$] (7), 483.9 [$(M+2H^+)^{2+}$] (100), 323.0 [$(M+3H^+)^{3+}$] (10); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3431, 2961, 2938, 2810, 1736, 1651, 1610, 1531, 1426, 1340, 751; **MF**: $C_{45}H_{81}N_{17}O_7Cl_6$; **MW** = 1184.96 g/mol;




2-(Bis-ethoxycarbonylmethyl-amino)-6-[3-(1-{2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1H-[1,2,3]triazol-4-yl)-propionylamino]-hexanoic acid methyl ester hexa-hydrochloride (**88**):

Synthesis followed **GP 10** using **83** (1000 mg, 0.66 mmol) and 26 ml of HCl/$Et_2O$. This yielded 749 mg of **88** (0.66 mmol, quant.) as colourless hygroscopic solid.
**Mp**: > 250 °C; **$^1$H-NMR** (400 MHz, $D_2O$): δ [ppm] = 1.17 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.16 – 1.28 (m, 2 H, $C^2H_2$), 1.33 – 1.40 (m, 2 H, $C^3H_2$), 1.61 – 1.77 (m, 2 H, $C^1H_2$), 2.49 (t, $^3J$ = 7.3 Hz, 2 H, $C^5H_2$), 2.91 (t, $^3J$ = 7.3 Hz, 2 H, $C^6H_2$), 3.03 (t, $^3J$ = 6.8 Hz, 2 H, $C^4H_2$), 3.17 – 3.21 (m, 16 H, cyclen-$CH_2$), 3.28 – 3.32 (m, 4 H, cyclen-$CH_2$), 3.35 – 3.40 (m, 4 H, cyclen-$CH_2$), 3.67 (s, 3 H, Me-ester), 3.69 – 3.73 (m, 1 H, C*H), 3.80 – 3.86 (m, 12 H, cyclen-$CH_2$ + N-$CH_2$), 3.90 – 3.93 (m, 2 H, $C^8H_2$), 4.12 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.60 – 4.62 (m, 2 H, $C^7H_2$), 7.92 (s, 1 H, triazole); **$^{13}$C-NMR** (100.6 MHz, $D_2O$): δ [ppm] = 13.3 (+, 2 C, Et-ester-$CH_3$), 20.7 (–, 1 C, $C^6$), 22.5 (–, 1 C, $C^2$), 28.9 (–, 1 C, $C^3$), 28.2 (–, 1 C, $C^1$), 34.8 (–, 1 C, $C^5$), 38.8 (–, 1 C, $C^4$), 40.7 (–, 1 C, $C^8$), 44.1 (–, 2 C, cyclen), 44.4 (–, 4 C, cyclen), 44.6 (–, 2 C, cyclen), 46.4 (–, 4 C, cyclen), 47.8 (–, 2 C, cyclen), 48.4 (–, 2 C, cyclen), 50.2 (–, 1 C, $C^7$), 52.7 (+, 1 C, Me-ester), 53.5 (–, 2 C, N-$CH_2$), 62.7 (–, 2 C, Et-ester), 65.7 (+, 1 C, C*H), 124.8 (+, 1 C, triazole), 145.8 ($C_{quat}$, 1 C, triazole), 155.7 ($C_{quat}$, 1 C, triazine), 156.1 ($C_{quat}$, 1 C, triazine), 163.4 ($C_{quat}$, 1 C, triazine), 171.2 ($C_{quat}$, 2 C, Et-ester), 172.8 ($C_{quat}$, 1 C), 174.3 ($C_{quat}$, 1 C); **MS** (ESI, MeCN/$H_2O$/TFA): m/z (%) = 918.7 [$MH^+$] (4), 460.0 [$(M+2H^+)^{2+}$] (100), 307.0 [$(M+3H^+)^{3+}$] (30); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] =

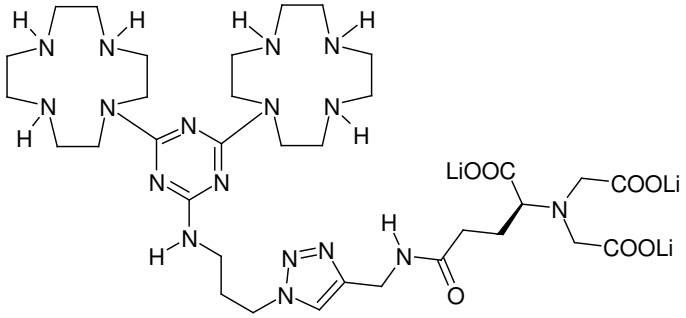
3428, 2964, 2940, 2811, 1734, 1651, 1609, 1530, 1426, 1342; **MF**: $C_{41}H_{81}N_{17}O_7Cl_6$; **MW** = 1136.92 g/mol;




2-(Bis-ethoxycarbonylmethyl-amino)-6-[4-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-butyrylamino]-hexanoic acid methyl ester hexa-hydrochloride (**89**):

Synthesis followed **GP 10** using **84** (790 mg, 0.52 mmol) and 21 ml of HCl/$Et_2O$. This yielded 591 mg of **89** (0.52 mmol, quant.) as colourless hygroscopic solid.

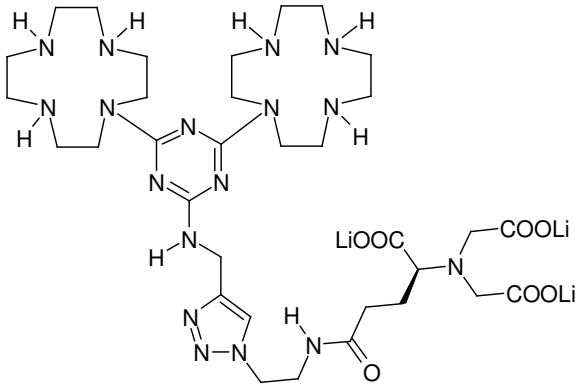
**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 1.12 (t, $^3J$ = 7.1 Hz, 6 H, Et-ester-$CH_3$), 1.23 – 1.42 (m, 4 H, $C^2H_2$ + $C^3H_2$), 1.66 – 1.86 (m, 2 H, $C^1H_2$), 2.00 – 2.15 (m, 4 H, $C^5H_2$ + $C^6H_2$), 2.98 (t, $^3J$ = 6.7 Hz, 2 H, $C^4H_2$), 3.08 – 3.33 (m, 24 H, cyclen-$CH_2$), 3.65 (s, 3 H, Me-ester-$CH_3$), 3.78 – 3.84 (m, 8 H, cyclen-$CH_2$), 3.93 – 3.98 (m, 1 H, C*H), 4.03 – 4.04 (m, 4 H, N-$CH_2$), 4.11 (q, $^3J$ = 7.1 Hz, 4 H, Et-ester-$CH_2$), 4.32 (t, $^3J$ = 6.5 Hz, 2 H, $C^7H_2$), 4.67 (s, 2 H, $C^8H_2$), 7.95 (bs, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 13.3 (+, 2 C, Et-ester-$CH_3$), 22.6 (–, 1 C, $C^2$), 25.8 (–, 1 C, $C^6$), 27.5 (–, 1 C, $C^3$), 27.9 (–, 1 C, $C^1$), 32.5 (–, 1 C, $C^5$), 35.8 (–, 1 C, $C^8$), 38.8 (–, 1 C, $C^4$), 44.4 (–, broad signal, 8 C, cyclen), 46.2 (–, 4 C, cyclen), 47.9 (–, broad signal, 4 C, cyclen), 50.2 (–, 1 C, $C^7$), 53.2 (+, 1 C, Me-ester), 53.9 (–, 2 C, N-$CH_2$), 63.3 (–, 2 C, Et-ester), 66.3 (+, 1 C, C*H), 124.4 (+, 1 C, triazole), 144.1 ($C_{quat}$, 1 C, triazole), 155.6 ($C_{quat}$, 2 C, triazine), 163.8 ($C_{quat}$, very broad signal, 1 C, triazine), 169.3 ($C_{quat}$, 2 C, Et-ester), 171.0 ($C_{quat}$, 1 C), 174.7 ($C_{quat}$, 1 C); **MS** (ESI, MeCN/$H_2O$): m/z (%) = 918.7 [$MH^+$] (5), 460.0 [$(M+2H^+)^{2+}$] (100), 307.0 [$(M+3H^+)^{3+}$] (40); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3428, 2964, 2940, 2811, 1734, 1651, 1609, 1530, 1426, 1342; **MF**: $C_{41}H_{81}N_{17}O_7Cl_6$; **MW** = 1136.92 g/mol;



$Li_3$[2-(Bis-carboxylate-methyl-amino)-4-[(1-{3-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-carbamoyl]-butanoate] (**90**):

Synthesis followed **GP 11** using **85** (730 mg, 0.4 mmol), 7.1 ml of ion-exchanger resin (6.4 mmol) and LiOH (29 mg, 1.2 mmol). This yielded 335 mg of **90** (0.4 mmol, quant.) as colourless frothy solid.

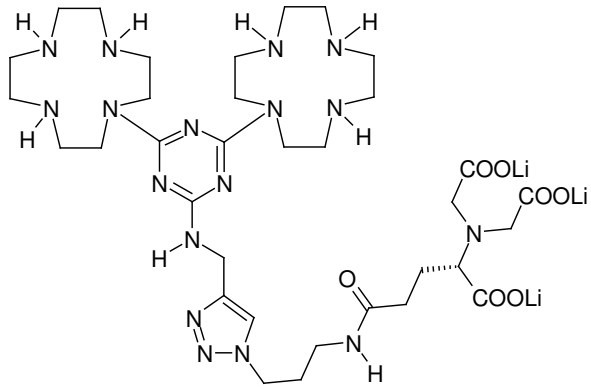
**MS** (pos. ESI, $H_2O$/MeCN/TFA): m/z (%) = 410.8 [$(A^{3-}+5H^+)^{2+}$] (60), 413.8 [$(A^{3-}+Li^+ +4H^+)^{2+}$] (100), 416.8 [$(A^{3-}+2Li^+ +3H^+)^{2+}$] (80), 419.8 [$(A^{3-}+3Li^+ +2H^+)^{2+}$] (40), 820.7 [$(A^{3-}+4H^+)^+$] (55), 826.7 [$(A^{3-}+Li^+ +3H^+)^+$] (40), 832.7 [$(A^{3-}+2Li^+ +2H^+)^+$] (35), 826.7 [$(A^{3-}+3Li^+ +H^+)^+$] (25); **MS** (neg. ESI, $H_2O$/MeCN/TFA): m/z (%) = 932.7 [$(A^{3-}+CF_3COO^- +3H^+)^-$] (100), 938.7 [$(A^{3-}+CF_3COO^- +Li^+ +2H^+)^-$] (25), 1046.7 [$(A^{3-}+2CF_3COO^- +Li^+ +3H^+)^-$] (30), 1052.6 [$(A^{3-}+2CF_3COO^- +2Li^+ +2H^+)^-$] (20), 1058.6 [$(A^{3-}+2CF_3COO^- +3Li^+ +H^+)^-$] (10); **MF**: $C_{34}H_{58}N_{17}O_7Li_3$; **MW** = 837.76 g/mol;



$Li_3$[2-(Bis-carboxylate-methyl-amino)-4-[2-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethylcarbamoyl]-butanoate] (**91**):

Synthesis followed **GP 11** using **86** (471 mg, 0.4 mmol), 7.1 ml of ion-exchanger resin (6.4 mmol) and LiOH (29 mg, 1.2 mmol). This yielded 329 mg of **91** (0.4 mmol, quant.) as colourless frothy solid.

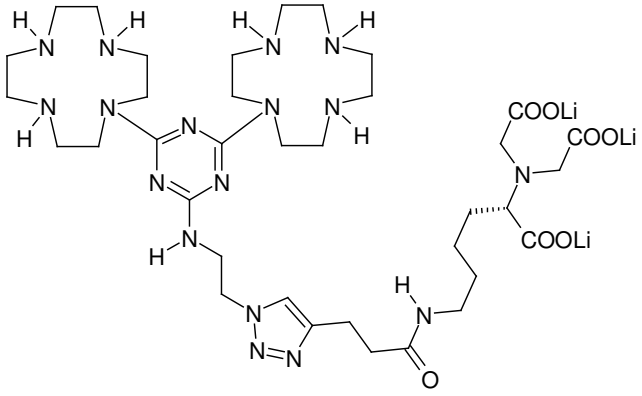
**MS** (pos. ESI, $H_2O$/MeCN/TFA): m/z (%) = 403.8 [$(A^{3-}+5H^+)^{2+}$] (65), 406.8 [$(A^{3-}+Li^+4H^+)^{2+}$] (100), 409.8 [$(A^{3-}+2Li^+3H^+)^{2+}$] (85), 412.8 [$(A^{3-}+3Li^+2H^+)^{2+}$] (35), 806.7 [$(A^{3-}+4H^+)^+$] (50), 812.7 [$(A^{3-}+Li^+3H^+)^+$] (35), 818.7 [$(A^{3-}+2Li^+2H^+)^+$] (30), 824.7 [$(A^{3-}+3Li^+H^+)^+$] (20); **MS** (neg. ESI, $H_2O$/MeCN/TFA): m/z (%) = 918.7 [$(A^{3-}+CF_3COO^-+3H^+)^-$] (100), 924.7 [$(A^{3-}+CF_3COO^-+Li^++2H^+)^-$] (20), 1032.7 [$(A^{3-}+2CF_3COO^-+Li^++3H^+)^-$] (25), 1038.6 [$(A^{3-}+2CF_3COO^-+2Li^++2H^+)^-$] (30), 1044.6 [$(A^{3-}+2CF_3COO^-+3Li^++H^+)^-$] (15); **MF**: $C_{33}H_{56}N_{17}O_7Li_3$; **MW** = 823.74 g/mol;



$Li_3$[2-(Bis-carboxylate-methyl-amino)-4-[3-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propylcarbamoyl]-butanoate] (**92**):

Synthesis followed **GP 11** using **87** (510 mg, 0.43 mmol), 7.6 ml of ion-exchanger resin (6.88 mmol) and LiOH (31 mg, 1.29 mmol). This yielded 360 mg of **92** (0.43 mmol, quant.) as colourless frothy solid.

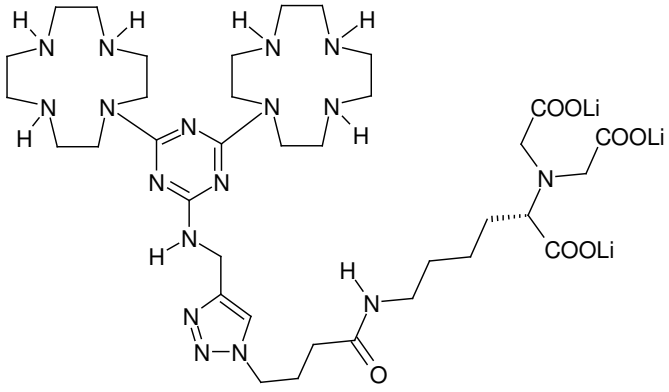
**MS** (pos. ESI, $H_2O$/MeCN/TFA): m/z (%) = 410.8 [$(A^{3-}+5H^+)^{2+}$] (65), 413.8 [$(A^{3-}+Li^+4H^+)^{2+}$] (100), 416.8 [$(A^{3-}+2Li^+3H^+)^{2+}$] (85), 419.8 [$(A^{3-}+3Li^+2H^+)^{2+}$] (35), 820.7 [$(A^{3-}+4H^+)^+$] (50), 826.7 [$(A^{3-}+Li^+3H^+)^+$] (35), 832.7 [$(A^{3-}+2Li^+2H^+)^+$] (30), 826.7 [$(A^{3-}+3Li^+H^+)^+$] (20); **MS** (neg. ESI, $H_2O$/MeCN/TFA): m/z (%) = 932.7 [$(A^{3-}+CF_3COO^-+3H^+)^-$] (100), 938.7 [$(A^{3-}+CF_3COO^-+Li^++2H^+)^-$] (20), 1046.7 [$(A^{3-}+2CF_3COO^-+Li^++3H^+)^-$] (25), 1052.6 [$(A^{3-}+2CF_3COO^-+2Li^++2H^+)^-$] (30), 1058.6 [$(A^{3-}+2CF_3COO^-+3Li^++H^+)^-$] (15); **MF**: $C_{34}H_{58}N_{17}O_7Li_3$; **MW** = 837.76 g/mol;



$Li_3$[2-(Bis-carboxylate-methyl-amino)-6-[3-(1-{2-[4,6-bis-(1,4,7,10tetraaza-cyclo-dodec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1H-[1,2,3]triazol-4-yl)-propionylamino]-hexanoate] (**93**):

Synthesis followed **GP 11** using **88** (749 mg, 0.66 mmol), 11.7 ml of ion-exchanger resin (10.56 mmol) and LiOH (48 mg, 2.00 mmol). This yielded 571 mg of **93** (0.66 mmol, quant.) as colourless frothy solid.

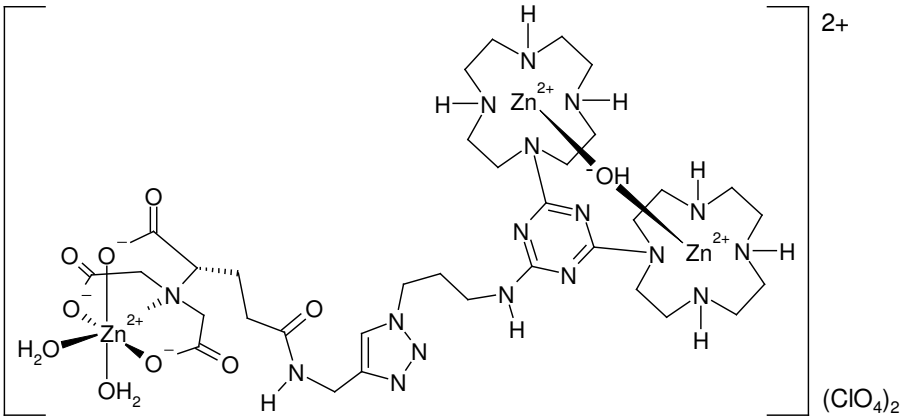
**MS** (pos. ESI, MeCN/$H_2O$): m/z (%) = 424.8 [$(A^{3-}+5H^+)^{2+}$] (15), 427.8 [$(A^{3-}+Li^+ +4H^+)^{2+}$] (25), 430.9 [$(A^{3-}+2Li^+ +3H^+)^{2+}$] (100), 433.9 [$(A^{3-}+3Li^+ +2H^+)^{2+}$] (30), 848.7 [$(A^{3-}+4H^+)^+$] (5), 854.7 [$(A^{3-}+Li^+ +3H^+)^+$] (4), 860.8 [$(A^{3-}+2Li^+ +2H^+)^+$] (4), 866.8 [$(A^{3-}+3Li^+ +H^+)^+$] (3); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 422.9 [$(A^{3-}+H^+)^{2-}$] (20), 846.8 [$(A^{3-}+2H^+)^-$] (100), 852.8 [$(A^{3-}+Li^+ +H^+)^-$] (30), 858.8 [$(A^{3-}+2\ Li^+)^-$] (20); **MF**: $C_{36}H_{62}N_{17}O_7Li_3$; **MW** = 865.82 g/mol;



$Li_3$[2-(Bis-carboxylate-methyl-amino)-6-[4-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)butyrylamino]hexanoate] (**94**):

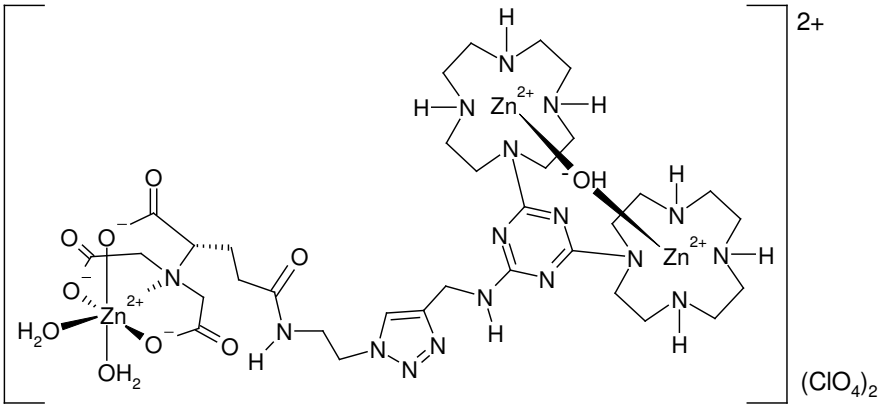
Synthesis followed **GP 11** using **89** (591 mg, 0.52 mmol), 9.2 ml of ion-exchanger resin (8.32 mmol) and LiOH (38 mg, 1.59 mmol). This yielded 450 mg of **94** (0.52 mmol, quant.) as colourless frothy solid.

**MS** (pos. ESI, MeCN/$H_2O$): m/z (%) = 424.8 [$(A^{3-}+5H^+)^{2+}$] (100), 427.8 [$(A^{3-}+Li^+ +4H^+)^{2+}$] (75), 430.9 [$(A^{3-}+2Li^+ +3H^+)^{2+}$] (70), 433.9 [$(A^{3-}+3Li^+ +2H^+)^{2+}$] (20), 848.6 [$(A^{3-}+4H^+)^+$] (15), 854.6 [$(A^{3-}+Li^+ +3H^+)^+$] (10), 860.7 [$(A^{3-}+2Li^+ +2H^+)^+$] (5); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 422.9 [$(A^{3-}+H^+)^{2-}$] (20), 846.8 [$(A^{3-}+2H^+)^-$] (100), 852.8 [$(A^{3-}+Li^+ +H^+)^-$] (30), 858.8 [$(A^{3-}+2\ Li^+)^-$] (20); **MF**: $C_{36}H_{62}N_{17}O_7Li_3$; **MW** = 865.82 g/mol;



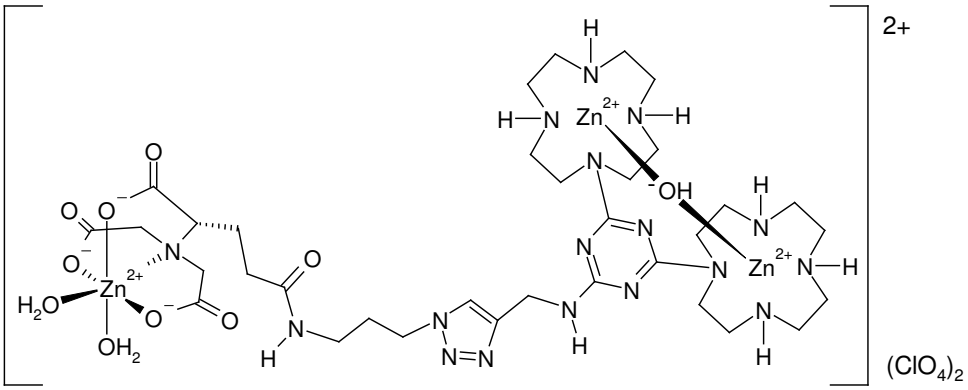

<u>$Zn_3$[2-(Bis-carboxylate-methyl-amino)-4-[(1-{3-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-carbamoyl]-butanoate] te] di-perchlorate hydroxide di-hydrate (**95**):</u>

Synthesis followed **GP 12** using **90** (335 mg, 0.40 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (492 mg, 1.32 mmol). This yielded **95** (364 mg, 0.29 mmol, 72 %) as colourless solid. **Mp**: > 250 °C; **MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 503.8 [$(K^{3+}-H^+)^{2+}$] (100), 512.8 [$(K^{3+}+OH^-)^{2+}$] (70), 553.8 [$(K^{3+}+ClO_4^-)^{2+}$] (20); **UV/Vis** ($H_2O$): λ (lg ε) = 222 nm (4.663); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3422, 3272, 2934, 2889, 2362, 2341, 1596, 1561, 1425, 1347, 1286, 1090, 978, 805; **MF**: $[C_{34}H_{58}N_{17}O_7Zn_3]^{3+}(ClO_4)_2(OH)$ x 2 $H_2O$; **MW** = 1265.02 g/mol;



$Zn_3$[2-(Bis-carboxylate-methyl-amino)-4-[3-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethylcarbamoyl]-butanoate] di-perchlorate hydroxide di-hydrate (**96**):

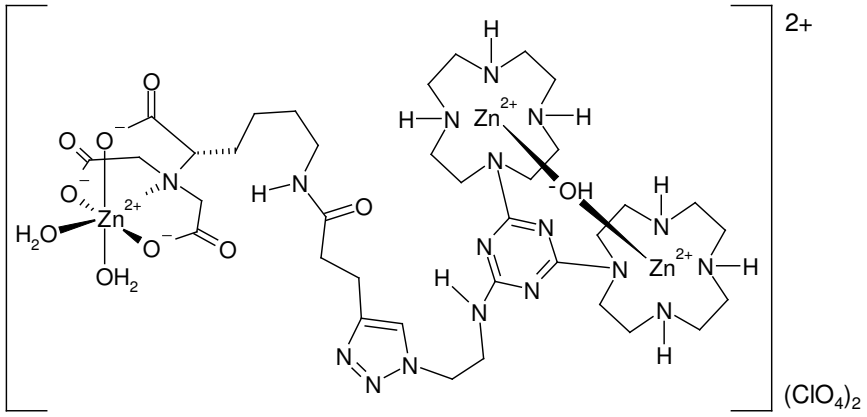
Synthesis followed **GP 12** using **91** (329 mg, 0.40 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (492 mg, 1.32 mmol). This yielded **96** (370 mg, 0.30 mmol, 74 %) as colourless solid. **Mp**: > 250 °C; **MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 496.8 $[(K^{3+}\text{-}H^{+})^{2+}]$ (100), 505.8 $[(K^{3+}+OH^{-})^{2+}]$ (25), 992.4 $[(K^{3+}\text{-}2H^{+})^{+}]$ (5), 1092.4 $[(K^{3+}+ClO_4^{-}\text{-}H^{+})^{+}]$ (12); **MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 544.7 $[(K^{3+}+ClO_4^{-}\text{-}4\ H^{+})^{2-}]$ (100); **UV/Vis** ($H_2O$): λ (lg ε) = 222 nm (4.655); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3424, 3273, 2933, 2890, 2361, 2340, 1596, 1562, 1426, 1346, 1286, 1091, 978, 806; **MF**: $[C_{33}H_{56}N_{17}O_7Zn_3]^{3+}(ClO_4)_2(OH)$ x 2 $H_2O$; **MW** = 1251.00 g/mol;



$Zn_3$[2-(Bis-carboxylate-methyl-amino)-4-[3-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propylcarbamoyl]-butanoate] di-perchlorate hydroxide di-hydrate (**97**):

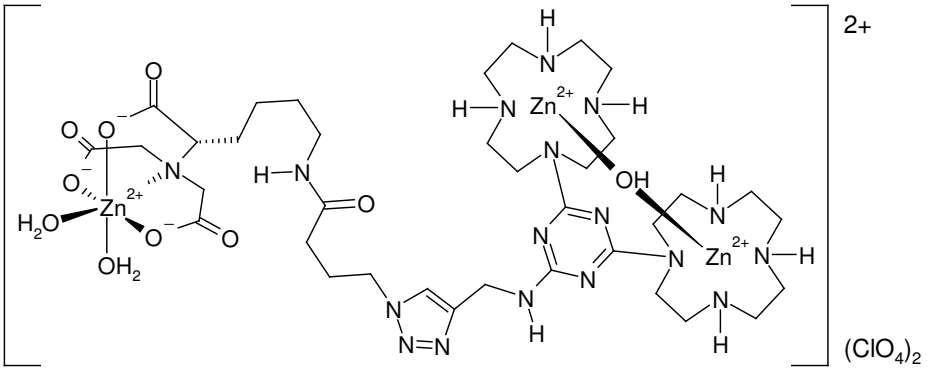
Synthesis followed **GP 12** using **92** (360 mg, 0.43 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (528 mg, 1.42 mmol). This yielded **97** (408 mg, 0.32 mmol, 75 %) as colourless solid.

**Mp**: > 250 °C; **MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 503.8 $[(K^{3+}\text{-}H^{+})^{2+}]$ (100), 512.8 $[(K^{3+}+OH^{-})^{2+}]$ (100), 553.8 $[(K^{3+}+ClO_4^{-})^{2+}]$ (15), 1124.3 $[(K^{3+}+OH^{-}+ClO_4^{-})^{+}]$ (8); **UV/Vis** ($H_2O$): λ (lg ε) = 222 nm (4.653); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3423, 3272, 2934, 2889, 2361, 2341, 1596, 1561, 1426, 1347, 1286, 1090, 978, 805; **MF**: $[C_{34}H_{58}N_{17}O_7Zn_3]^{3+}(ClO_4)_2(OH)$ x 2 $H_2O$; **MW** = 1265.02 g/mol;



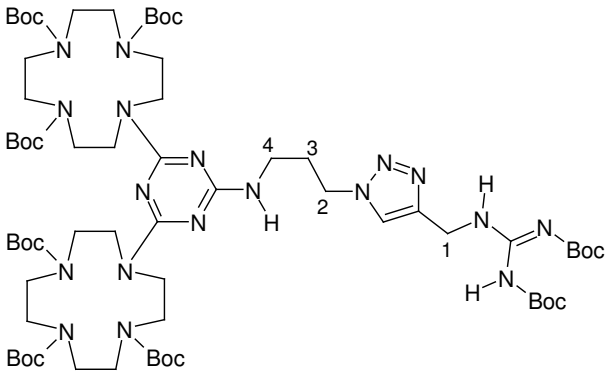

$Zn_3$[2-(Bis-carboxylate-methyl-amino)-6-[3-(1-{2-[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl}-1H-[1,2,3]triazol-4-yl)-propionylamino]-hexanoate] di-perchlorate hydroxide di-hydrate (**98**):

Synthesis followed **GP 12** using **93** (571 mg, 0.66 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (811 mg, 2.18 mmol). This yielded **98** (648 mg, 0.50 mmol, 76 %) as colourless solid. **Mp**: > 250 °C; **MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 517.9 $[(K^{3+}\text{-}H^{+})^{2+}]$ (100), 526.9 $[(K^{3+}+OH^{-})^{2+}]$ (25), 547.9 $[(K^{3+}+CH_3COO^{-})^{2+}]$ (80), 567.9 $[(K^{3+}+ClO_4^{-})^{2+}]$ (30); **UV/Vis** ($H_2O$): λ (lg ε) = 222 nm (4.656); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3423, 3272, 2934, 2889, 2361, 2341, 1596, 1561, 1426, 1347, 1286, 1090, 978, 805; **MF**: $[C_{36}H_{62}N_{17}O_7Zn_3]^{3+}(ClO_4)_2(OH)$ x 2 $H_2O$; **MW** = 1293.08 g/mol;



$Zn_3$[2-(Bis-carboxylate-methyl-amino)-6-[4-(4-{[4,6-bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-butyrylamino]-hexanoate] di-perchlorate hydroxide di-hydrate (**99**):

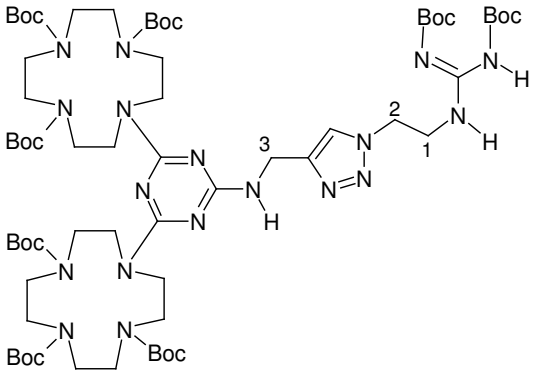
Synthesis followed **GP 12** using **94** (450 mg, 0.52 mmol) and $Zn(ClO_4)_2$ x 6 $H_2O$ (639 mg, 1.72 mmol). This yielded **99** (497 mg, 0.38 mmol, 74 %) as colourless solid. **Mp**: > 250 °C; **MS** (pos. ESI, $H_2O$/MeCN): m/z (%) = 517.9 $[(K^{3+}\text{-}H^{+})^{2+}]$ (100), 526.9 $[(K^{3+}+OH^{-})^{2+}]$ (30), 547.9 $[(K^{3+}+CH_3COO^{-})^{2+}]$ (20), 567.9 $[(K^{3+}+ClO_4^{-})^{2+}]$ (20); **UV/Vis** ($H_2O$): λ (lg ε) = 222 nm (4.658); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3423, 3272, 2934, 2889, 2361, 2341, 1596, 1561, 1426, 1347, 1286, 1090, 978, 805; **MF**: $[C_{36}H_{62}N_{17}O_7Zn_3]^{3+}(ClO_4)_2(OH)$ x 2 $H_2O$; **MW** = 1293.08 g/mol;



*N,N'*-Di-Boc-*N*-(1-{3-[4,6-Bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-guanidine (**100**):

Synthesis followed **GP 13** using **54** (159 mg, 0.54 mmol), **17** (600 mg, 0.54 mmol), sodium ascorbate (212 mg, 1.07 mmol, 2 eq) and $CuSO_4$ x 5 $H_2O$ (134 mg, 0.54 mmol, 1 eq). This yielded 692 mg (0.49 mmol, 91 %) of **100** as a colourless solid. $R_f$ (EE:PE = 4:1) = 0.54.

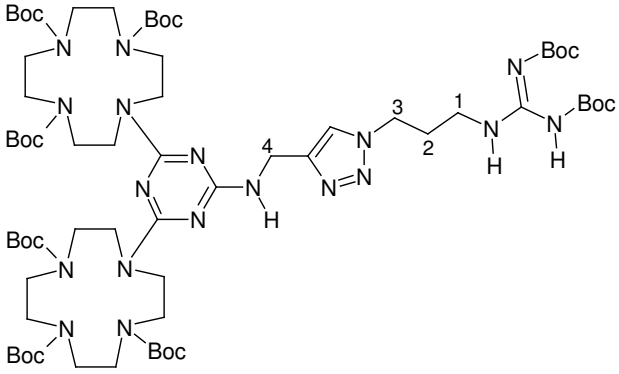
**Mp**: 205 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.36 (bs, 36 H, Boc-$CH_3$), 1.38 (s, 18 H, Boc-$CH_3$), 1.41 (s, 9 H, Boc-$CH_3$), 1.44 (s, 9 H, Boc-$CH_3$), 2.09 (tt, $^3$J = 6.5 Hz, 2 H, $C^3H_2$), 3.24 – 3.59 (m, 34 H, cyclen-$CH_2$ + $C^4H_2$), 4.38 (t, $^3$J = 6.5 Hz, 2 H, $C^2H_2$), 4.63 (d, $^3$J = 5.1 Hz, 2 H, $C^1H_2$), 4.83 – 4.88 (m, 1 H, NH), 7.54 (bs, 1 H, triazole), 8.72 (t, $^3$J = 5.0 Hz, 1 H, NH), 11.38 (s, 1 H, Boc-NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C, Boc-$CH_3$), 28.3 (+, 3 C, Boc-$CH_3$), 28.5 (2 close signals, +, 18 C, Boc-$CH_3$), 30.8 (–, 1 C, $C^3$), 36.5 (–, 1 C, $C^4$), 37.5 (–, 1 C, $C^1$), 47.8 (–, 1 C, $C^2$), 50.3 (–, 16 C, cyclen), 79.3 ($C_{quat}$, 1 C, Boc), 79.7 ($C_{quat}$, 4 C, Boc), 79.9 ($C_{quat}$, 2 C, Boc), 83.2 ($C_{quat}$, 1 C, Boc), 122.3 (+, 1 C, triazole), 143.8 ($C_{quat}$, 1 C, triazole), 152.9 ($C_{quat}$, 1 C, urethane), 156.0 ($C_{quat}$, 1 C, urethane), 156.3 ($C_{quat}$, 6 C, urethane), 163.4 ($C_{quat}$, 1 C, guanidine), 165.9 ($C_{quat}$, 1 C, triazine), 166.4 ($C_{quat}$, 2 C, triazine); **MS** (ESI, DCM/MeOH): m/z (%) = 1418.2 [$MH^+$] (100), 709.7 [$(M+2H^+)^{2+}$] (75), 659.7 [$(M+3H^+-CO_2-(C_4H_8)^+)^{2+}$] (60), 609.7 [$(M+4H^+-2CO_2-2(C_4H_8)^+)^{2+}$] (60), 559.7 [$(M+5H^+-3CO_2-3(C_4H_8)^+)^{2+}$] (40); **EA** ($C_{66}H_{116}N_{18}O_{16}$) calc.: C 55.91, H 8.25, N 17.78, found: C 55.61, H 8.49, N 17.53; **UV/Vis** ($CH_3CN$): λ (lg ε) = 230 nm (4.803); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3437, 3340, 3137, 2974, 2933, 1693, 1632, 1540, 1478, 1412, 1367, 1250, 1165; **MF**: $C_{66}H_{116}N_{18}O_{16}$; **MW** = 1417.76 g/mol;




<u>*N*,*N*'-Di-Boc-*N*-[2-(4-{[4,6-Bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclo-dodec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethyl]-guanidine (**101**):</u>

Synthesis followed **GP 13** using **50** (214 mg, 0.65 mmol), **22** (700 mg, 0.65 mmol), sodium ascorbate (258 mg, 1.30 mmol, 2 eq) and $CuSO_4$ x 5 $H_2O$ (163 mg, 0.65 mmol, 1 eq). This yielded 821 mg (0.58 mmol, 90 %) of **101** as a colourless solid. $R_f$ (EE:PE = 4:1) = 0.53.

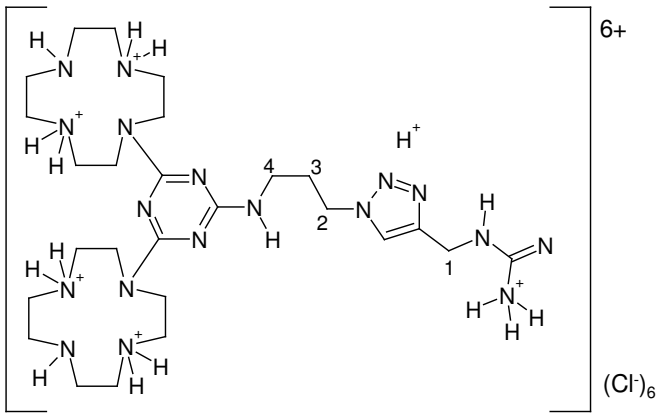
**Mp**: 211 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.36 (bs, 36 H, Boc-$CH_3$), 1.38 (s, 18 H, Boc-$CH_3$), 1.40 (s, 9 H, Boc-$CH_3$), 1.43 (s, 9 H, Boc-$CH_3$), 3.22 – 3.57 (m, 32 H, cyclen-$CH_2$), 3.86 (dt, $^3$J = 5.8 Hz, 2 H, $C^1H_2$), 4.48 (t, $^3$J = 5.9 Hz, 2 H, $C^2H_2$), 4.55 – 4.57 (m, 2 H, $C^3H_2$), 5.14 – 5.23 (m, 1 H, NH), 7.63 (bs, 1 H, triazole), 8.51 (t, $^3$J = 5.6 Hz, 1 H, NH), 11.38 (s, 1 H, Boc-NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C, Boc-$CH_3$), 28.2 (+, 3 C, Boc-$CH_3$), 28.4 (2 close signals, +, 18 C, Boc-$CH_3$), 36.3 (–, 1 C, $C^3$), 40.5 (–, 1 C, $C^1$), 49.1 (–, 1 C, $C^2$), 50.2 (–, 16 C, cyclen), 79.4 ($C_{quat}$, 1 C, Boc), 79.7 ($C_{quat}$, 4 C, Boc), 79.8 ($C_{quat}$, 2 C, Boc), 83.3 ($C_{quat}$, 1 C, Boc), 122.5 (+, 1 C, triazole), 146.0 ($C_{quat}$, 1 C, triazole), 152.8 ($C_{quat}$, 1 C, urethane), 156.3 ($C_{quat}$, 7 C, urethane), 163.3 ($C_{quat}$, 1 C, guanidine), 165.6 ($C_{quat}$, 1 C, triazine), 166.4 ($C_{quat}$, 2 C, triazine); **MS** (ESI, MeCN/$H_2O$): m/z (%) = 1404.2 [$MH^+$] (100), 702.7 [$(M+2H^+)^{2+}$] (40), 652.7 [$(M+3H^+-CO_2-(C_4H_8)^+)^{2+}$] (70), 602.6 [$(M+4H^+-2CO_2-2(C_4H_8)^+)^{2+}$] (90), 552.6 [$(M+5H^+-3CO_2-3(C_4H_8)^+)^{2+}$] (50), 502.5 [$(M+6H^+-4CO_2-4(C_4H_8)^+)^{2+}$] (50); **EA** ($C_{65}H_{114}N_{18}O_{16}$) calc.: C 55.62, H 8.19, N 17.96, found: C 55.37, H 8.43, N 17.69; **UV/Vis** ($CH_3CN$): λ (lg ε) = 230 nm (4.796); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3437, 3340, 3139, 2976, 2936, 1694, 1633, 1541, 1478, 1412, 1367, 1250, 1165; **MF**: $C_{65}H_{114}N_{18}O_{16}$; **MW** = 1403.73 g/mol;




*N,N'*-Di-Boc-*N*-[3-(4-{[4,6-Bis-(4,7,10-tri-tert-butoxycarbonyl-1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propyl]-guanidine (**102**):

Synthesis followed **GP 13** using **51** (172 mg, 0.50 mmol), **22** (541 mg, 0.50 mmol), sodium ascorbate (199 mg, 1.01 mmol, 2 eq) and $CuSO_4$ x 5 $H_2O$ (126 mg, 0.50 mmol, 1 eq). This yielded 654 mg (0.46 mmol, 92 %) of **102** as a colourless solid. $R_f$ (EE:PE = 4:1) = 0.54.

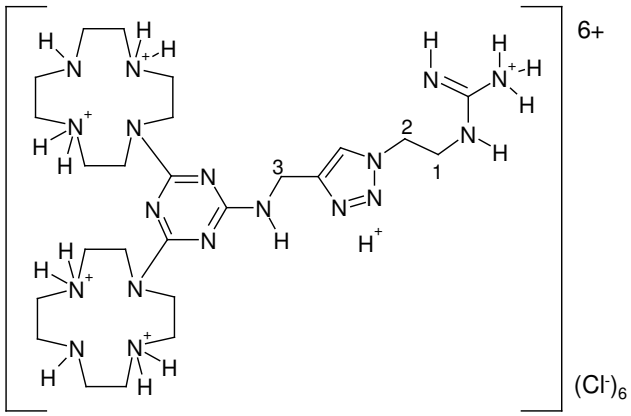
**Mp**: 205 °C; **$^1$H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.35 (bs, 36 H, Boc-$CH_3$), 1.38 (s, 18 H, Boc-$CH_3$), 1.42 (s, 9 H, Boc-$CH_3$), 1.42 (s, 9 H, Boc-$CH_3$), 2.13 (tt, $^3$J = 6.7 Hz, 2 H, $C^2H_2$), 3.24 – 3.56 (m, 34 H, cyclen-$CH_2$ + $C^1H_2$), 4.35 (t, $^3$J = 6.8 Hz, 2 H, $C^3H_2$), 4.54 – 4.56 (m, 2 H, $C^4H_2$), 5.21 – 5.24 (m, 1 H, NH), 7.82 (bs, 1 H, triazole), 8.41 (t, $^3$J = 5.6 Hz, 1 H, NH), 11.41 (s, 1 H, Boc-NH); **$^{13}$C-NMR** (75.5 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C, Boc-$CH_3$), 28.2 (+, 3 C, Boc-$CH_3$), 28.4 (2 close signals, +, 18 C, Boc-$CH_3$), 30.2 (–, 1 C, $C^2$), 36.3 (–, 1 C, $C^4$), 37.5 (–, 1 C, $C^1$), 47.5 (–, 1 C, $C^3$), 50.2 (–, 16 C, cyclen), 79.2 ($C_{quat}$, 1 C, Boc), 79.7 ($C_{quat}$, 4 C, Boc), 79.8 ($C_{quat}$, 2 C, Boc), 83.2 ($C_{quat}$, 1 C, Boc), 122.4 (+, 1 C, triazole), 145.7 ($C_{quat}$, 1 C, triazole), 153.1 ($C_{quat}$, 1 C, urethane), 156.3 ($C_{quat}$, 6 C, urethane), 156.4 ($C_{quat}$, 1 C, urethane), 163.4 ($C_{quat}$, 1 C, guanidine), 165.5 ($C_{quat}$, 1 C, triazine), 166.1 ($C_{quat}$, 2 C, triazine); **MS** (ESI, DCM/MeOH): m/z (%) = 1418.2 [$MH^+$] (80), 709.7 [$(M+2H^+)^{2+}$] (100), 659.7 [$(M+3H^+-CO_2-(C_4H_8)^+)^{2+}$] (70), 609.7 [$(M+4H^+-2CO_2-2(C_4H_8)^+)^{2+}$] (60), 559.7 [$(M+5H^+-3CO_2-3(C_4H_8)^+)^{2+}$] (30), 509.7 [$(M+6H^+-4CO_2-4(C_4H_8)^+)^{2+}$] (25); **EA** ($C_{66}H_{116}N_{18}O_{16}$) calc.: C 55.91, H 8.25, N 17.78, found: C 55.71, H 8.51, N 17.67; **UV/Vis** ($CH_3CN$): λ (lg ε) = 230 nm (4.809); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3437, 3340, 3137, 2974, 2933, 1693, 1632, 1540, 1478, 1412, 1367, 1250, 1165; **MF**: $C_{66}H_{116}N_{18}O_{16}$; **MW** = 1417.76 g/mol;




*N*-(1-{3-[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-guanidinium hexa-hydrochloride (**103**):

Synthesis followed **GP 10** using **100** (612 mg, 0.43 mmol) and 23 ml of HCl-saturated diethylether. This gave **103** as hygroscopic, colourless solid (361 mg, 0.43 mmol, 100 %).

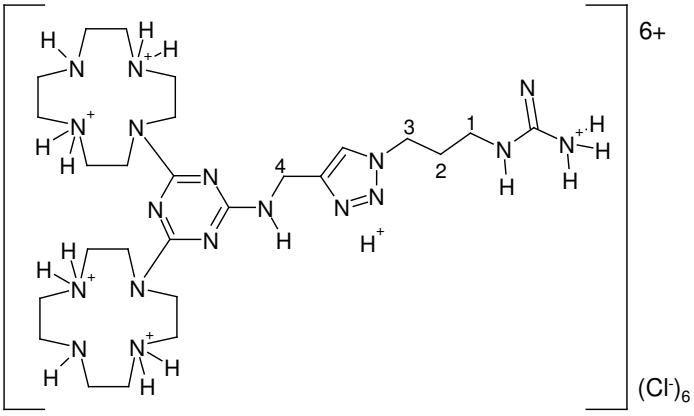
**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 2.14 (tt, $^3J$ = 6.7 Hz, 2 H, $C^3H_2$), 3.14 – 3.35 (m, 24 H, cyclen-$CH_2$), 3.40 (t, $^3J$ = 6.7 Hz, 2 H, $C^4H_2$), 3.78 – 3.82 (m, 8 H, cyclen-$CH_2$), 4.42 (t, $^3J$ = 6.7 Hz, 2 H, $C^2H_2$), 4.42 (s, 2 H, $C^1H_2$), 7.95 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 28.8 (–, 1 C, $C^3$), 36.3 (–, 1 C, $C^4$), 37.9 (–, 1 C, $C^1$), 44.4 (–, 8 C, cyclen), 46.5 (–, 4 C, cyclen), 48.1 (–, 5 C, cyclen + $C^2$), 124.3 (+, 1 C, triazole), 143.1 ($C_{quat}$, 1 C, triazole), 155.5 ($C_{quat}$, 2 C, triazine), 157.0 ($C_{quat}$, 1 C, guanidine), 163.2 (very broad signal, $C_{quat}$, 1 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 309.3 [$(M+2H^+)^{2+}$] (65), 220.2 [$(M+MeCN+3H^+)^{3+}$] (45), 206.6 [$(M+3H^+)^{3+}$] (100); **MF**: $C_{26}H_{58}N_{18}Cl_{16}$; **MW** = 835.59 g/mol;




*N*-[2-(4-{[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethyl]-guanidinium hexa-hydrochloride (**104**):

Synthesis followed **GP 10** using **101** (600 mg, 0.43 mmol) and 23 ml of HCl-saturated diethylether. This gave **104** as hygroscopic, colourless solid (355 mg, 0.43 mmol, 100 %).

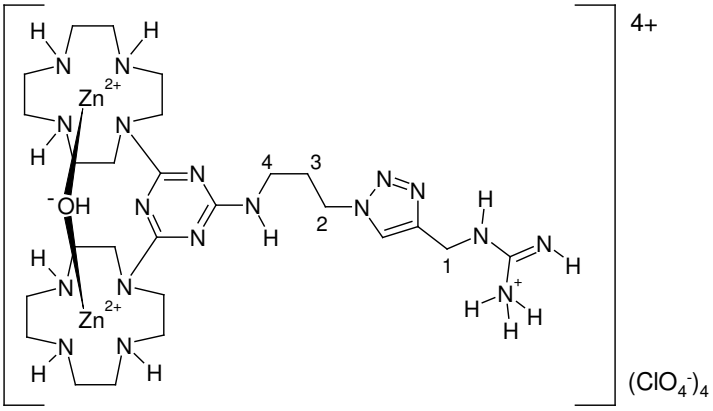
**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 3.12 – 3.33 (m, 24 H, cyclen-$CH_2$), 3.60 (t, $^3J$ = 5.5 Hz, 2 H, $C^1H_2$), 3.80 – 3.85 (m, 8 H, cyclen-$CH_2$), 4.50 (t, $^3J$ = 5.5 Hz, 2 H, $C^2H_2$), 4.67 (s, 2 H, $C^3H_2$), 7.97 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 35.8 (–, 1 C, $C^3$), 41.0 (–, 1 C, $C^1$), 44.4 (–, 8 C, cyclen), 46.3 (–, 4 C, cyclen), 48.1 (–, 4 C, cyclen), 49.4 (–, 1 C, $C^2$), 124.7 (+, 1 C, triazole), 144.6 ($C_{quat}$, 1 C, triazole), 155.6 ($C_{quat}$, 2 C, triazine), 156.9 ($C_{quat}$, 1 C, guanidine), 163.7 (very broad signal, $C_{quat}$, 1 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 302.3 [$(M+2H^+)^{2+}$] (90), 215.6 [$(M+MeCN+3H^+)^{3+}$] (100), 201.9 [$(M+3H^+)^{3+}$] (50); **MF**: $C_{25}H_{56}N_{18}Cl_{16}$; **MW** = 821.56 g/mol;




*N*-[3-(4-{[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propyl]-guanidinium hexa-hydrochloride (**105**):

Synthesis followed **GP 10** using **102** (620 mg, 0.44 mmol) and 24 ml of HCl-saturated diethylether. This gave **105** as hygroscopic, colourless solid (365 mg, 0.44 mmol, 100 %).

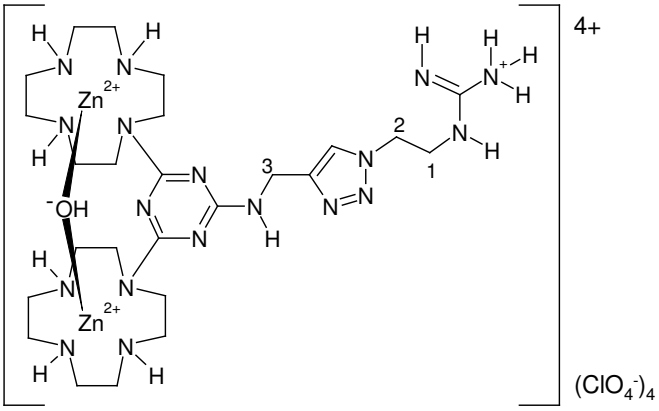
**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 2.10 (tt, $^3J$ = 6.7 Hz, 2 H, $C^2H_2$), 3.10 (t, $^3J$ = 6.7 Hz, 2 H, $C^1H_2$), 3.11 – 3.40 (m, 24 H, cyclen-$CH_2$), 3.82 – 3.88 (m, 8 H, cyclen-$CH_2$), 4.41 (t, $^3J$ = 6.7 Hz, 2 H, $C^3H_2$), 7.95 (s, 1 H, triazole), the signal of $C^4H_2$ was overlaid by the $H_2O$-signal (proved by HSQC); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 28.4 (–, 1 C, $C^2$), 35.9 (–, 1 C, $C^4$), 38.3 (–, 1 C, $C^1$), 43.9 – 44.5 (–, 8 C, cyclen), 46.2 (–, 4 C, cyclen), 47.8 (–, 1 C, $C^3$), 48.0 (–, 4 C, cyclen), 124.1 (+, 1 C, triazole), 144.7 ($C_{quat}$, 1 C, triazole), 156.3 ($C_{quat}$, 2 C, triazine), 156.8 ($C_{quat}$, 1 C, guanidine), 164.0 (very broad signal, $C_{quat}$, 1 C, triazine); **MS** (ESI, $H_2O$/MeCN/TFA): m/z (%) = 309.2 [$(M+2H^+)^{2+}$] (100), 220.1 [$(M+MeCN+3H^+)^{3+}$] (10), 206.4 [$(M+3\ H^+)^{3+}$] (15); **MF**: $C_{26}H_{58}N_{18}Cl_{16}$; **MW** = 835.59 g/mol;



$Zn_2$[*N*-(1-{3-[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-propyl}-1H-[1,2,3]triazol-4-ylmethyl)-guanidinium] tetra-perchlorate hydroxide (**106**):

Synthesis followed **GP 14**, using **103** (350 mg, 0.42 mmol). This gave **106** as a hygroscopic, colourless solid (423 mg, 0.35 mmol, 84 %).

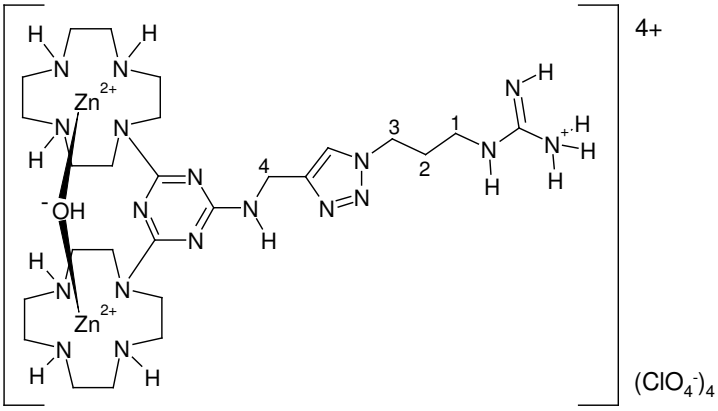
**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.15 (tt, $^3J$ = 6.7 Hz, 2 H, $C^3H_2$), 2.70 – 3.15 (m, 24 H, cyclen-$CH_2$), 3.29 – 3.39 (m, 4 H, cyclen-$CH_2$), 3.42 (dt, $^3J$ = 6.1 Hz, $^3J$ = 6.7 Hz, 2 H, $C^4H_2$), 3.50 – 3.57 (bs, 6 H, cyclen-NH), 4.22 – 4.34 (m, 4 H, cyclen-$CH_2$), 4.42 – 4.46 (m, 4 H, $C^1H_2$ + $C^2H_2$), 6.34 (bs, 4 H, guanidine-NH), 6.36 (t, $^3J$ = 6.1 Hz, 1 H, NH), 6.76 (t, $^3J$ = 5.9 Hz, 1 H, NH), 7.88 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 29.4 (–, 1 C, $C^3$), 36.4 (–, 1 C, $C^4$), 37.4 (–, 1 C, $C^1$), 43.1 (–, 4 C, cyclen), 45.0 (–, 2 C, cyclen), 45.2 (–, 2 C, cyclen), 46.0 (–, 2 C, cyclen), 46.1 (–, 2 C, cyclen), 46.4 (–, 2 C, cyclen), 46.5 (–, 2 C, cyclen), 47.7 (–, 1 C, $C^2$), 123.3 (+, 1 C, triazole), 142.1 ($C_{quat}$, 1 C, triazole), 157.1 ($C_{quat}$, 1 C, guanidine), 165.6 ($C_{quat}$, 1 C, triazine), 169.4 ($C_{quat}$, 1 C, triazine), 169.9 ($C_{quat}$, 1 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 247.7 [$(K^{4+}\text{-}H^{+}\text{-}H_2O)^{3+}$] (20), 253.7 [$(K^{4+}\text{-}H+)^{3+}$] (20), 421.2 [$(K^{4+}\text{-}H^{+}\text{-}H_2O+ClO_4^{-})^{2+}$] (100); **UV/Vis** ($CH_3CN$): λ (lg ε) = 222 nm (4.643); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3375, 3292, 2940, 2890, 2717, 2022, 1662, 1562, 1474, 1346; **MF**: $[C_{26}H_{54}N_{18}OZn_2]^{4+}$ $(ClO_4^{-})_4$ x 2 $H_2O$; **MW** = 1199.43 g/mol;




$Zn_2$[*N*-[2-(4-{[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-ethyl]-guanidinium] tetra-perchlorate hydroxide (**107**):

Synthesis followed **GP 14**, using **104** (350 mg, 0.42 mmol). This gave **107** as a hygroscopic, colourless solid (423 mg, 0.36 mmol, 85 %).

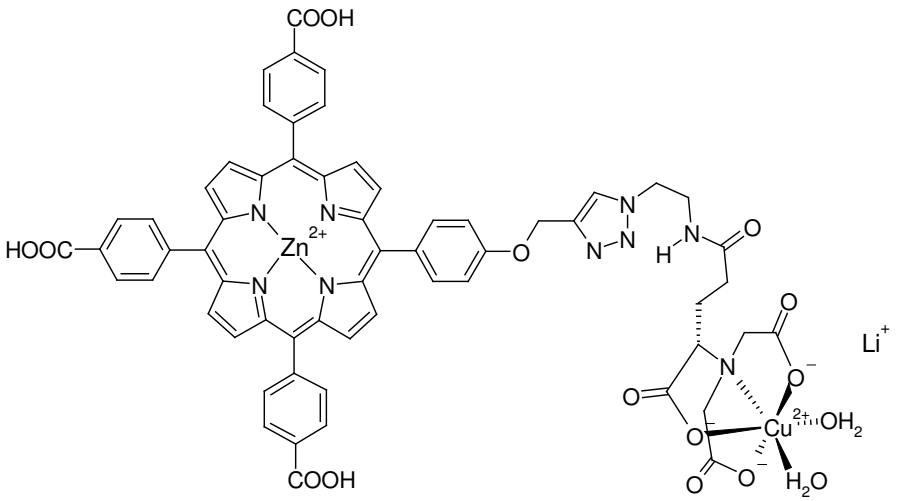
**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.68 – 3.45 (m, 30 H, cyclen-NH + cyclen-$CH_2$), 3.60 – 3.70 (m, 4 H, cyclen-$CH_2$ + $C^1H_2$), 3.98 – 4.06 (m, 2 H, cyclen-$CH_2$), 4.37 – 4.68 (m, 8 H, cyclen-$CH_2$ + $C^2H_2$ + $C^3H_2$), 6.20 (bs, 4 H, guanidine-NH), 6.51 (t, $^3$J = 6.1 Hz, 1 H, NH), 6.63 (t, $^3$J = 5.9 Hz, 1 H, NH), 7.84 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 35.3 (–, 1 C, $C^3$), 41.0 (–, 1 C, $C^1$), 42.9 (–, 2 C, cyclen), 43.0 (–, 2 C, cyclen), 45.7 (–, 2 C, cyclen), 45.9 – 46.4 (–, 8 C, cyclen), 46.6 (–, 2 C, cyclen), 48.4 (–, 1 C, $C^2$), 123.5 (+, 1 C, triazole), 145.2 ($C_{quat}$, 1 C, triazole), 156.7 ($C_{quat}$, 1 C, guanidine), 165.6 ($C_{quat}$, 1 C, triazine), 169.4 ($C_{quat}$, 2 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 243.1 [$(K^{4+}\text{-}H^+\text{-}H_2O)^{3+}$] (25), 249.1 [$(K^{4+}\text{-}H+)^{3+}$] (20), 414.2 [$(K^{4+}\text{-}H^+\text{-}H_2O+ClO_4^-)^{2+}$] (100); **UV/Vis** ($CH_3CN$): λ (lg ε) = 222 nm (4.653); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3375, 3296, 2942, 2893, 2719, 2023, 1663, 1562, 1475, 1346; **MF**: $[C_{25}H_{52}N_{18}OZn_2]^{4+}$ $(ClO_4^-)_4$ x 2 $H_2O$; **MW** = 1185.40 g/mol;



$Zn_2$[*N*-[3-(4-{[4,6-Bis-(1,4,7,10tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propyl]-guanidinium] tetra-perchlorate hydroxide (**108**):

Synthesis followed **GP 14**, using **105** (365 mg, 0.44 mmol). This gave **108** as a hygroscopic, colourless solid (459 mg, 0.38 mmol, 87 %).

**Mp**: > 250 °C; **$^1$H-NMR** (300 MHz, $CD_3CN$): δ [ppm] = 2.10 (tt, $^3J$ = 6.7 Hz, 2 H, $C^2H_2$), 2.65 – 3.20 (m, 26 H, $C^1H_2$ + cyclen-$CH_2$), 3.29 – 3.39 (m, 4 H, cyclen-$CH_2$), 3.50 – 3.57 (bs, 6 H, cyclen-NH), 4.22 – 4.34 (m, 4 H, cyclen-$CH_2$), 4.43 (t, $^3J$ = 6.7 Hz, 2 H, $C^3H_2$), 4.71 – 4.85 (m, 2 H, $C^4H_2$), 6.34 (bs, 4 H, guanidine-NH), 6.48 (t, $^3J$ = 5.9 Hz, 1 H, NH), 6.96 (t, $^3J$ = 5.7 Hz, 1 H, NH), 7.81 (s, 1 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $CD_3CN$): δ [ppm] = 28.8 (–, 1 C, $C^2$), 37.2 (–, 1 C, $C^4$), 38.4 (–, 1 C, $C^1$), 43.2 (–, 4 C, cyclen), 45.1 (–, 2 C, cyclen), 45.3 (–, 2 C, cyclen), 46.0 (–, 2 C, cyclen), 46.2 (–, 2 C, cyclen), 46.4 (–, 2 C, cyclen), 46.5 (–, 2 C, cyclen), 47.9 (–, 1 C, $C^3$), 123.5 (+, 1 C, triazole), 142.2 ($C_{quat}$, 1 C, triazole), 157.2 ($C_{quat}$, 1 C, guanidine), 165.5 ($C_{quat}$, 1 C, triazine), 169.5 ($C_{quat}$, 1 C, triazine), 169.8 ($C_{quat}$, 1 C, triazine); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 247.7 [$(K^{4+}\text{-}H^{+}\text{-}H_2O)^{3+}$] (25), 253.7 [$(K^{4+}\text{-}H+)^{3+}$] (25), 421.2 [$(K^{4+}\text{-}H^{+}\text{-}H_2O+ClO_4^{-})^{2+}$] (100); **UV/Vis** ($CH_3CN$): λ (lg ε) = 222 nm (4.648); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3375, 3292, 2940, 2890, 2717, 2022, 1662, 1562, 1474, 1346; **MF**: $[C_{26}H_{54}N_{18}OZn_2]^{4+}$ $(ClO_4^{-})_4$ x 2 $H_2O$; **MW** = 1199.43 g/mol;



Li[Cu(2-(Bis-carboxylate-methyl-amino)-4-{2-[4-(4-(Zn[10,15,20-tris-(4-carboxy-phenyl)-porphyrin])-5-yl-phenoxymethyl)-2,3-dihydro-[1,2,3]triazol-1-yl]-ethylcarbamoyl}-butanoate)] di-perchlorate hydroxide di-hydrate (**109**):

The Zn(II)-porphyrin **62** (140 mg, 0.16 mmol) was suspended in water (4 ml). A sat. aq. solution of $NaHCO_3$ was added dropwise until **62** was dissolved completely. Then, the Cu(II)-NTA-complex **41** (78 mg, 0.18 mmol, 1.1 eq) was added. Aqueous stock solutions of $CuSO_4$ x 5 $H_2O$ (c = 0.01 mol/l) and sodium ascorbate (c = 0.04 mol/l) were prepared. Aliquots of 270 µl of both solutions were added to the reaction mixture three times in intervals of 3 h. This corresponded to an altogether addition of 5 mol% of copper sulfate (0.7 mg, 2.7 µmol) and 20 mol% sodium ascorbate (2.1 mg, 10.8 µmol). The mixture was stirred at r.t. for an altogether time of 24 h and then acidified carefully with 1 N aq. HCl until **109** was precipitated completely. Fast addition and excesses of aq. HCl were avoided as this could result in a cleavage of the phenyl ether. The product was separated from the solution after centrifugation, washed with water and MeOH and dried in vacuum. This yielded 191 mg (0.15 mmol, 90 %) of **109** as dark red solid.

**MS** (neg. ESI, $H_2O$/MeCN): m/z (%) = 626.3 $[(M\text{-}H^+)^-]$ (40), 644.3 $[(M+Cl^-)^-]$ (50), 656.3 $[(M+CH_3COO^-)^-]$ (100), 1253.6 $[A^-]$ (40); **UV/Vis** (Hepes-buffer, pH 7.5, 50 mM): λ (lg ε) = 308 nm (4.342), 423 nm (5.656), 558 nm (4.205), 599 nm (3.969); **FI** (Hepes-buffer, pH 7.5, $\lambda_{ex}$= 423 nm): $\lambda_{em}$ (a.u.) = 609 nm (59), 660 nm (27); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3406, 3071, 2966, 2926, 2802, 2523, 2081, 1604, 1384, 1244, 995; **MF**: $C_{61}H_{44}N_9O_{14}ZnCuNa$ x 2 $H_2O$; **MW** = 1314.97 g/mol;

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# 3. Novel Bidentate Triazole Ligands and Complexes

## 3.1. Introduction

A large number of transition metal complexes (especially ruthenium(II)- and platinum(II)-complexes), based on the 2,2'-bipyridine (bpy) ligand and its derivatives have been in the focus of many investigations due to their potential as redox- and photo-catalysts.[1] Because of their kinetic inertness, a large number of well defined dinuclear and polynuclear Ru(II)-complexes have been described.[2]

Commonly, the $[Ru(bpy)_3]^{2+}$ and related octahedral $d^6$ transition metal complexes are employed in studies about the properties of their excited state e.g. in photochemistry, chemiluminescence, electrochemiluminescence and electron-transfer chemistry.[3] These complexes are coordinatively saturated and therefore, their interactions and photochemical reactions are usually restricted to the outer-sphere.

One of the most actual scientific investigation of these complexes is their application in the OLED-technology (**O**rganic **L**ight **E**mitting **D**iods).[4] OLEDs have recently received increased attention due to their economic and fabrication advantages when compared to their inorganic semiconductor counterparts. Especially tris-chelated 1,2-diimine Ru(II)- and Os(II)-complexes possess attractive advantages over e.g. polymers, which have been used for OLED applications so far.[5] These advantages are:

- high photoluminescence (PL) quantum yields
- a large electronic stability
- long excited state lifetimes
- and further a facile synthesis and good chemical stability

For example, the PL quantum yield of $[Ru(bpy)_3]^{2+}$ is $\Phi = 0.061$ in MeCN solution at room temperature. This value increases up to 0.25, when the complex is used in electro-generated chemiluminescence (ECL) cells.[6]

Recently, De Cola *et. al* reported about a white light emitting assembly of Ir(III)- and Eu(III)-complexes.[7] The combination of the blue light emitting hetero-leptic

Ir(III)-pyridine-triazolyl complex[8] **1** with the red light emitting Eu(III)-terpyridine complex **2** in a 2:1 stoichiometry led to the white light emitting complex **3** (fig. 1).

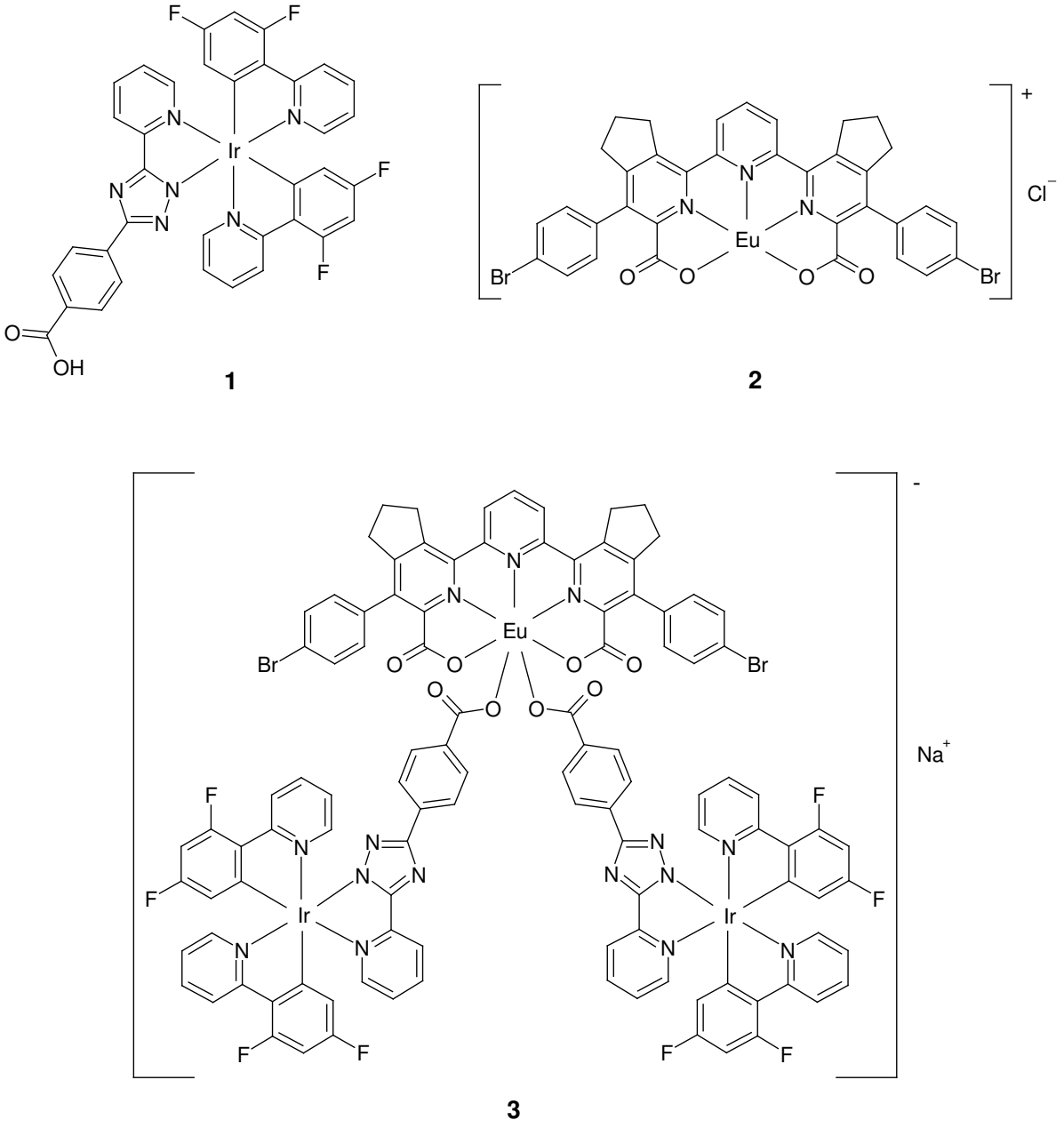
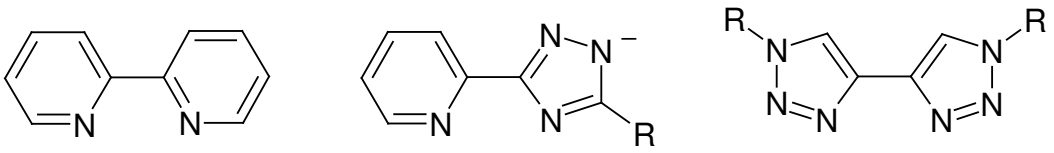



Figure 1: Combination of the blue light emitting Ir(III)-complex **1** with the red light emitting Eu(III)-complex **2** yields the white light emitting complex **3**.

As a white light emitting system is highly desired for applications in OLED-applications,[9] this kind of combination of two different metal complexes seems to be a promising approach. In particular, the exchange of a py- or bpy-ligand by a 1,2,4-triazolyl-pyridine ligand (like in **1**) is highly interesting, as this allows to shift the wavelength of the emitted light into the blue region (461 nm). Due to the structural

similarity, 1,4-disubstituted 1,2,3-triazole-ligands seem to be promising alternatives in these kinds of complexes, with interesting photophysical properties (fig. 2).




bpy triazolyl-py bitriazole

Figure 2: The bpy, a triazolyl-py (1 of 5 mesomeric structures) and a bitriazole ligand.

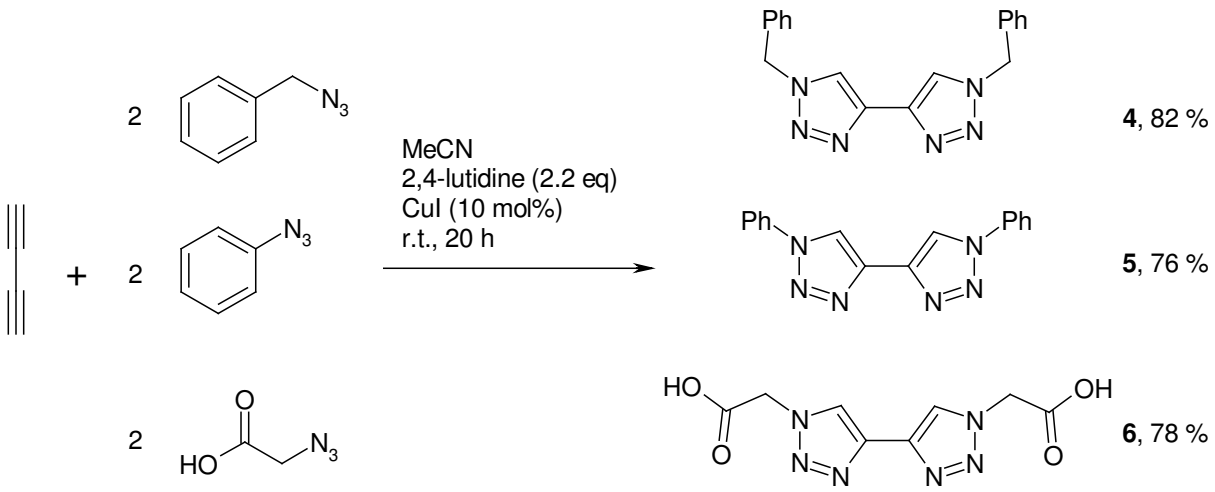
For this reason, novel bitriazole ligands and corresponding Ru(II)-complexes have been synthesized, using the "click-chemistry" approach.[10] Further, the spectroscopical behaviour of these ligands and complexes has been investigated with UV/Vis- and fluorescence spectroscopy.

## 3.2. Synthesis of [4,4']-Bitriazole Ligands[a]

The desired bitriaziole-ligands were synthesized by a twofold Cu(I)-catalyzed cycloaddition between 1,3-butadiyne and organic azides (β-azido-acetic acid, benzylazide and phenylazide). The 1,3-butadiyne was freshly prepared from 1,4-dichloro-butyne[11] and reacted immediately with the azide compound in acetonitrile solution at basic conditions with catalytic amounts of copper iodide (scheme 1).

In order to remove the present $Cu^+$, which could form a complex with the synthesized bitriazole, a workup with aq. $H_2O_2$ (3%) and sat. aq. EDTA was necessary. After purification the bitriazole-ligands **4** – **6** were obtained in acceptable yields.

[a] Complex **7** and X-ray crystallographic data of **4** were obtained by Dr. Uwe Monkowius.




Scheme 1: Synthesis of the bitriazoles **4**, **5** and **6**.

Investigation of the spectroscopic properties of **4** – **6** with UV/Vis and fluorescence spectroscopy showed no noticeable results. Compounds **5** and **6** showed absorption in the UV region between 220 – 260 nm. UV/Vis-spectrum of **4** was not detected as its solubility was limited to $CHCl_3$. Compound **6** showed a very weak emission signal at 414 nm ($\lambda_{Ex}$: 267 nm) in the fluorescence spectrum.

Ligand **4** gave crystals of two different modifications – prisms (monoclinic space proup $P2_1/c$ [Z=2]) and needles (monoclinic space group *Cc* [Z=4]). Crystallographic data for the first modification showed an asymmetric unit consisting of a half structural unit as this modification had an inversion center (fig 3). Data of the latter modification showed the whole molecule as asymmetric unit, as there was no inversion symmetry (fig. 4).

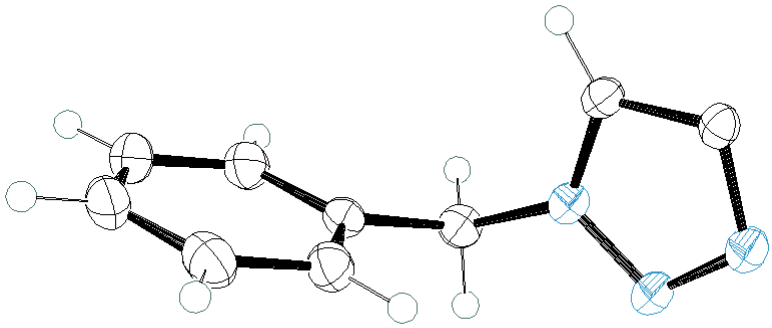


Figure 3: X-ray structure of **4** (prismatic crystals). Carbon is coloured black, nitrogen is blue.

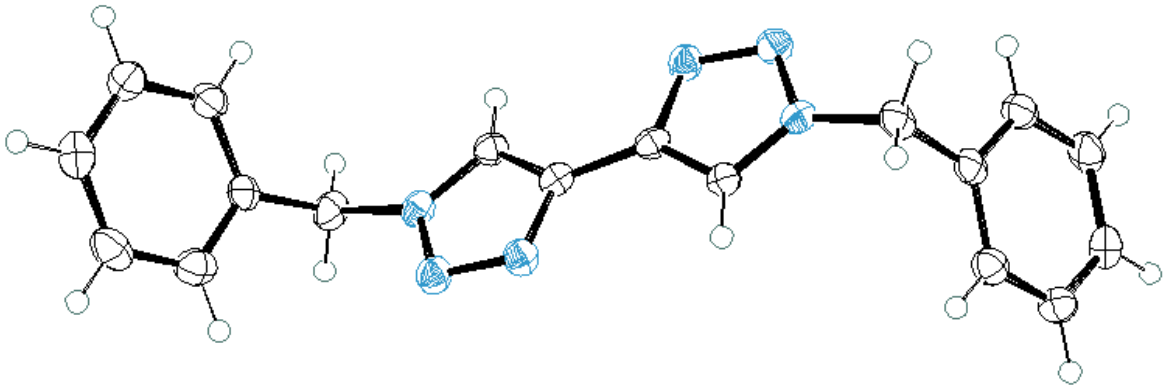


Figure 4: X-ray structure of **4** (needle crystals).

Compounds **5** and **6** were obtained as colourless needles. The X-ray structure of **5** showed the whole molecule as asymmetric unit (fig. 5), while the crystallographic data of **6** revealed an inversion center (fig. 6).

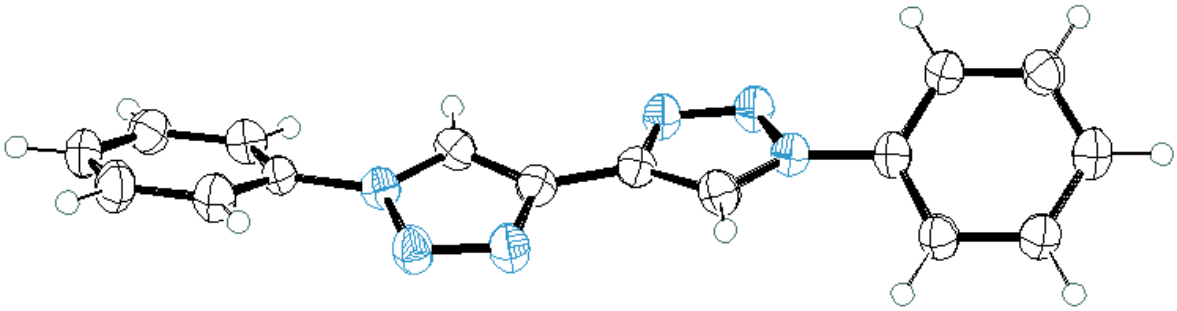


Figure 5: X-ray structure of **5** (needle crystals).

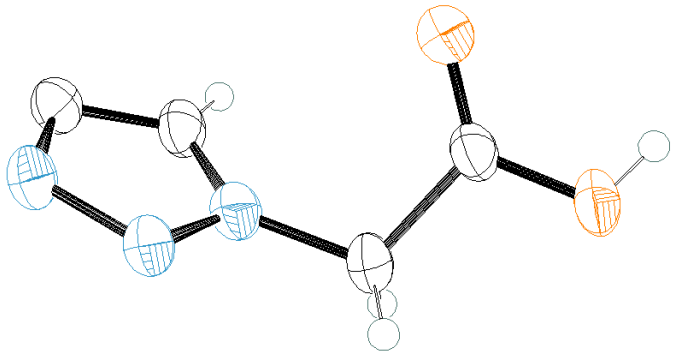
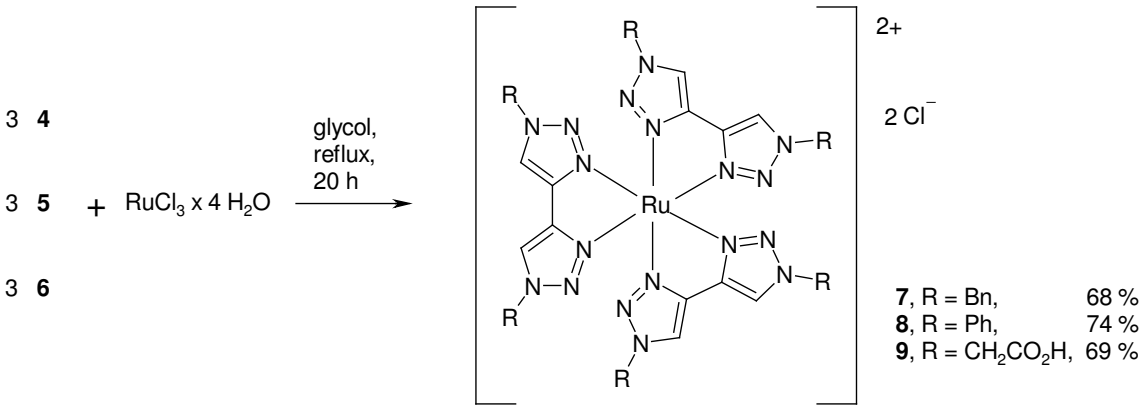


Figure 6: X-ray structure of **6** (needle crystals). Oxygen is coloured orange.

## 3.3. Synthesis of Complexes

The synthesis of homoleptic Ru(II)-complexes from ligands **4** – **6** was carried out with $RuCl_3$ x 4 $H_2O$ in boiling ethylene glycol solution. Precipitation of the complexes **7** – **9** was achieved by addition of ethanol (scheme 2).




Scheme 2: Synthesis of homoleptic complexes **7**, **8** and **9**.

Recrystallisation in ethylene glycol / ethanol yielded the complexes **7** – **9** as light yellow crystals, but only the crystals of compound **7** showed sufficient quality for X-ray crystallography (fig. 7).

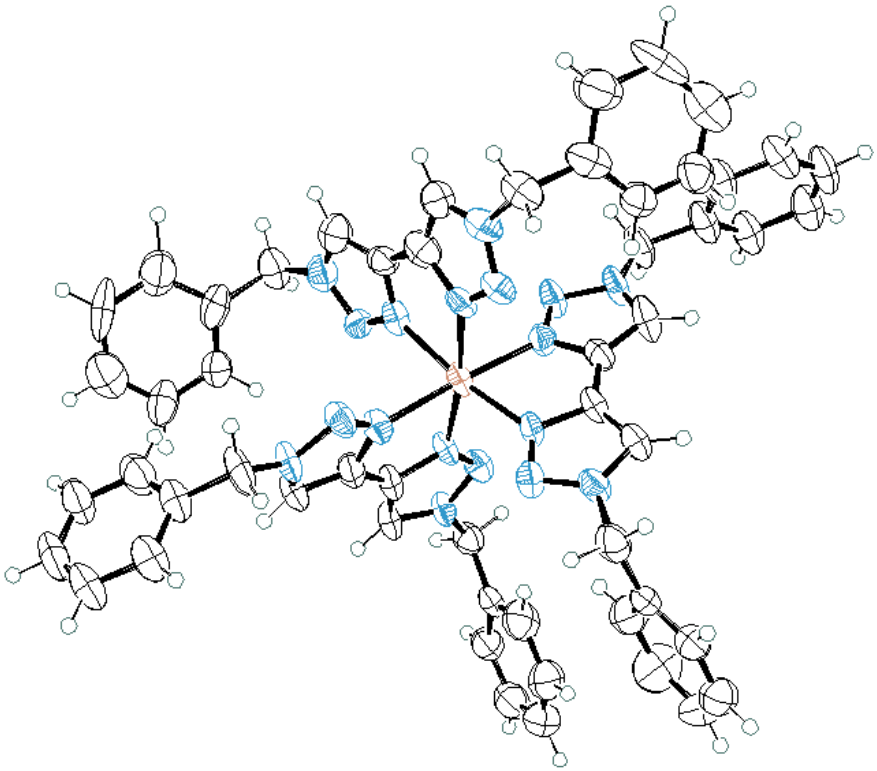


Figure 7: X-ray structure of **7** (counterions and solvent molecules are not shown for better clarity).

The UV/Vis-spectrum of compounds **8** and **9** in $H_2O$ showed two absorption signals between 225 – 240 nm and at approx. 300 nm (see exp. section for details). A weak emission signal was detected at 355 nm after excitation at 215 nm or 270 nm (fig. 8 shows exemplary the spectra of **9**).

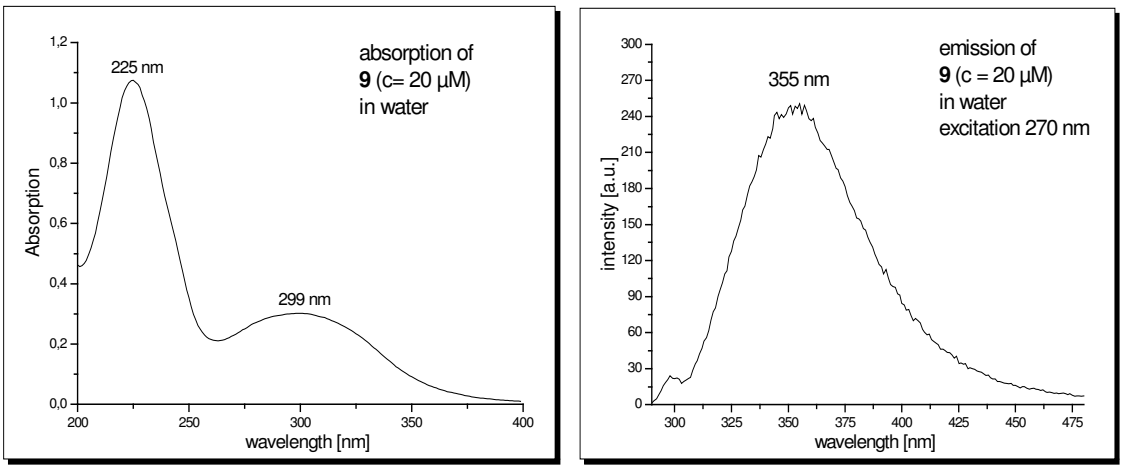



Figure 8: On the left: absorption spectrum of **9** (c = 2.0 x $10^{-5}$ mol/L) in water.
On the right: corresponding emission spectrum ($\lambda_{Ex}$= 270 nm).

## 3.4. Summary and Outlook

New 1,1'-disubstituted [4,4']-bitriazole compounds have been synthesized using the Cu(I)-catalyzed cycloaddition between 1,4-butadiyne and various alkyl- and aryl-triazoles.

Ru(II)-complexes of the obtained ligands have been synthesized and their characteristics in the UV- and Fl-spectrum have been determined.

In order to vary the properties of this class of ligands and complexes, several synthetical procedures are available:

- Monosubstituted [4,4']-bitriazoles can be obtained from 1,4-butadiyne and a mixture of alkyl azide and TMS-azide.[12] This allows the formation of a triazolyl-anion after deprotonation of the unsubstituted triazole.
- The use of 2,6-diethynyl-pyridine instead of 1,4-butadiyne would yield ligands which are comparable to terpyridines
- The substitution of the triazole-CH allows further functionalisations. [13]

These procedures allow modifications of the ligands and the spectroscopic properties. Their transition metal complexes may be of interest for many applications.

## 3.5. Experimental Part

### 3.5.1. General Information

For all general information about synthesis and spectroscopy see *chapter 2.6.1.*!

### 3.5.2. Synthesis of Triazole Ligands and Complexes

#### *3.5.2.1. General Procedures*

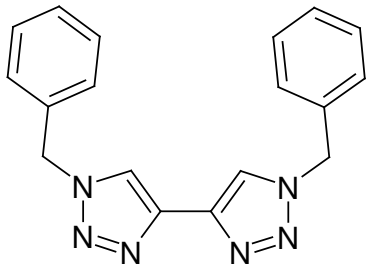
**GP 1** – Twofold "click-reaction" with 1,3-butadiyne:

The freshly prepared butadiyne was condensed into 5 ml of acetonitrile at – 40 °C. Following, a solution of the azide (2eq), 2,4-lutidine (2.2 eq) and CuI (10 mol %) in 10 ml of acetonitrile was cooled to – 40 °C and added. The reaction mixture was allowed to warm to room temperature. The reaction vessel was stored in a closed autoclave to prevent evaporation of the butadiyne. After stirring for 20 h, the mixture was poured into 50 ml of an aq. solution of $H_2O_2$ (3 %). The mixture was stirred until the generation of gas had ended. Following, 50 ml of a sat. aq. solution of EDTA was added and the mixture was stirred for further 30 min. Further purification was individual for each compound.

**GP 2** – Synthesis of homoleptic Ru(II)-complexes:

In a round bottom flask (25 ml) the bitriazole ligand (3 eq) and $RuCl_3$ x 4 $H_2O$ were suspended in 10 ml of ethylene glycol. A reflux condenser was put on and the mixture was heated (oil bath temperature: 200 °C). This yielded a dark red solution which slowly turned to light orange within 20 h. The solution was concentrated in vacuum upon heating to 3 ml. Following, boiling ethanol was added to the solution at 80 °C, until a white precipitate appeared. The mixture was stored in the freezer at - 20 °C during the night to complete precipitation. The solid was separated by centrifugation and dried in vacuum.

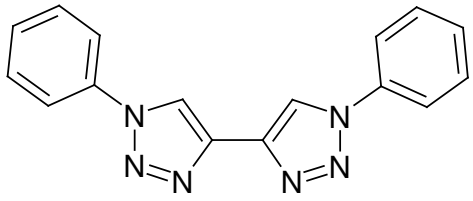
*3.5.2.2. Synthesis of New Compounds*



1,1'-Dibenzyl-1*H*,1'*H*-[4,4']bi[[1,2,3]bitriazolyl] **4**:

Synthesis followed **GP 1** using butadiyne (563 mg, 11.3 mmol), benzyl azide (2996 mg, 22.5 mmol, 2 eq), 2,4-lutidine (2652 mg, 2.86 ml, 24.8 mmol, 2.2 eq) and CuI (214 mg, 1.13 mmol, 10 mol %). After workup, the aqueous solution was extracted with $CHCl_3$ (3 x 100 ml). The organic phase was dried with $Na_2SO_4$, filtered and concentrated at reduced pressure. The crude product was purified by CC with DCM:MeOH from 99:1 to 96:4. This yielded 2931 mg of **4** as colourless crystals (9.3 mmol, 82 %).

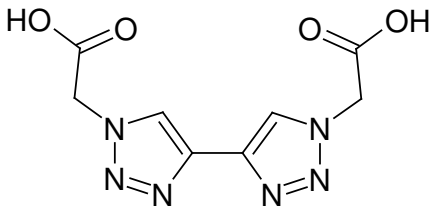
**Mp**: 228 °C; **$^1$H-NMR** (300 MHz, DMSO-d6): $\delta$ [ppm] = 5.67 (s, 4 H, $CH_2$), 7.31 – 7.40 (m, 10 H, CH), 8.58 (s, 2 H, triazole); **$^{13}$C-NMR** (75.5 MHz, DMSO-d6): $\delta$ [ppm] = 52.8 (–, 2 C), 121.7 (+, 2 C, triazole), 127.9 (+, 4 C), 128.1 (+, 2 C), 128.7 (+, 4 C), 139.2 ($C_{quat}$, 1 C), 142.2 ($C_{quat}$, 1 C); **MS** (ESI, DCM/MeOH): m/z (%) = 317.1 [$MH^+$] (100); **EA** ($C_{18}H_{16}N_6$) calc.: C 68.34, H 5.10, N 26.56, found: C 68.21, H 5.21, N 26.42; **UV/Vis** ($CHCl_3$): no signal detectable; **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3133, 3101, 3031, 2984, 2946, 2858, 1673, 1494, 1439, 1410, 1364, 1293, 1231, 1076, 1058, 949, 836, 722; **MF**: $C_{18}H_{16}N_6$; **MW** = 316.37 g/mol;



1,1'-Diphenyl-1*H*,1'*H*-[4,4']bi[[1,2,3]bitriazolyl] (**5**):

Synthesis followed **GP 1** using butadiyne (335 mg, 6.7 mmol), phenyl azide (1598 mg, 13.4 mmol, 2 eq), 2,4-lutidine (1579 mg, 1.70 ml, 14.8 mmol, 2.2 eq) and CuI (127 mg, 0.67 mmol, 10 mol %). After workup, the aqueous solution was heated to 40 °C and extracted with warm $CHCl_3$ (40 °C, 8 x 80 ml). The organic phase was dried with $Na_2SO_4$, filtered, concentrated at reduced pressure and dried in vacuum. The residue was dissolved in boiling $CHCl_3$, filtered over charcoal and cellite and concentrated again. The crude product was purified by recrystallisation from $CHCl_3$/PE. This yielded 1468 mg of **5** as colourless crystals (5.1 mmol, 76 %).

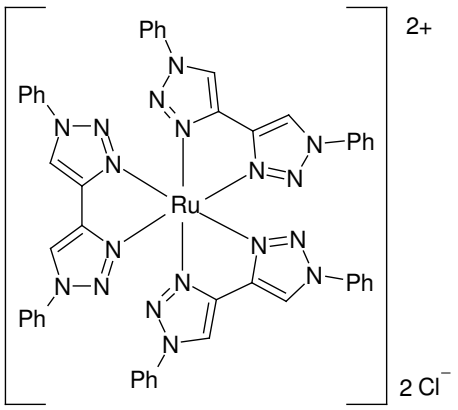
**Mp**: 236 °C; **$^1$H-NMR** (300 MHz, $CHCl_3$): $\delta$ [ppm] = 7.49 (t, $^3J$ = 7.7 Hz, 2 H, CH), 7.53 – 7.59 (m, 4 H, CH), 7.82 (d, $^3J$ = 7.6 Hz, 4 H, CH), 8.59 (s, 2 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $CHCl_3$): $\delta$ [ppm] = 118.9 (+, 2 C, triazole), 120.6 (+, 4 C), 129.0 (+, 2 C), 129.9 (+, 4 C), 136.9 ($C_{quat}$, 1 C), 140.5 ($C_{quat}$, 1 C); **MS** (ESI, DCM/MeOH): m/z (%) = 289.1 [$MH^+$] (100); **EA** ($C_{16}H_{12}N_6$) calc.: C 66.66, H 4.20, N 29.15, found: C 66.57, H 4.31, N 29.01; **UV/Vis** ($CH_3CN$): $\lambda$ (lg $\varepsilon$) = 232 nm (4.480), 257 nm (4.366); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] = 3132, 3093, 3065, 1654, 1597, 1504, 1464, 1396, 1257, 1234, 1034, 825, 756; **MF**: $C_{16}H_{12}N_6$; **MW** = 288.31 g/mol;



(1'-Carboxymethyl-1'H-[4,4']bi[[1,2,3]triazolyl]-1-yl)-acetic acid (**6**):

Synthesis followed **GP 1** using butadiyne (541 mg, 10.8 mmol), β-azidoacetic acid (2183 mg, 21.6 mmol, 2 eq), 2,4-lutidine (2546 mg, 2.75 ml, 23.8 mmol, 2.2 eq) and CuI (206 mg, 1.08 mmol, 10 mol %). After workup, the aqueous solution was acidified with 6 N aq. HCl to pH 2. The precipitate was filtered, washed with water and suspended in 10 ml water. A 0.1 N aq. solution of NaOH was added dropwise until the solid was dissolved completely. The solution was lyophilized to yield the sodium salt of 6 (2502 mg, 8.4 mmol, 78 %). NMR-, MS- and UV-spectroscopy were performed with this salt. The solid was dissolved in water (20 ml), the solution was heated to boiling and 0.5 N aq. HCl was added dropwise until a precipitate appeared. After cooling to room temperature, 2 N aq. HCl was added until no further product precipitated. The solid was filtered, washed with water and dried in vacuum. This yielded 2116 mg of **6** as colourless crystals (8.4 mmol, 78 %).

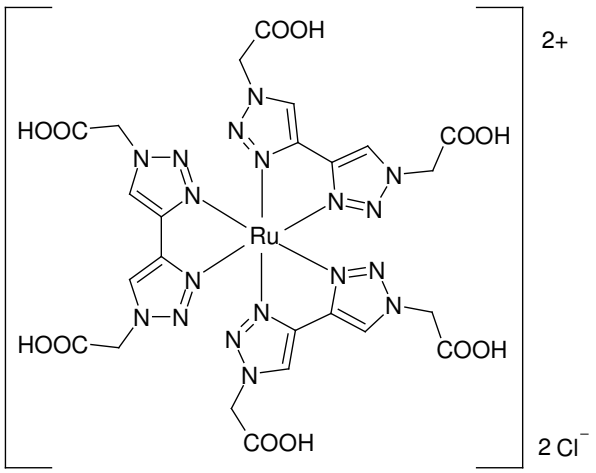
**Mp**: > 215 °C (decomp.); **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 5.05 (s, 4 H, $CH_2$), 8.20 (s, 2 H, triazole); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 53.3 (–, 2 C), 123.9 (+, 2 C, triazole), 138.6 ($C_{quat}$, 2 C), 173.1 ($C_{quat}$, 2 C); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 253.1 [$MH^+$] (100); **EA** ($C_8H_8N_6O_4$) calc.: C 38.10, H 3.20, N 33.32, found: C 37.97, H 3.32, N 33.17; **UV/Vis** ($H_2O$): λ (lg ε) = 225 nm (4.092); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3435, 3151, 3117, 3013, 2947, 2792, 2708, 2594, 2511, 1930, 1794, 1724, 1496, 1420, 1347, 1295, 1257, 1223, 1151, 1109, 1064, 1011, 976, 896, 795; **MF**: $C_8H_8N_6O_4$; **MW** = 252.16 g/mol;



Ru[tri-(1,1'-Diphenyl-1*H*,1'*H*-[4,4']bi[[1,2,3]bitriazolyl])] di-chloride (**8**):

Synthesis followed **GP 2** using **5** (250 mg, 0.87 mmol) and $RuCl_3$ x 4 $H_2O$ (78 mg, 0.29 mmol). This yielded 665 mg of **8** as colourless crystals (0.64 mmol, 74 %).

**Mp**: >210 °C (decomp.); **$^1$H-NMR** (300 MHz, DMSO-d6): δ [ppm] = 7.54 – 7.66 (m, 18 H, CH), 7.89 (d, $^3$J = 7.1 Hz, 12 H, CH), 9.76 (s, 6 H, triazole); **$^{13}$C-NMR** (75.5 MHz, DMSO-d6): δ [ppm] = 120.1 (+, 2 C, triazole), 120.5 (+, 4 C), 129.9 (+, 2 C), 130.0 (+, 4 C), 135.6 ($C_{quat}$, 1 C), 140.9 ($C_{quat}$, 1 C); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 483.2 [$(L_3Ru^{2+})^{2+}$] (100), 1115.4 [$(L_3Ru^{2+}+TfO^-)^+$]; **UV/Vis** ($H_2O$): λ (lg ε) = 307 nm (4.517), 239 (5.012); **IR** (KBr): $\tilde{\nu}$ [$cm^{-1}$] = 3102, 3062, 1595, 1499, 1464, 1422, 1313, 1271, 1065, 999, 760; **MF**: $C_{48}H_{36}N_{18}RuCl_2$; **MW** = 1036.91 g/mol;




Ru[tri-((1'-Carboxymethyl-1'H-[4,4']bi[[1,2,3]triazolyl]-1-yl)-acetic acid)] di-chloride (**9**):

Synthesis followed **GP 2** using **6** (250 mg, 0.99 mmol) and $RuCl_3$ x 4 $H_2O$ (90 mg, 0.33 mmol). This yielded 634 mg of **8** as colourless crystals (0.68 mmol, 69 %).

**Mp**: >205 °C (decomp.); **$^1$H-NMR** (300 MHz, $D_2O$): δ [ppm] = 5.22 (s, 12 H, $CH_2$), 8.50 (s, 6 H, CH); **$^{13}$C-NMR** (75.5 MHz, $D_2O$): δ [ppm] = 51.5 (–, 6 C), 122.0 (+, 6 C, triazole), 138.2 ($C_{quat}$, 6 C), 171.0 ($C_{quat}$, 6 C); **MS** (ESI, $H_2O$/MeCN): m/z (%) = 478.2 (100), 507.2 (100), 521.3 (100), 955.3 (65); **UV/Vis** ($H_2O$): λ (lg ε) = 299 nm (4.169), 225 nm (4.721); **IR** (KBr): $\bar{\nu}$ [$cm^{-1}$] =3429. 3136, 2925, 1746, 1629, 1454, 1377, 1288, 1221, 1021; **MF**: $C_{24}H_{24}N_{18}O_{12}RuCl_2$; **MW** = 928.54 g/mol;

For details about the synthesis of complex **7**, please contact Dr. Uwe Monkowius at J. Kepler University, Linz, Austria. (email: uwe.monkowius@jku.at).

*3.5.2.3. Crystal Data*

Crystal data of **4** (prisms):

Monoclinic; space group: $P2_1/c$; cell dimensions: a = 8.1669 (10) Å, $\alpha$ = 90°, b = 9.4871 (13) Å, $\beta$ = 94.135° (14), c = 10.3595 (12) Å, $\gamma$ = 90°; V = 800.57 (17) Å$^3$; Z = 2, $D_x$ = 1.311 Mg/m$^3$; $\mu$ = 0.083 mm$^{-1}$; F(000) = 332. Data collection: T = 123 K; graphite monochromator. A translucent colorless prismatic crystal with dimensions of 0.32 x 0.22 x 0.18 mm was used to measure 5365 reflections (1589 unique reflections, $R_{int}$ = 0.0337) from 2.50° to 26.80° 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 of 0.913 for all reflections and 109 parameters. Final R indices: [I≥2σ(I)] = 0.0333, $wR_2$ = 0.0805. Absolute structure parameters: $\sigma_{fin}$(max/min)[eÅ$^{-3}$] = 0.238/-0.137.

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

| Atom | x | y | z | U(eq) |
|---|---|---|---|---|
| N(8) | 1128(1) | 2840(1) | 4667(1) | 22(1) |
| N(9) | 739(1) | 2801(1) | 5909(1) | 25(1) |
| N(10) | 200(1) | 1519(1) | 6131(1) | 25(1) |
| C(1) | 3573(2) | 3887(1) | 3761(1) | 24(1) |
| C(2) | 4691(2) | 3083(1) | 4530(1) | 28(1) |
| C(3) | 6293(2) | 2899(1) | 4173(2) | 35(1) |
| C(4) | 6791(2) | 3529(2) | 3058(2) | 39(1) |
| C(5) | 5693(2) | 4346(2) | 2306(2) | 40(1) |
| C(6) | 4083(2) | 4521(1) | 2648(1) | 32(1) |
| C(7) | 1837(2) | 4122(1) | 4143(1) | 25(1) |
| C(11) | 259(1) | 740(1) | 5028(1) | 21(1) |
| C(12) | 849(1) | 1580(1) | 4083(1) | 22(1) |

Table of bond legths [Å] and angles [deg]:

| Bond | Length | Bond | Angle | Bond | Angle |
|---|---|---|---|---|---|
| N(8)-N(9) | 1.3476(15) | N(9)-N(8)-C(7) | 120.17(10) | C(1)-C(2)-H(2) | 120.00 |
| N(8)-C(7) | 1.4677(16) | N(9)-N(8)-C(12) | 111.05(10) | C(3)-C(2)-H(2) | 120.00 |
| N(8)-C(12) | 1.3522(15) | C(7)-N(8)-C(12) | 128.70(11) | C(2)-C(3)-H(3) | 120.00 |
| N(9)-N(10) | 1.3195(15) | N(8)-N(9)-N(10) | 107.26(10) | C(4)-C(3)-H(3) | 120.00 |
| N(10)-C(11) | 1.3643(17) | N(9)-N(10)-C(11) | 108.71(10) | C(3)-C(4)-H(4) | 120.00 |
| C(1)-C(2) | 1.3954(18) | C(2)-C(1)-C(6) | 119.15(12) | C(5)-C(4)-H(4) | 120.00 |
| C(1)-C(6) | 1.3910(19) | C(2)-C(1)-C(7) | 121.15(12) | C(4)-C(5)-H(5) | 120.00 |
| C(1)-C(7) | 1.5156(18) | C(6)-C(1)-C(7) | 119.67(11) | C(6)-C(5)-H(5) | 120.00 |
| C(2)-C(3) | 1.3957(19) | C(1)-C(2)-C(3) | 120.31(13) | C(1)-C(6)-H(6) | 120.00 |
| C(3)-C(4) | 1.388(2) | C(2)-C(3)-C(4) | 120.21(13) | C(5)-C(6)-H(6) | 120.00 |
| C(4)-C(5) | 1.383(2) | C(3)-C(4)-C(5) | 119.61(13) | N(8)-C(7)-H(7A) | 109.00 |
| C(5)-C(6) | 1.396(2) | C(4)-C(5)-C(6) | 120.51(14) | N(8)-C(7)-H(7B) | 109.00 |
| C(11)-C(12) | 1.3764(17) | C(1)-C(6)-C(5) | 120.20(13) | C(1)-C(7)-H(7A) | 109.00 |
| C(11)-C(11)#1 | 1.4666(16) | N(8)-C(7)-C(1) | 112.10(10) | C(1)-C(7)-H(7B) | 109.00 |
| C(2)-H(2) | 0.9500 | N(10)-C(11)-C(12) | 108.54(10) | H(7A)-C(7)-H(7B) | 108.00 |
| C(3)-H(3) | 0.9500 | N(10)-C(11)-C(11)#1 | 121.62(11) | N(8)-C(12)-H(12) | 128.00 |
| C(4)-H(4) | 0.9500 | C(11)#1-C(11)-C(12) | 129.85(11) | C(11)-C(12)-H(12) | 128.00 |
| C(5)-H(5) | 0.9500 | N(8)-C(12)-C(11) | 104.45(11) | | |
| C(6)-H(6) | 0.9500 | | | | |
| C(7)-H(7A) | 0.9900 | | | | |
| C(7)-H(7B) | 0.9900 | | | | |
| C(12)-H(12) | 0.9500 | | | | |

Crystal data of **4** (needles):

Monoclinic; space group: *Cc*; cell dimensions: a = 34.414 (3) Å, $\alpha$ = 90°, b = 5.5439 (5) Å, $\beta$ = 102.466° (10), c = 8.4506 (7) Å, $\gamma$ = 90°; V = 1574.3 (2) Å$^3$; Z = 4, $D_x$ = 1.335 Mg/m$^3$; $\mu$ = 0.085 mm$^{-1}$; F(000) = 664. Data collection: T = 123 K; graphite monochromator. A translucent colorless crystal with dimensions of 0.48 x 0.28 x 0.02 mm was used to measure 6381 reflections (3264 unique reflections, $R_{int}$ = 0.0854) from 2.42° to 26.80° 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 of 0.951 for all reflections and 217 parameters. Final R indices: [I≥2σ(I)] = 0.0565, $wR_2$ = 0.1401. Absolute structure parameters: $\sigma_{fin}$(max/min)[eÅ$^{-3}$] = 0.294/-0.248.

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

| Atom | x | y | z | U(eq) |
|---|---|---|---|---|
| N(1) | 876(1) | 6980(4) | -2258(3) | 25(1) |
| N(2) | 864(1) | 4560(5) | -2200(3) | 28(1) |
| N(3) | 1154(1) | 3870(5) | -978(3) | 26(1) |
| N(4) | 1863(1) | 7664(4) | 1914(3) | 26(1) |
| N(5) | 2158(1) | 6927(4) | 3104(3) | 26(1) |
| N(6) | 2162(1) | 4489(4) | 3033(3) | 24(1) |
| C(1) | -4(1) | 9615(6) | -2434(3) | 30(1) |
| C(2) | -393(1) | 9321(6) | -2235(4) | 33(1) |
| C(3) | -618(1) | 7378(6) | -2952(4) | 34(1) |
| C(4) | -454(1) | 5729(6) | -3853(4) | 36(1) |
| C(5) | -67(1) | 5997(5) | -4038(3) | 32(1) |
| C(6) | 163(1) | 7958(5) | -3327(3) | 25(1) |
| C(7) | 589(1) | 8297(6) | -3509(3) | 29(1) |
| C(8) | 1170(1) | 7889(6) | -1077(3) | 24(1) |
| C(9) | 1347(1) | 5885(5) | -274(3) | 23(1) |
| C(10) | 1684(1) | 5674(5) | 1109(3) | 22(1) |
| C(11) | 1873(1) | 3640(5) | 1814(3) | 23(1) |
| C(12) | 2457(1) | 3098(6) | 4205(3) | 27(1) |
| C(13) | 2856(1) | 2877(5) | 3740(3) | 25(1) |
| C(14) | 2938(1) | 900(5) | 2828(3) | 27(1) |
| C(15) | 3309(1) | 665(6) | 2459(3) | 31(1) |
| C(16) | 3603(1) | 2370(6) | 3006(4) | 32(1) |
| C(17) | 3523(1) | 4327(6) | 3897(4) | 34(1) |
| C(18) | 3151(1) | 4592(6) | 4253(3) | 30(1) |

Table of bond legths [Å] and angles [deg]:

| Bond | Length | Bond | Angle | Bond | Angle |
|---|---|---|---|---|---|
| N(1)-N(2) | 1.344(4) | N(2)-N(1)-C(7) | 120.0(2) | C(13)-C(18)-C(17) | 120.5(3) |
| N(1)-C(7) | 1.474(4) | N(2)-N(1)-C(8) | 111.6(2) | C(2)-C(1)-H(1) | 120.00 |
| N(1)-C(8) | 1.356(4) | C(7)-N(1)-C(8) | 128.4(2) | C(6)-C(1)-H(1) | 120.00 |
| N(2)-N(3) | 1.328(4) | N(1)-N(2)-N(3) | 107.0(2) | C(1)-C(2)-H(2) | 120.00 |
| N(3)-C(9) | 1.368(4) | N(2)-N(3)-C(9) | 108.4(3) | C(3)-C(2)-H(2) | 120.00 |
| N(4)-N(5) | 1.331(4) | N(5)-N(4)-C(10) | 108.4(2) | C(2)-C(3)-H(3) | 120.00 |
| N(4)-C(10) | 1.371(4) | N(4)-N(5)-N(6) | 106.7(2) | C(4)-C(3)-H(3) | 120.00 |
| N(5)-N(6) | 1.353(3) | N(5)-N(6)-C(11) | 111.6(2) | C(3)-C(4)-H(4) | 120.00 |
| N(6)-C(11) | 1.354(4) | N(5)-N(6)-C(12) | 120.4(2) | C(5)-C(4)-H(4) | 120.00 |
| N(6)-C(12) | 1.473(4) | C(11)-N(6)-C(12) | 128.0(2) | C(4)-C(5)-H(5) | 120.00 |
| C(1)-C(2) | 1.392(4) | C(2)-C(1)-C(6) | 120.6(3) | C(6)-C(5)-H(5) | 120.00 |
| C(1)-C(6) | 1.389(4) | C(1)-C(2)-C(3) | 119.9(3) | N(1)-C(7)-H(7A) | 109.00 |
| C(2)-C(3) | 1.388(5) | C(2)-C(3)-C(4) | 119.8(3) | N(1)-C(7)-H(7B) | 109.00 |
| C(3)-C(4) | 1.385(5) | C(3)-C(4)-C(5) | 120.5(3) | C(6)-C(7)-H(7A) | 109.00 |
| C(4)-C(5) | 1.382(5) | C(4)-C(5)-C(6) | 120.2(3) | C(6)-C(7)-H(7B) | 109.00 |
| C(5)-C(6) | 1.401(4) | C(1)-C(6)-C(5) | 119.0(3) | H(7A)-C(7)-H(7B) | 108.00 |
| C(6)-C(7) | 1.520(4) | C(1)-C(6)-C(7) | 119.6(3) | N(1)-C(8)-H(8) | 128.00 |

| | | | | | |
|---|---|---|---|---|---|
| C(8)-C(9) | 1.374(4) | C(5)-C(6)-C(7) | 121.4(3) | C(9)-C(8)-H(8) | 128.00 |
| C(9)-C(10) | 1.463(4) | N(1)-C(7)-C(6) | 112.2(2) | N(6)-C(11)-H(11) | 128.00 |
| C(10)-C(11) | 1.372(4) | N(1)-C(8)-C(9) | 104.1(3) | C(10)-C(11)-H(11) | 128.00 |
| C(12)-C(13) | 1.514(4) | N(3)-C(9)-C(8) | 108.9(3) | N(6)-C(12)-H(12A) | 109.00 |
| C(13)-C(14) | 1.404(4) | N(3)-C(9)-C(10) | 120.6(2) | N(6)-C(12)-H(12B) | 109.00 |
| C(13)-C(18) | 1.390(4) | C(8)-C(9)-C(10) | 130.6(3) | C(13)-C(12)-H(12A) | 109.00 |
| C(14)-C(15) | 1.382(4) | N(4)-C(10)-C(9) | 121.7(2) | C(13)-C(12)-H(12B) | 109.00 |
| C(15)-C(16) | 1.390(5) | N(4)-C(10)-C(11) | 109.0(2) | H(12A)-C(12)-H(12B) | 108.00 |
| C(16)-C(17) | 1.382(5) | C(9)-C(10)-C(11) | 129.2(3) | C(13)-C(14)-H(14) | 120.00 |
| C(17)-C(18) | 1.385(4) | N(6)-C(11)-C(10) | 104.3(2) | C(15)-C(14)-H(14) | 120.00 |
| C(1)-H(1) | 0.9500 | N(6)-C(12)-C(13) | 113.5(2) | C(14)-C(15)-H(15) | 120.00 |
| C(2)-H(2) | 0.9500 | C(12)-C(13)-C(14) | 120.5(3) | C(16)-C(15)-H(15) | 120.00 |
| C(3)-H(3) | 0.9500 | C(12)-C(13)-C(18) | 120.4(3) | C(15)-C(16)-H(16) | 120.00 |
| C(4)-H(4) | 0.9500 | C(14)-C(13)-C(18) | 119.0(3) | C(17)-C(16)-H(16) | 120.00 |
| C(5)-H(5) | 0.9500 | C(13)-C(14)-C(15) | 120.2(3) | C(16)-C(17)-H(17) | 120.00 |
| C(7)-H(7A) | 0.9900 | C(14)-C(15)-C(16) | 120.2(3) | C(18)-C(17)-H(17) | 120.00 |
| C(7)-H(7B) | 0.9900 | C(15)-C(16)-C(17) | 119.9(3) | C(13)-C(18)-H(18) | 120.00 |
| C(8)-H(8) | 0.9500 | C(16)-C(17)-C(18) | 120.3(3) | C(17)-C(18)-H(18) | 120.00 |
| C(11)-H(11) | 0.9500 | | | | |
| C(12)-H(12A) | 0.9900 | | | | |
| C(12)-H(12B) | 0.9900 | | | | |
| C(14)-H(14) | 0.9500 | | | | |
| C(15)-H(15) | 0.9500 | | | | |
| C(16)-H(16) | 0.9500 | | | | |
| C(17)-H(17) | 0.9500 | | | | |
| C(18)-H(18) | 0.9500 | | | | |

Crystal data of **5**:

Triclinic; space group: P-1; cell dimensions: a = 5.729 (4) Å, $\alpha$ = 101.38(11)°, b = 11.019 (18) Å, $\beta$ = 93.17° (7), c = 16.744 (14) Å, $\gamma$ = 102.51 ° (11); V = 1006 (2) $Å^3$; Z = 3, $D_x$ = 1.428 $Mg/m^3$; $\mu$ = 0.741 $mm^{-1}$; F(000) = 450. Data collection: T = 150 K; graphite monochromator. A translucent colorless crystal with dimensions of 0.42 x 0.06 x 0.04 mm was used to measure 5716 reflections (2062 unique reflections, $R_{int}$ = 0.0263) from 2.71° to 50.31° on a Oxford Diffraction Gemini Ultra with the omega-scan method. Structure refinement: The $F^2$ value was refined using the full-matrix least squares refinement method, with a goodness-of-fit of 0.881 for all reflections and 298 parameters. Final R indices: [I≥2σ(I)] = 0.0338, $wR_2$ = 0.0754. Absolute structure parameters: $\sigma_{fin}$(max/min)[$eÅ^{-3}$] = 0.146/-0.213.

Table of atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters ($A^2$ x $10^3$) of **5**. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor:

| Atom | x | y | z | U(eq) |
|---|---|---|---|---|
| N(1) | 4727(3) | 5087(2) | 3299(1) | 32(1) |
| N(2) | 7099(3) | 5203(2) | 3510(1) | 40(1) |
| N(3) | 7354(3) | 5157(2) | 4285(1) | 39(1) |
| C(1) | 3939(4) | 5139(2) | 2484(1) | 31(1) |
| C(2) | 5573(4) | 5696(2) | 2012(1) | 38(1) |
| C(3) | 4851(4) | 5730(2) | 1223(1) | 41(1) |
| C(4) | 2490(4) | 5224(2) | 903(1) | 41(1) |
| C(5) | 859(4) | 4691(2) | 1381(1) | 40(1) |
| C(6) | 1568(4) | 4637(2) | 2174(1) | 37(1) |
| C(7) | 3474(4) | 4967(2) | 3945(1) | 34(1) |
| C(8) | 5154(4) | 5023(2) | 4575(1) | 33(1) |
| N(4) | 4082(3) | 1779(2) | 4064(1) | 32(1) |
| N(5) | 1707(3) | 1673(2) | 3862(1) | 38(1) |
| N(6) | 1436(3) | 1715(2) | 3085(1) | 40(1) |
| N(7) | 6065(3) | 2150(2) | 1641(1) | 37(1) |
| N(8) | 5739(3) | 2074(2) | 851(1) | 38(1) |
| N(9) | 3324(3) | 1759(2) | 632(1) | 32(1) |
| C(9) | 4883(4) | 1740(2) | 4880(1) | 30(1) |
| C(10) | 3216(4) | 1197(2) | 5351(1) | 39(1) |
| C(11) | 3938(4) | 1160(2) | 6140(1) | 45(1) |
| C(12) | 6305(4) | 1657(2) | 6463(1) | 44(1) |
| C(13) | 7945(4) | 2176(2) | 5988(1) | 40(1) |
| C(14) | 7257(4) | 2227(2) | 5190(1) | 36(1) |
| C(15) | 5324(4) | 1895(2) | 3411(1) | 34(1) |
| C(16) | 3632(4) | 1852(2) | 2787(1) | 32(1) |
| C(17) | 3873(4) | 1887(2) | 1935(1) | 32(1) |
| C(18) | 2128(4) | 1641(2) | 1290(1) | 36(1) |
| C(19) | 2429(4) | 1566(2) | -210(1) | 32(1) |
| C(20) | 3650(4) | 971(2) | -808(1) | 35(1) |
| C(21) | 2820(4) | 800(2) | -1620(1) | 39(1) |
| C(22) | 806(4) | 1208(2) | -1836(1) | 40(1) |
| C(23) | -393(4) | 1783(2) | -1234(1) | 36(1) |
| C(24) | 396(4) | 1959(2) | -416(1) | 36(1) |

Table of bond legths [Å] and angles [deg] of **5**:

| Bond | Length | Bond | Angle | Bond | Angle |
|---|---|---|---|---|---|
| N(1)-N(2) | 1.357(3) | N(2)-N(1)-C(1) | 119.4(2) | C(11)-C(12)-C(13) | 119.6(2) |
| N(1)-C(1) | 1.429(4) | N(2)-N(1)-C(7) | 110.5(2) | C(12)-C(13)-C(14) | 120.9(2) |
| N(1)-C(7) | 1.342(4) | C(1)-N(1)-C(7) | 130.2(2) | C(9)-C(14)-C(13) | 118.8(2) |
| N(2)-N(3) | 1.310(3) | N(1)-N(2)-N(3) | 107.4(2) | N(4)-C(15)-C(16) | 105.0(2) |
| N(3)-C(8) | 1.365(4) | N(2)-N(3)-C(8) | 108.7(2) | N(6)-C(16)-C(15) | 108.3(2) |
| N(4)-C(9) | 1.429(4) | N(5)-N(4)-C(15) | 110.6(2) | N(6)-C(16)-C(17) | 121.2(2) |
| N(4)-C(15) | 1.348(4) | C(9)-N(4)-C(15) | 130.4(2) | C(15)-C(16)-C(17) | 130.5(2) |
| N(4)-N(5) | 1.358(3) | N(5)-N(4)-C(9) | 119.1(2) | N(7)-C(17)-C(16) | 122.2(2) |
| N(5)-N(6) | 1.312(3) | N(4)-N(5)-N(6) | 107.3(2) | N(7)-C(17)-C(18) | 108.1(2) |
| N(6)-C(16) | 1.368(4) | N(5)-N(6)-C(16) | 108.9(2) | C(16)-C(17)-C(18) | 129.7(2) |
| N(7)-C(17) | 1.368(4) | N(8)-N(7)-C(17) | 108.9(2) | N(9)-C(18)-C(17) | 105.3(2) |
| N(7)-N(8) | 1.310(3) | N(7)-N(8)-N(9) | 107.3(2) | N(9)-C(19)-C(20) | 118.6(2) |
| N(8)-N(9) | 1.362(3) | N(8)-N(9)-C(19) | 119.7(2) | N(9)-C(19)-C(24) | 120.1(2) |
| N(9)-C(19) | 1.433(4) | N(8)-N(9)-C(18) | 110.4(2) | C(20)-C(19)-C(24) | 121.3(2) |
| N(9)-C(18) | 1.342(4) | C(18)-N(9)-C(19) | 129.9(2) | C(19)-C(20)-C(21) | 118.9(2) |
| C(1)-C(6) | 1.380(4) | C(2)-C(1)-C(6) | 120.4(2) | C(20)-C(21)-C(22) | 120.5(2) |
| C(1)-C(2) | 1.380(4) | N(1)-C(1)-C(6) | 120.3(2) | C(21)-C(22)-C(23) | 119.8(2) |
| C(2)-C(3) | 1.373(4) | N(1)-C(1)-C(2) | 119.3(2) | C(22)-C(23)-C(24) | 120.9(2) |
| C(3)-C(4) | 1.379(4) | C(1)-C(2)-C(3) | 119.9(2) | C(19)-C(24)-C(23) | 118.7(2) |
| C(4)-C(5) | 1.374(4) | C(2)-C(3)-C(4) | 120.3(2) | C(9)-C(10)-H(10) | 120.00 |
| C(5)-C(6) | 1.384(4) | C(3)-C(4)-C(5) | 119.6(2) | C(11)-C(10)-H(10) | 120.00 |
| C(7)-C(8) | 1.372(4) | C(4)-C(5)-C(6) | 120.7(2) | C(10)-C(11)-H(11) | 120.00 |
| C(8)-C(8)#1 | 1.452(4) | C(1)-C(6)-C(5) | 119.0(2) | C(12)-C(11)-H(11) | 120.00 |
| C(2)-H(2) | 0.9500 | N(1)-C(7)-C(8) | 105.1(2) | C(11)-C(12)-H(12) | 120.00 |
| C(3)-H(3) | 0.9500 | C(7)-C(8)-C(8)#1 | 129.6(2) | C(13)-C(12)-H(12) | 120.00 |
| C(4)-H(4) | 0.9500 | N(3)-C(8)-C(7) | 108.3(2) | C(12)-C(13)-H(13) | 120.00 |
| C(5)-H(5) | 0.9500 | N(3)-C(8)-C(8)#1 | 122.1(2) | C(14)-C(13)-H(13) | 120.00 |
| C(6)-H(6) | 0.9500 | C(1)-C(2)-H(2) | 120.00 | C(9)-C(14)-H(14) | 121.00 |
| C(7)-H(7) | 0.9500 | C(3)-C(2)-H(2) | 120.00 | C(13)-C(14)-H(14) | 121.00 |
| C(9)-C(10) | 1.384(4) | C(4)-C(3)-H(3) | 120.00 | N(4)-C(15)-H(15) | 127.00 |
| C(9)-C(14) | 1.379(4) | C(2)-C(3)-H(3) | 120.00 | C(16)-C(15)-H(15) | 128.00 |
| C(10)-C(11) | 1.373(4) | C(5)-C(4)-H(4) | 120.00 | N(9)-C(18)-H(18) | 127.00 |
| C(11)-C(12) | 1.383(4) | C(3)-C(4)-H(4) | 120.00 | C(17)-C(18)-H(18) | 127.00 |
| C(12)-C(13) | 1.369(4) | C(4)-C(5)-H(5) | 120.00 | C(19)-C(20)-H(20) | 121.00 |
| C(13)-C(14) | 1.388(4) | C(6)-C(5)-H(5) | 120.00 | C(21)-C(20)-H(20) | 121.00 |
| C(15)-C(16) | 1.372(4) | C(1)-C(6)-H(6) | 120.00 | C(20)-C(21)-H(21) | 120.00 |
| C(16)-C(17) | 1.449(4) | C(5)-C(6)-H(6) | 121.00 | C(22)-C(21)-H(21) | 120.00 |
| C(17)-C(18) | 1.374(4) | N(1)-C(7)-H(7) | 127.00 | C(21)-C(22)-H(22) | 120.00 |
| C(19)-C(20) | 1.392(4) | C(8)-C(7)-H(7) | 127.00 | C(23)-C(22)-H(22) | 120.00 |
| C(19)-C(24) | 1.377(4) | N(4)-C(9)-C(10) | 118.3(2) | C(22)-C(23)-H(23) | 120.00 |
| C(20)-C(21) | 1.379(4) | N(4)-C(9)-C(14) | 120.9(2) | C(24)-C(23)-H(23) | 120.00 |
| C(21)-C(22) | 1.381(4) | C(10)-C(9)-C(14) | 120.8(2) | C(19)-C(24)-H(24) | 121.00 |
| C(22)-C(23) | 1.379(4) | C(9)-C(10)-C(11) | 119.3(2) | C(23)-C(24)-H(24) | 121.00 |
| C(23)-C(24) | 1.381(4) | C(10)-C(11)-C(12) | 120.6(2) | | |
| C(n)-H(n) | | | | | |
| [n=10-24] | 0.9500 | | | | |

Crystal data of **6**:

Monoclinic; space group: $P2_1/c$; cell dimensions: a = 7.4088 (4) Å, $\alpha$ = 90°, b = 4.5388 (3) Å, $\beta$ = 95.767° (5), c = 14.5860 (9) Å, $\gamma$ = 90°; V = 488.00 (5) Å$^3$; Z = 2, $D_x$ = 1.716 Mg/m$^3$; $\mu$ = 1.221 mm$^{-1}$; F(000) = 260. Data collection: T = 123 K; graphite monochromator. A translucent colorless crystal with dimensions of 0.44 x 0.02 x 0.01 mm was used to measure 1756 reflections (748 unique reflections, $R_{int}$ = 0.0298) from 6.10° to 62.10° on a Oxford Diffraction Gemini Ultra with the omega-scan method. Structure refinement: The $F^2$ value was refined using the full-matrix least squares refinement method, with a goodness-of-fit of 1.062 for all reflections and 85 parameters. Final R indices: [I≥2σ(I)] = 0.0773, $wR_2$ = 0.2273. Absolute structure parameters: $\sigma_{fin}$(max/min)[eÅ$^{-3}$] = 1.021/-0.299.

Table of atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters (Å$^2$ x $10^3$) of **6**. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor:

| Atom | x | y | z | U(eq) |
|---|---|---|---|---|
| O(1) | 6329(4) | -1046(6) | 3132(2) | 39(1) |
| O(2) | 8158(4) | 2644(7) | 2785(2) | 46(1) |
| N(1) | 7524(4) | 4376(8) | -159(2) | 36(1) |
| N(2) | 6519(4) | 2632(8) | 315(2) | 35(1) |
| N(3) | 7674(4) | 1282(8) | 945(2) | 34(1) |
| C(1) | 9291(5) | 4167(9) | 188(2) | 32(1) |
| C(2) | 9397(5) | 2156(9) | 898(2) | 34(1) |
| C(3) | 6952(6) | -688(10) | 1597(2) | 37(1) |
| C(4) | 7213(5) | 541(9) | 2575(2) | 32(1) |

Table of bond legths [Å] and angles [deg] of **6**:

| Bond | Length | Bond | Angle | Bond | Angle |
|---|---|---|---|---|---|
| O(1)-C(4) | 1.310(5) | C(4)-O(1)-H(1O) | 112(4) | O(1)-C(4)-C(3) | 110.8(3) |
| O(2)-C(4) | 1.205(5) | N(2)-N(1)-C(1) | 109.5(3) | O(2)-C(4)-C(3) | 122.9(3) |
| O(1)-H(1O) | 0.80(6) | N(1)-N(2)-N(3) | 106.1(3) | O(1)-C(4)-O(2) | 126.3(3) |
| N(1)-N(2) | 1.328(5) | N(2)-N(3)-C(2) | 112.0(3) | N(3)-C(2)-H(2) | 128.00 |
| N(1)-C(1) | 1.359(5) | C(2)-N(3)-C(3) | 129.2(3) | C(1)-C(2)-H(2) | 128.00 |
| N(2)-N(3) | 1.339(4) | N(2)-N(3)-C(3) | 118.7(3) | N(3)-C(3)-H(3A) | 109.00 |
| N(3)-C(2) | 1.345(5) | N(1)-C(1)-C(2) | 107.9(3) | N(3)-C(3)-H(3B) | 109.00 |
| N(3)-C(3) | 1.447(5) | C(1)#1-C(1)-C(2) | 130.0(3) | C(4)-C(3)-H(3A) | 109.00 |
| C(1)-C(1)#1 | 1.446(5) | N(1)-C(1)-C(1)#1 | 122.1(3) | C(4)-C(3)-H(3B) | 109.00 |
| C(1)-C(2) | 1.377(5) | N(3)-C(2)-C(1) | 104.6(3) | H(3A)-C(3)-H(3B) | 108.00 |
| C(3)-C(4) | 1.526(4) | N(3)-C(3)-C(4) | 111.6(3) | | |
| C(2)-H(2) | 0.9500 | | | | |
| C(3)-H(3A) | 0.9900 | | | | |
| C(3)-H(3B) | 0.9900 | | | | |

Crystal data of **7**:

Monoclinic; space group: $P2_1/a$; cell dimensions: a = 16.3417 (3) Å, $\alpha$ = 90°, b = 23.1347 (4) Å, $\beta$ = 107.787° (3), c = 18.0784 (5) Å, $\gamma$ = 90°; V = 6508.0 (3) Å$^3$; Z = 4, $D_x$ = 1.134 Mg/m$^3$; $\mu$ = 2.968 mm$^{-1}$; F(000) = 2286. Data collection: T = 123 K; graphite monochromator. A translucent colorless crystal with dimensions of 0.36 x 0.11 x 0.06 mm was used to measure 31311 reflections (6840 unique reflections, $R_{int}$ = 0.0455) from 3.20° to 51.54° on a Oxford Diffraction Gemini Ultra with the omega-scan method. Structure refinement: The $F^2$ value was refined using the full-matrix least squares refinement method, with a goodness-of-fit of 15.585 for all reflections and 677 parameters. Final R indices: [I≥2σ(I)] = 0.1981, $wR_2$ = 0.3470. Absolute structure parameters: $\sigma_{fin}$(max/min)[eÅ$^{-3}$] = 3.112/-1.520.

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## 4. Abbreviations

| | |
|---|---|
| A | anion |
| Ala | alanine |
| Asp | aspartic acid |
| aq | aqueous |
| Bn | benzyl |
| BnOH | benzyl alcohol |
| Boc | *tert*-Butoxycarbonyl |
| Bu | butyl |
| $^{t}$BuOH | *tert*-butanol |
| Bzl | benzyl |
| c | concentration |
| calc | calculated |
| CC | column chromatography |
| CD | circular dichroism |
| d | days |
| DCM | dichloromethane |
| DIEA | ethyl diisopropyl amine |
| DMAP | 4-(dimethylamino) pyridine |
| DMF | dimethylformamide |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| EA | elemental analysis |
| EDC | 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide |
| EDTA | ethylenediaminetetraacetic acid disodium salt |
| EE | ethyl acetate |
| EI | electronic ionisation |
| eq | equivalents |
| ESI | electronic spray ionisation |
| Et | ethyl |
| $Et_2O$ | diethyl ether |
| EtOAc | ethyl acetate |
| EtOH | ethanol |
| FAB | fast atom bombardment |
| Fc | ferrocene |
| fig | figure |
| FI | fluorescence |
| FRET | fluorescent resonance energy transfer |
| Glu | glutamic acid |
| Gly | glycine |
| GP | general procedure |
| h | hour |
| HEL | hen eggwhite lysozyme |
| Hepes | 4-(2-hydroxyethyl)-piperazine-1-ethane sulfonic acid |
| His | histidine |
| $HNEt_2$ | diethyl amine |
| HOBt | 1-Hydroxybenzotriazole |
| HPLC | high performance liquid chromatography |

| | |
|---|---|
| HR | high resolution |
| IDA | iminodiacetic acid |
| IR | infrared spectroscopy |
| J | coupling constant |
| K | cation |
| LC | liquid chromatography |
| Leu | leucine |
| Lys | lysine |
| M | molecule |
| Me | methyl |
| MeCN | acetonitrile |
| MeOH | methanol |
| min | minutes |
| MF | molecular formular |
| Mp | melting point |
| MS | mass spectroscopy |
| MW | molecular weight |
| Myo | myoglobine |
| $NEt_3$ | triethyl amine |
| NIR | near infrared |
| NMR | nuclear magnetic resonance |
| NTA | nitrilotriacetic acid |
| OAc | acetate |
| PE | petrol ether |
| Ph | phenyl |
| Phe | phenylalanine |
| $R_f$ | retention factor |
| RNA | ribonucleic acid |
| r.t. | room temperature |
| sat | saturated |
| Ser | serine |
| $S_N$ | nucleophilic substitution |
| TBABr | tetrabutyl ammonium bromide |
| TBTU | 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium tetrafluoro borate |
| TFA | trifluoroacetic acid |
| TGS | target guided synthesis |
| THF | tetrahydrofurane |
| TLC | thin layer chromatography |
| TMS | trimethylsilyl |
| TPP | tetraphenyl porphyrin |
| UV | ultraviolet |
| Val | valine |
| Vis | visible |
| x | mole fraction |

# 5. Appendix

## PUBLICATIONS

- *Chiral NADH Model Systems Functionalized with Zn(II)-cyclen as Flavin Binding Site*; Stefan C. Ritter, Martin Eiblmaier, Veronika Michlova, Burkhard König, *Tetrahedron* **2005**, *61*, 5241 – 5251.
- *Zum Forschen nach China*; Michael Kruppa, Stefan C. Ritter, Burkhard König, *Nachrichten aus der Chemie* **2005**.
- *Amplification and Transduction of a Light Signal by Regulated Photo-Activated Transition Metal Catalysis*; Stefan C. Ritter, Burkhard König, *Chem. Commun.* **2006**, *45*, 4694 – 4696.
- *Novel Bitriazole Ligands and Metal Complexes*; Uwe Monkowius, Stefan C. Ritter, Manfred Zabel, Hartmuth Yersin, Burkhard König, in Vorbereitung.

## Conferences and Presentations

- Workshop *Supramolecular Chemistry*; Institute of Chemical Technologie in Prague, Czech Republic, May **2003**.
- Stefan C. Ritter, Burkhard König; **New Chiral NADH-Model-Systems**; Symposium *Intra- and Intermolecular Electron-transfer* of the Volkswagen-Foundation in Walberberg, Deutschland, Oct. **2003**.
- Stefan C. Ritter, Burkhard König; **Molecular Recognition of Protein Surfaces**; International Conference *Reaction Mechanisms VII* in Dublin, Ireland, July **2004**.
- Stefan C. Ritter, Burkhard König; **„Click-Chemistry" in Target-Guided-Synthesis**; *Summer School Medicinal Chemistry* of the International Quality Network (IQN) in Regensburg, Germany, Oct. **2004**.
- Stefan C. Ritter, Burkhard König; **A Light-Induced Cu(I)-Catalyzed Cycloaddition Reaction**; International Conference *Central European Conference On Photochemistry* in Bad Hofgastein, Austria, March **2006**.

# Curriculum Vitae

## Personal Details

| | |
|---|---|
| Name: | Stefan Christian Ritter |
| Date of birth: | 30.08.1977 |
| Place of Birth: | Schongau |

## Tertiary Education

| | |
|---|---|
| 01.04 – 03.07 | PhD in Organic Chemistry under Prof. Dr. Burkhard König at the University of Regensburg. |
| 01.03 – 09.03 | Diploma-Thesis in Organic Chemistry under Prof. Dr. Burkhard König at the University of Regensburg, thesis on the “synthesis of chiral NADH-derivatives and their reaction behaviour with riboflavine-tetraacetate”. |
| 11.98 – 12.02 | Chemistry studies at the University of Regensburg. |

## Civilian Service

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|---|---|
| 08.97 – 09.98 | “Diakonie in Oberbayern” of the Protestant Church in Herzogsaegmuehle. |

## School Education

| | |
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| 05.97 | Abitur (A levels), majors in Chemistry and Maths. |
| 09.89 – 06.97 | Gymnasium (grammar school) in Schongau. |
| 09.85 – 07.89 | Volksschule (primary school) in Altenstadt. |

**Internship**

| | |
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| 08.01 – 09.01 | Internship at Bayer AG Pharma Research, Department for chemical research in Wuppertal. |

**Stay Abroad**

| | |
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| 01.05 – 03.05 | Research at the „Shanghai Institute Of Organic Chemistry“ (SIOC) in Shanghai, China, in the working group of Prof. Dr. Dawei Ma. |

**Scholarships**

| | |
|---|---|
| 02.06 – 02.07 | Scholarship (BayEFG) of the Bavarian State. |
| 01.05 – 03.05 | Scholarship of the European Union within the context of the "Asia-Link Program". |