Synthesis of amphiphilic, fluorescent metal complexes – an approach towards binding assays

Organic Chemistry Research Report

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1. Introduction

Dynamic interactions of artificial functionalized vesicles have been investigated in the master thesis of Stefan Balk.[1] Biological membranes are built up by different membrane lipids like phospholipids, glycerolipds, sphingolipids and cholesterol with different proteins bound in or at the membrane. So far it is very difficult to investigate physical interactions in such complex systems. Therefore an approach with model membranes and synthetic receptor molecules was made to minimize the complexity and to mimic processes at the surface of biomembranes. [1]

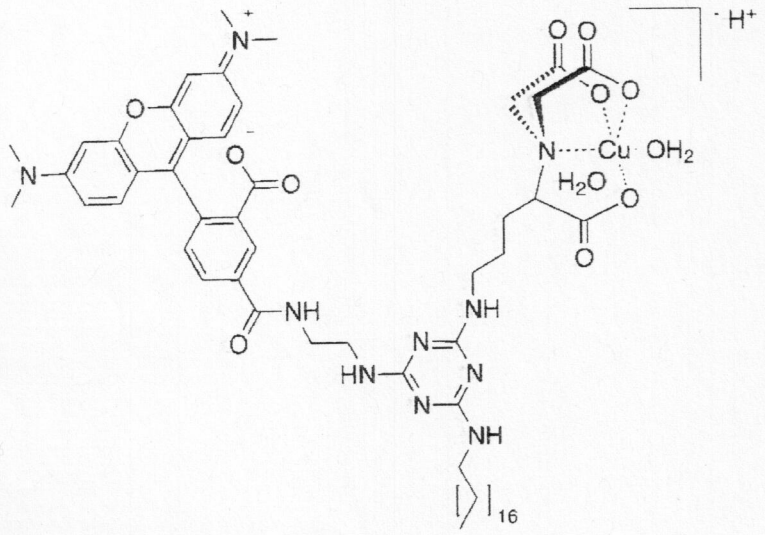
Synthetic receptor molecules are functionalized with transition metal complexes with vacant coordination sites for selective binding of specific ions. Mn(II)- or Zn(II)-dipicolin (Dpa) or Cu(II)-nitrilotriacetic acid (NTA) complexes are known to mimic the active sites of metalloenzyms. [1]

A synthetic receptor is built up by a fluorescent dye as a reporter, a metal complex as binding site which are connected via an amphiphilic linker with an alkyl chain. The hydrophobic moiety of the linker enables the assembly of the receptor in the vesicle membrane and connects the fluorescent dye and the ligand. [1]

Fluorescent labeling is a useful way to detect binding events of metal-complexes in vesicular membranes due to a change of emission intensity. Fluorescence Resonance Energy Transfer (FRET) between two fluorophores is a distance-dependent process.[2] The electronic state of a donor fluorophore is excited by a certain wavelength and transfers its energy to an acceptor fluorophore by radiationless dipole-dipole coupling.[3] This results in a fluorescence emission of the acceptor.

Energy transfer is dependent on the distance between a donor-acceptor pair and therefore measuring fluorescence is used for determining the spatial proximity between FRET-partners. A common used donor-acceptor pair for this method is Carboxyfluorescein (CF) as a donor and Tetramethylrhodamin (TAMRA) as an acceptor.[4] The synthetic receptor **1** is a FRET partner for already synthesized amphiphilic Zn^{2+}-/Mn^{2+}-Dpa complexes with a CF dye.[1]

Aim of this work was to obtain reactants for the synthesis of the amphiphilic fluorescent Cu(II)-NTA receptor **1** consisting of an amphiphilic triazine-linker, TAMRA as fluorescent dye and a Cu(II)-NTA-complex that binds small molecules with imidazole or histidine moieties.

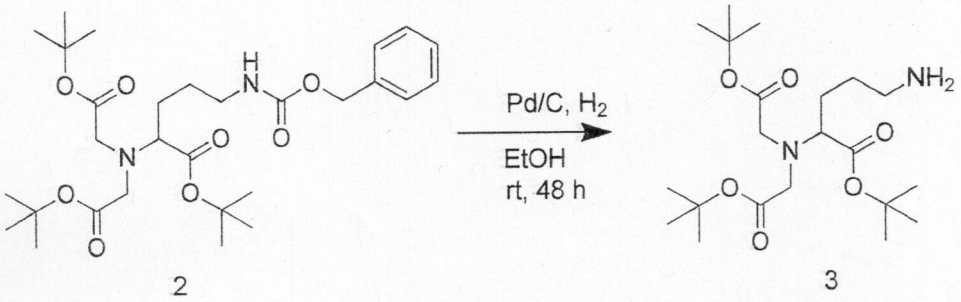


Scheme 1. Final target molecule: Amphiphilic fluorescent Cu(II)-NTA receptor. [1]

2. Synthetic approach

Synthesis of the target molecule **1** is carried out by connecting TAMRA dye and NTA-ligand via an amphiphilic cyanuric chloride linker and a following complexation with Cu(II). Modular synthesis of subunits is advantageous because it provides an easy interchangeability of the fluorescent labels and the binding sites.[1]

2.1 Deprotection of the NTA-complex precursor

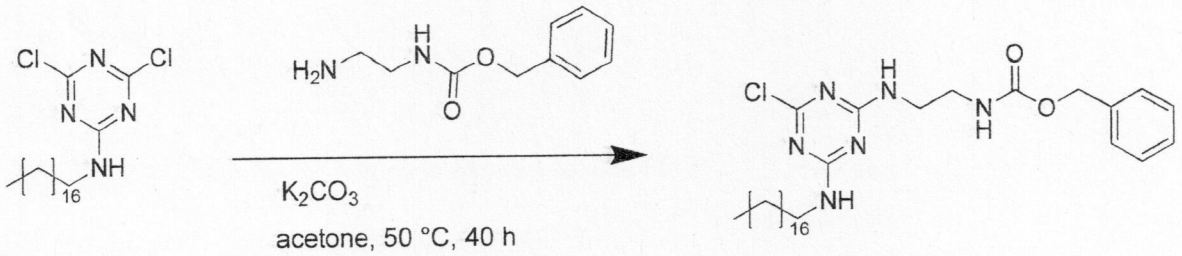


2 3

Scheme 2. Deprotection of NTA-precursor to yield the NTA-complex.

Hydrogenolytic deprotection of the NTA-complex precursor **2** leads to the cleavage of the carbamate moiety in order to obtain the final *tert*-butyl protected NTA-complex with a terminal amine.

2.2 Second substitution of the linker group

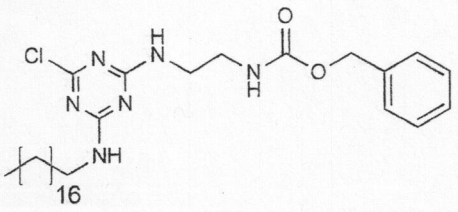


4 5 6

Scheme 3. Second substitution with mono-Cbz-protected ethylendiamine.

Cyanuric chloride as a linker is chosen because its degree of substitution is dependent on the temperature. Octadodecanylamine is tethered to the linker at 10 °C in a first substitution step yielding **4**. Second nucleophilic substitution is carried out at 50 °C in order to attach Cbz-protected ethyldiamine **5** to **4** yielding **6**.

2.3 Third substitution of the linker group



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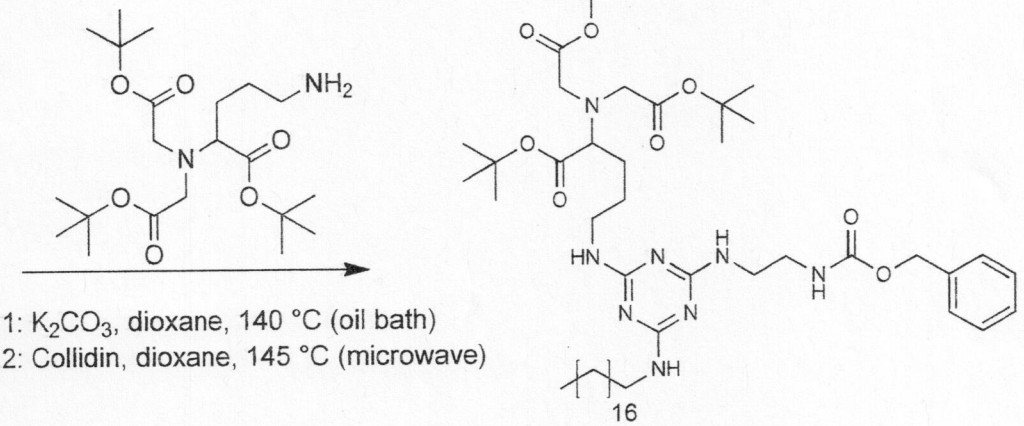


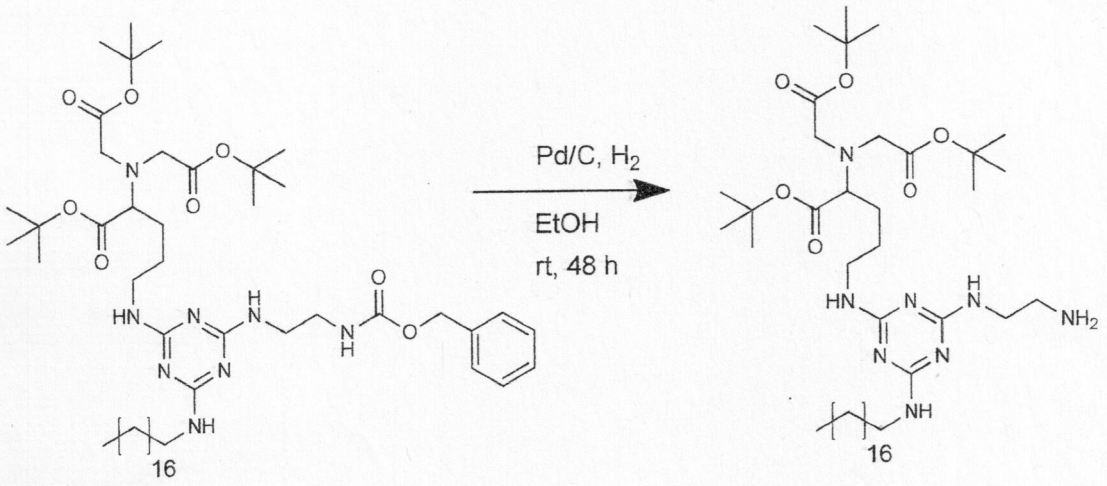
Pathway 1: K_2CO_3, dioxane, 140 °C (oil bath)
Pathway 2: Collidin, dioxane, 145 °C (microwave)

3 7

Scheme 4. Third substitution yielding the final Cbz-protected linker with the NTA-ligand.

Third substitution of the cyanuric chloride linker is carried out at 140 °C towards pathway 1 or 145°C towards pathway 2. The terminal amine of the NTA precursor **3** is connected with the amphiphilic linker **6** via nucleophilic substitution yielding **7**.

2.4 Deprotection of the cyanuric chloride linker connected with the NTA-ligand 7



7 8

Scheme 5. Deprotection of the linker-group.

Cbz-groups of the reagent **7** are hydrogenolytical cleaved off yielding a spacer with a terminal amine for fluorescent labeling.

3. Summary and outlook

In this work compounds *tert*-butyl protected NTA-complex **3** and double substituted amphiphilic triazine linker **6** have been synthesized. In a following step linker **6** was attached to the NTA-precursor **3** yielding **7** and spacer moiety compound **8** with a terminal amine after deprotection.

Next steps would be coupling compound **8** with the luminescent reporter TAMRA, its deprotection and complexation of the NTA-precursor with $Cu_2(OH)_2CO_3$ leading to final target receptor molecule **1**.

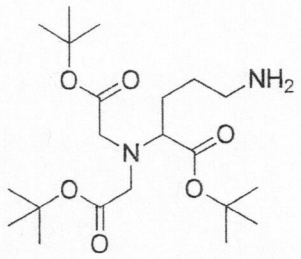
Future work will focus on the investigation of fluorescence properties of TAMRA labeled Cu(II)-NTA-complex **1**. Main goal will be the incorporation of **1** and CF labeled Zn(II)-DPA-complex in a single vesicle and to induce a FRET by bringing both luminescent receptors in close proximity in the presence of divalent ligands binding to both compounds.[1]

Investigations of these vesicular dynamic interactions give an approach to multivalent functionalized vesicles that perfectly match a biological structure to give some kind of synthetic antibodies.

4. Experimental section: Synthesis of compounds

4.1 Synthesis of di-*tert*-butyl 2,2'-((5-amino-1-(tert-butoxy)-1-oxopentan-2-yl)azanediyl)diacetate 3

NTA-precursor with a terminal amino group



4.1.1 Synthesis of the NTA-precursor 3

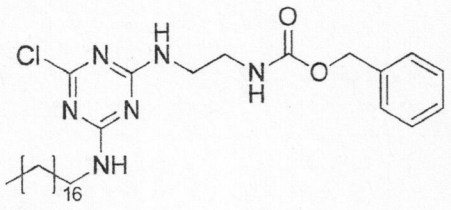
Cbz-protected NTA-precursor **2** (2.27 g, 4.12 mmol) was dissolved in ethanol (15 mL) and Pd/C (400 mg) was added. After 1 day additional Pd/C (350 mg) was added. The reaction was was carried out at rt in a H_2 atmosphere of 40 bar and stirred for further 4 d. Pd/C was filtered off via celite and the solvent was evaporated. Yellow, resinous oil was obtained (1.38 g, 3.34 mmol, 81%). R_f 0.00 (hexane/EA 3:1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ=5.00 (s, 1 H, *NH*), 3.41 (s, 2 H, CH_2-N), 3.38 (s, 2 H, CH_2-N), 3.31 (m, 1 H, *CH*-N), 2.85 (m, 2H, CH_2-NH_2), 1.52- 1.87 (m, 4 H, CH_{2alkyl}), 1.41- 1.38 (m, 27 H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ=171.82 (*COO*), 170.68 (*COO*), 81.40 (CO_{quart}), 81.03 (CO_{quart}), 65.21 (*CH*-N), 57.79 (CH_2-N), 40.36 (CH_2-NH_2), 28.13 (CH_{2alkyl}), 28.04 (CH_3), 27.04 (CH_2-CH), 18.30 (CH_3).

4.1.2 Improved synthesis of the NTA-precursor 3

Cbz-protected NTA-precursor **2** (3.20 g, 5.81 mmol) was dissolved in ethanol (15 mL) and Pd/C (670 mg) was added. The reaction was carried out at 40 bar H_2 pressure and was stirred at rt for 48 h. Pd/C was filtered off via celite and the solvent was evaporated. Yellow, resinous oil was obtained (2.42 g, 5.81 mmol, 100%). R_f 0.00 (hexane/EA 3:1).

4.2 Synthesis of benzyl (2-((4-chloro-6-(octadecylamino)-1,3,5-triazin-2-yl)amino)ethyl)carbamate 6

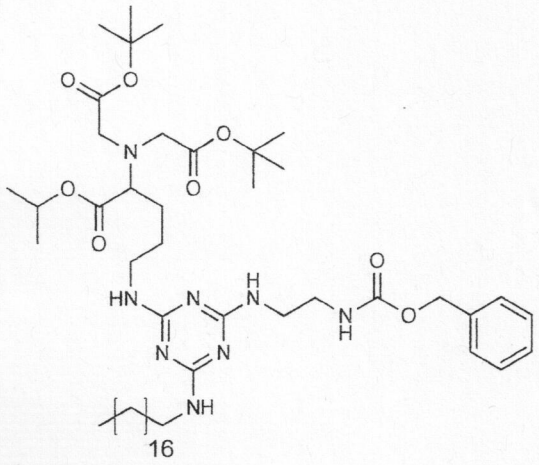
Double-substituted triazine



Amphiphilic triazine **4** (4.50 g, 10.80 mmol) was dissolved in acetone (50 mL), subsequently a solution of Cbz-protected ethylendiamine **5** (2.10 g, 10.80 mmol) in acetone (10 mL) was added dropwise and followed by addition of K_2CO_3 (7.46 g, 54.00 mmol). After stirring at 50 °C for 40 h, acetone was evaporated. The residue was dissolved in $CHCl_3$ (100 mL) and washed three times with a water/brine solution (2:1, 100 mL). The unified organic phases were dried over $MgSO_4$. The obtained raw product was recrystallized from EA_{pa} (100 mL). The precipitate was washed with EA (4 °C, 20 mL). An ochre powder was yielded as product (3.46 g, 5.84 mmol, 55 %). R_f 0.16 (hexane/EA 2:1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ= 7.29 (m, 5 H, CH_{arom}), 5.08 (s, 2 H, CH_2-Cbz), 3.56 (m, 2 H, CH_{2alkyl}), 3.27- 3.45 (m, 4 H, CH_2-CH_2), 1.53 (t, 2 H, CH_{2alkyl}), 1.25 (br s, 30 H, CH_{2alkyl}), 0.88 (t, 3 H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ= 136,53 (CH_{arom}), 128.07- 128.54 (CH_{arom}), 66.72 (CH_2-CH_2), 41.05 (CH_{2alkyl}), 31.94 (CH_{2alkyl}), 29,38-29.72 (CH_{2alkyl}), 22.71 (CH_{2alkyl}), 14.14 (CH_3).

4.3 Synthesis of di-tert 2,2'-((4-((4-((2-(((benzyloxy)carbonyl)amino(ethyl) amino)-6-(octadecylamino)-1,6dihydro-1,3,5-triazin-2-yl)amino-1-(tert-butoxy)-1-oxopentan-2-yl)azanediyl)diacetate 7

Third substitution of the amphiphilic triazine



4.3.1 Pathway 1

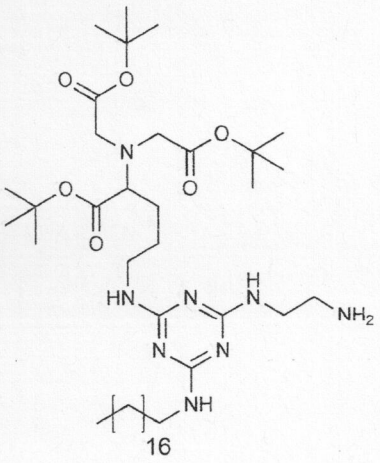
Double-substituted triazine **6** (1.35 g, 2.35 mmol) and NTA-precursor **3** (0.98 g, 2.35 mmol) were suspended in dioxane (50 mL). Subsequently K_2CO_3 (1.50 g, 3.62 mmol) was added and the mixture was stirred at 140 °C for 24 h. After TLC showed no conversion, the organic base triethylamine (330 µL, 2.35 mmol) was added and the reaction mixture was stirred at 140 °C for further 42 h. Then solvent was removed in vacuo and the residue was dissolved in $CHCl_3$ (100 mL) and washed three times with a water/brine solution (2:1, 100 mL).The unified organic phases were dried with $MgSO_4$ and the solvent was evaporated. The raw product was purified via flash silica colum (EA/hexane 2:1) and a yellow, resinous oil was obtained (0.24 g, 0.26, 11 %). R_f 0.19 (EA/hexane 2:1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.29- 7.33 (m, 5 H, CH_{arom}), 5.07 (s, 2 H, CH_2-Cbz), 3.26- 3,50 (m, 13 H, *CH* und 6 CH_{2alkyl}), 1.70 (m, 2 H, CH_{2alkyl}), 1.64 (m, 2 H, CH_{2alkyl}), 1.50 (t, 2 H, CH_{2alkyl}), 1.43 (bs, 30 H, CH_{2alkyl}), 1.24 (br s, 27 H, CH_3), 0.88 (t, 3 H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 172.221 (*COO*), 170.66 (*COO*), 136,73 (CH_{arom}), 128.45 (CH_{arom}), 127.96 (CH_{arom}), 81.11 (CO_{quart}), 80.67 (CO_{quart}), 66.49 (*CHCOO*), 65,1 (CH_2-Cbz), 53.82 (CH_2-N), 40.68 (CH_2-NH), 40.39 (CH_2-NH), 31.39- 26.22 (CH_{2alkyl}), 26.97 (CH_3-C), 22.69 (CH_2-CH_3), 14.12 (CH_3), ESI-MS (MH^+): 955.7.

4.3.2 Pathway 2

The NTA-precursor **3** (0.56 g, 1.35 mmol) was suspended in dioxane (5 mL) in a special micro wave vessel and the double-substituted linker **6** (1.42 g, 2.47 mmol) and collidine (1.63 µL, 12.37 mmol) were added. The reaction mixture was heated in the microwave at 145 °C for 2 h. Then further NTA-precursor **3** (0.44 g, 1.12 mmol) was added and the suspension was stirred in the microwave at 145 °C for further 30 min. The crude product (3.00 g) was purified via flash silica column (EA/hexane 2:1) and a brown to yellow oil was obtained (0.48 g, 37 %). R_f 0.19 (EA/hexane 2:1). ESI-MS (MH^+): 955.8.

4.4 Synthesis of di-tert-butyl 2,2'-(5-((4-((2-aminoethyl)amino)-6-(octodecylamino)-1,3,5-triazin-2-yl)amino)1-(tert-butoxy)-1-oxopentan-2-yl)azanediyl)diacetate 8

Spacer moiety compound



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7 (0.19 g, 0.20 mmol) was solved in ethanol (15 mL) and Pd/C (0.50 g) was added. The reaction mixture was stirred at rt in a H_2 atmosphere of 40 bar for 2 d. After that Pd/C was filtered off via celite and the solvent was evaporated. A light yellow, resinous oil was obtained (0.16 g, 0.20 mmol, 100%). R_f 0.19 (EA/hexane 2:1).

5. Abbreviations

Cbz	benzyloxycarbonyl
CF	carboxyfluorescin
Cu	copper
d	day
Dpa	di-(2-picolyl)amine
EA	ethylacetate
FRET	fluorescence resonance energy transfer
g	gram
h	hour
mg	milligram
min	minutes
Mn	manganese
NEt_3	triethylamine
NMR	nuclear magnetic resonance
NTA	nitriloacetic acid
R_f	retention factor
TAMRA	tetramethyl rhodamine
TLC	thin layer chromatography
Zn	zinc

6. Literature

[1] S. Balk, Master Thesis, Universität Regensburg, **2010**.

[2] Stryer L: *Fluorescence energy transfer as a spectroscopic ruler*. In *Annu. Rev. Biochem*. **1978**, 47, S. 819–46.

[3] D. L. Andrews, *"A unified theory of radiative and radiationless molecular energy transfer", Chem. Phys*. **1989**, 135, 195-201

[4] Integrated DNA Technologies, Fluorescence and Fluorescence Applications, **2005**, www.idtdna.com.