## 2. Constituents of Yoctowells:

2.1 Introduction

Bolaamphiphilic molecules contain a hydrophobic skeleton (e.g. one, two, three alkyl chains, a steroid, or a porphyrin) and two water-soluble groups on both ends.[2,7,25-28] Bolaamphiphiles are related to and often combined with "edge amphiphiles", where one flank of a hydrophobic core carries hydrophilic groups whereas the other edge is hydrophobic (Figure 1). Synthetic bolaamphiphiles try to reproduce the unusual architecture of monolayered membranes found in archaebacteria but commonly do not use the same building blocks, which are difficult to synthesize.[29-31] The ester bonds found in membrane lipids of most other organisms as well as in common model bolas are replaced there by ether bonds, which let the archaebacteria survive in a volcanic environment, e.g., in hot sulfuric acid. The hydrophobic core usually contains several chiral methine groups with methyl substituents. They help to stiffen the membrane by helix formation within the macrocycles. If more fluid membrane parts are needed, contemporary biological organisms as well as synthetic chemists introduce *cis*-configured C=C double bonds into the hydrophobic core. This is not allowed for the volcano inhabitants who have to survive in highly acidic environments.
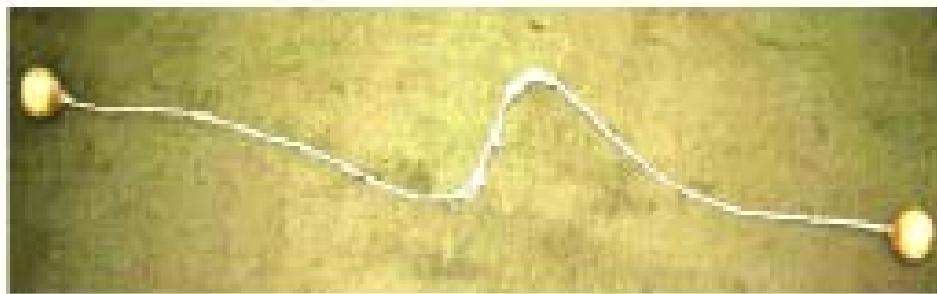

(a)
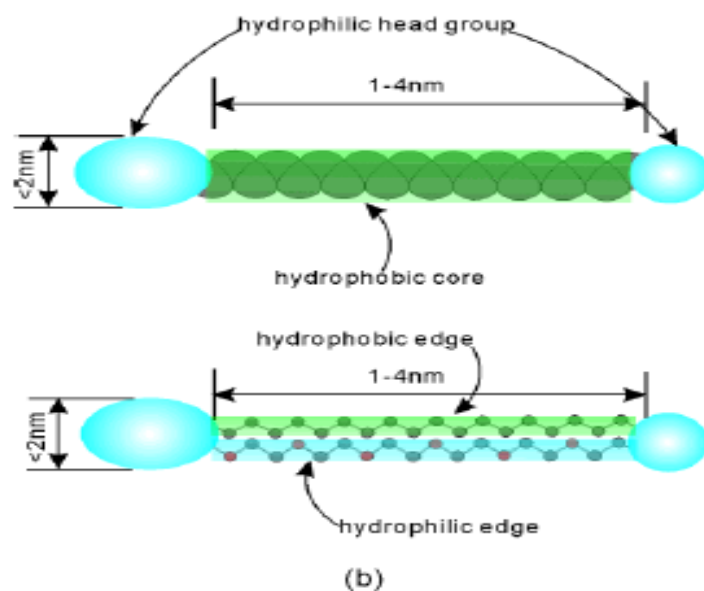
Figure 1. (a) Model of an argentine bola with leather balls; (b) schematic drawings of a bolaamphiphile (abbreviation, bola). Green coloring indicates hydrophobic parts; blue means hydrophilic. Red will be used for conjugated systems, usually dyes, as well as for oxygen atoms in structural formula.

Membrane asymmetry is more important because it may allow the localization of electron donor-acceptor pairs at distances between 1 nm to 3 nm, which is ideal for light-induced charge separation and related energy conversions. In artificial systems bolas may allow photosynthetic processes from nature to be copied without using membrane proteins. If one adds 1 mol of α, ω-bis-viologen tetrabromide amphiphile to 1 mol of iodide ions, one induces a one-sided aggregation of the symmetrical bola and obtains again an unsymmetrical membrane: all pyridinium iodides are inside the vesicle and all pyridinium bromides are outside (Figure 2a).[32] Complexation of the viologen headgroup with benzidine has a similar effect: the blue benzidine-viologen will only form at the outer surface (Figure 2b).[26]

(a)

(b)
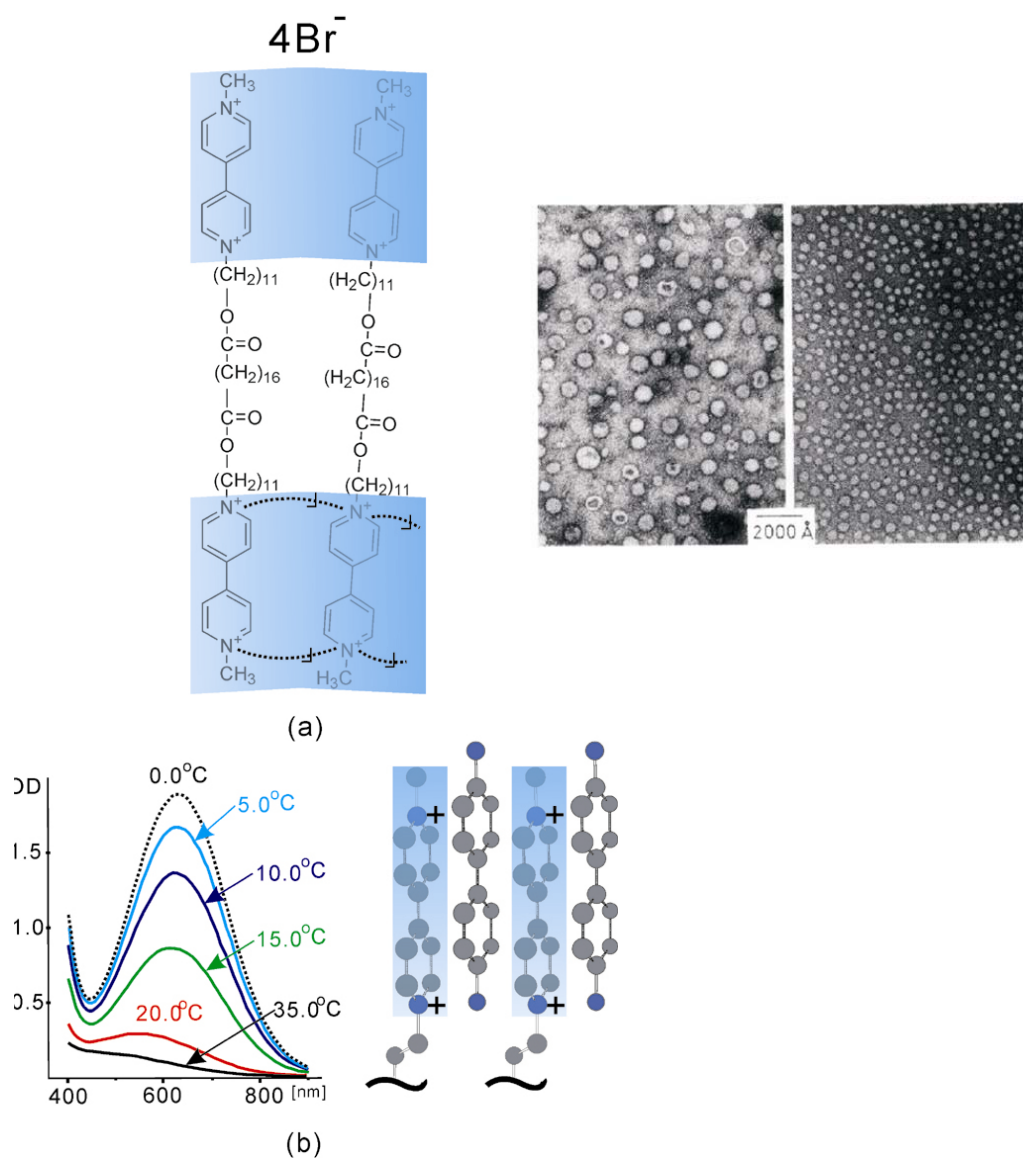
Figure 2. (a) One-sided connection of bipyridinium headgroups with iodides leads to a spontaneous vesiculation of the water-soluble tetrabromide. (b) Benzidine intercalates into the outer region of viologen vesicles and forms blue polymeric charge-transfer complexes at low temperature.

Synthetic bolas tend to form extended planar monolayers on the surface of water or of smooth solids (Figure 3a).[2,7,8] Multilayers may be formed by the combination of two cationic or two anionic headgroups or more common, by combination of a dianionic bola and a cationic polymer or vice versa. Ultrasonication of aqueous dispersions of bola monolayers yields spherical lipid particles made of monolayer lipid membranes (MLMs). Long-chain bolas produce vesicles. Short-chain water-soluble bolas give micelles.

Bolas were then preferably used as coatings of smooth solid materials: one end of bolas was covalently attached to the surface of electrodes (Figure 3a), where as the other head-groups were used for solubilization membrane in water and for interactions with solutes. Fluid or rigid monolayers covered with reactive end groups were thus obtained and applied to yield electron-conducting materials or machinery based on molecular recognition processes.[7,8]

(a)
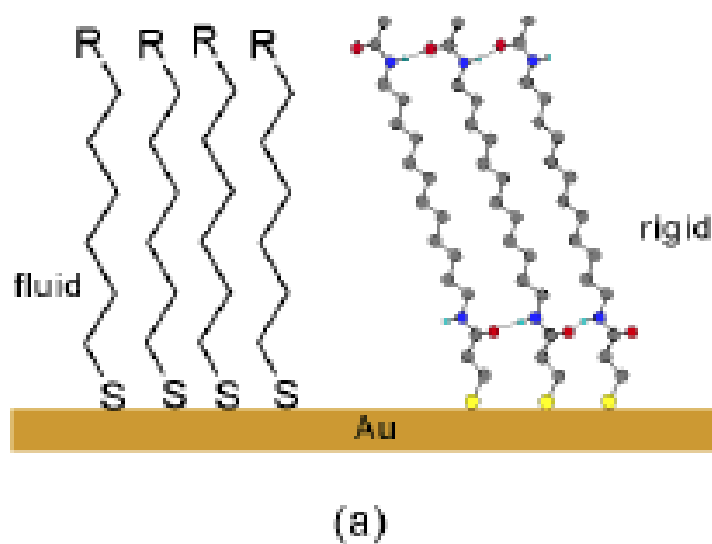
Figure 2. Once ordered, a monolayer lipid membrane of a vesicles with charged headgroups does not rearrange (no flip-flop).

Biological organisms select hydrophobic clefts on enzyme surfaces or within membrane proteins as sites for chemical reactions. Light- and redox-active sites often contain metalloporphyrins. Amphiphiles containing two secondary amide links at the ends of the hydrophobic core form rigid monolayers on smooth surfaces because two hydrogen bond

chains prevent conformational changes. Membrane-soluble amines, for example, do not permeate such a membrane.[25] Böhme *et. al.*[34] used bolaamphiphile to modified PAN surface, two parallel running amide hydrogen bond form rigid membrane and even-numbered connecting oligomethylene chain between two amide bond rigidified monolayer, if upright standing odd-numbered oligomethylene chain form fluid monolayer. The stiffness may be reached by either two parallel-running hydrogen-bond chains between two secondary amide groups at the end of hydrophobic cores made of oligomethylene chains[13,34-35] or stiff hydrophobic units such as bridged carbocycles, stilbene, or biphenylene[36-43](Figure 3 a-b)

(a)

(b)
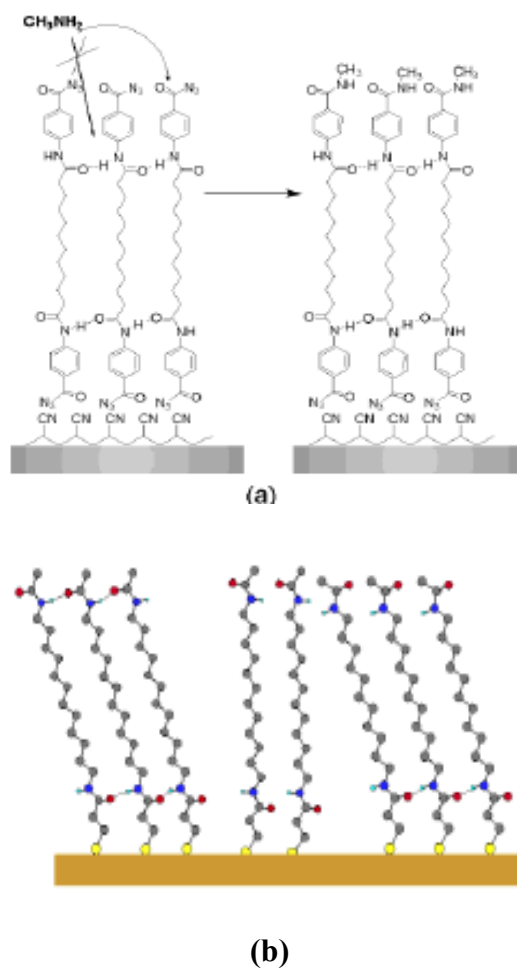
Figure 3. (a) bolaamphiphile used to modified PAN surface, two parallel running amide hydrogen bond form rigid membrane.(b) even-numbered connecting oligomethylene chain between two amide bond rigidified monolayer, if upright standing odd-numbered oligomethylene chain form fluid monolayer.

A 15 nm lipid bilayer is totally impermeable to methylamine, if two parallel running amide hydrogen-bond chains rigidify the monolayer. Outside azide groups are quantitatively aminated; inside azide groups are not changed within the following hours (Figure 3a). The formation of two parallel-running amide hydrogen-bond chains depends on the scant angle of about 20° and an even-numbered connecting oligomethylene chain. Upright standing and odd-numbered diamido bolas form fluid monolayers because only one amide-hydrogen-bond chain can be formed (Figure 3b).

Bolas have also been used routinely as a flexible connecting link between haptenes and membranes or between gold and membranes or between haptenes and proteins. All three types of linkers were applied in a molecular machinery in which a gramicidine pore is closed for ion transport if an antigen arrives. Gramicidine pores open if two helices combine end-on in both parts of a fluid bilayer lipid membrane. The pore closes if one of the gramicidine molecules diffuses away. Immobilization of the inner gramicidine as an end group of a gold-bound bola still allows the outer membrane α-grmicidine-ω-biotin-streptavidin bola to move freely and to open the ion pore. If, however, the streptavidin is bound twice via a biotin-binding peptide bola to an antigen molecule, the gramicidine molecule becomes totally immobilized and the ion flow stops(Figure 64).[44]
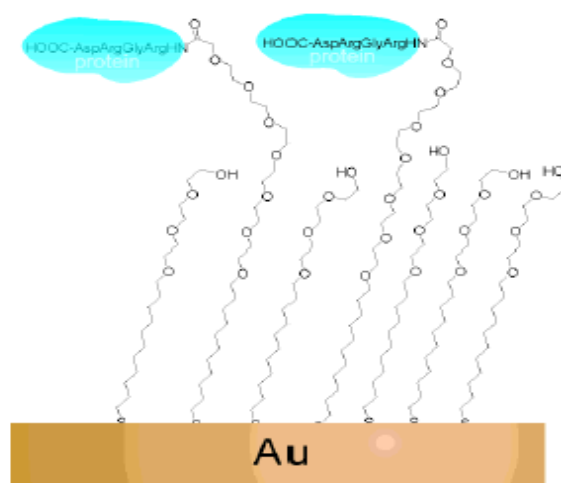

Figure 4. Schematic picture of a SAM with triethyleneglycol areas, which repulse proteins and cells and a receptor peptide, which promotes cell and protein adhesion.

Thus the classical application of bolas is the formation of monolayer membrane (MLM) vesicles. These vesicles are robust with respect to fusion and flip-flop of headgroups, and their membrane may be one-half as thick as a bilayer. The major contribution of asymmetric arrangements of α,ω-headgroups and the formation of cooperative hydrogen-bond of π-π interaction chains. The headgroups differentiate between binding to a solid surface and interactions with solvents or solutes; the internal connectivity produces an astonishing chemical and physical stability of solid surfaces. Inorganic silica gel and gold particles, for example become much more stable under an organic bola monolayer. Very strong odd-even and chain length effects allow one to manipulate the rigidity of the molecular assemblies. Bolaamphiphiles are molecules for the plain. Formation of fluid or rigid monolayers on smooth surfaces of low overall curvature and the selectivity interactions with molecules in the environment via the outer headgroups cooperate to create useful surface proprieties. Perfect protection against corrosion, form-stable nanowells which may be filled with water or chloroform and nanosized printings are typical bola success stories.

Yoctowells are form-stable gaps in rigid lipid monolayers which are bound to smooth solid surfaces or colloidal particles.[13-15,53] The form of the container stability leads to the name "well", the diameter of its porphyrin bottom between one and two nanometer makes it a "yoctowell". They were produced by two-step self-assembly procedures: first, a flat porphyrin molecule was bound in an orientation parallel to the surface; second, upright-standing bolas were self-assembled to the remaining free reaction sites of the same surface. This leads to the formation of yoctowells with the shape of a porphyrine and a height corresponding to the bola length. This will, however, only be the case if the hydrophobic bolas are rigid or if they are connected by two hydrogen-bond chains between secondary amide groups.
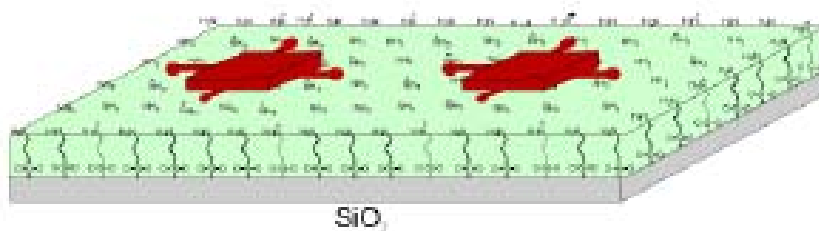
A
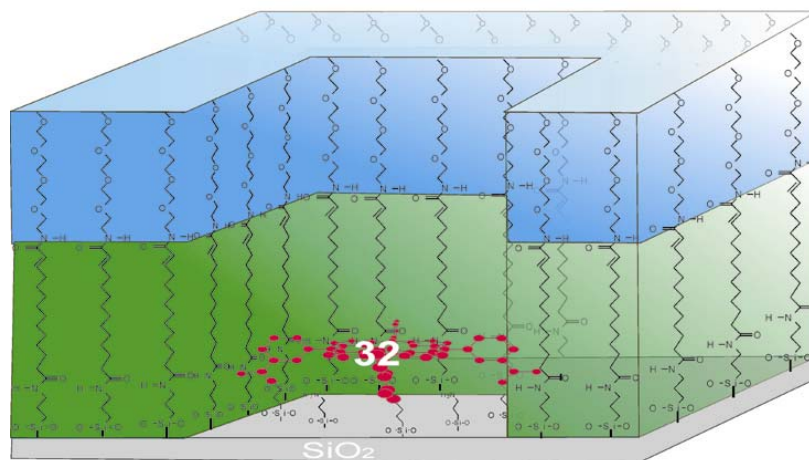
Figure 6. (A) Model of an amide-coated silica particle with covalently attached porphyrine molecules. The diameter of the smooth particles was 100 nm; their surface is, on a molecular scale, close to planar.(B) In a second self- assembly step a rigid bola wall was raised around the porphyrin a yoctowell with bolaamphiphile and a porphyrin bottom was thus established.

The construction of form-stable yoctowells on smooth surface was based on the following experimental findings:

(I) A bolaamphiphile with one head group in α-position binding to a solid colloidal sub phase and another head group in ω-position being in contact with a bulk solvent and fulfills two tasks. First it protects the surface by a monolayer coat from corroding agents in the bulk fluid phase and secondly it provides intense contact to the fluid medium.

(II) Bolaamphiphiles with two secondary amide groups close to the α- and ω- positions form two parallel hydrogen bond chains and made the monolayer impermeable two water and amines.

(III) The same amide hydrogen bonds render the monolayer extremely rigid. It now forms rigid walls around objects which are deposited on the smooth surface, in due case yoctowells around flat-lying porphyrins.

In the following we describe the synthesis of diamide bolaamphiphiles with an activated carboxyl group on one end and an oligoethylene (OEG) group at the other end. The activated acid will then be fixed on aminated silica gel particles, the OEG-group will make these particles soluble in water and solvents.

Synthesis and Characterization of Bolaamphiphiles

2.2 (OEG)2-bolaamphiphile (6)

The aim of synthesizing such lipid membrane was to have a long tetra diamido chain made of triethylene glycol (TEG) separating one OEG and one carboxylic acid group. The use of polar chains within the yoctowells have never been reported before and allows binding studies within the well provided this walls are stable in water and in organic solvents. Synthesis of ethyl 14-hydroxy-3,6,9,12-tetraoxatetradeconate **1** was achieved by direct generation of alkoxide from TEG without using protecting group.[57-59] An excess of TEG was used, which also acts as solvent. When we used toluene as a solvent for this reaction, use of toluene as a solvent created viscosity problems during the reaction. The reaction was carried out in presence of sodium metal and at high temperature. The reaction temperature had to be maintained high to avoid viscosity problem during reaction. The monosubstituted and unreacted TEG were separated by washing with dichloromethane. The obtained monosubstituted TEG was collected by evaporating solvent on rotavap. at reduced pressure. Flash silica gel column chromatography made the title compound ethyl 14-hydroxy-3,6,9,12-tetraoxatetradeconate **1** as pure. The yield was 74% with respect to ethyl bromoacetate.


1
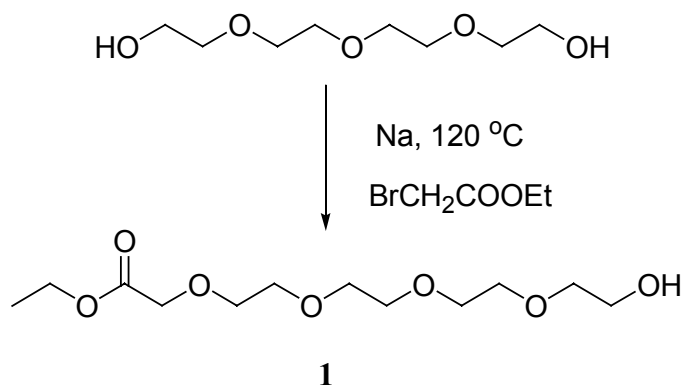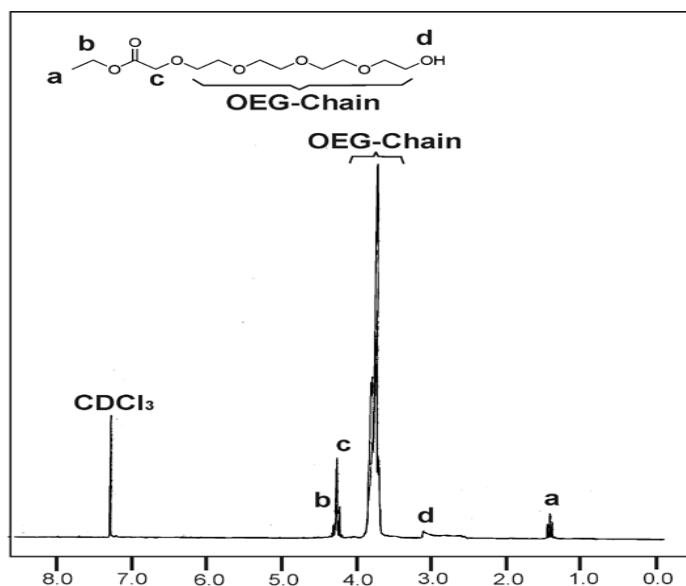
^{1}H NMR-spectrum (CDCl$_3$) of ethyl 14-hydroxy-3,6,9,12-tetraoxatetradecanote **1**

Introduction of the azido function in to ethyl 14-hydroxy-3,6,9,12- tetraoxatetradeconate **1** was carried out by mesylation followed by treatment with sodium azide in ethanol. The reaction was carried out by mesylation since sulfonate esters and halides have been extensively used as good leaving groups for substitution reaction at the terminal end of ethylene glycols. For the preparation of a variety of functionalised oligo- and poly(ethylene glycols) mesylates and tosylates have been used as starting materials. Recently it was shown that silver (I) oxide could be used for selective monoalkylation of symmetrical diols.[59] In our strategy we used compound **1** as monoalkylated so use of silver (I) oxide was not necessary. The mesylation of **1** was carried out in presence of triethylamine. After completion of reaction the reaction mixture was filtered off and the solvent was removed at reduced pressure. To remove trace amount of dichloromethane the reaction mixture was treated three times with toluene and removed at reduced pressure. The obtained pure mesylated product was treated with sodium azide at 90 ^{o}C . Thus the ethyl 14-azido-3,6,9,12-tetroxatetradeconate **2** was prepared in 90% yield from the mesylate of **1** by using sodium azide according to established method.[44,45] The yield of the compound **2** was 90%. The m/z: 306 [M+H]$^+$ obtained from pos-FAB confirmed the title compound **2**.

2
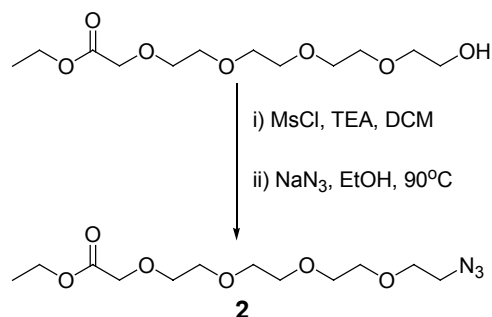
The reduction of azide group of ethyl 14-azido-3,6,9,12-tetroxatetradeconate **2** may be carried out with different methods. The conversion can be readily effected under hydrogen in the presence of palladium on carbon,[63,64] but if azide is not pure then the impurities cause loss of catalyst activity. It thus becomes necessary to add catalyst frequently to derive the reaction to completion. However, addition of palladium catalyst in presence of hydrogen atmosphere is dangerous. To overcome these difficulties Dhawan *et. al.* used formic acid as a hydrogen source, which allowed to carry the hydrogenation in open vessel. The obtained amine was in the 60-70% range. To obtain the excellent yield of amine from azide, the reduction of azide was carried out with triphenyl posphine[45,48] (Staudinger reduction) in dry THF. To the reaction mixture water was added and washed with toluene. All impurities was removed from the reaction mixture by toluene. To get the pure amine several times washing by toluene was needed. The obtained yield of ethyl 14-amino-3,6,9,12-tetraoxatetradecanoate **3** was 85% of a yellow oil.


3
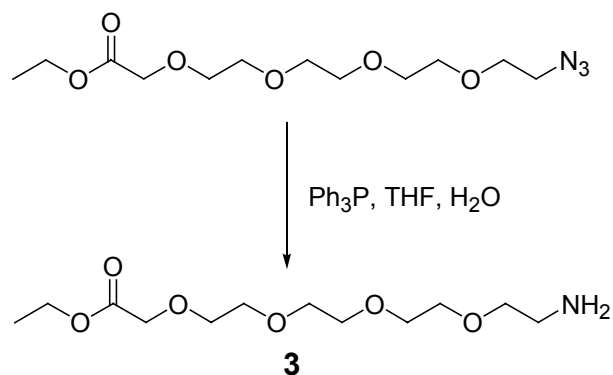
The expected molecular weight m/z 279 confirmed by mass spectrum (pos-FAB): [m/z] 279.8(M+H)$^{+}$.

Using standard peptide chemistry[66] the synthesized ethyl 14-amino-3,6,9,12-tetraoxatetradecanoate **3** (3.5 mmol) derivative was connected with trioxadecan-acid (3.5 mmol) via amide linkages to give the title compound (2-{2-[2-(-2{-2[2-(2-methoxy-ethoxy)-ethoxy-acetylamino}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-acetic acid ethyl ester **4**. The above OEG acid was treated with N-hydroxy succinimide (NHS) followed by dicyclohexyl carbodimide (DCC) in methylene chloride to yield a urethane. It is highly versatile method.[67] In this case N-hydroxy succinimide (NHS) ester was used on catalytic amount. NHS are stable at room temperature and neither air, moisture nor light sensitive. The use of NHS minimizes the side products. The urethane prepared was coupled with **3** in presence of triethylamine[51] to give the (2-{2-[2-(-2{-2[2-(2-methoxy-ethoxy)-ethoxy-acetylamino}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-acetic acid ethyl ester **4** in good yield. Dicyclohexylurea (DHU) was removed by filtration, and solvent was removed by evaporation. The residue was taken up in water, acidified, filtered and extracted with dichloromethane. During the work up of the reaction, the reaction mixture was washed by water to remove the water soluble by-product. Thus by water washing compound was made pure. The presence of remaining small impurities was removed by column chromatography (MeOH / CH_2Cl_2 2:15) on silica gel : yields 76% as a yellow oil of **4**.


4
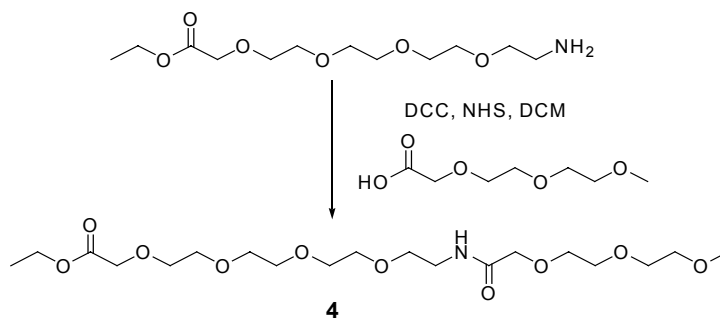
The ^{1}H NMR, ^{13}C NMR, IR and mass spectral data confirmed the title compound **4**.
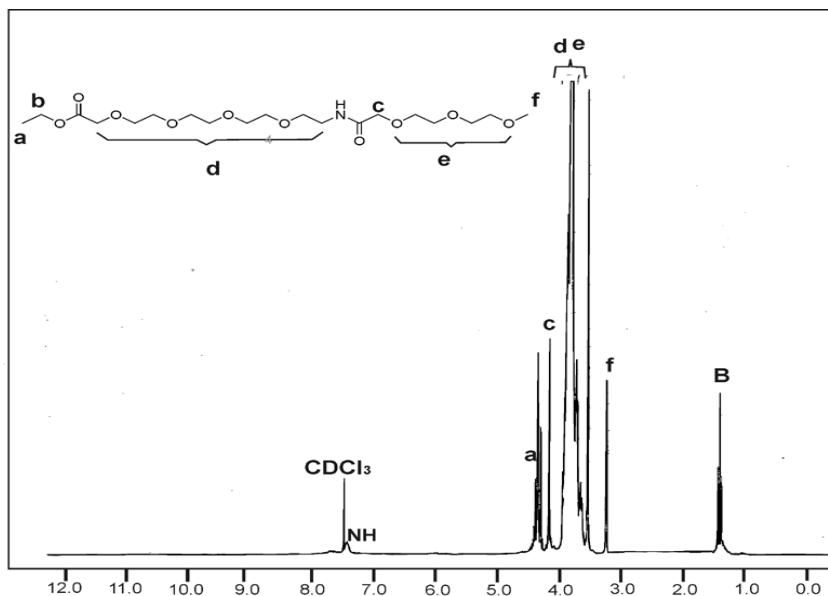
^{1}H NMR-spectrum (CDCl$_3$) of (2-{2-[2-(-2{-2[2-(2-methoxy-ethoxy)-ethoxy-acetylamino}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-acetic acid ethyl ester **4**

The obtained amide was further subjected for cleavage of ester group.[58] The ethyl ester was then easily cleaved without any disturbance of the peptide bonds by the use of sodium carbonate in water to yield the acid (2-{2-[2-(-2{-2[2-(2-methoxy-ethoxy)-ethoxy-acetylamino}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-acetic acid **5**. After completion of reaction the reaction mixture was washed with dichloromethane. The obtained product was in its salt form. Then the reaction mixture was made acidic by using 1 N HCl, followed by extraction by dichloromethane. The dichloromethane was removed on rotavapo. This method is mild, highly effective method for the hydrolysis of an ester group. The yellow oil **5** (95%) was obtained.
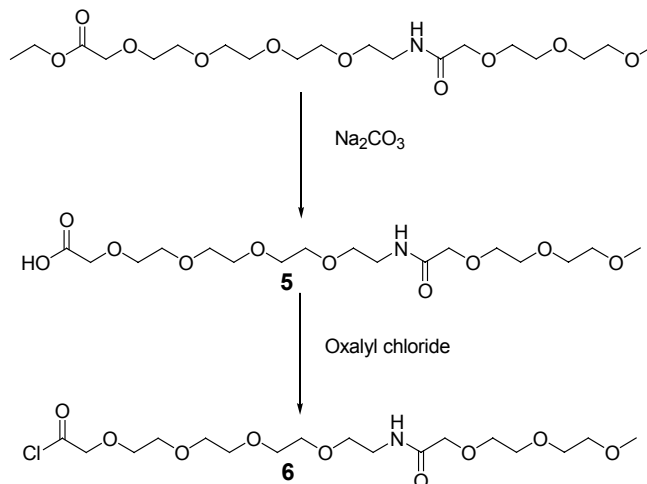
The prepared (2-{2-[2-(-2{-2[2-(2-methoxy-ethoxy)-ethoxy-acetylamino}-ethoxy)-ethox -y]-ethoxy}-ethoxy)-acetic acid **5** was modified at the acid end to have a chloride tail group. These bolaamphiphiles binds covalently to modified amino silicate particles to constitute nanowells along with *meso*-tetraphenyl meta-tetracarboxy porphyrin **32**. The terminal chloride group would normally be introduced by reaction of the free acid with oxalyl chloride.[69] This method is simple and efficient, since the removal of solvent under low pressure afforded product were used such as for self-assembly on aminated silica colloidal particles.

2.3 (2-(2-(2-(2-(2-methoxy-ethoxy)-ethoxy)-acetylamino)-acetylamino)-acetyl chloride (10)

Bolaamphiphile **10** with an OEG head group was synthesized to study the usefulness of yoctowells as the receptor and to compare these hydrogen bonds in water and solvents. The reaction steps of the synthesis of lipid membrane **10** includes protection, coupling, deprotection and introduction of chloride group at the acidic end of membrane forming bolaamphiphile. The peptide used was triglycine, which contains like all peptides an amino group at one end and acid at the other.

The synthesis of free amino acid benzyl ester[70-76] was reported by Bergmann, Zervas and Ross[70] via N-carboxy amino acid anhydrides. This method is mostly employed in the preparation of benzyl esters of amino acids. By using the method with free amino acid,[54] Kopple *et.al.*[76] prepared triglycine benzyl ester *p*- toluenesulfonate in good yield by direct esterification of the commercially available free tripeptide.

By keeping all aspects in mind the method used by us was treatment of triglycine with benzyl alcohol and PTSA in presence of toluene. Due to the carcinogenic nature of benzene it was replaced by toluene as solvent. The reaction mixture obtained by removal of water azeotropically treated with ether which leads to the precipitation of the desired (2-(2-amino-acetylamino)-acetylamino)-acetic acid benzyl ester as its *p*-toluenesulfonic acid salt **7**. The yield was raised to 77 %.

The neutralization of prepared triglycine benzyl ester in water or dimethylformamide or suspended in chloroform resulted in the formation of hexaglycine benzyl ester, in 20-40 % yield.[76] Because of rapidity of its dimerization it did not seem good to use triglycine benzyl ester as the amine component in the coupling process. The coupling of (2-(2-amino-acetylamino)-acetylamino)-acetic acid benzyl ester *p*-toluenesulfonate with trioxadecan acetic acid was carried out in presence of 1-(3-dimethylaminopropyl)-3-ethyl -carbodiimide hydrochloride at room temperature.[77] The reaction was monitored by TLC. After completion of reaction the reaction mixture was successively washed with water, 0.5 M HCl followed by water , 10% sodium carbonate and brine to remove side products. The obtained product **[PEG-GGGOCH$_2$Ph] 8** was purified by crystallization in methanol. The yield was satisfactory above 86%.
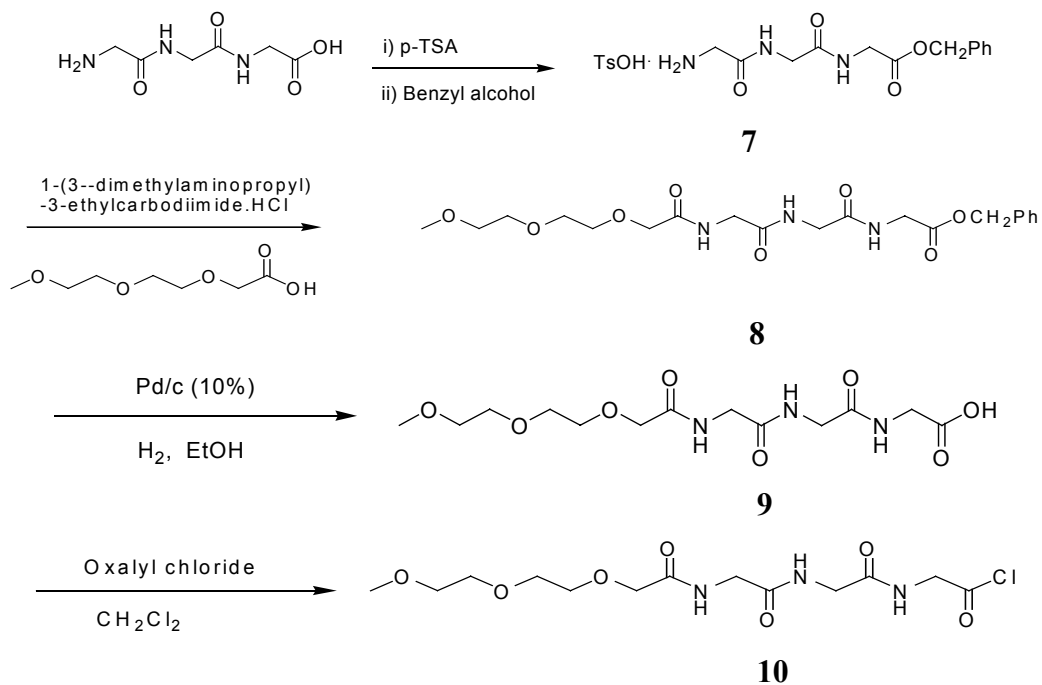
The expected benzyl group of bolaamphiphile **PEG-GGG-OCH₂Ph 8** removed by hydrogenation over 10% palladium on carbon in presence of hydrogen atmosphere. The yield of product [**PEG-GGG-OH**] **9** was 95%.

In order to use it in self assembly the terminal acid end of the [**PEG-GGG-OH**] **9** had to be converted to acid chloride [**PEG-GGG-Cl**] **10**.[69] The prepared peptide bola **10** with OEG head group at the one end and chloride group at the other end was ready to use in self assembly which made yoctowells innovative. The prepared model was used to study yoctowells as receptor in water and in various organic solvents e.g. chloroform, ethanol, etc.

2.4 (9-Chlorocarbonyl –nonanoylamino)-acetic acid methyl ester (14)

Selective monoesterification of diacids is of great importance from a synthetic point of view. Esterification reaction of diacid was performed with alkyl halide in the presence of caustic base under phase-transfer catalyst.[78] Phase- transfer catalysis method was proven to be useful to achieve high selectivity in the preparation of monoesters from diacids and alkyl halides. Sebacic acid was treated with equimolar ratio of potassium hydroxide in water stirring for one hour at room temperature. Toluene was added to it and was dried azeotropically. Potassium sebacate was obtained, dissolved in toluene and was treated with tetrabutylammonium bisulfate (TBAB) followed by benzyl bromide. The mixture was refluxed for five hours. A mixture of monoester (97.9%) and diester (2.1%) was obtained, purified on silica gel column (hexane/ ethylacetate 2:1), 65% white shiny crystals of compound decanedioic acid monobenzyl ester **11**. The expected molecular weight m/z 292 was conformed by pos- FAB mass spectrum.
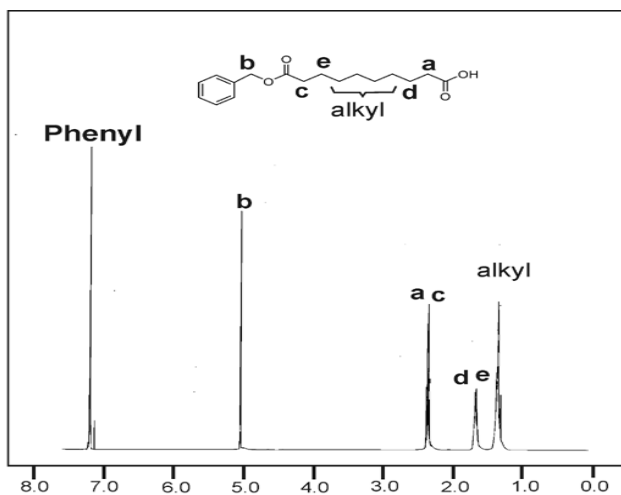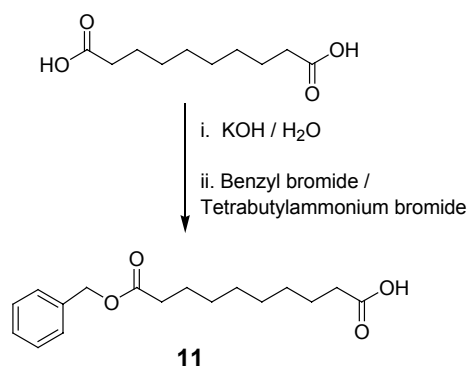
^{1}H NMR-spectrum (CDCl$_3$) of decanedioic acid monobenzyl ester **11**

In order to activate the carboxy group at one of the end of hydrophobic chain the monoprotected sebacic acid **11** was reacted with ethylchloroformate[79-80] in dry chloroform at −10 to 0 °C. Which was further treated with glycine methyl ester hydrochlorie and yielded 85% of title compound 9-(methoxy carbonyl methyl carbamoyl) -nonanoic acid benzyl ester **12** as white powder.
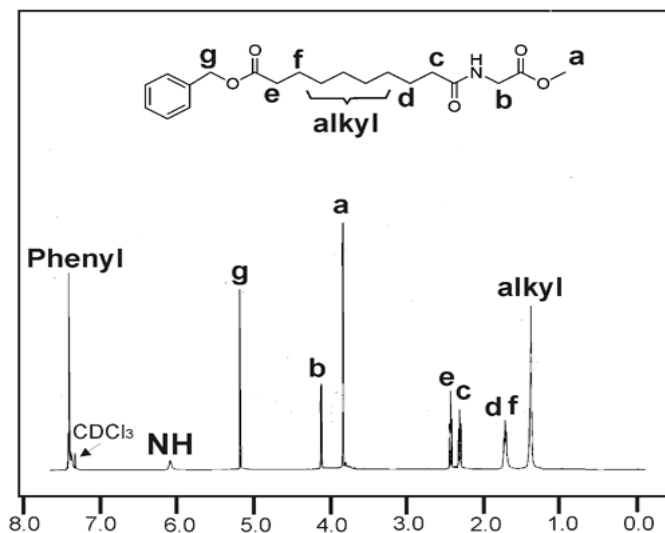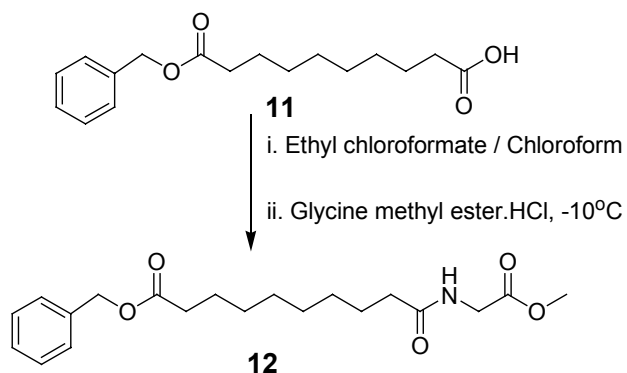
^{1}H NMR-spectrum (CDCl$_3$) of 9-(methoxy carbonyl methyl –carbamoyl) nananoic acid benzyl ester **12**
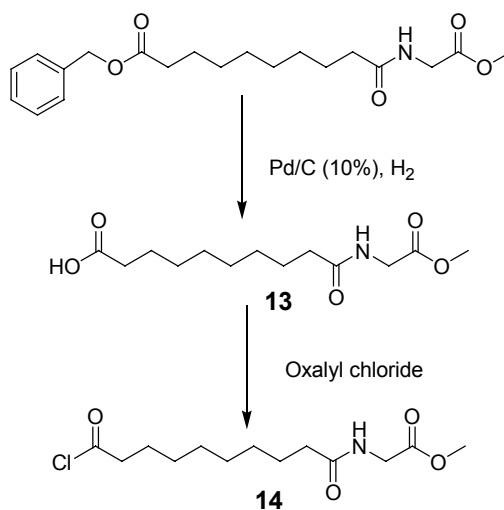
In the next step of synthesis was removal of the benzyl group from the 9-(methoxy carbonyl methyl–carbamoyl) nananoic acid benzyl ester **12**. This was carried out by using palladium on carbon in presence of hydrogen atmosphere.[81] The reaction mixture was filtered hot through a celite pad. The removal of solvent under reduced pressure followed by recrystallisation gave 9-(methoxy carbonylmethyl-carbamoyl) nonanoic acid **13**. At this stage of the synthesis the new lipid is almost complete. It could be bound to the silica surface in the form of an acid chloride which was again introduced by reaction of the free acid with oxalyl chloride.[69]

2.5 [3-(9-Chlorocarbonyl-nonanoylamino)-propyl]-trimethyl ammonium bromide (18)

Scheme:
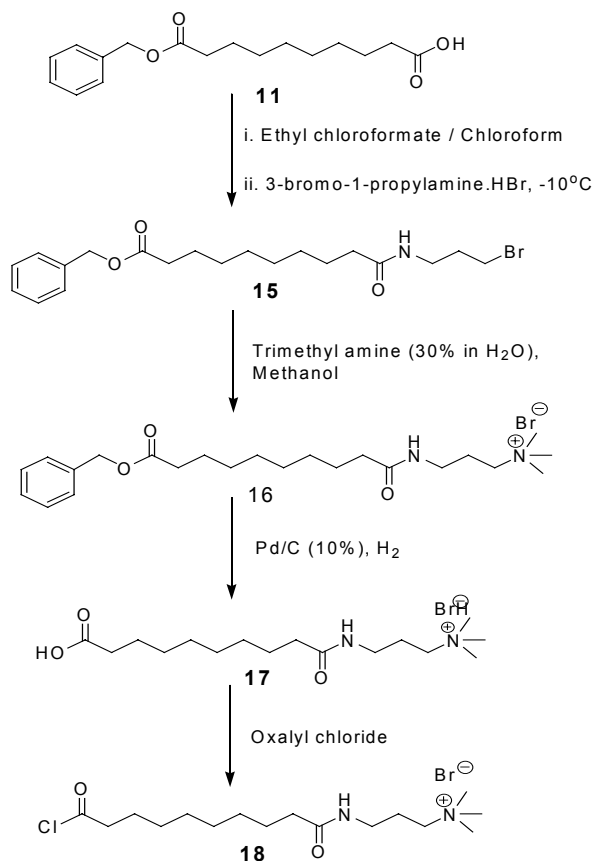
In dry chloroform at −10 °C temperature decanedioic acid monobenzyl ester **11** was activated with ethylchloroformate.[81] The reaction was carried out at −10 °C and maintained till the completion of the reaction. In situ the obtained anhydride was treated with 3-bromo-1-propylamin hydrobromide dissolved in ethanol / triethylamine / water. The reaction mixture was partitioned between water and chloroform. The organic layer was washed with water to remove impurities. The obtained product of 9-(3-Bromo-propylcarbamoyl)-nonanoic acid benzyl ester **15** was 85%.

To achieve positive charge at the end of the bolaamphiphile, 9-(3-Bromo-propyl carbamoyl)-nonanoic acid benzyl ester **15** was treated with trimethylamine (30% in water/methanol).[82] After completion of reaction solvent was removed under reduced pressure and the solid was washed with hexane followed by chloroform. The obtained

product [3-(9-Benzyloxycarbonyl nonanoylamino)propyl] trimethyl ammonium bromide **16** was white solid with 85 % yield. The ^{1}H NMR of compound **16** confirms the presence of nine proton belongs to quaternary group at 3.30 ppm.
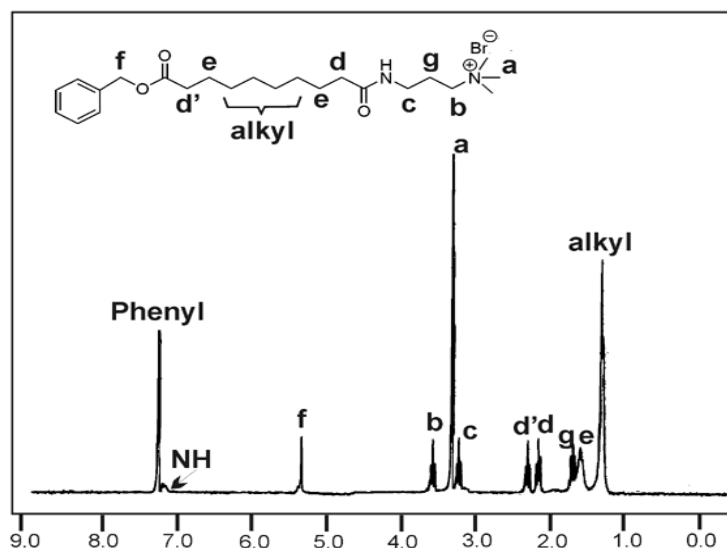
^{1}H NMR-spectrum (CDCl$_3$) of [3-(9-benzyloxycarbonyl- nonanoylamino)-propyl]- trimethyl-ammonium bromide **16**

The benzyl ester was easily cleaved from [3-(9-Benzyloxycarbonyl nonanoylamino) propyl] trimethyl ammonium bromide **16** by Pd/C (10%) in THF/ methanol at room temperature. The conversion can be readily effected under hydrogen in the presence of palladium/charcole.[81] After completion of the reaction, the reaction mixture was filtered hot through a celite pad. In the ^{1}H-NMR of compound [3-(9-carboxy-nonanoylamino)-propyl]-trimethyl-ammonium bromide **17**, similar to that of compound **16**, the peaks from benzyl and aromatic protons had disappeared. The obtained lipid was activated at the acid end by treatment with oxalyl chloride[69] to give [3-(9-Chlorocarbonyl-nonanoylamino)-propyl]-trimethyl ammonium bromide **18**. Thus the synthesized bolaamphiphile was quite good with introduced alkyl ammonium head group, which is equivalent to positively charged particles.

2.6 Synthesis of Bolaamphiphile (27)

The aim of the synthesis of the bolaamphiphile 17-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylcarbamoyl}-heptydec-16-enoyl chloride **27** was to have a long hydrophobic skeleton separating one carboxylic acid group and another ethylene glycol chain. The ethylene glycol chain makes the system water soluble as well as soluble in several organic solvents. Similar bolaamphiphiles were with connecting were obtained earlier in our group.[15] The C_{18}-alkyl chain in **27** make yoctowells deeper to study the diffusion of dye in it, as well as to do charge separation over distance about 1.5 nm. The observed results was exciting and the bola itself was much more useful in the study.

Commercial 16-hydroxydodecanoic acid was first protected by benzyl bromide in presence of potassium bicarbonate.[83-84] Benzyl esters are stable in presence of many mild acids, oxidants and reductants and are removed selectively by catalytic hydrogenolysis and also with LiOH without affecting other commonly used protecting groups.[84] The 16-hydroxyhexadecanoic acid was dissolved in dry DMF and was treated with potassium bicarbonate and benzyl bromide stirred overnight, the resulting reaction mixture poured in ice-water, filtered off the solid product and re-crystallized from methanol to offered 16- hydroxyhexadecanoic-acid benzyl ester **19** as a solid compound. The obtained ester **19** was 86% in yield.

Pyridinium chlorochromate was used to oxidize the primary alcoholic group of 16-hydroxy hexadecanoic-acid benzyl ester **19** to 16-oxo-hexydecanoic acid benzyl ester **20**.[85] Pyridinium chlorochromate was prepared by the addition of pyridine to a solution of chromium trioxide to 6M HCl followed by filtration to obtain a yellow-orange, air-stable solid. The purification of aldehyde was achieved by filtration through florisil. The inorganic byproducts were remain on florisil. The obtained product **20** was pure with 91.8% yield. The ^{1}H-NMR and mass spectral data confirmed the structure of **20,** which appeared as an oil.
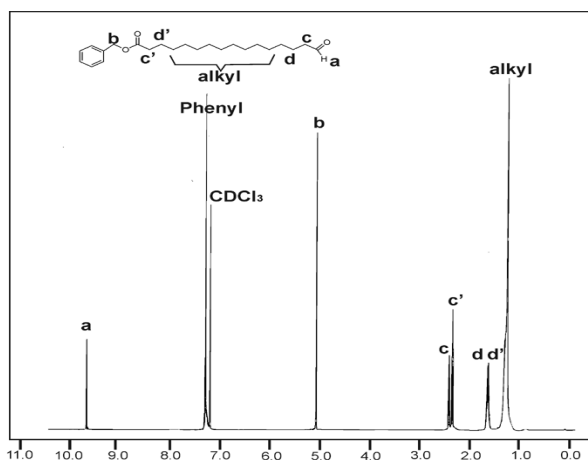
^{1}H NMR-spectrum (CDCl$_3$) of 16-oxo-hexydecanoic acid benzyl ester **20**

The next step of the synthesis was carried out to introduce the Michael double bond within the lipid. The double bond is essential to do in situ addition reaction on the particles, which modify the membrane at this site. Introduction of a trans C=C bond was carried out by Horner-Wadsworth-Emmons method[86-88] using *tert*-butyl *p,p*-dimethyl phosphono acetate. Use of *tert*-butyl *p,p*-dimethylphosphono acetate was much alternative to the triphenyl phosphine. The phosphorous by-product is water soluble, in contrast to Ph$_3$PO, which makes it easy to separate easily from the olefin. The obtained yield of *trans*-octadec-2-enedioic acid 18-benzyl ester 1-*tert*-butyl ester **21** was 74%. The trans double bond was characterized by ^{1}H-NMR the α-vinyl proton gives doublet near 5.73 ppm and β- proton gives triplet of a doublet at 6.87 ppm and the coupling constant shows 11Hz indicate a trans relationship.
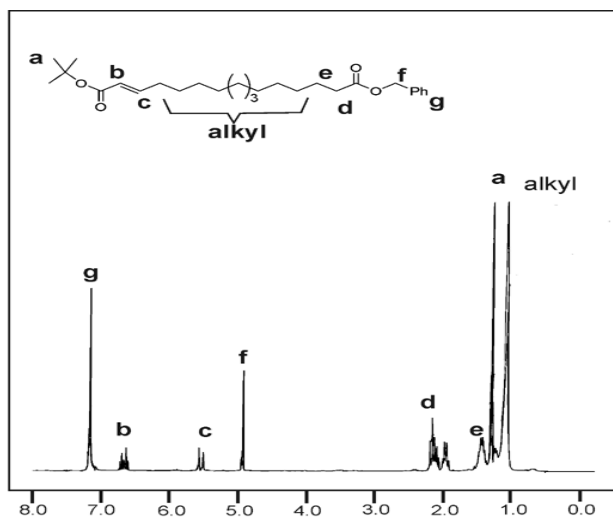
^{1}H NMR-spectrum (CDCl$_3$) of *trans*-octadec-2-enedioic acid 18-benzyl ester 1-*tert*-butyl ester **21**

The *tert*-butyl group was selectively hydrolysed by *para*-toluene sulfonic acid without affecting the benzyl ester group.[89] PTSA is efficient, mild and selective in the cleavage of the *tert*- butyl group. Thus the *tert*- butyl group was selectively deprotected in presence of benzyl ester. The resulting solid was recrystallized from chloroform/hexane to give white powder. The obtained yield of octadec-2-enedioic acid 18-benzyl ester **22** was 96%. The ^{1}H-NMR of **22** is similar to that of compound **21**, except the disappearance of signal for *tert*- butyl group at 1.47 ppm.

For the purpose of OEG-amine head group:
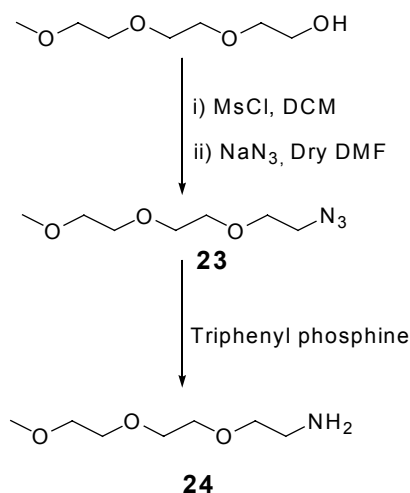
At First protection of -OH end group was achieved by using mesylate[62] in dry dichloromethane. 2-[-(2- metoxy-ethoxy)-ethoxy]–ethanol was treated with triethylamine followed by methylsulfonyl chloride in methylene chloride. The obtained mesylated product was further treated with sodium azide in dry DMF,[625] the yielded product of OEG azide 1-[2-(2-azido-ethoxy)-ethoxy]-2-methoxy-ethane **23** was 92%. 2-[-2-(2-metoxy-ethoxy)-ethoxy]–ethylamine **24** was synthesized by using Staudinger reduction method.[65] The conversion of azide **23** to amine **24** by this method is much more effective than the other methods.

The Staudinger reaction occurs between a phosphine and an azide to produce an aza-ylide[65] as shown below. In the presence of water, this intermediate hydrolyzes spontaneously (aza ylide is not stable in water) to yield a primary amine and

corresponding phosphine oxide. The phosphine and the azide react with each other rapidly in water at room temperature in high yield.

The free acid octadec-2-enedioic acid 18-benzyl ester **22** reacted with OEG amine **24** in prescence of N,N'-dicyclohexylcarbodiimide (DCC) and DMAP via the active ester method.[66,89-90] In contrast to other schemes carboxyl activation involving mixed anhydride formation, the reaction is not sensitive to moisture. The co-product, N,N'-dicyclohexylurea, has a very low solubility in most organic solvents and easily separated by filtration. The yield of obtained product 17-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylcarbamoyl}-heptadec-16-enoicacid benzyl ester **25** was 90%.

^{1}H NMR-spectrum (CDCl₃) of 17-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylcarbamoyl}-heptadec-16-enoicacid benzyl ester **25**

Benzyl ester group of 17-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylcarbamoyl}-heptadec-16-enoicacid benzyl ester **25** was hydrolyzed under mild, efficient conditions by lithium hydroxide in a heterogeneous reaction to yield 17-{2-[2-(2-methoxy-ethoxy)-ethoxy] ethylcarbamoyl}-heptydec-16-enoic acid **26**.[92] This is mild and effective method for the base hydrolysis of an ester. For the preparation of 17-{2-[2-(2-methoxy-ethoxy)-ethoxy] ethylcarbamoyl}-heptydec-16-enoiyl chloride **27** oxalyl chloride was used. Thus the 17-{2-[2-(2-methoxy-ethoxy)-ethoxy]ethylcarbamoyl}-heptydec-16-enoic acid **26** modified to acid chloride by using oxalyl chloride.[69]

2.7 Synthesis of porphyrin (31)

The synthesis of *meta*-tetracarboxy 4,8,12,18-tetra-(3-ethoxycarbonyl-propyl)-3,7,13,17-tetramethyl-22,24-dihydro-porphin-2-yl]-butyric acid ethyl ester **31** was achieved along the following pathway:
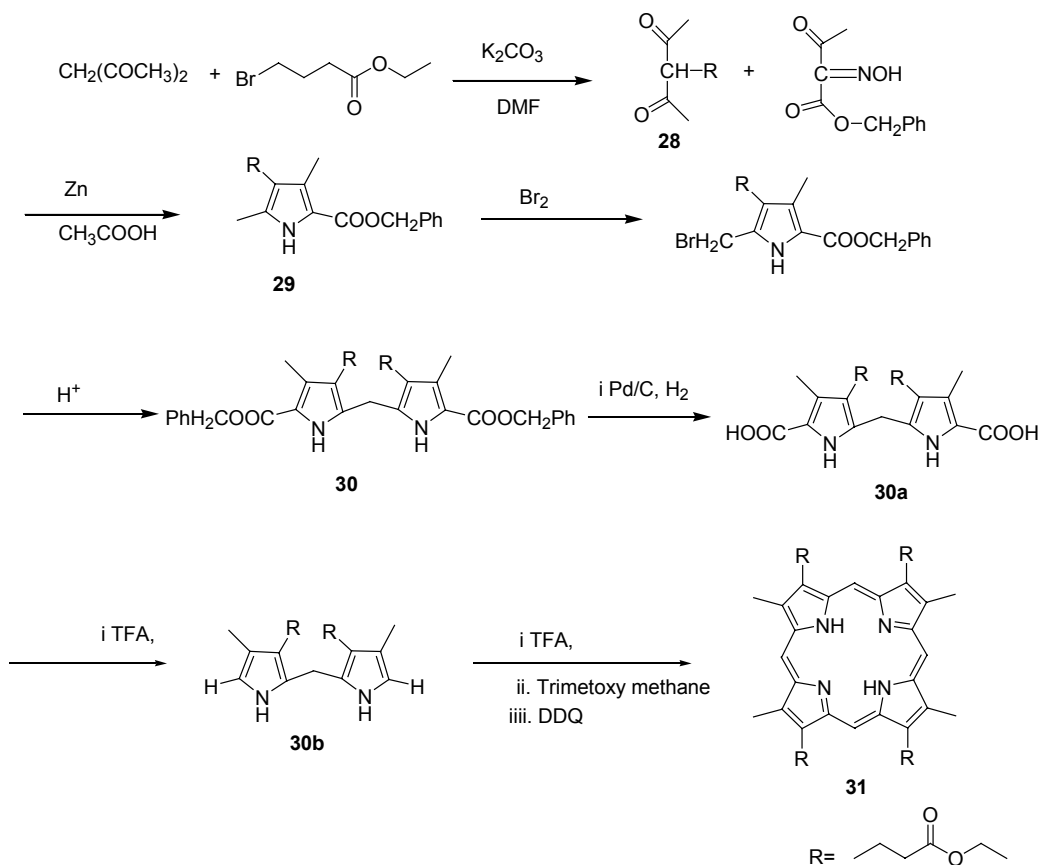
The first step includes the treatment of ethyl-4-bromobutyrate with acetylacetone in presence of anhydrous potassium carbonate at 90 °C for six hours.[93] The purification of crude product was carried out by steam distillation at 150-157 °C and 15-17 mbar. The yield of collected product 3-ethoxycarbonylpropyl-2,4-pentanedione **28** was 65%.
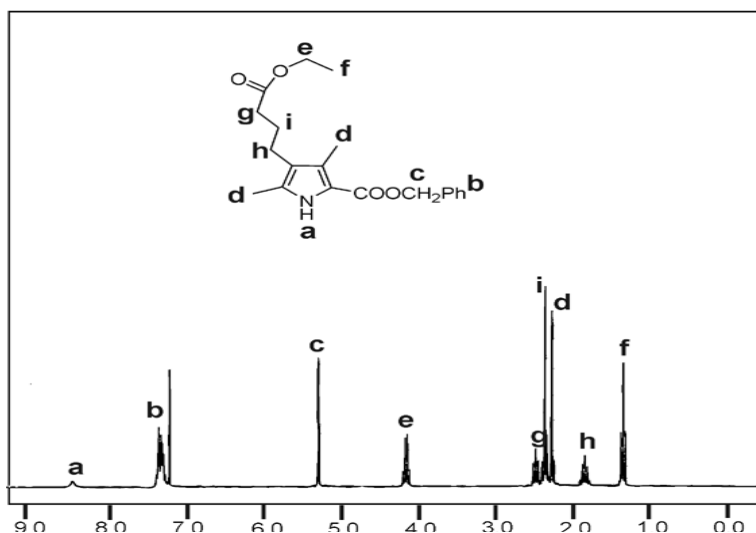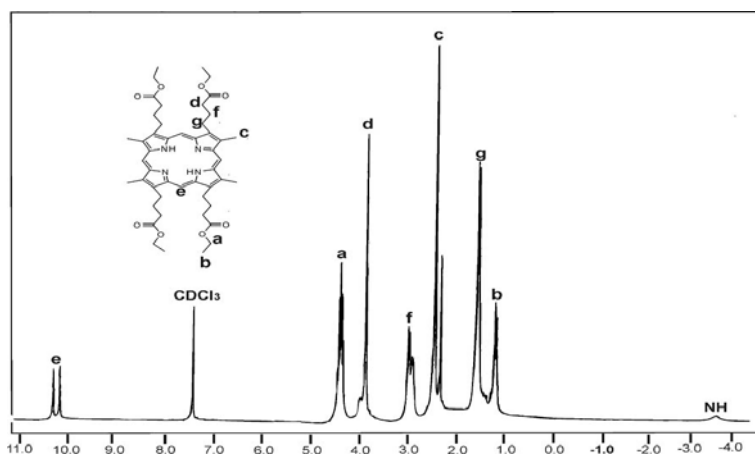

^{1}H NMR-spectrum(CDCl$_3$) of 2,4-dimethyl-3-ethoxycarbonylpropyl-5-benzyloxycarbonyl pyrrole **29**

The Knorr[73] reaction of asymmetric β-diketones is useful to prepare functionalised porphyrin compounds because the reaction can introduce various substituents into the pyrrole at two β-positions.[95-96] A regioselective Knorr pyrrole synthesis starting with an asymmetric β-diketone **28** was applied. β-Diketone **28** was condensed with phenyl α-oximinoacetoacetate in the presence of zinc dust in acetic acid. The isolated yield of 2,4-dimethyl-3-ethoxycarbonylpropyl-5-benzyloxy carbonyl pyrrole **29** was 58%. Pyrrole **29** was then treated with bromine in dry diethyl ether to yield bromomethylpyrrole **29a**. Which was refluxed with 1 mL HBr for one hour to give dibenzyloxycarbonyl-3,3'-di(3-ethoxycarbonylpropyl)-4,4'-dimethyl-2, 2'-pyrromethane **30** (95%).[69,97]
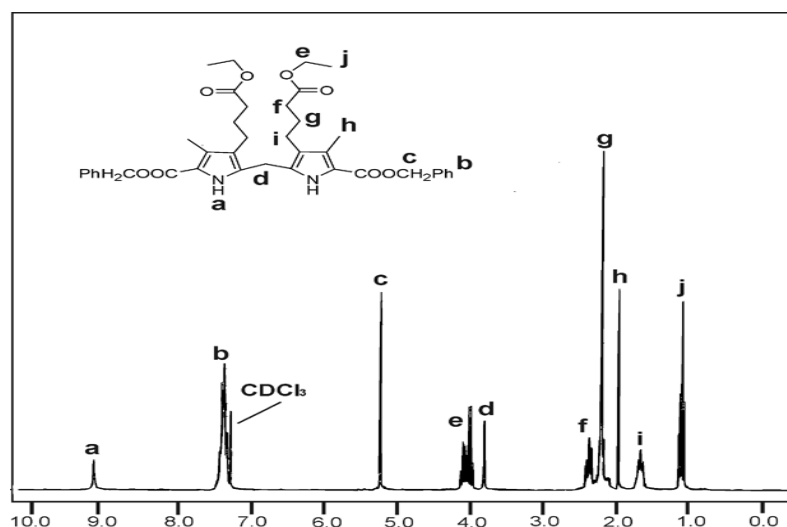
^{1}H NMR-spectrum(CDCl$_3$) of 5,5'- dibenzyloxycarbonyl-3,3'-di (3-
ethoxycarbonylpropyl)-4,4'-dimethyl-2,2'-pyrromethane **30**

The 5,5'-dibenzyloxycarbonyl-3,3'-di(3-ethoxycarbonylpropyl)-4,4'-dimethyl-2,2'-pyrro
-methane **30** was subjected for palladium-carbon catalyzed hydrogenolysis. The
decarboxylation of **30a** to give **30b** was achieved by different methods. Decarboxylation
of **30a** in presence of aqueous alkali[98] was carried out but the obtained yield was not
satisfactory. The preparation of **30b** by decarboxylation of **30a** in 2-aminoethanol also
failed; the amino reacted with the ester group.[99] This problem was overcome by
attempting one-pot decarboxylation and porphyrine synthesis. Thus the dihydrodipyrrin
30b was synthesized by acid catalyzed decarboxylation of **30a**. In situ the obtained **30b**
was treated with trimethoxy methane to yield porphyrin **31**.[99]

^{1}H NMR-spectrum(CDCl$_3$) of 4,8,12,18-tetra-(3-ethoxycarbonyl-propyl)-3,7,13,17-
tetramethyl-22,24-dihydro-porphin-2-yl]-butyric acid ethyl ester **31**

2.8 Synthesis of *meso*-5,10,15,20-tetrakis(3-carboxylatophenyl) porphyrin (32)

For the preparation of the yoctowells on silica colloidal particles *meso*-5,10,15,20-tetrakis-(3-carboxylatophenyl)porphyrin was applied (**32 a**, and **32 b**). Synthesis of *meso*-5,10,15,20-tetrakis-(3-carboxylatophenyl)porphyrin **32** was carried out starting with 3-formyl-benzoic acid ethyl esters described in references 13 and 35. At first porphyrin molecule are bound in an orientation parallel to the amino silicate particle's surface. For this purpose we used *meso*-5,10,15,20-tetrakis-(3-carboxylato phenyl)porphyrin, was preferable used. Application of *meso*-5,10,15,20-tetrakis(4-carboxylato phenyl)porphyrin or *meso*-5,10,15,20-tetrakis(2-carboxylatophenyl)porphyrin can not bind tightly, because ortho-substituents caused disturbances of porphyrin planarity, four para-substituents cannot be attached to the subphase for steric reason. We finally only applied the *meso*-5,10,15,20-tetrakis (3-carboxylatophenyl) porphyrin **32b**.
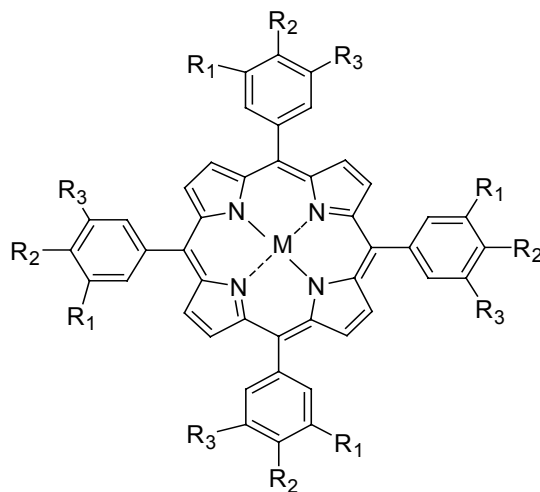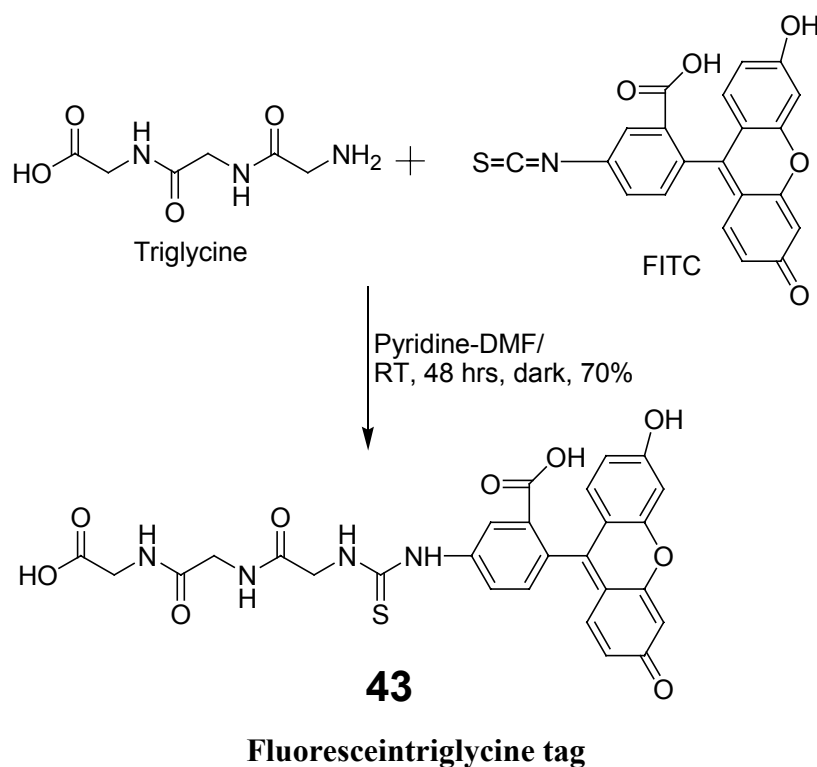

32   R$_1$ = COOEt, R$_2$,R$_3$=H, M =2H

a' R$_1$ = COOH, R$_2$,R$_3$=H, M =2H

a   R$_1$ = COCl, R$_2$,R$_3$=H, M =2H

b R$_1$ = COCOOEt, R$_2$,R$_3$=H, M =2H

2.9 Synthesis of fluoresceintriglycine tag (43)[113]

43

Fluoresceintriglycine tag

A fluorescent ligand for ex. Fluorescein isothiocyanate (FITC) **43** used because 1) it is a very commonly used dye in biology; 2) its excitation wavelength (488 nm) doesn't interfere with that of porphyrin; 3) it is easily accessible and 4) it does not require either sophisticated microscopic instruments or special filters for fluorescence recording. The fluoresceintriglycine tag was synthesized starting with triglycine and FITC in dry pyridine: DMF (1:1) at room temperature for 48 hrs in dark obtained fluoresceintriglycine tag **43** in 70%.[113]

2.10 Synthesis of bolaamphiphile: 6-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl carbamoyl}-4-oxo-hexanoic chloride (50)

The hydrolysis of 4-oxoheptandiacid diethyl ester (purchased from Aldrich) was carried out utilizing 2 M methanolic KOH.[114] After refluxing at 85°C for 4 hrs obtained 92% of 4-oxo-heptanedioic acid **48.** Which was further subjected for amidation coupling reaction with oligoethylene-amine (OEG-amine) **20** utilizing DCC and catalytic amount of NHS (N-hydroxy succinamide) in dry THF.[67] The product was purified by column chromatography obtained 90 % yield of 6-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethylcarbamoyl}-4-oxo-hexanoic acid **49**. The acidic end of the bolaamphiphile was converted to acid chloride **50**[69] utilizing oxalyl chloride in dry DCM.