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PhD Thesis

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Cluj-Napoca
March 2012

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Synthesis, Structure and Properties of Some New Macrocyclic Compounds and New Functionalized Lipid Derivatives for Biophysical Applications

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March 2012

Keywords:

Suzuki cross-coupling
Macrocycles
Rotaxanes
Cholesteryl-nucleoside, nucleobase
Amphiphilic lipid
Lipid microtubes
Nanocarrier

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Cluj-Napoca
March 2012

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Key words:

Suzuki cross-coupling
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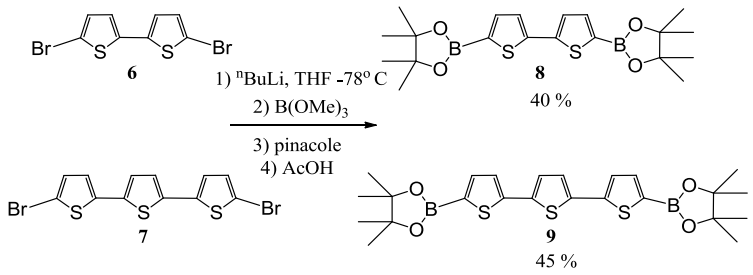
1.2.4.1. Synthesis of precursors

Our target was to synthesized new macrocyclic compounds by intermolecular Suzuki cross coupling reaction. For the synthesis of target macrocycles we vary reaction conditions (substrates, bases or solvent) to identify the suitable method of macrocyclization. A new synthetic method of one-pot macrocyclization in which the lithiation and coupling reactions were performed *in situ* was approached.

The synthesis of target macrocycles was started from their precursors. These precursors are derivatives of bithiophene, terthiophene and thiophene-phenothiazine compounds.

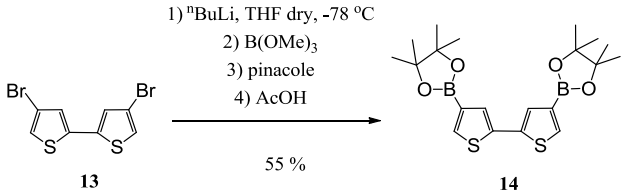
The first precursors which we synthesized were derivatives of bi- and ter-thiophene. Bithiophene **4** and terthiophene **5** were synthesized by a Kumada coupling reaction, which were submitted to their dibromo-derivatives **6** and **8**. This two dibrominated thiophene derivatives were submitted further to a lithiation reaction (^{n}BuLi, in dry THF) for a bromine-lithium exchange. This step was followed by addition of trimethylborate as electrophile.[1]. For a better stability and to avoid photodeborylation, the methyl boronic ester was converted in situ to the pinacolyl boronic ester[2], scheme 5.

[1] C. Krämer,T. J. Zimmermann, M. Sailer, T. J. J. Müller *Synthesis* **2002**, *9*, 1163.
[2] R. W. Hoffmann, S. Dresely *Synthesis* **1998**, 103.



Scheme 5.

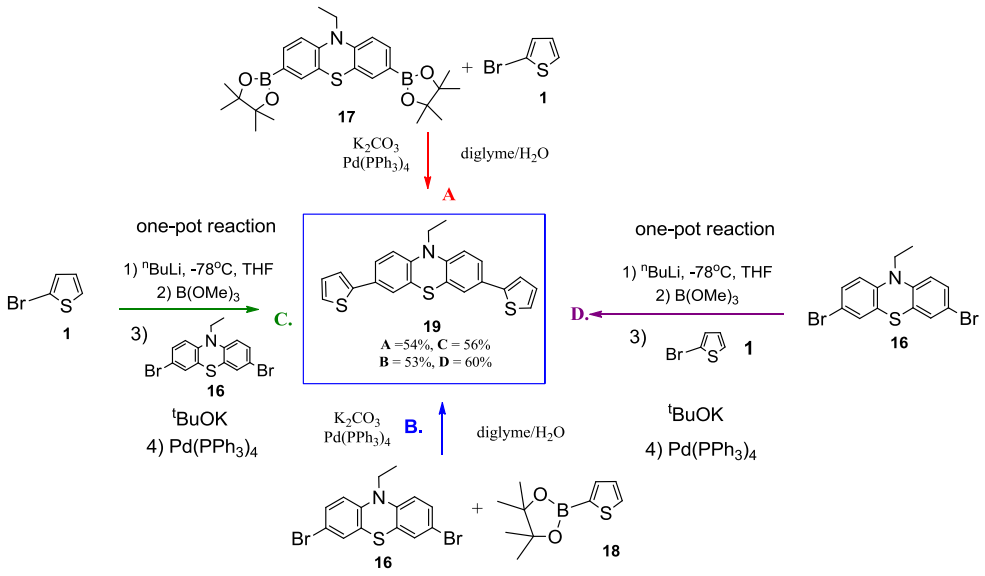
Another bithiophene derivative which we want to use as rigid unit for macrocyclization reaction was 4,4'-dibromo-2,2'-thiophene, which was reacted to give bis-pinacole boronic ester **14**, scheme 7.



Scheme 7.

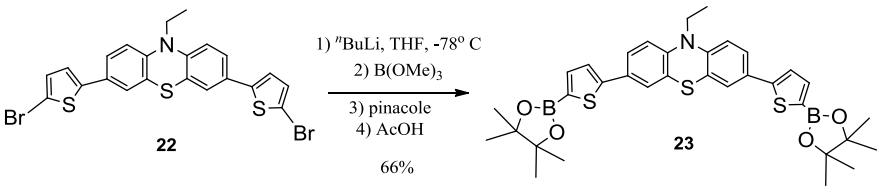
A thiophene-phenothiazine podand was used for Suzuki macrocyclization. This derivative was synthesized to see if the size or planarity of the central unit influences the closing of the cycle. For this reason we chose as central unit phenothiazine, a planar and rigid structure.

An important precursor for different target compounds which we synthesized was 10-ethyl-3,7-di(thien-2-yl)-10*H*-phenothiazine (derivative **19**), which was synthesized in four different ways by classical Suzuki cross coupling reaction between a boronic ester and a bromine derivative (routes **A** and **B**), and by one-pot reaction in which the borate derivative was generated *in situ* followed by Suzuki coupling (routes **C** and **D**), scheme 10.



Scheme 10.

Derivative **19** was submitted to a bromination reaction with NBS to give 3,7-bis(5-bromothiophen-2-yl)-10-ethyl-10H-phenothiazine **22**. Which was transformed to its corresponding pinacole-boronic ester **23** by a lithiation reaction, followed by electrophilic addition, scheme 13.

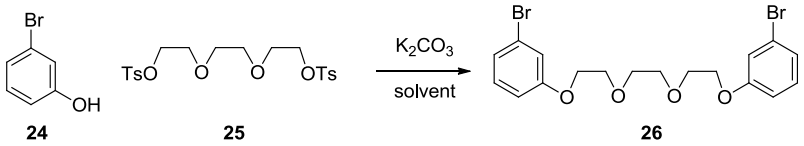


Scheme 13.

For macrocyclization reaction, which we aboard, we needed to synthesize a flexible moiety. This moiety contains a chain of n-ethylene glycol and meta-bromophenol at its terminus. This synthesis can be performed variety of solvents[3,4,5]. The reaction between triethylene glycole ditosylate **25** and 3-

[3] H.-Y. Kim, W.-J. Lee, H.-M. Kang C.-G. Cho *Org. Lett.* **2007**, *9*, 3185.
[4] L. Sheeney-Haj-Ichia, I. Willner *J. Phys. Chem. B* **2002**, *106*, 13094.
[5] S. J. Rowan, J. F. Stoddart *Org. Lett.* **1999**, *1*, 1913.

bromophenol **24**, using potassium carbonate as base, gives 1,2-bis(2-(3-bromophenoxy)ethoxy)ethane **26**, scheme 14. We investigated four different solvents (isopropanol, DMF, acetone and acetonitrile), the best reaction yields were obtained when acetonitrile is used. By similar reaction procedure using acetonitrile as solvent synthesis of 3,3'-((((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(bromobenzene) ,derivative **28**, was synthesized.



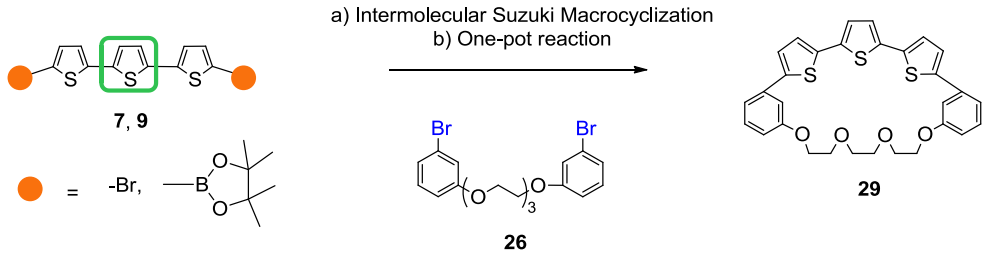
24 **25** **26**

Scheme 14.

1.2.4.2. Synthesis of macrocycles

For Suzuki macrocyclization reaction we used rigid compounds containing thiophene and thiophene-phenothiazine units (**6**, **7**, **8**, **9**, **13**, **14**, **22**, **23**) and as flexible unit dibromo-derivatives **26** and **28**. For all these reactions the catalyst was $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0).

For macrocycle **29** several reactions conditions (solvent mixture or base) were varied. Three different solvent base combinations were tried using classical Suzuki macrocyclization. Additional two ways in which lithiation and coupling reactions were performed in situ as one-pot reactions, were also investigated, scheme 17. The base used for classical coupling reactions were Cs_2CO_3 or K_2CO_3 and the solvents used were mixture of diglyme : water and DMF : water. For one pot reactions was used THF as solvent and ^tBuOK or Cs_2CO_3 as base, the yields for one-pot reactions were lower than we expected.



Scheme 17.

Results for macrocyclization reaction of new compound **29** are presented in table 2. The best reaction yield after purification was obtained for Cs_2CO_3 as base and a mixture of diglyme:H_2O as solvent.

Reaction conditions for macrocycle **29**	Yield %
diglyme:H_2O, K_2CO_3	22%
diglyme:H_2O, Cs_2CO_3	30%
DMF:H_2O, Cs_2CO_3	26%
One pot reaction, ^{t}BuOK	10%
One pot reaction, Cs_2CO_3	12%

Table 2.

The structure of the target macrocycle **29** was confirmed by ^{1}H-NMR (Figure 7), ^{13}C-NMR and MS-ESI(+) spectroscopy. Part of the ^{1}H-NMR spectrum confirms the symmetry of macrocycle **29**, the alkyl protons of triethylene glycol moiety appear as two triplet signals at 3.88 ppm (orange) and at 3.63 ppm (green), respectively and as one singlet for protons at 3.57 ppm (blue). In the aromatic region it can be seen two specific doublets for protons of thiophene unit (ring **B**) at 7.40 ppm (red) and 6.94 ppm (purple) with a coupling constant J^3= 7.3 Hz, for rings **A** and **C** can be seen overlapped peaks in the region between 7.80-7.00 ppm (grey).

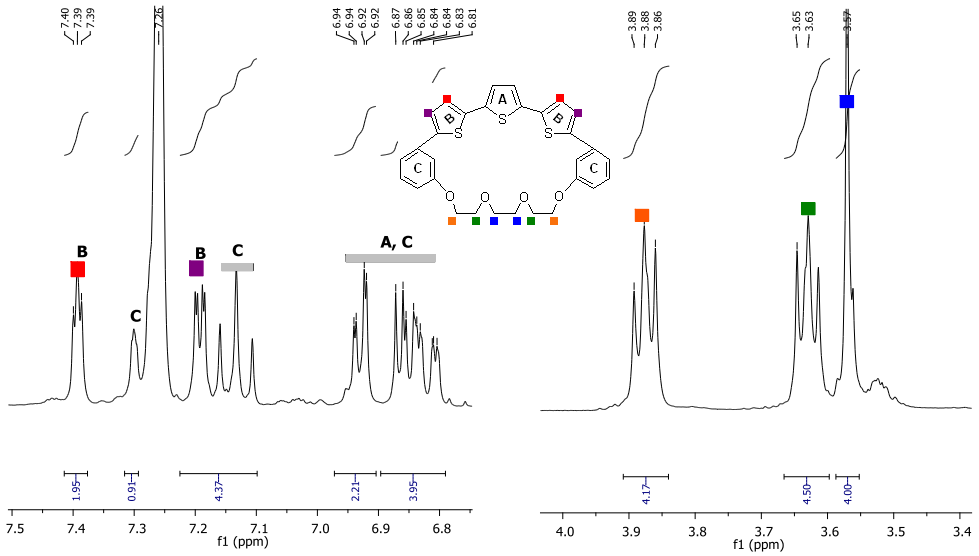
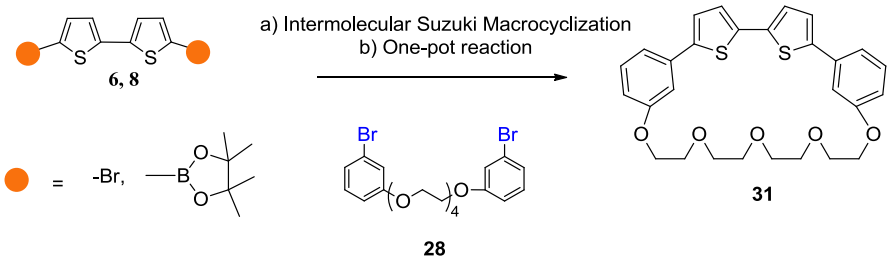


Figure 7. Part of the ^{1}H-NMR spectrum of macrocycle **29** (C_6D_6, 300 MHz).

The non-cyclization of derivative **8** with chain **26** suggests us to change the size of the flexible chain, and we used derivative **28**. For the cyclization reaction were used 2,2'-bithiophene-5,5'-diboronic acid bis-(pinacol) ester **8** or 5,5'-dibromo-2,2'-bithiophene **6** with flexible chain **28**, in same reaction procedures as described for macrocycle **29**, scheme 20.



Scheme 20.

The macrocycle structure was confirmed by NMR and EI-MS. The ^{1}H-NMR spectrum of derivative **31** is presented in figure 9. The symmetry of the macrocycle is confirmed by the alkyl signals of the ethylene glycol moiety as four triplets at 3.97 ppm, 3.73 ppm, 3.37 and 3.35 ppm. In the aromatic region the most deshielded signals are for protons of thiophene unit (red) and *ortho* protons of benzene unit (mauve), for the other aromatic protons, ring **A** and **B**, the NMR signals are overlapped from 7.19 to 6.82 ppm.

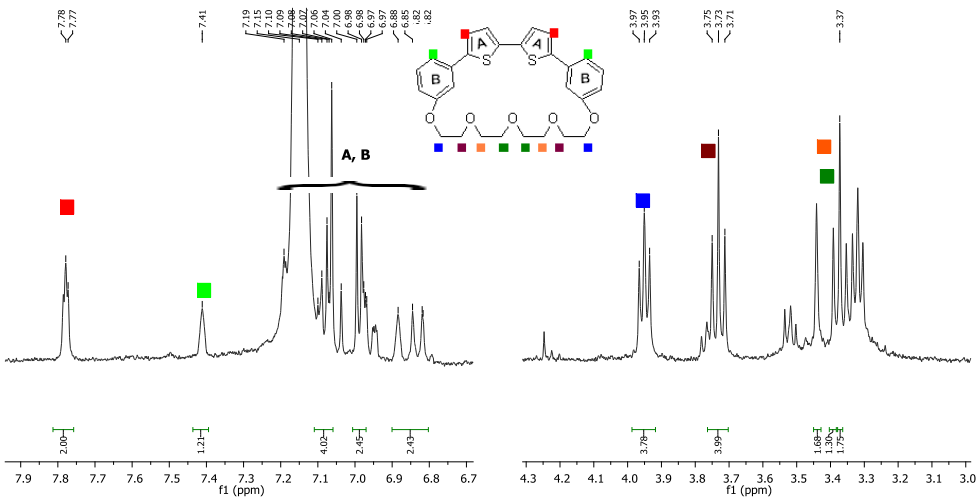
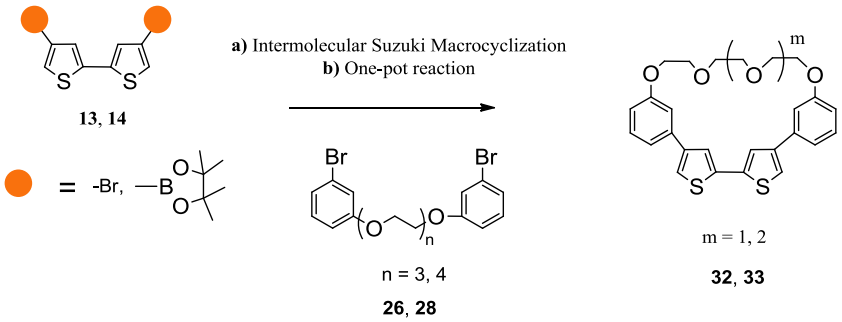


Figure 9. Fragment of the ^{1}H-NMR spectrum of derivative **31** (C_6D_6, 300 MHz).

It is well known that different thiophene derivatives substituted at position 3 can easily polymerize. This was one of the reasons why we tried to obtained macrocycles containing 4,4'-disubstituted-2,2'-bithiophene (**13** and **14**). Another reason was to compare reactivity of 4,4'-disubstituted-2,2'-bithiophene with 5,5'-disubtituted one with two types of dibrominated chains. Derivatives **13** and **14** were reacted with dibrominated chains **26** and **28**, in two reaction conditions Cs_2CO_3 as base and diglyme : H_2O mixture as solvent and one-pot method with Cs_2CO_3 as base. The general scheme of reaction is presented in scheme 21.

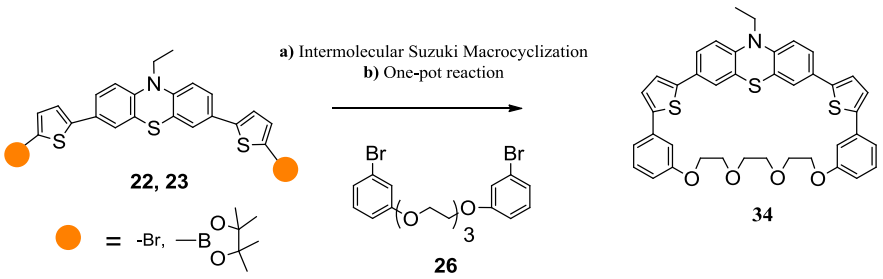


Scheme 21.

For these two macrocycles (**32** and **33**) the reaction yields were lower than we expected. In a similar manner as the macrocycles indicated above, the best

yields obtained were by using Cs_2CO_3 as base and a mixture of diglyme:H_2O as solvent.

By same type of macrocyclization methods: classical Suzuki crosses coupling (a) and one-pot (b) reactions, we tried to synthesize a new macrocycle **34**, scheme 22.



Scheme 22.

The best reaction yields for macrocycle **34** were obtained with Cs_2CO_3 base and diglyme : H_2O mixture solvent as previous, table 6.

Reaction conditions for macrocycle **34**	Yield %
diglyme:H_2O, K_2CO_3	17 %
diglyme:H_2O, Cs_2CO_3	31 %
DMF:H_2O, Cs_2CO_3	20 %
One pot reaction, ^{t}BuOK	< 5 %
One pot reaction, Cs_2CO_3	8%

Table 6.

The structure of macrocycle **34** was confirmed by ^{1}H-NMR, APT-NMR and EI-MS. The ^{1}H-NMR of target macrocycle in C_6D_6 is presented in figure 11. In the ^{1}H-NMR spectrum the symmetry of the new macrocycle **34** was confirmed by signals of the alkyl protons of triethylenoxy moiety as two triplets at 3.85 ppm (blue) and 3.53 (burgundy) and one singlet at 3.30 ppm (dark green). In the aromatic region the most deshielded signals are a doublet from protons of thiophene at 7.71 ppm (red) and a doublet from phenothiazine at 7.36 ppm (light

green). The signals for the protons of aromatic rings **A**, **B** and **C**, are overlapped peaks between 7.20 and 6.80 ppm.

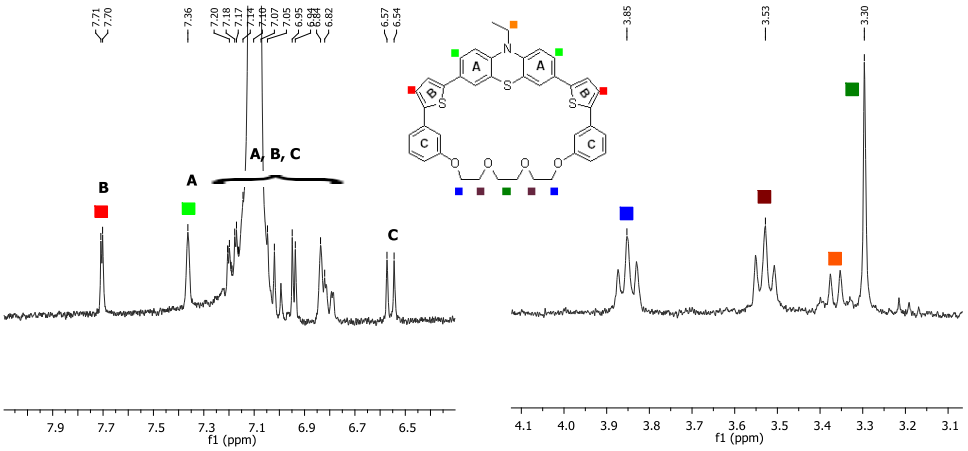


Figure 11. Fragments of the ^{1}H-NMR spectrum of macrocycle **34** (C_6D_6, 300 MHz).

Five new macrocycles **29**, **31**, **32**, **33** and **34** were synthesized and characterized. The synthesis for these new macrocycles was tried in three classical intermolecular Suzuki cross coupling reactions and a novel approach by one-pot synthesis, unfortunately the one-pot reaction didn't give the expected yields. For the classical intermolecular Suzuki macrocyclization reactions we used Cs_2CO_3 or K_2CO_3 as bases and diglyme : water or DMF : water as solvents mixtures. The best reaction yields were obtained using Cs_2CO_3 as base and diglyme : water as solvent.

1.2.5. Conclusions

In this chapter we aboard two ways for macrocyclization using intermolecular Suzuki cross coupling reaction, a classical way between a diboronic ester and a dibrmominated derivative and a novel approach in which we performed lithiation and coupling reaction in situ. For all coupling reactions $Pd(PPh_3)_4$ catalyst was used.

For our target macrocycles we synthesized six new podands, derivatives: **14**, **19**, **21**, **22**, **23** and **32**, which were analyzed and characterized by NMR and MS.

By our aboard macrocyclization reaction we synthesized five new macrocycles containing bithiophene, terthiophene and thiophene-phenothiazine units, **29**, **31**, **32**, **33** and **34**.

The best yields obtained for all these macrocycles was by using Cs_2CO_3 as base and a mixture of diglyme : water as solvent.

For these five new macrocycles a new synthetic method was tried, a one-pot reaction, but the reaction yields were lower than we expected.

1.3. Macrocycles with Possible Application in Rotaxanes Synthesis

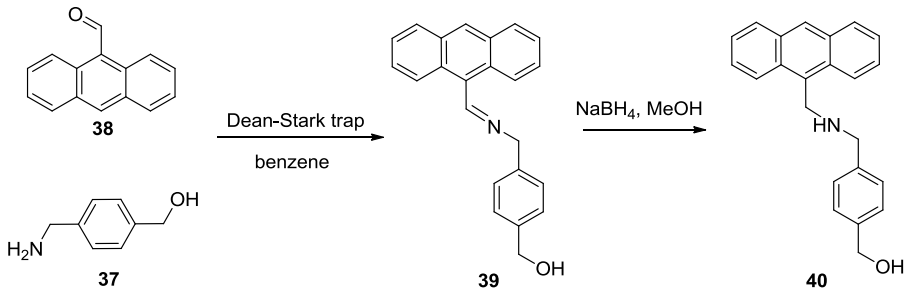
1.3.5. Results and Discussions

Our first target was to synthesize some new [n+1]-rotaxanes from [2]-rotaxane for monoaxle derivative. The axle units have as base unit an amine salt and the wheel is a functionalized macrocycle. The final step for the [2]-rotaxane with different functionalized wheel we wanted to be a reaction with different linker units.

1.3.5.1. Synthesis of the axles

First we have synthesized the amine unit (**37**), common for all axles. *p*-Cyanobenzyl bromide was hydrolyzed to the corresponding alcohol **36**, and then reduced to corresponding amino alcohol[6]. The amine **37** is sensitive to air and humidity and was used further without purification for axles synthesis.

For monoaxle, diaxle and triaxle synthesis the next step was a condensation reaction of **37** with three types of aldehydes[7], which were used further without any purification to give corresponding amines. For monoaxle the antracene aldehyde, as one side stopper, was reacted with amine **37** to give imine **39**, which was submitted by a hydrogenation reaction to **40**, with 65% overall yield, scheme 24. The final salt **41** was obtained from amine **40** in acid media and further treatment with 20% $NaPF_6$ to give the desired monoaxle in 75 % yield.

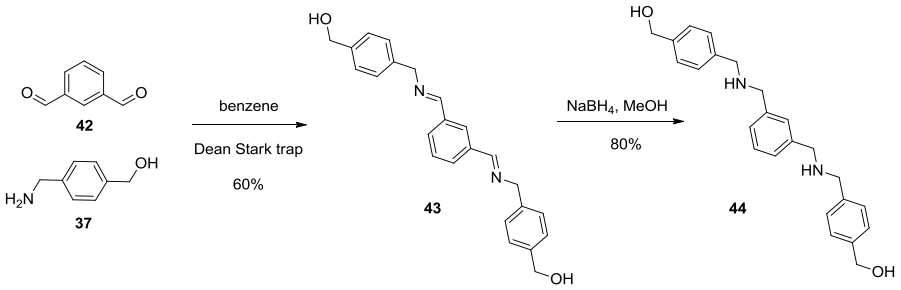


[6] (a) B. F. Glisin *Helv. Chim. Acta* **1973**, *56*, 1476; (b) J. A. Garvin, M. E. Garcia, A. J. Benesi, T. E. Mallouk *J. Org. Chem.* **1998**, *63*, 7663; (c) S. Vassilioiu, M. Xeillari, A. Yiotakis, J. Grembecka, M. Pawelczak, P. Kafarski, A. Mucha *Bioorg. Med. Chem.* **2007**, *15*, 3187.

[7] J. D. Badjic, V. Balzani, A. Credi, J. N. Lowe, S. Silvi, J. F. Stoddart *Chem. Eur. J.* **2004**, *10*, 1926.

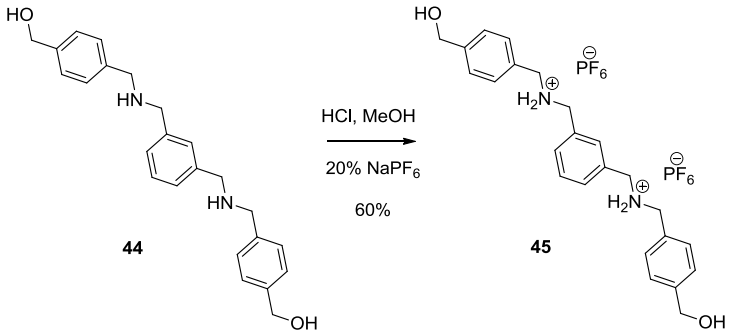
Scheme 24.

The same chemical procedure used for monoaxle such as condensation reaction between aldehyde and amine to give imine (**43**) and reduction to amine (**44**) was followed also for the new diaxle derivative **44**. Isophtalaldehyde was used for as linker unit in diaxle synthesis, scheme 26.



Scheme 26.

The diaxle **44** was submitted to its corresponding 2-hexafluorophosphate salt, **45**, which makes it more soluble in ordinary solvents, scheme 27.



Scheme 27.

For the new diaxle derivative **45** the presence of counter ion PF_6^- was confirmed by the ^{31}P-NMR spectrum which shows the coupling of phosphorous with fluorine as a heptet and the ^{19}F-NMR spectrum which shows the coupling of fluorine with phosphorous as a doublet, figure 19.

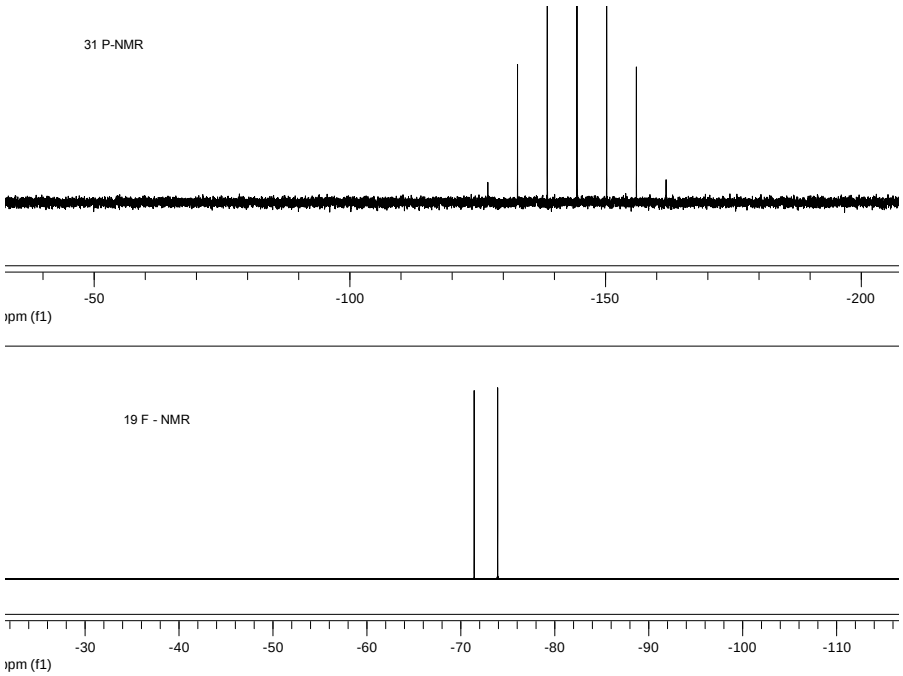
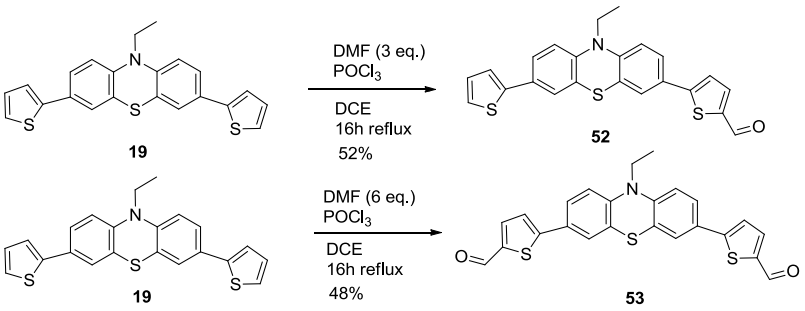


Figure 19. ^{31}P-NMR (CD_3CN, 121 MHz) and ^{19}F-NMR (CD_3CN, 282 MHz) spectra of diaxle **45**

1.3.5.2. Synthesis of macrocycles

Molecules which contain thiophene and phenothiazine units present interesting opto-electronic properties, as described in chapter 1.2.1, that's why we wanted to synthesize new macrocycle compounds containing these building blocks and use their properties in rotaxane synthesis.

For the macrocycles which we want to synthesize, the base unit are 3,7-dibromo-10-ethyl-10H-phenothiazine **16** and pinacole boronic ester derivative **17**. One important podand is 10-ethyl-3,7-di(thien-2-yl)-10*H*-phenothiazine **19**, synthesis described in scheme 10. Derivative **19** was submitted by a Vilsmeier Haack formylation to aldehydes **52** and **53** depending on equivalent number of Vilsmeier reagent added, scheme 31.



Scheme 31.

The aromatic part of the ^{1}H-NMR spectra for monoaldehyde **52** and dialdehyde **53** are presented in figure 20. For monoaldehyde the NMR was submitted in C_6D_6 and for dialdehyde in DMSO-d_6. In the ^{1}H-NMR can be seen that the most deshielded signals are for the protons of carbonyl moiety. For dialdehyde **53** can be seen symmetry of the molecule as two doublets for the thiophene moiety nuclei **A** and one doublet and overlapped peaks for phenothiazine moiety **B**.

For monoaldehyde **52** the signals are more expanded. The most deshielded signals correspond to phenothiazine moiety, nuclei **B**. For formyl-thiophene moiety, nuclei **A**, proton signals appear as two doublets. The three protons of nuclei **D** appear as one doubled and overlapped peaks at around 6.8 ppm.

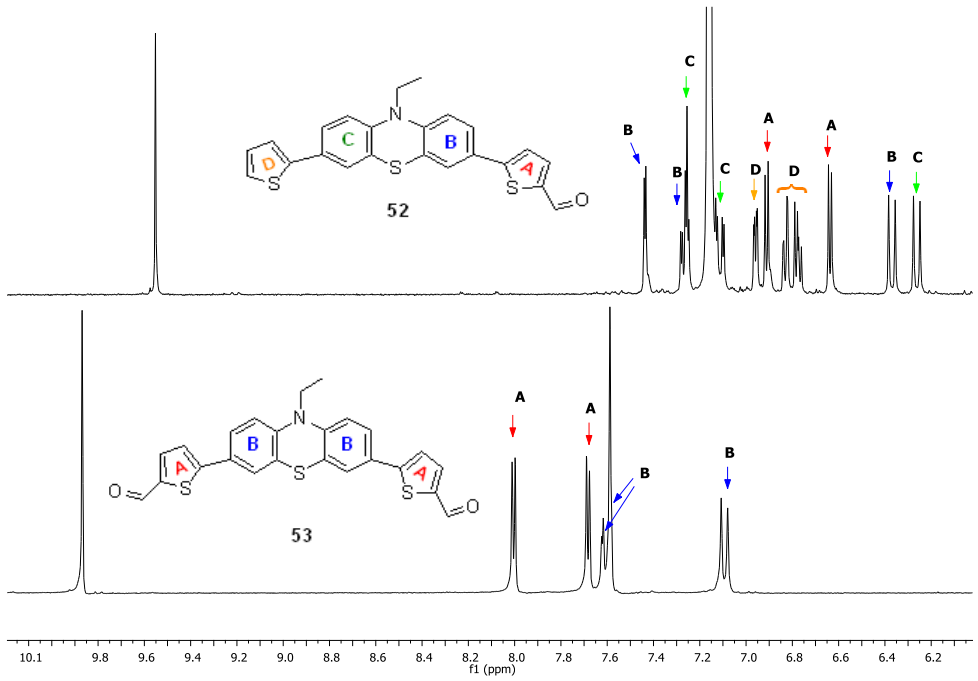
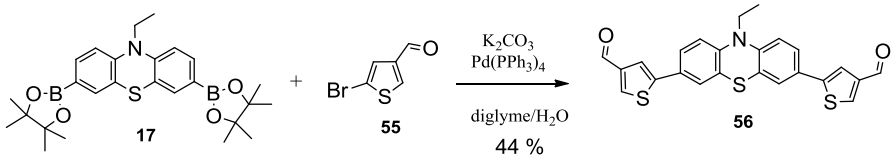


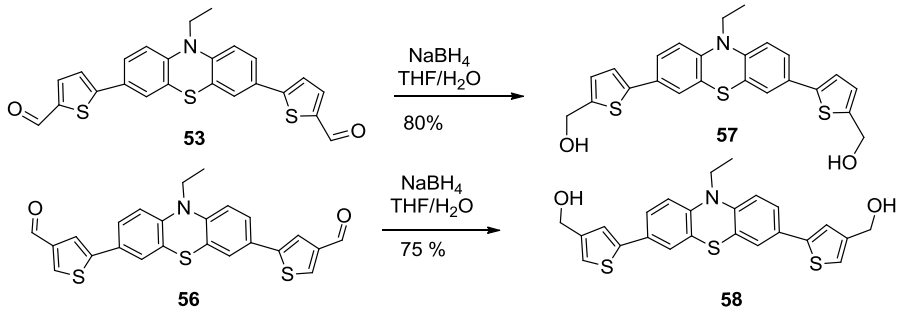
Figure 20. Part of the ^{1}H-NMR spectrum of monoaldehyde **52** (C_6D_6 at 300 MHz) and dialdehyde **53** (d^6-DMSO, 300 MHz).

By changing position of formyl moiety from positions 5,5' (**53**) to 4,4' we synthesized a new dialdehyde **56** from 5-bromothiophene-3-carbaldehyde **55** and 10-ethyl-3,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-10H-phenothiazine **17**, scheme 33.



Scheme 33.

The two new dialdehyde derivative **53** and **56** were submitted to their corresponding alcohols **57** and **58** by a reduction reaction using $NaBH_4$ in a mixture of THF : water as solvent, scheme 34.

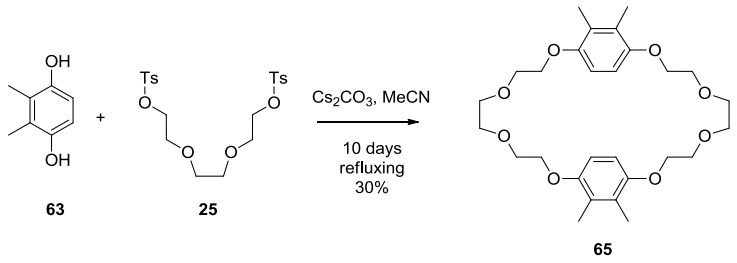


Scheme 34.

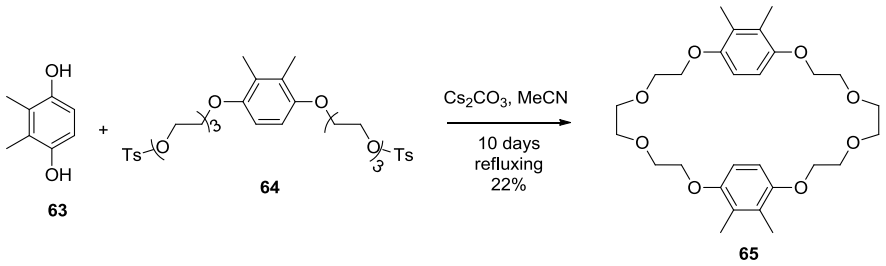
In order to get different types of macrocycles which contain crown ether moiety to form hydrogen-bonding with the thread, we synthesized a new symmetric macrocycle, with the inner ring of 28 atoms. [8]

The ditosylated derivative **25** was reacted with 2,3-dimethyl-hydroquinone **63** in ultra dilution conditions to give the new podand **64**[9].

By a similar procedure using Cs_2CO_3 instead of K_2CO_3 as a base, in ultradilution conditions, and adding drop wise in 3 days ditosylated derivative **25** on the solved quinine **63**, a new macrocycle **65** was synthesized. The reaction mixture was refluxed another 7 days, than purified on chromatographic column to give **65** in 30% yield. The same macrocycle was obtained by reacting ditosylated **64** with 2,3-dimethyl-hydroquinone **63** and Cs_2CO_3, in ultradilution conditions, macrocycle **65** was obtained in 22% yield, scheme 41.



[8] M. V. Circu, A. Petran, A. S. Gaz, E. Bogdan, A. Terec, C. I. Rat, R. A. Varga, I. Grosu *J. Mol. Struct.* **2011**, *996*, 17.
[9] Ichia, L.; Willner, I. *J. Phys. Chem. B* **2002**, *106*, 13094.



Scheme 41.

The structure of macrocycle **65** was investigated by the solid state molecular structure obtained by single crystal X-ray diffractometry. The ORTEP diagram shows the almost planar structure of the macrocycle; the angle between the two planes described by the aromatic units is α=0.00°; the distance between the two planes described by the aromatic units is d' = 6.430 Å (figure 24).

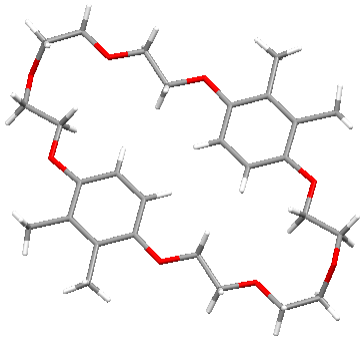


Figure 24. ORTEP diagram of macrocycle **65**.

The view of the lattice along the b axis reveals the formation of columns, figure 25.

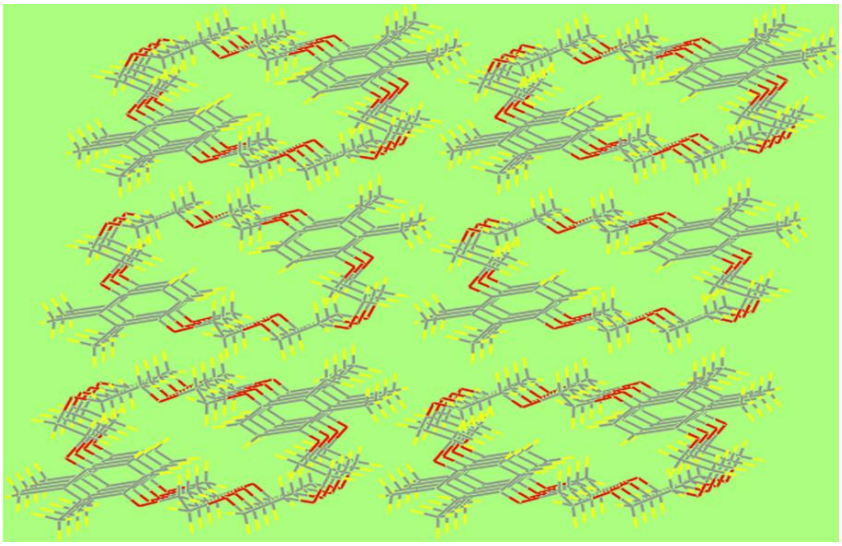


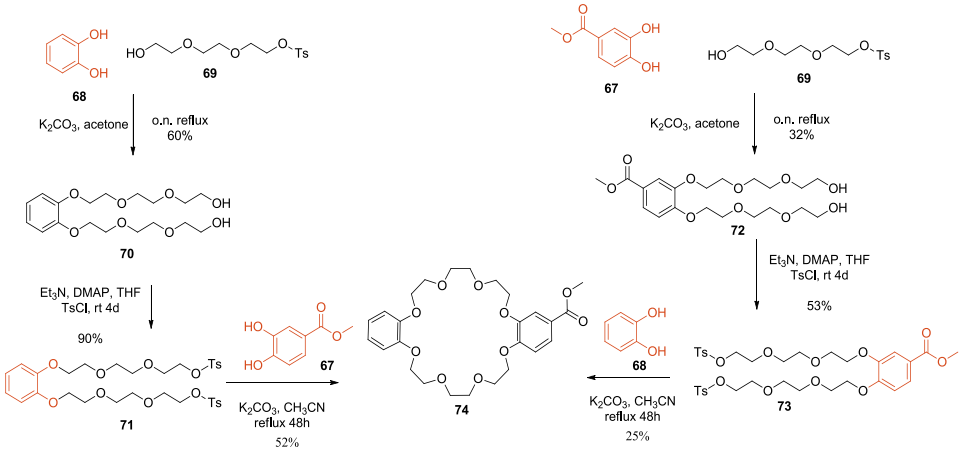
Figure 25. View of the lattice along b crystallographic axis.

To get functionalized macrocycles for intermolecular reaction between the macrocycle and the linker we synthesized first different substituted macrocyles, having OH or COOH units.

For asymmetric macrocycles which we want to synthesize the first step was synthesis of podands. As asymmetric part of the macrocycle we used methyl 3,4-dihydroxybenzoate[10] **67**, which was synthesized from its corresponding acid **66**, scheme 42.

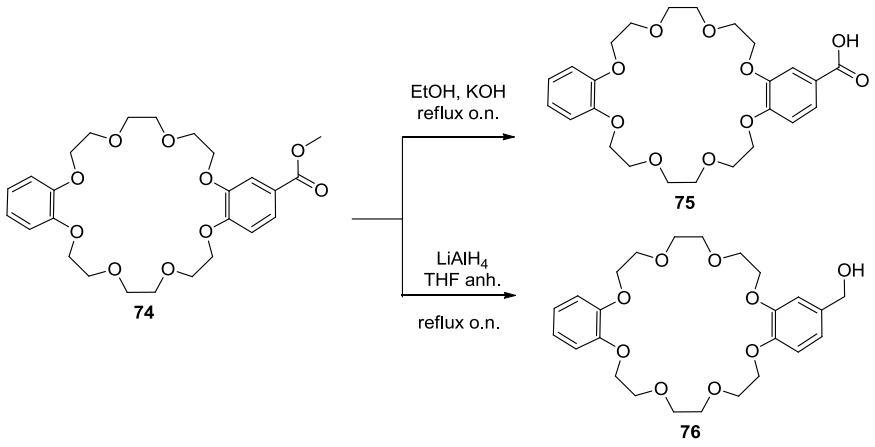
To obtain macrocycle **74** [11,12] two synthetic paths were followed; the first starts from triethyleneglycol monotosylate **69** and pyrocatechol **68**, and the other way employs methyl-3,4-dihydrobenzoate **67** with the same monotosylate and later catechol, scheme 43.

[10]Y. Kita, M. Arisawa, M. Gyoten, M. Nakajima, R. Hamada, H. Tohma, T. Takada *J.Org.Chem.* **1998**, *63*, 6625.
[11] K. Yamabuki, Y. Isobe, K. Onimura, T. Oishi *Chem. Lett.* **2007**, *36*, 1196.
[12] D.-J. Feng, X.-Q. Li, X.-Z. Wang, X.-K. Jiang, Z.-T. Li *Tetrahedron* **2004**, *60*, 6137.



Scheme 43.

The asymmetric macrocycle **95** was synthesized by hydrolysis in alkaline environment to an acid functionalized macrocycle, **75**, and by reduction with lithium aluminum hydrate to an alcohol macrocycle[13] **76**, scheme 44.



Scheme 44.

1.3.5.4. Pseudorotaxanes and rotaxanes formation

For rotaxane synthesis the first step was formation of pseudorotaxane. The first pseudorotaxane, **83**, which we synthesized was between monoaxle **62** and commercially available dibenzo-24-crown ether (DB24).

[13] S. J. Cantrill, G. J. Youn, J. F. Stoddart *J. Org. Chem.* **2001**, *66*, 6857.

The axle **41** has antracene as one stopper moiety, so we decide to form a pseudorotaxane by *threading* this axle in macrocycle DB24.

To see the formation of pseudorotaxane **83** we used ^{1}H-NMR, figure 27. It can be easily seen that two protons of benzene ring of the macrocycle are shielded at around 6.5 ppm (purple arrow) and alkyl protons of monoaxle involved in hydrogen bonding are more deshielded, at around 5.3 ppm (red and blue arrows).

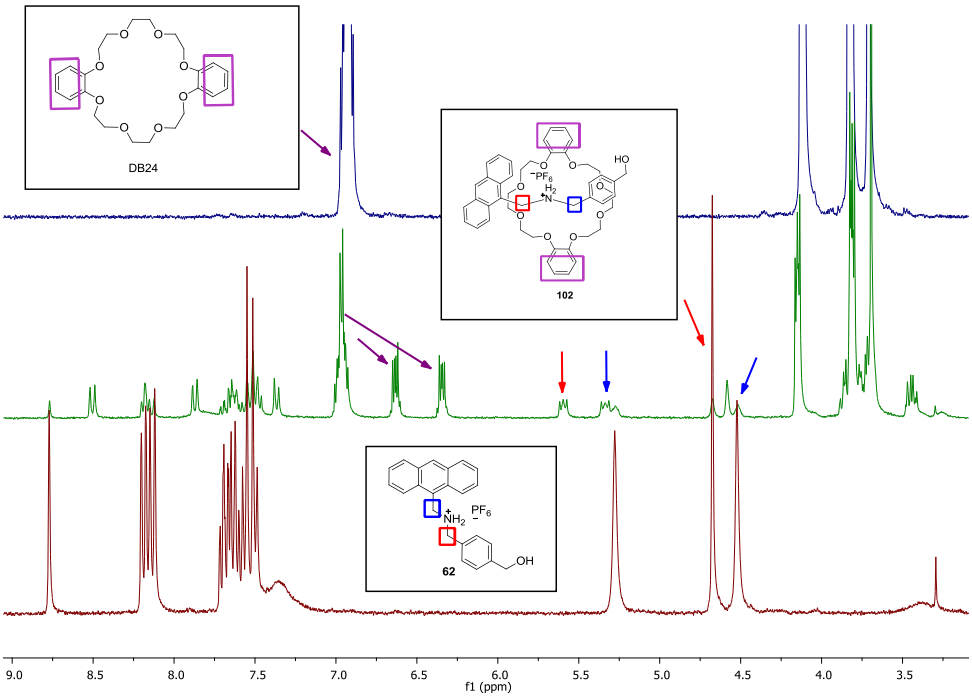
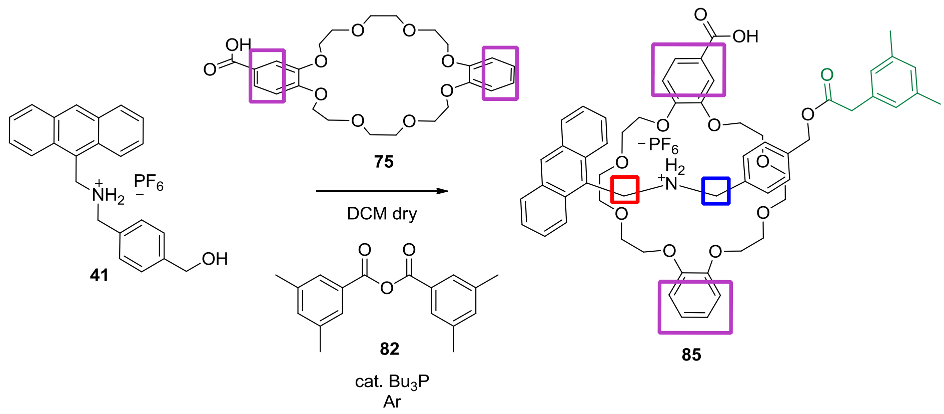


Figure 27. Comparable ^{1}H-NMR spectra for pseudorotaxane **83** with DB24 and monoaxle **41** (CD_3CN, 300 MHz).

Our first rotaxane that we synthesized was by using monoaxle **41** as thread, macrocycle **75** as wheel and 3,5-dimethylbenzoic anhydride as precursor for stopper unit, scheme 49.



Scheme 49.

The 1H-NMR spectrum of target rotaxane **85**, figure 28, present specific signals of protons involved in hydrogen bond between the axle and asymmetric macrocycle. Parts of the hydrogen atoms from benzene ring (violet), appearing as a multiplet, shielded from 6.87 ppm to 6.57 and 6.37 ppm, specific signals for aromatic protons involved in hydrogen bond. The protons of methylene units of axle (blue and red arrows) are deshielded from 4.65 and 4.50 at around 5.48 and 5.34 ppm as broad peaks. The attachment of the stopper it's confirmed by the presence of two singlet's of methyl units at around 2.40 ppm.

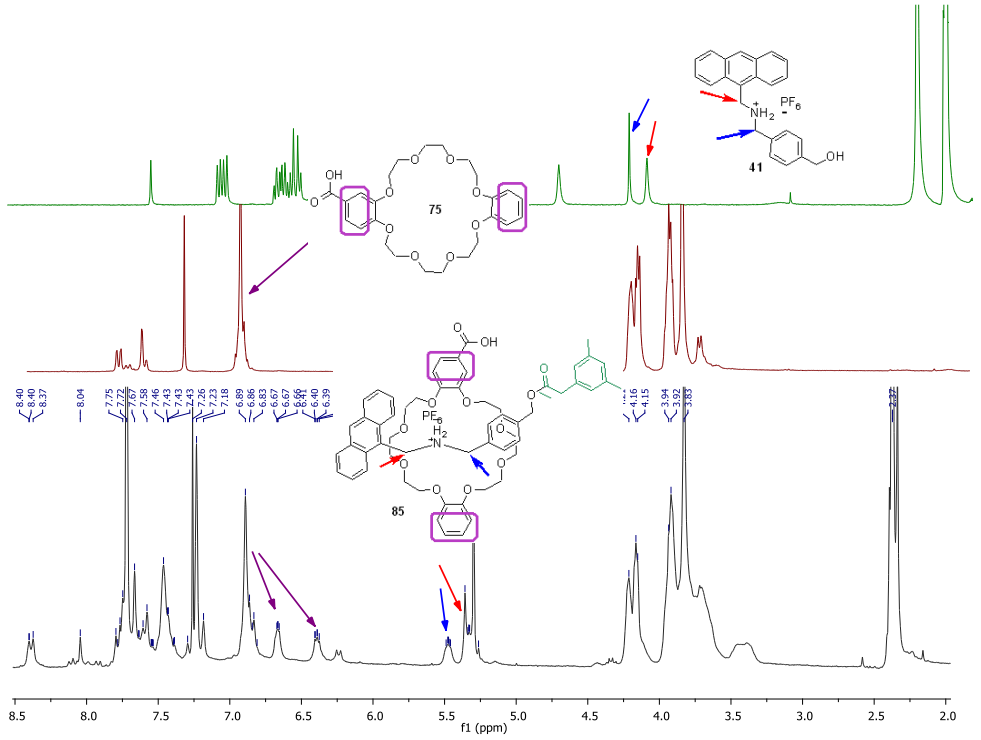


Figure 28. Superpose ^{1}H-NMR spectra of monoaxle **41** (CD_3CN, 300 MHz), macrocycle **75** and rotaxane **85** ($CDCl_3$, 300 MHz).

This new [2]-rotaxane, **85**, is the first precursor for intermolecular reactions with different linker units to form [n+1]-rotaxane.

1.3.6. Conclusions

As a new thread compound we synthesized imine **43**, amine **44** and amine hexafluoro phosphate salt **45**.

As precurors for target macrocycle compounds containing thiophene-phenothiazine units we synthesized monoaldehyde **52**, dialdehydes **53**, **56** and **61**, diols **57** and **58** and a new ditosylated chain **64**, all these new compounds were analyzed by NMR and MS.

A new macrocycle **65** was synthesized in two different ways and analyzed by NMR, MS and X-ray crystallography.

A new rotaxane **85** was synthesized and characterized by NMR and MS.

2. New Functionalized Lipid Derivatives for Biophysical Applications

Key words:

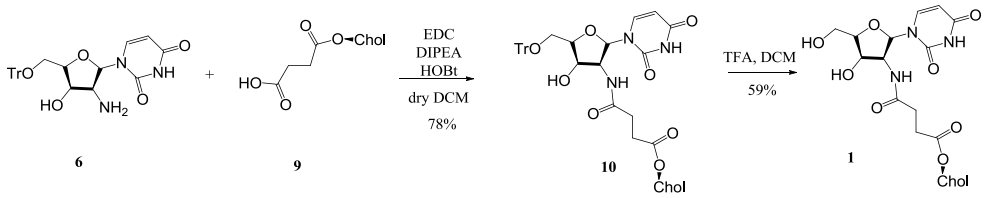
Cholesteryl-nucleoside
Amphiphilic lipid
Lipid microtubes
Nanocarrier

2.2. Cholesterol-Modified Nucleosides as Precursors for Microtubes Self-Assembly

2.2.3. Results and discussions

Our target was to synthesize new cholesteryl-nucleosides, -nucleobase derivatives with varied chain length, type of nucleoside, nucleobase or site of attachment, figure 7, and analyzing there possible self assembled arrangement as micro tubes by mixing with phospholipids.

First of all we synthesized derivative **1**, the most important derivatives for this synthesis are compounds **6** and **9**. The coupling reaction was mediated by 1-ethyl 3, 3 dimethylaminopropyl carbodiimide (EDC), hydroxybenzotriazole (HOBt) and N,N diisopropylethylamine (DIPEA) in dry dichloromethane at room temperature scheme 3.[14] The last step was deprotection with trifluoroaceticacid (TFA) in DCM giving **1** in 55%.



Scheme 3.

The self assembly of 2'-*N*-(2-(cholesteryl)-succinyl)-2'-deoxy-2'-aminouridine **1** in combination with dioleoylphosphatidylcholine (DOPC) gives formation of tubules (figure 8) which were visualized by an Olympus IX-81 inverted fluorescence microscope equipped with a cooled CCD camera (SPOT slider, Visitron Systems, Puchheim, Germany). Images were acquired using 100x Plan-APO oil immersion objective with the appropriate differential interference contrast (DIC) optics and fluorescence filter sets: BP 470–490, FT 505, and BP 510–550 (NBD, FITC). In addition to vesicles and aggregates, tubular structures of 2-3 µm and under 1 µm diameter were observed with light microscopy (Figure 8).

[14] (a) G. Hofle, W. Steglich, H. Vorbriiggen *Angew. Chem. Int. Ed.* **1978**, *17*, 569; (b) A. D. Abell, B. K. Nabbs *Bioorg. Med. Chem.* **2001**, *9*, 621.

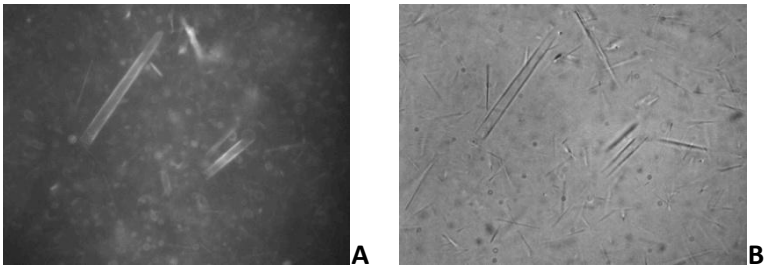
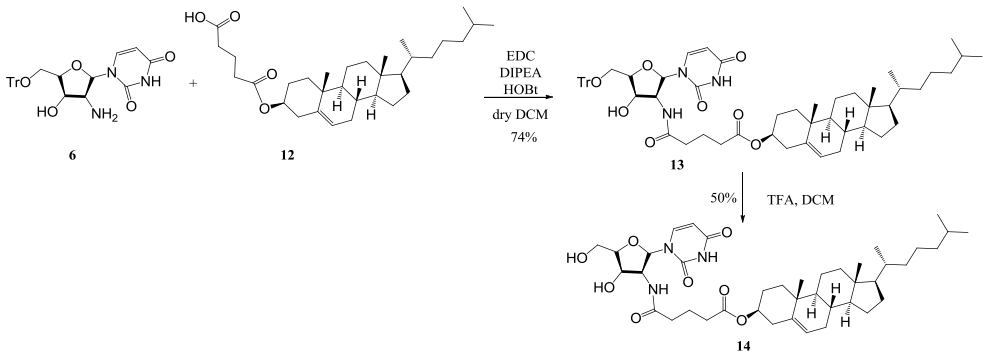


Figure 8. Tubules self assembly images of *N*-(2-(cholesteryl)-succinyl)-2'-deoxy-2'-aminouridine **1**, in 69 :30:1 mol% DOPC/conjugate/NBD-DPPE (all lipids from Avanti Polar Lipids Inc.) conditions, with an Olympus IX-81 inverted fluorescence microscope (Olympus, Hamburg, Germany). **A)** NBD fluorescence image; **B)** differential interference contrast image

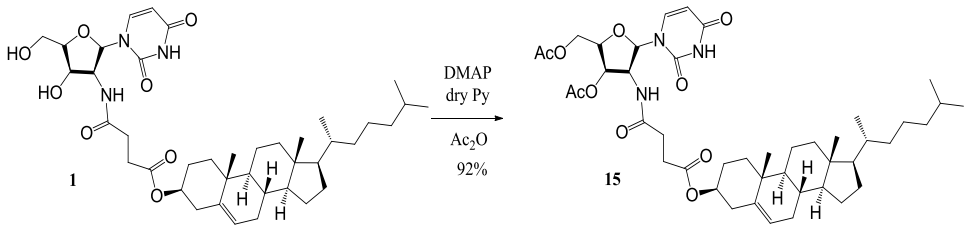
A similar procedure as the one described for compound **1** was used for the synthesis of compound **14**, i. e. reaction of glutaric anhydride with cholesterol. The next step was a condensation reaction between derivative **6** and **12**, and deprotection of trithyl protected uridine **13**, scheme 4.



Scheme 4.

To see if the OH units of the sugar moiety are responsible for microtube self-assembly we protect these units with acetyl. Derivative **1** was acetylated using catalytic amounts of DMAP in pyridine with acetic anhydride[15] to afford **15** in 92% yield, scheme 5.

[15] S. K. Mahto, C. S. Chow *Bioorg. Med. Chem.* **2008**, *16*, 8795.



Scheme 5.

For the new nucleoside derivative **15**, fluorescence images with an Olympus IX-81 inverted fluorescence microscope showed that needle like crystals were formed with DOPC and most of them were aggregated, figure 10.

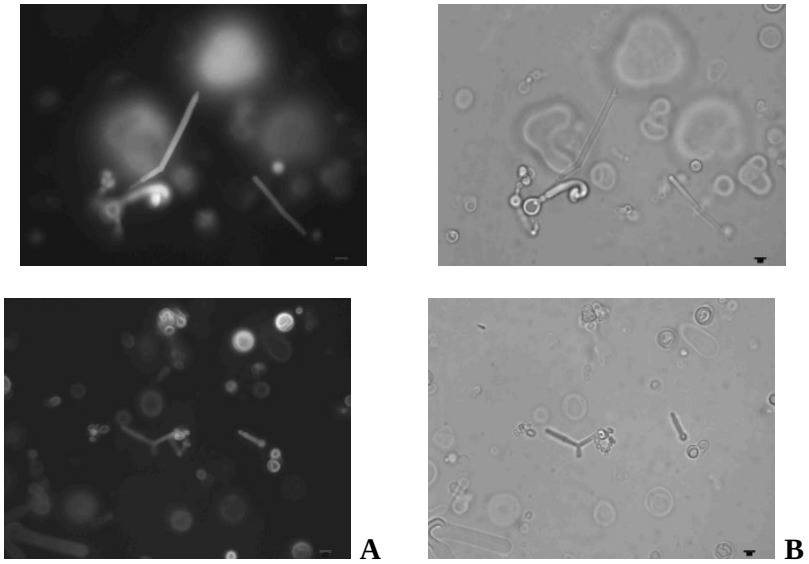
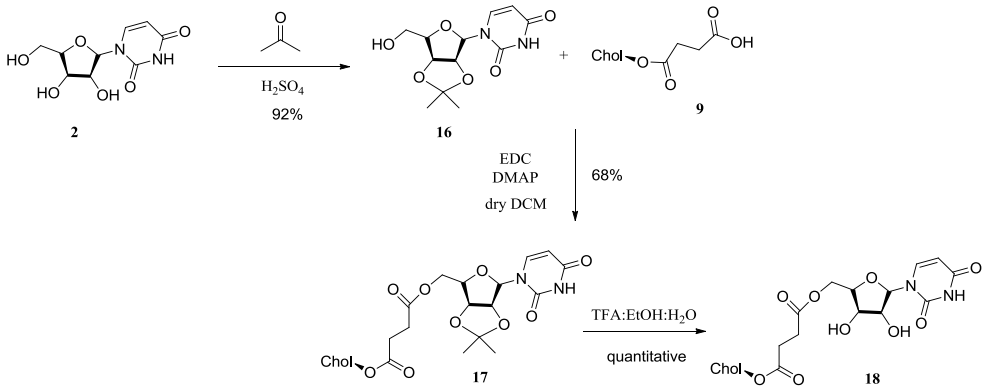


Figure 10. Tethers self assembly imagies of derivative **15**, in 69 :30:1 mol% DOPC/conjugate/NBD-DPPE (All lipids from Avanti Polar Lipids Inc.) conditions, with an Olympus IX-81 inverted fluorescence microscope (Olympus, Hamburg, Germany). **A)** NBD fluorescence images; **B)** differential interference contrast images.

First step of the synthesis of cholesteryl-nucleobase derivative **18** was protection of uridine OH units in 2´ and 3´ positions with acetone and catalytic amount of H_2SO_4.[16] The next step was a coupling reaction between **16** and

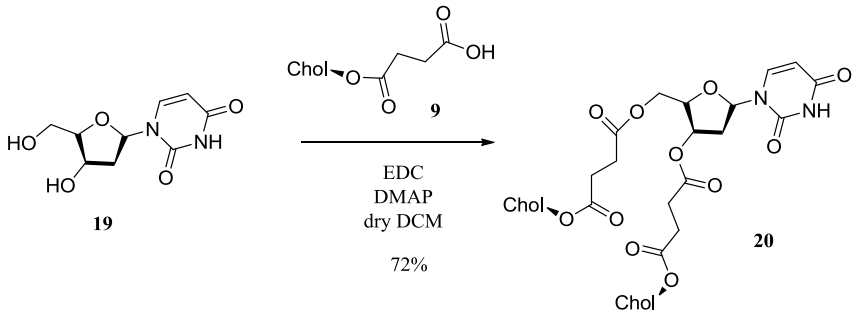
[16] P. Tarasconi, S. Capacchi, G. Pelosi, M. Cornia, R. Albertini, A. Bonati, P. P. Dall'Aglio, P. Lunghi, S.

cholesteryl succinate **9**[17] in the presence of 4-dimethyl-aminopyridine (DMAP). Final deprotection reaction[18] gave **18** in quantitative yield, scheme 6.



Scheme 6.

To investigate self-assembling of a uridine derivative with two attached cholesteryl units, we decided to synthesize compound **20**. By a simple reaction from 2'-deoxyuridine **19** in dichloromethane with DMAP and EDC we synthesized a new dicholesteryl-uridine derivative **20** in 72% yield, scheme 7.



Scheme 7.

For a mixture of the dicholesteryl derivative **20** and DOPC the image of fluorescence microscope presents an aggregate of branches, figure 13. Derivative **20** were well mixable with the phospholipids and it incorporated into the bilayer

Pinelli *Biooorg. Med. Chem.* **2000**, *8*, 157.
[17] A. Thesis, H. Ritter *Macromolecules* **2003**, 7552.
[18] D. W. Gammon, R. Hunter, S. Wilson *Tetrahedron Letters* **2002**, *43*, 3141.

structures. In the control sample prepared from 69:30:1 DOPC/cholesterol/NBD-DPPE no angled structures were observed.

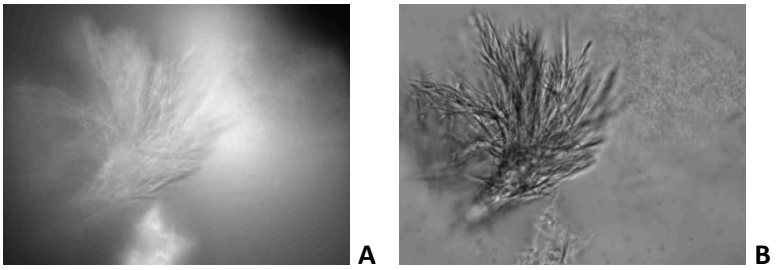
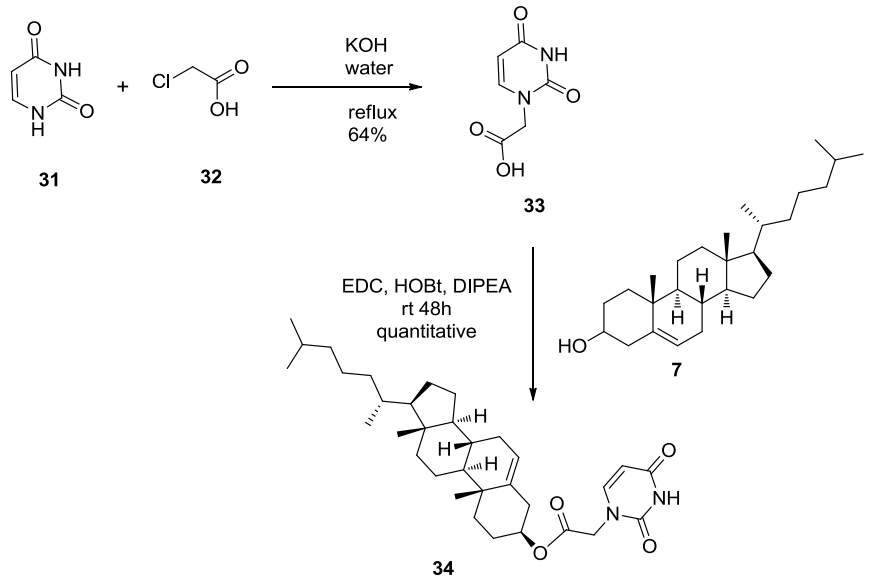


Figure 13. Branches self assembly arrangement of derivative **20**, in 69 :30:1 mol% DOPC/conjugate/NBD-DPPE (All lipids from Avanti Polar Lipids Inc.) conditions, with an Olympus IX-81 inverted fluorescence microscope (Olympus, Hamburg, Germany). **A)** NBD fluorescence image; **B)** differential interference contrast image

Another new cholesteryl derivative which we want to synthesize was a cholesteryl-nucleobase as cholesteryl-uracil **34**. Derivative **33** was synthesized starting from uracil and chloroacetic acid.[19] After N-alkylation the resulting acetic acid **33** was submitted to esterification with cholesterol, scheme 12.



[19] J. R. Jacobsen, A. G. Cochran, J. C. Stephans, D. S. King, P. G. Schultz *J. Am. Chem. Soc.* **1995**, *117*, 5453.

Scheme 12.

In the control sample prepared from 69:30:1 DOPC/cholesterol/NBD-DPPE the self-assembly of derivative **34** occurred almost entirely inside the lipid vesicles: flat and needle like structured were observed, figure 15.

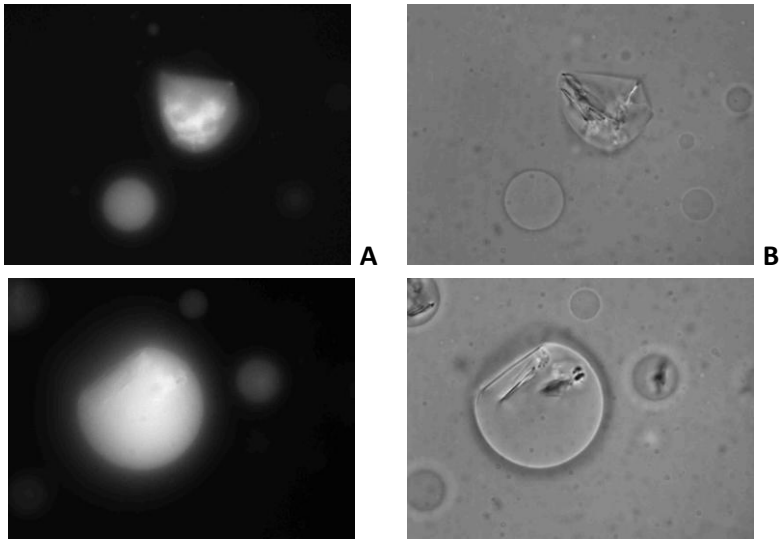


Figure 15. Flat and needle like structured images of derivative **34**, in 69 :30:1 mol% DOPC/conjugate/NBD-DPPE (all lipids from Avanti Polar Lipids Inc.) conditions, with an Olympus IX-81 inverted fluorescence microscope (Olympus, Hamburg, Germany). **A)** NBD fluorescence image; **B)** differential interference contrast image

For the synthesized target nucleoside the bio-physicians will study there self-assemble arrangements.

2.2.4. Conclusions

As new cholesteryl derivatives, four new cholesteryl-nucleosides (**14**, **15**, **18** and **20**) and a new cholesteryl-uracil **34** were synthesized and analyzed by NMR spectroscopy, MS-spectrometry and elemental analysis.

The target cholesteryl derivatives were self assembled in combination with dioleoylphosphatidylcholine (DOPC) by the group of Dr. A. Abruzova. The microscope images show different arrangements for all these compounds.

The self assembly of 2'-*N*-(2-(cholesteryl)-succinyl)-2'-deoxy-2'-aminouridine **1** show formation of microtubules with an average diameter of ~300 nm and 2-3 μm sometimes with a thinner tubule sticking out of the open end.

For derivative **15** the microscope images show only lamellar structures as vesicles and tethers and most of them are aggregates arrangement.

For dicholesteryl **20** the self-assembly images show formation of aggregate of branches and for uracil-derivative **34** needle like structured were observed.

2.3. Synthesis of Novel Amphiphilic Conjugates with a Biological Recognition Function for Developing Targeted Triggered Liposomal Delivery Systems.

2.3.3. Results and Discussions

The target of this research was to synthesized and analyzed new amphiphilic lipids with potential of triggering drug release from liposomes by cleavable linker group and enzymatic glucose cleavage[20], the general chemical structure of this type of molecule is presented in figure 18.

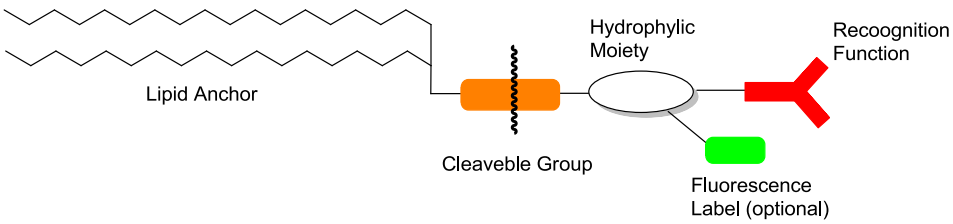
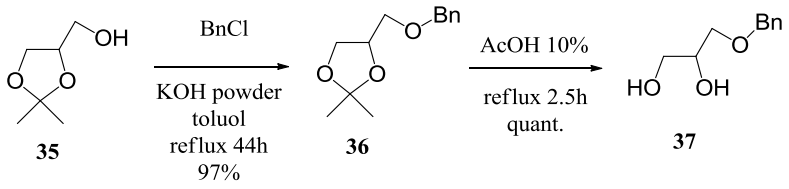


Figure 18. Amphiphilic lipids with biological recognition function, a cleavable linker group and an optional fluorescence label useful for incorporation into liposomes

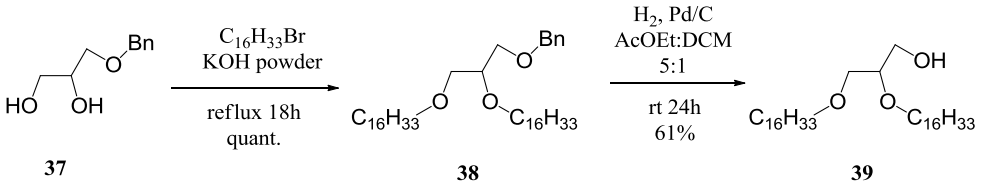
The first step in the synthesis of target compound starts from protection of hydroxyl group of racemic 2,3-*O*-isopropylidene glycerol **35**[21], followed by deprotection of glycerol derivative **36**[22] to give diol **37** in quantitative yield, scheme 14.

[20] N. Brodersen PhD. Thesis, Humboldt-University Berlin, Germany **2009**.
[21] M. Kates, T. H. Chan, N. Z. Stanacev *Biochemistry* **1963**, *2*, 394.
[22] R. J. Howe, T. Malkin *J. Chem. Soc.* **1951**, 2663.



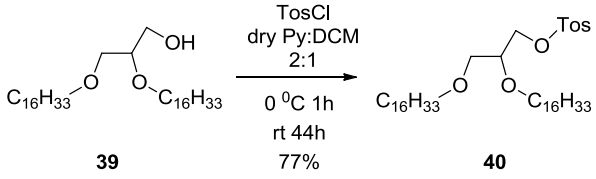
Scheme 14.

Electrophilic substitution with 1-bromohexadodecane gave benzyl protected ether **38,** which was further reduced to the corresponding alcohol **39** in hydrogen atmosphere[139a], scheme 15.



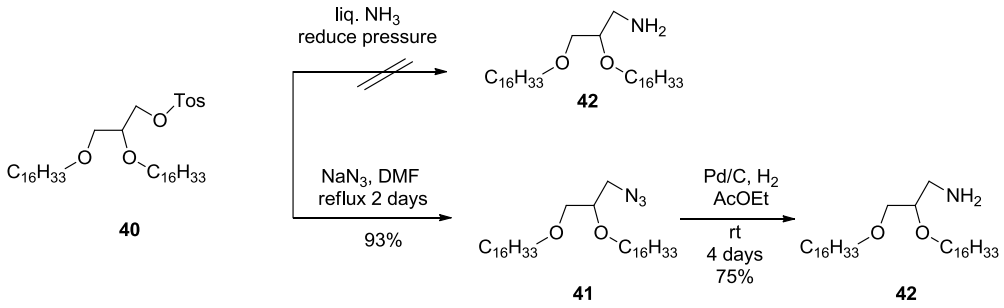
Scheme 15.

The alcohol **39** was reacted with tosyl chloride to give tosyl derivative **40**. Treatment of **40** with liquid ammonia in methanol at low temperature, under elevated pressure[23] did not result the corresponding amine. Pleasingly, transformation of **40** into the azide **41**[24] followed by catalytic hydrogenation [139a], provided the anticipated amine **42** in high yields (scheme 16).



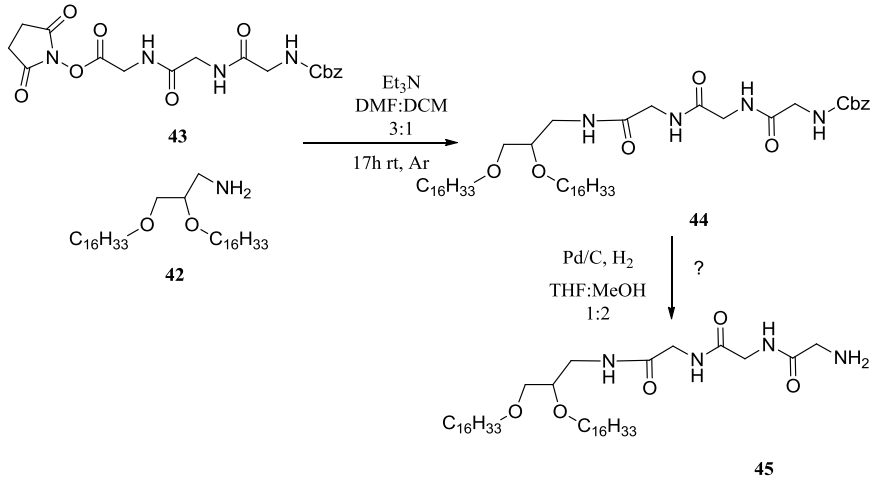
[23] (a) S. Bhattacharya, P.V. Dileep *Tetrahedron Letters* **1999**, *40*, 8167; (b) I. A. Godunov, A. V. Abramenkov, V. A. Bataev, V. I. Pupysev *Russian Chemical Bulletin* **1999**, *48*, 1369; (c) O. Seitz, I. Heinemann, A. Mattes, H. Waldmann *Tetrahedron* **2001**, *57*, 4365.

[24] (a) N. Madhavan, E. C. Robert, M. S. Gin *Angew. Int. Ed.* **2005**, 7584; (b) C. D. Pointer-Keenan, D.-K. Lee, K. Hallock, A. M. Tan, R. Zand, A. Ramamoorthy *Chem. Phys. Lip.* **2004**, *127*, 47.



Scheme 16.

Amine **42** was acylated in good yield with commercially available benzyloxycarbonyl triglycine succinate **43** using triethylamine as base[25]. Cbz protected derivative **44** was further reduced in a stainless still reactor in hydrogen atmosphere to give amine **45**, scheme 17. The yield was lower than expected; we supposed that this was due to sticking of the deprotected derivative **45** on the Pd/C catalyst.

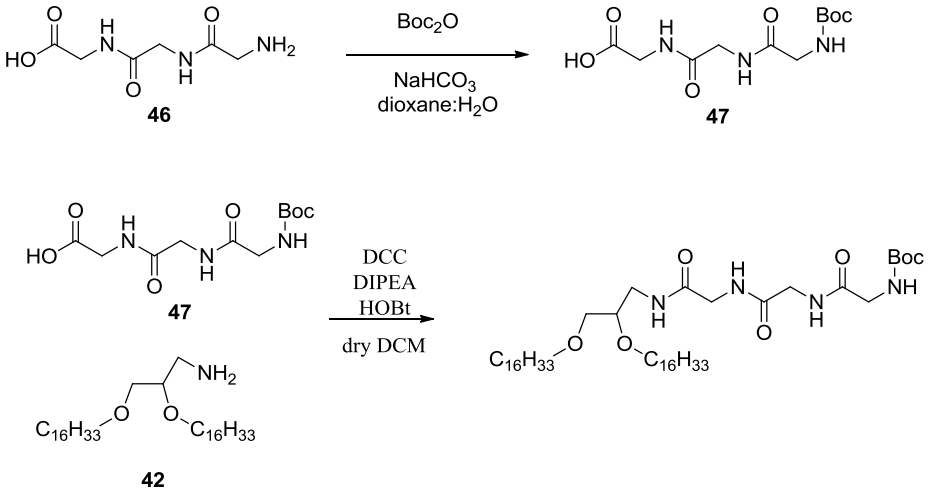


Scheme 17.

Because of the low yield of deprotection reaction we change the strategy for the synthesis of the oligopeptide-"breaking point". The commercially available GlyGlyGly was protected on the amino side with di-*tert*-butyl dicarbonate to give

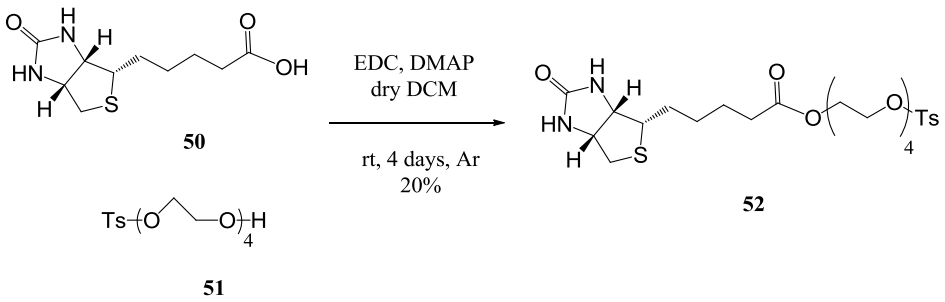
[25] N. Kameta, G. Mizuno, M. Masuda, H. Minamikawa, M. Kogiso, T. Shimizu *Chemistry Letters* **2007**, *36*, 896.

47 which was further reacted with amine **42** to give the coupling derivative **48**. The deprotection reaction was realized in a mixture of dichloromethane and TFA (trifluoro acetic acid) but the analysis for deprotected product **49** were not performed yet, scheme 18.



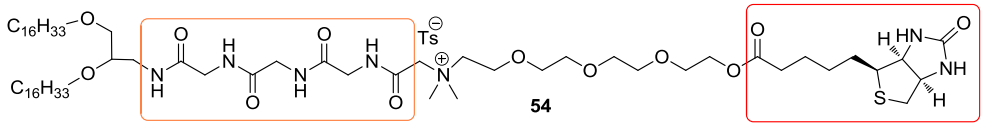
Scheme 18.

The tetraehtyleneglycol **52** containing biotin as recognition function was synthesized starting from biotin **50** and tetraethyleneglycol monotosylate in dry DCM using EDC and DMAP as typical reagents for ester synthesis, scheme 19.



Scheme 19.

The final two steps for the synthesis of target amphiphilic lipid, scheme 20 are the reactions of triglycine **49** with dimethylamino-glycine to give **53** and the formation of target compound **54**, reaction between derivative **53** and **52**. These last two steps will be realized in the early future.



Scheme 20.

The target compound **54** which contain tetraglycine as cleavable group was designed for building liposomes filled with cargo and directing these liposomes to a biological/pharmacological target were this tetraglycine moiety will be cleaved.

2.3.4. Conclusions

For the synthesis of target amphiphilic lipid **54** we synthesized and analyzed by NMR spectroscopy, MS-spectrometry three new precursors **44**, **45** and **48**.

The target compound **54** with tetraglycine as cleavable point will be study as potential building liposome filled with cargo for triggering drug release by cleavable linker of group.