Synthesis of Novel Calix[4]arene Based Dendrimers and their Biological Applications

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Declaration

This thesis is submitted to the University of East Anglia for the Degree of Doctor of Philosophy and has not been previously submitted at this, or any other university for assessment or for any other degree. Except where stated, and reference or acknowledgement is given, this work is original and has been carried out by the author alone.

Abstract

This thesis describes the development and synthesis of multicalixarenes and their application in gene and drug delivery.

Chapter 1 provides an introduction to gene therapy and calixarenes. The synthesis of two families of amino functionalised multicalixarenes are described. Evaluation of their toxicity and transfection studies are reported and a number of candidates for development identified.

Chapter 2 describes the development of calixarene glycoconjugates using a wide range of approaches. The synthesis of calixarene glycoconjugates through click chemistry is reported and the development of the first multicalixarene glycoconjugate is discussed.

Chapter 3 reviews the development of large multicalixarenes assemblies. The synthesis of large (9 and 21 member) click based multicalixarenes is described.

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Chapter 1: Synthesis of multicalixarene based transfection agents

1.1. Introduction

Deoxyribonucleic acid (DNA) is a macromolecule which encodes the genetic information in all living organism and also in some viruses. Most DNA molecules are double-stranded helices formed of two long polymer chains. Each chain is composed of a sequence of simple units called nucleotides. Each unit is made up of three components: a heterocyclic base, a sugar and one or more phosphate groups. The heterocyclic bases present in the DNA, can be classified into two families: purines and pyrimidines.[1] Purine bases are bicyclic molecules containing four nitrogen atoms, formed by the fusion of six and five member heteroaromatic rings (Figure 1.1). Adenine (A) and guanine (G) belong to this family. The pyrimidine bases present in DNA are called thymine (T) and cytosine (C). These are heterocyclic aromatic molecules containing two nitrogen atoms and four carbon atoms. Purines and pyrimidines are bound to a molecule of 2-deoxyribose. This sugar is a pentose monosaccharide which differs from ribose in the lack of an oxygen in position 2. The nucleotide is completed by one or more or more phosphate groups which bind the sugar in position 5' and connect to the phosphate groups of other nucleotides forming a strand held by a negatively charged phosphate back bone. The nitrogenous bases adenine and guanidine can selectively form hydrogen bonds with thymine and cytosine respectively forming the base pairs AT and GC.

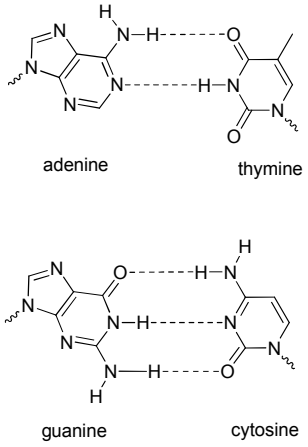


Figure 1. 1: Complementary base pairs adenine-thymine and guanine-cytosine

In double helix DNA each member of the base pair is on a different strand of nucleotides, which run in opposite directions and it is therefore complementary and anti-parallel. In cells the DNA is located in the nucleus and is organised in chromosomes. The set of chromosomes in a cell forms the genome. The human genome is composed of 23 sets of chromosomes which consist of approximately 3 billion base pairs. The Human Genome Project, started in 1990, aimed to determine the base pairs which form the human genes and to identify and map those genes. Since the completion of the project on 2003[2] the use of nucleic acid as a therapeutic agent has become an area of great interest for the scientific community.

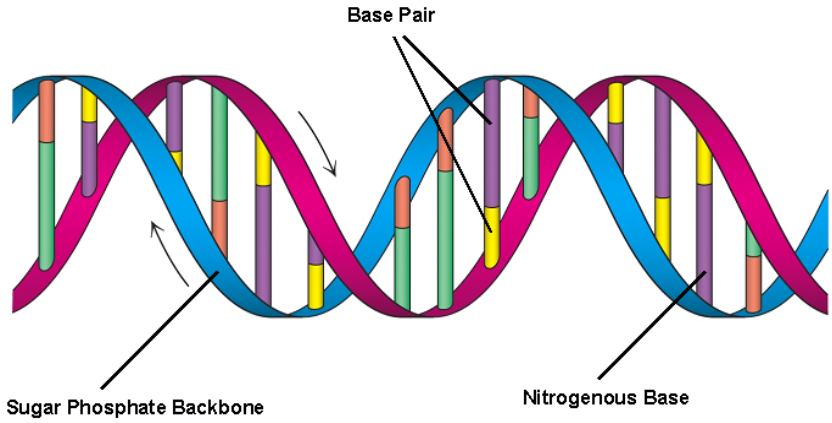


Figure 1. 2: Helical structure of double stranded DNA[3]

1.1.1. Gene therapy

Excluding those caused by traumatic incidents, virtually all diseases have a hereditary component.[4] The predisposition to a disease can be found in misspellings (mutations) of the genetic code. The likelihood of developing the disease then depends on environmental factors such as chemicals, viruses, stress etc.

Gene therapy aims to treat human diseases by transferring genetic material into specific cells of the patient.[5, 6]The therapeutic effect of gene therapy is obtained by replacing defective genes with DNA containing the correct sequence.[7] It is straightforward to imagine the treatment of diseases strictly related to a genetic deficiency such as haemophilia,[8] muscular dystrophy[9] or cystic fibrosis[10] through substitution of the faulty genes within the affected cells with correct ones. However research is focused also in developing genetic therapies for the treatment of cardiovascular,[11] neurological[12, 13] and infectious diseases[14] wound healing[15] and cancer[16, 17, 18]

through enhancing naturally occurring proteins by the introduction of genes which alter the expression of the existing genes.

In 1987 Flegner *et al.* were the first to succeed in transfecting DNA in to a mammalian cell using unilamellar liposomes.[19] The first human gene therapy trial started in the USA in 1990.[20] Blaese *et al* .transfected, the adenosine deaminase gene using a modified retrovirus as transfection agent into the T cells of two children affected with severe combined immunodeficiency. Two years after the therapy ended the gene expression still persisted. This first clinical trial demonstrated that gene therapy could be safe and efficient in the treatment of some immunodeficiency diseases.

The two main methods to introduce nucleic acids into the cell are *ex-vivo* and *in-vivo*.[21] In the *ex-vivo* approach, the target cells are removed from the patient, purified and re-inoculated after being genetically modified.[22] This method has the disadvantage of being invasive for the patient. With the *in-vivo* approach the genes are directly delivered into the patient's tissues using vectors. [23] The delivery of nucleic acid directly in to the patient cells can be achieved using two main categories of vectors: recombinant viral vectors or synthetic vectors. Each delivery approach has some disadvantages.

1.1.1.1. Viral vectors

The use of various types of viral vectors has been widely used to transfect genes in to cells.[24] The primary function of a virus is to carry its genetic material from one host cell to another. Once entered into the new host cell, the virus navigates to the cell nucleus where it begins the expression of its genome. Replacing part of the viral genome with therapeutic genes makes it possible to exploit this property creating gene-delivery vehicles. In order to be employed the pathogenic effect of the virus needs to be disabled.[25] The most used viral carriers are the retroviruses and the adenovirus.[24] The use of a virus is a powerful tool for the delivery of genes because nature has evolved them as specific machinery to deliver genetic material into cells. Despite their efficiency in transfecting the target cells the use of viral vectors has some drawbacks. The human immune system fights off the virus and therefore the viral gene delivery has always to face the host response. Although the recombinant viral vectors are rendered non-replicative and non pathogenic there is still the risk that the virus reverts into a wild-type virion or co-purify with replication-competent virions.[24]

1.1.1.2. Synthetic vectors

Another approach towards the cellular delivery of nucleic acids involves the use of synthetic carriers. These vectors have the advantage of being safer because they do not trigger immune reactions in the host organism.[24] Their manufacturing is more facile compared to the preparation of viral vectors. Synthetic vectors need to match a number of requirements to be suitable candidates for gene delivery. The ideal vector for gene therapy must fulfil several criteria: bind nucleic acid electrostatically, condense the genetic material, preserve the genes from enzymatic degradation, mediate the cellular entry and release the nucleic acid load and have low toxicity. In order to be able to bind the negatively charged nucleic acid the vectors need to be positively charged. Several independent groups have reported that a minimum of six to eight charges are required for efficient DNA condensation and consequent transfection.[26, 27, 28] Complexes of plasmid DNA with cationic lipid or cationic polymers are called lipoplexes and polyplexes respectively. Although synthetic vectors have been employed for *in-vitro* studies since the 1960s, the development of *in-vivo* transfection has to confront several problems such as low gene-transfer efficiency, toxicity and the *in-vivo* stability.

Figure 1.3 summarizes all the steps involved in the gene delivery process and all the barriers that the complex DNA-synthetic vector must overcome in order to transfect the genetic material into the cell. It is now commonly accepted that polyplexes and lipoplexes are internalized *via* endocytosis.[29, 30, 31] Although clarithrin dependent endocytosis has been considered for a while to be the only pathway involved in the internalization of exogenous molecule into the cells, the past two decades have shown that also other routes, so-called “clathrin independed endocytosis”, may be involved. The mechanism of such pathways is still not completely understood, but seems to differ, for a given formulation, with the cell type and molecular composition of the cell surface.[32] The size and the global charge of the complex also seem to influence the internalisation process involved. Functionalisation of the complexes with motifs capable of binding cellular membrane receptors is expected to improve the transfection efficiency, *via* receptor-mediated internalisation which can be faster than non-specific uptake.

Once the DNA-carrier complex has penetrated the cell through endocytosis, it is confined in the endosome, which can either fuse with a lysosome or recycle its cargo back to the cell surface. Endosomal escape is considered one of the major limitations for non-viral transfection vectors. Cationic polymers bearing unportonated amino groups at neutral pH act upon protonation in the endosomes as a proton sponge leading to swelling, rupture of the vescicle and consequent release of its content.[33]

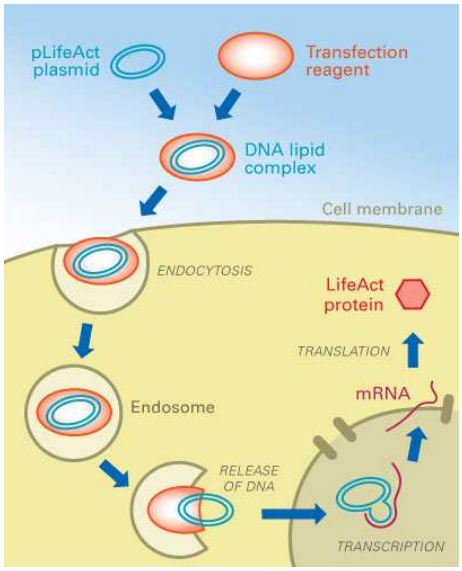


Figure 1. 3: Cellular gene delivery process

The movement of the plasmid DNA in the cytosol is another crucial step in the transfection process. The cytoskeleton components seem to be involved with the cytoplasmic motion of the nucleic acid either free or complexed with the carrier. In particular it has been found that unspecific binding of DNA with actin, vimentin and keratin impairs the movement of the plasmid in the cytosol.[34] On the other hand microtubules are required to trasnsport the DNA efficiently through the cytosol.[35] Inibition of histone deacetylase 6 (HDAC6), an enzyme which regulates the deacetylation of the microtubules, appears to be associated with an increase of plasmid trafficking and entry into the nucleus.[31] Therefore HDAC6 inhibitors could be good candidates to increase the efficiency of gene transfection by non viral vectors. Furthermore the migration of polyplexes and lipoplexes could be improved by functionalising the complexes with signal molecules capable of enhancing the binding to the microtubules.

The last barrier between the plasmid DNA and the transcription is nuclear import. The access to the nucleus in non-dividing cells is regulated by pores. Molecules larger than 40 kDa must display specific nuclear localization signals (NLS) to be actively transported. Wagstaff and Janson reported that only 0.1% of DNA microinjected to the cell was transcribed.[36] Nevertheless the entry of the genetic material into the nucleus could be improved by coupling of either the synthetic vector or the back bone of the DNA with NLS.

1.1.1.2.1. Lipid based vectors

The first synthetic vector was used in 1987 by Flegner *et al.* Small unilamellar liposome containing a synthetic cationic lipid interacted spontaneously with DNA to form a lipoplex.[19] The cationic lipid facilitated the fusion of the complex with the plasma membrane. The structure of the cationic lipid and of the neutral "helper" lipid forming the liposome has a great influence on the interactions between the lipids and the DNA and on the properties of the liposome membrane; both these factors have a major influence in the transfection efficiency.[37] Since Flegner's success, lipofection has been widely used *in-vitro* and *in-vivo* studies as well as in many human clinical trials. Despite the wide impact this gene delivery approach has some limitations which need to be overcome such as reproducibility in fabricating the lipoplex, toxicity towards some cell types and colloidal stability especially if administrated systemically.[37,38]

1.1.1.2.2. Polymer based vectors

Cationic polymers comprise linear, branched and dendritic structures.[24] Polymers such as polylysine,[39] branched polyethylenimine (PEI)[40] or polyamidoamine dendrimer (PAMAM)[41] have been used since the early stages of polymer based transfection research. Because of the flexibility of polymer chemistry it is possible to embroider the structures with functionalities required for efficient gene delivery while maintaining biocompatibility, facile manufacturing and robust and stable formulations.[24] Despite the great potential that cationic polymers have in human gene therapy, their use in clinical applications has been limited by their poor gene transfer efficiency.

1.1.1.2.3. Dendrimers in gene delivery

Among the polymer based vectors, dendrimers have unique properties which make them attractive materials for gene therapy applications.[42] The first polycationic dendrimer to find its use as a nucleic acid vector was a polyamidoamine (PAMAM) based dendrimer (figure 1.4).[43] Its *in-vitro* transfection property was first reported by Haensler and Szodka in 1993.[41] This kind of dendrimer is normally based on an ethylendiamine or ammonia core with four or three

branching point respectively,[43, 44] Polymers based on polypropyleneimine (PPI),[45, 46, 47] with similar features to PAMAM dendrimers, have also been widely explored as gene delivery systems. [48] The commercial availability of these polymers made them and their derivatives the most used in this research area.[42, 49, 50, 51]

Dendrimers interact with the nucleic acids on the base of electrostatic interactions[52] and lack sequence specifitity. [45] The resulting complex is called a dendriplex and protects the DNA from degradation.[53] Upon complexation of the DNA with PAMAM the formation of toroidal structure around 50 nm can be observed. [45] The binding between the polyplex and the cell membrane is initially based on an electrostatic attraction between the cationic complex and the negatively charged cell surface groups followed by an inclusion *via* endocytosis.[29, 42] The stability of the complexes and transfection properties were found to increase nearly exponentially with an increase in the number of generations.[54] On the other hand, regardless of the repeat unit structure, haemolysis and cytotoxity are also strictly dependant on the number or size and number of surface amino groups.[55]

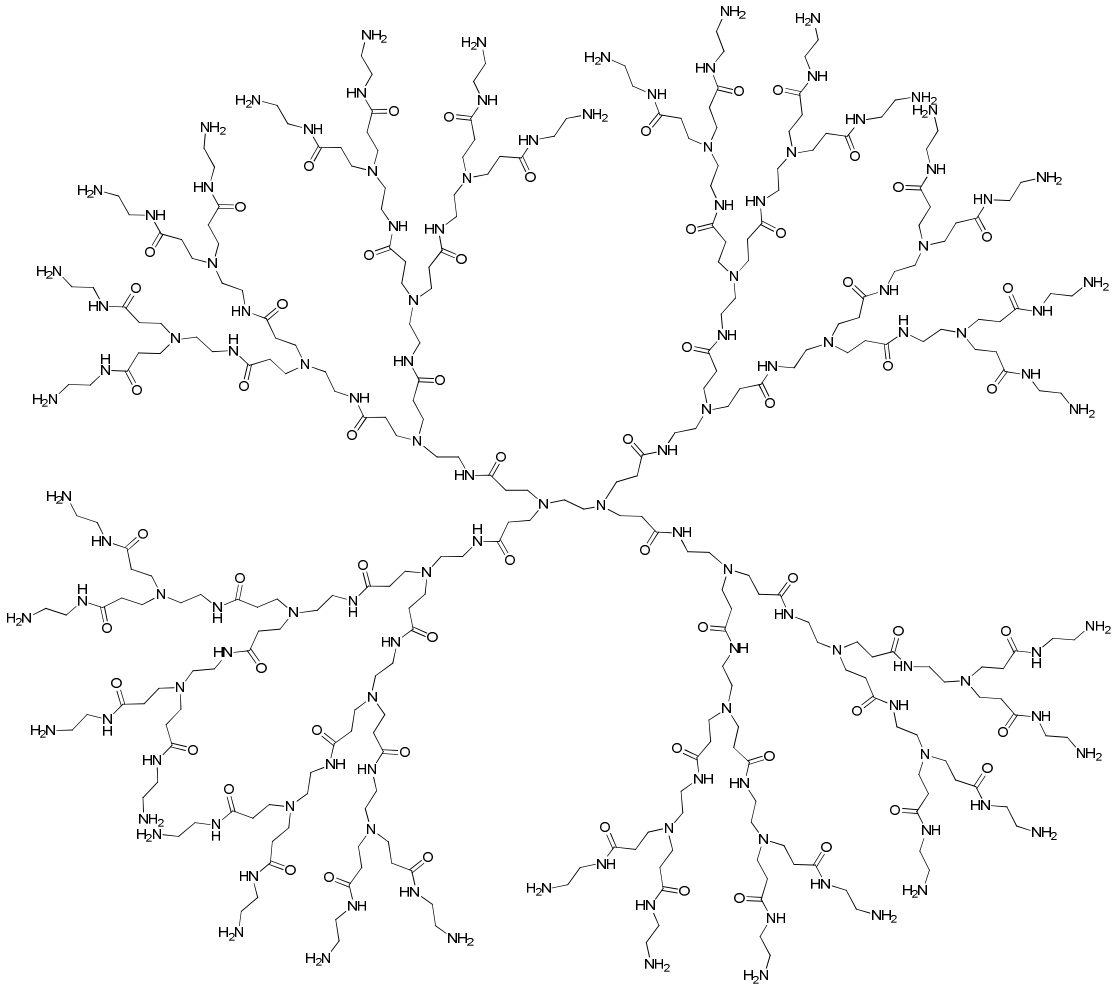


Figure 1. 4: PAMAM structure

1.1.2. Dendrimers

Dendrimers can be defined as a class of polymeric materials. They can be described as highly branched tridimensional macromolecules which develop from an inner central core with a series of repeating units. The name dendrimer originates from the Greek "*dendron*" which means tree or branch and "*meros*" meaning part. The unique properties such as such as uniform size, high degree of branching, water solubility, multivalency, well-defined molecular weight and available internal cavities has made these molecules a focus of interest in the scientific community since their conception in the late 1970s. They emerged from a new class of polymers called "cascade molecules" reported for the first time by Vogtle.[56] In the early 1980s pioneering work carried out by different groups developed these molecules to larger dendritic structures.[57, 57b, 58, 59]

1.1.2.1. Dendrimers structure and synthesis

Dendrimers are large and complex molecules generally characterised by well defined molecular structures.[60] They are built of three distinguishing architectural components: a central core, a number of layers of repeating units, called generations, readily attached to the central core and the exterior, the outermost generation which carry the terminal functionalities. For the purpose of this thesis we will call the central core G0, the second series of repeating units G1 and so on. Each individual "branch" of a dendrimer is named a dendron.[42] In contrast to linear polymers the dendrimer viscosity does not have a linear correlation with the mass but shows a maximum at a specific number of generations. This is explainable considering the shape change of a dendrimer with the increase in number of generations from a more open-planar elliptical to a more compact spherical shape.[61, 62] Because of their structure, dendrimers have the highest molecular density on the outer generation. These molecules are obtained with a stepwise synthesis which bring well defined sizes and structures.[42] The synthetic pathway can follow either a convergent or a divergent strategy.

1.1.2.2. Convergent dendrimer synthesis

The synthesis of dendrimers *via* a convergent approach exploits the symmetric nature of these polymers (figure 1.5). The molecule is built up layer after layer starting with the higher generation and terminating with the central core (G0).[63] A number of peripheral units are covalently linked with an equivalent number of corresponding binding sites into single joining units. A number of the branched molecules are covalently linked to an equivalent number of binding sites of another joining molecule and so on. Each cycle of reactions enables the growth of one generation. The synthesis ends when the branched polymeric dendrons of the target size are attached to the central core. The advantage of this strategy is that only a limited number of reactions take place at each step.[64] As result the reactions can be driven to completion with only a slight excess of reagent reducing to a minimum the formation of side products.

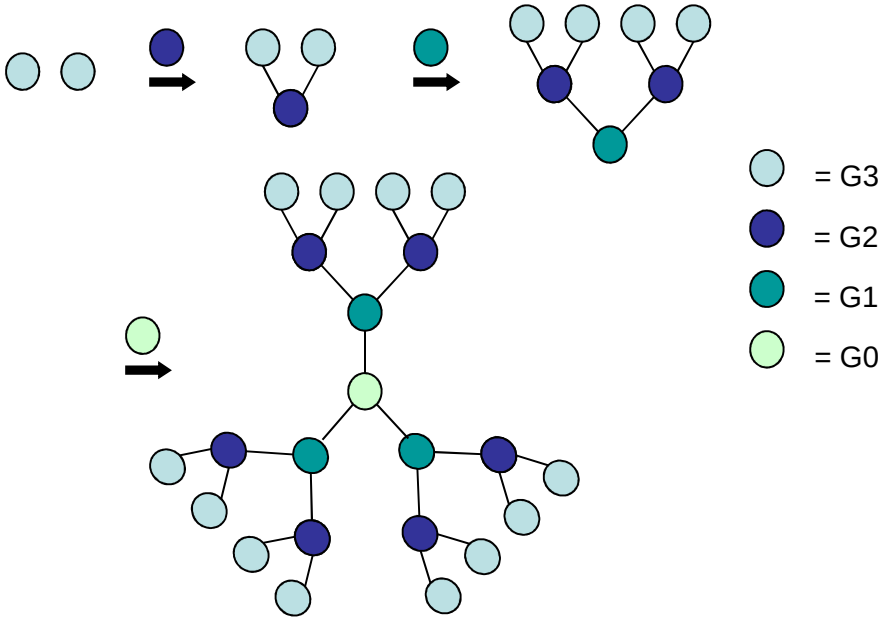


Figure 1. 5: Convergent dendrimer synthesis

1.1.2.3. Divergent dendrimer synthesis

In the divergent approach the synthesis starts from the multivalent central core G0 and builds up one monolayer at a time (figure 1.6).[58] In the first step the central core is allowed to react with G1 units bearing one active binding site and a number of inactive binding sites. Once the first

generation of the dendrimer is formed, the additional binding sites can be activated for the next step and can then be reacted with an equivalent number of next generation monomers. This process can be repeated for several generations until steric effects prevent the peripheral groups from reacting with a new generation of monomers.[42] Because of the high number of reactions occurring at one time, the majority of divergent dendrimer syntheses require an excess load of monomer for each generation growth and lengthy chromatographic purifications for each step, in particular for the higher generations.[60]

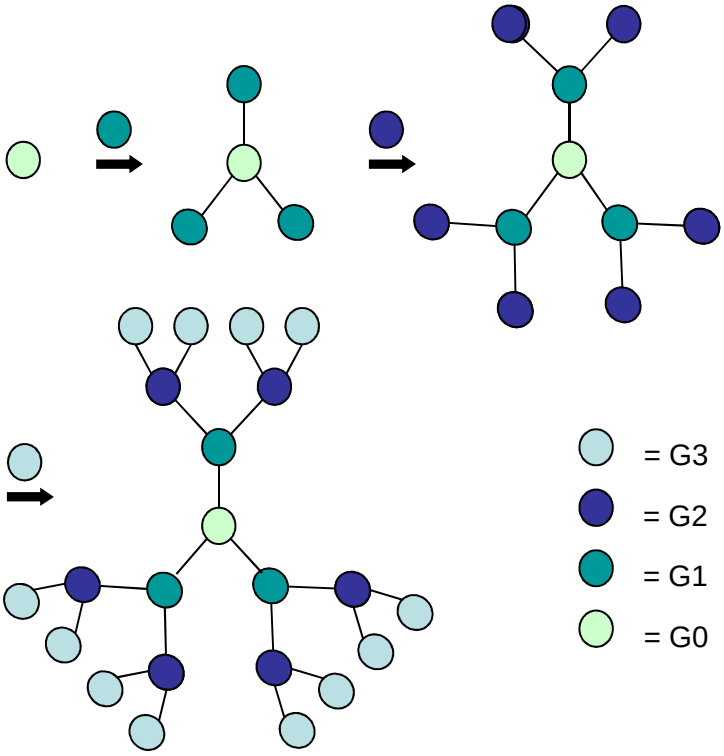


Figure 1. 6: Divergent dendrimer synthesis

1.2. Calixarenes

The calixarenes are macrocyclic molecules formed from the condensation of phenols and aldehydes.[65] This type of reaction was first performed by the Nobel Prize winner Adolph von Baeyer whom, in two papers published in 1872, described the product obtained by mixing formaldehyde and phenols in the presence of a strong acid as a "cement like substance".[66, 67] About twenty years later two other German chemists, namely L. Lederer and O. Manasse, working independently on the reaction between phenol and formaldehyde succeeded in isolating as crystalline compounds *o*-hydroxymethylphenol and *p*-hydroxymethylphenol.[68, 69] (Figure 1.7)

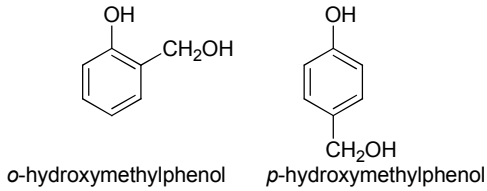
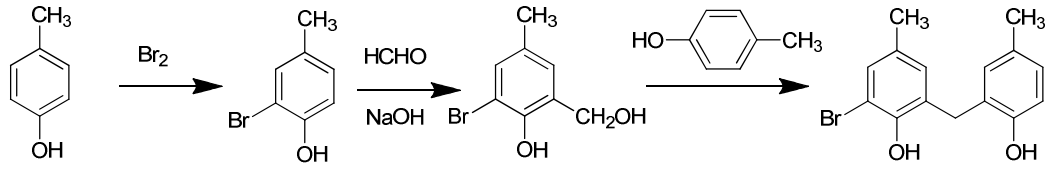


Figure 1. 7:. Lederer and Manasse product.

The Lederer-Manasse reaction did not lead to the formation of the tar like material because of the milder conditions used. In fact when the reaction is performed under more strenuous conditions, *e.g.* acid or base catalysed, the resinous tar is obtained.[65]

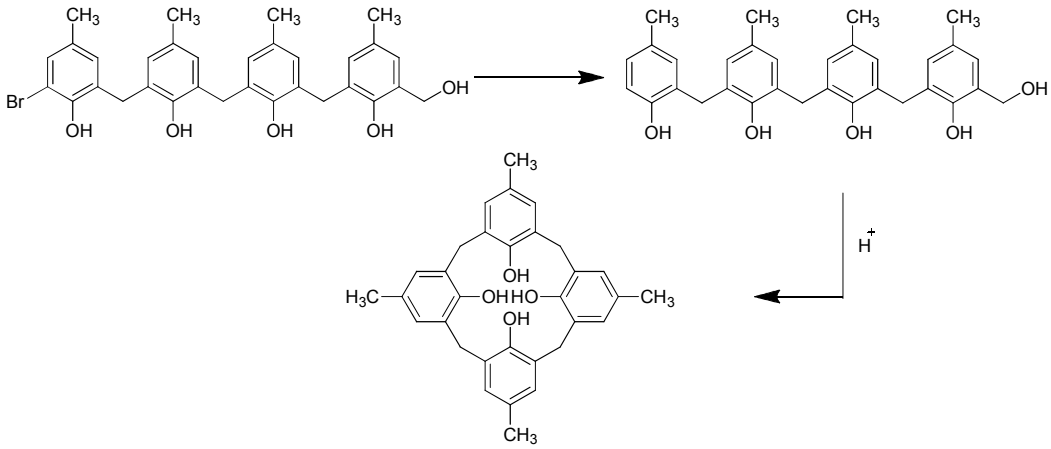
Research in this area was resumed in the beginning of the twentieth century by Leo Baekeland who patented the cement like substance with the name of Bakelite giving birth to the age of the synthetic plastics.[70] The Bakelite process has been a source of inspiration for many other scientists. Among them Zinke and Ziegler perceived that the resin obtained from the phenol-formaldehyde condensation was due to the cross linked polymerization which occurs in the reaction.[71, 72] Both the *ortho* and *para* positions are equally reactive in basic or acid conditions for electrophilic aromatic substitution. Thus the phenol ring reacts at all the three reactive positions to yield the polymer. Starting from this hypothesis they aimed to obtain a more tractable product using *para*-alkyl substituted phenols. These compounds can react only in the *ortho* position leading to the formation of a linear polymer. From their experiments they noted that the resin obtained on heating formaldehyde and *p*-tert-butylphenol was soluble in linseed oil at 100-120^{o}C. Upon further heating to 200-220^{o}C they noted the formation of a precipitate which once washed with EtOAc and precipitated from chloroform with methanol gave a crystalline compound which decomposed above 300^{o}C. Whilst, at this point, it was not postulated that this product was cyclic, further work by Niederl and Vogel on an analogous reaction between resorcinol and aldehydes led to the suggestion of a cyclic structure for both this condensation and for the Zinke product.[73]

Proof of this theory arrived only in the 1950s from the work of Hayes and Hunter,[74] whom with a stepwise synthesis obtained the structure postulated by Niederl. Starting from *p*-methylphenol they blocked one of the reactive *ortho* positions, allowing the other to react with formaldehyde under basic conditions to give 2-bromo-4-methyl-6-hydroxymethylphenol which when treated with HCl and *p*-cresol at 70^{o}C yielded the dimer: 3-bromo-2:2`-dihydroxy-5:5`-dimethyldiphenylmethane.



Scheme 1. 1: Dimer formation

After repeating this cycle two times, the tetramer obtained was de-protected *via* catalytic hydrogenation and cyclization of the oligomer was achieved by acid treatment (scheme 1.2).



Scheme 1.2: Cyclization of the linear oligomer

The light brown material obtained had a melting point above 300°C and did not undergo coupling with benzenediazonium chloride, showing the lack of *ortho* and *para* reactive positions. Another chemist involved in the history of the phenol-aldehyde condensation chemistry is the English Nobel Prize winner John Cornforth. On repeating the Zinke reaction he isolated two compounds,[75, 76] with different melting points, rather than one. To explain this result he postulated two hypotheses. In the first place he suggested that the condensation could yield macrocycles containing more than four members or alternatively the tetramer could be present in different rotational diastereoisomers. Now is it known that the product he isolated was the cyclic octamer, nevertheless his second suggestion of the presence of diastereoisomers has also been demonstrated to be correct.[65]

In the same period, in the USA, phenol-formaldehyde chemistry found a new application. Melvin DeGroote from the Petrolite Corporation, in Missouri, discovered that treatment of the product obtained by reacting *p*-alkyl-substituted phenols and formaldehyde, with ethylene oxide produces a surfactant with demulsifier characteristics. The product patented was thought to be a linear polymer rather than a cyclic oligomer. The real structure was discovered only twenty years later when as a consequence of customers' complaints of the formation of a sludgy precipitate, the product came back to the chemistry research laboratories. They simulated the

same conditions used in production plants; reflux of *p*-tert-butyl-phenol and formaldehyde in xylene in the presence of 50% KOH solution with *in-situ* removal of excess water. In order to determinate what was causing the sludge, the precipitate formed during the reaction was filtered and discovered to have a very high melting point and very low solubility. The "Petrolite" procedure was immediately patented in 1976 as a new route for the synthesis of cyclic oligomers.

Almost 40 years from their discovery, these macrocycles remained nameless, until 1978 when the name calixarene appeared in print for the first time. [77] This name was coined by Gutsche, an American chemist, who was studying the tetramers as potential enzyme mimics. The name "calix" was given as the three-dimensional structure of these molecules resembles the shape of the "calix crater" an ancient Greek vase, and the term "arene" indicates the presence of aryl moieties in the molecule.

1.2.1.Synthesis of calixarenes

As proposed by Cornforth the condensation of aldehydes and phenols leads the formation of macrocycles of different sizes. The most common products are the 4, 6, and 8 membered rings. It is possible to selectively form each ring size by using different synthetic conditions.

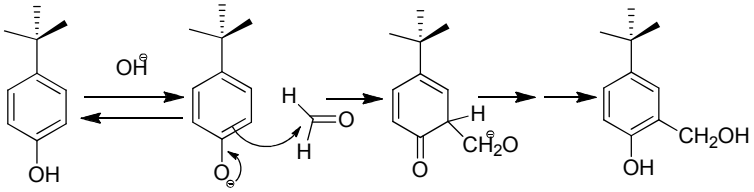
In the *Modified Zinke-Cornforth Procedure*, which leads selectively to the formation of the cyclic tetramer, *p*-tert-butyl phenol is heated for 2 hours at 110-120^{o}C in the presence of 37% formaldehyde and 0.045 equivalents of NaOH per phenol unit. The thick mass produced, called the "precursor" is then refluxed for a further two hours in diphenyl ether and the product collected by filtration and re-crystallized from toluene. The optimum amount of base that has to be used in order to obtain the cyclic tetramer is about 0.03-0.04 equivalent. The yield of the tetramer falls both below and above this range, in the former case, no reaction occurs and in the latter the reaction yields the cyclic hexamer.[78]

The hexamer can also be prepared according to the *Modified Pertrolite Procedure* a mixture of *p-tert*-butylphenol and formaldehyde is heated for two hours with 0.3 equivalent of KOH per phenol unit.[65] The formed precursor is then dissolved in xylene and refluxed for three hours. The crude product collected by filtration is then neutralized and re-crystallized from chloroform-methanol to yield calix[6]arene.

Preparation of calix[8]arene is described in the *Standard Petrolite Procedure*, in which paraformaldehyde and *p-tert*-butylphenol are heated for 4 hours in xylene with 0.3 equivalent of NaOH. And the crude product removed by filtration is crystallized from chloroform.

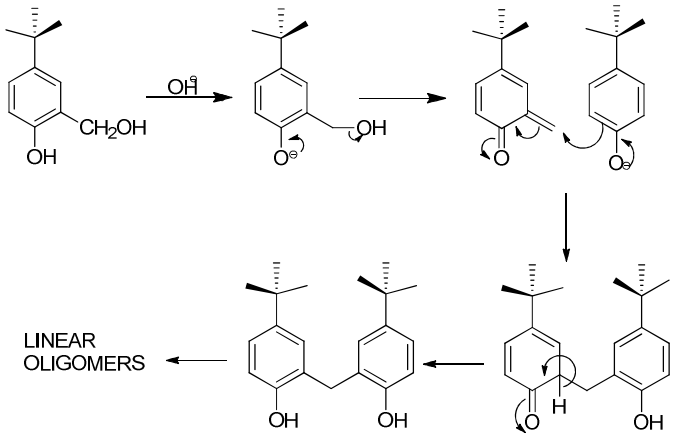
The syntheses of calix[5]- and calix[7]arene have also been described but these products have been isolated only in much lower yields[65].

The mechanism of the reaction has been studied for many years and proceeds as shown in scheme 1.3. The reaction in mild conditions can be stopped at this stage and the Lederer-Mannasse product can be isolated.[79]



Scheme 1.3: Base catalysed formaldehyde - *p-tert*-butylphenol condensation mechanism

However in more strenuous conditions the reaction proceeds with the reaction shown in scheme 1.4.



Scheme 1.4: Mechanism of the linear oligomer formation

The linear oligomers or "precursor" depending on the conditions used then cyclises to give the calix[4]arene, calix[6]arene or calix[8]arene (figure 1.8).

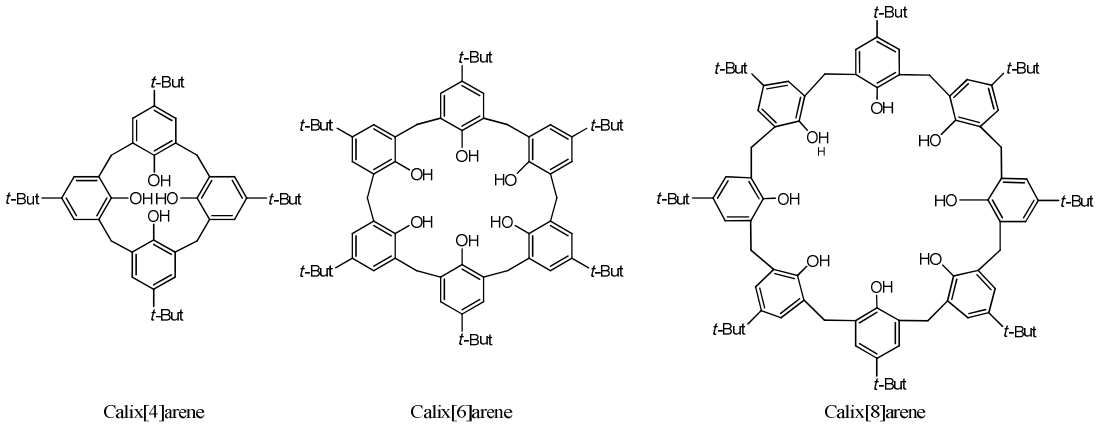


Figure 1. 8: Structure of calix[4]arene, calix[6]arene and calix[8]arene

Base catalysis is not the only route for condensing aldehydes and phenols, the reaction can occur also under acid catalysed conditions. However due to lower yields this method is not commonly used for the synthesis of phenol derived calixarenes but it remains a valid method for the synthesis of resorcinol derived calixarenes otherwise known as resorcinarenes.

1.2.2.Calix[4]arene conformations

As proposed by Cornforth, calix[4]arenes can exist in different conformations.[75] In the absence of bulky substituents (larger than ethyl) on the phenolic OH, the benzene rings can swing around the methylene bridges, resulting in four different conformations; the cone, partial cone 1,3 alternate and 1,2 alternate.

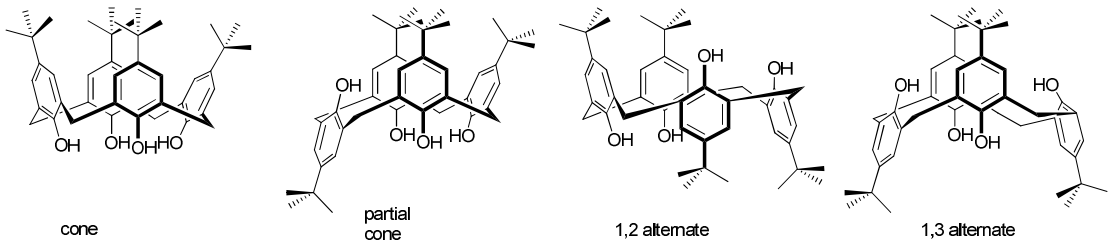
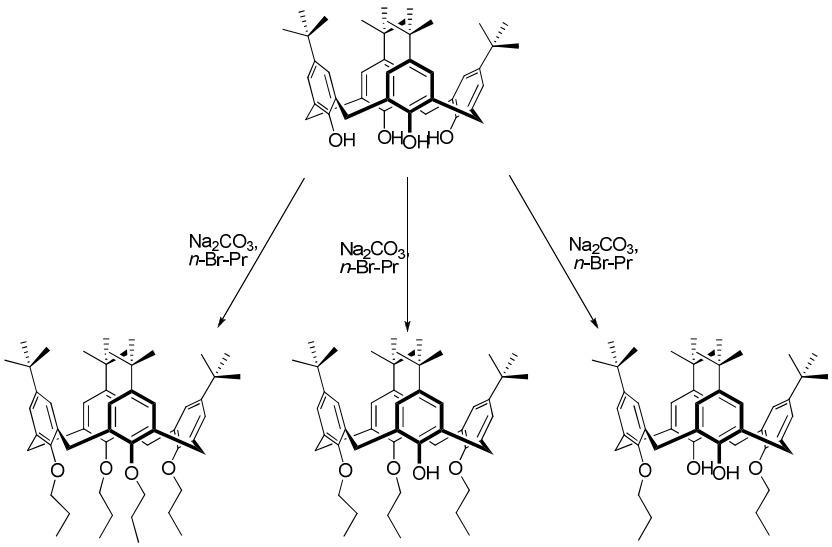


Figure 1.9: Calix[4]arene possible conformations

The rate of the rotation depends on the reaction conditions (temperature, solvent and base)[80] In non-polar solvents such as chloroform or benzene the rotational energy barrier is higher than in more polar solvents such as acetone or acetonitrile resulting in an increased rate of conformational changes in the latter case.[81]

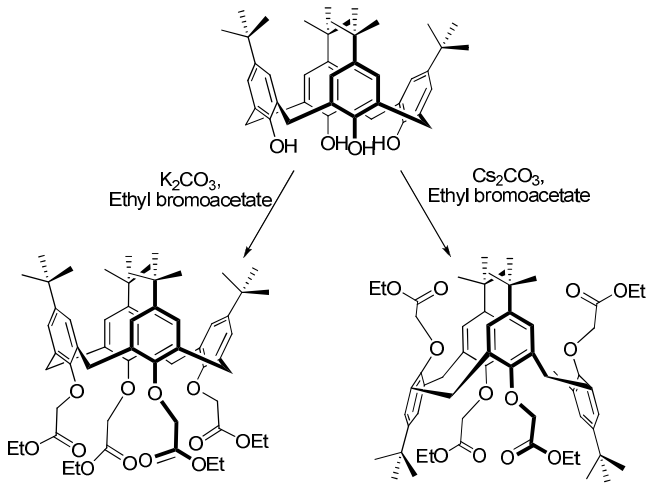
It is possible to lock selectively the conformation of the calix[4]arenes by alkylating the phenolic OH in presence of a suitable base and solvent. The choice of the right reaction conditions allows moreover the selective synthesis of mono-, di-, tri- and tetra substituted derivatives.

For example sodium hydride is the most common base used for full alkylation.[82] [83] [84] [85] [81] The use of this base at high temperature yields a mixture of cone and partial cone conformations,[86] whilst at lower temperature the cone conformer is the favoured product. In contrast, the use of bases such as lithium, potassium or sodium carbonate results in the formation of the di–alkylated product, whereas utilizing barium hydroxide combined with barium oxide it is possible to obtain the tri-alkylated product.



Scheme 1.5: Selective alkylation

An example of the importance of the counter ion of the base on determining the conformation of the calix[4]arene is shown by the reaction between *p*-t-butylcalix[4]arene and ethylbromoacetate. When the reaction is performed in the presence of potassium carbonate it gives the tetra alkylated derivative in the cone conformation, if caesium carbonate is used instead the tetra alkylated product is obtained in the 1,3 alternate conformation.[85]



Scheme 1.6: Effect of the counter ion on the conformation

In the calixarenes it is possible to identify three regions. The first is called the upper or wide rim, the second the methylene bridges and the third the lower or narrow rim.

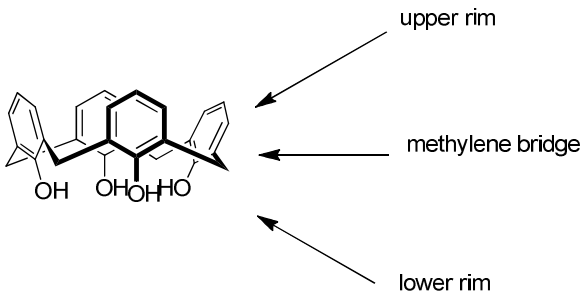


Figure 1.10: Calix[4]arene regions

It is possible to functionalise selectively these three regions with different functional groups. The phenolic oxygens at the narrow rim can be selectively linked to aryl or alkyl moieties either *via* ether or ester bonds. The upper rim is activated for electrophlic substitutions and reactions such as nitration can be performed in this position. Treatment of the acetates of *p-tert*-butylcalix[4,6,8]arenes with CrO_3 leads to the conversion of the methylene bridge to a carbonyl group,[87] which can be reduced with $NaBH_4$ to the corresponding alcohol.[88] The same position in the tetramethyl ether of *p-tert*-butylcalix[4]arenes can be brominated[89] or lithiated[89] using N-bromosuccinimide and *n*-butyllithium respectively.

1.2.3.Biological application of calixarenes

Although the antitubercular properties of calixarenes were known since 1954 thanks to the pioneering work of Cornforth,[75] for many years these molecules, have been mainly exploited as host for ions and neutral molecules.[90] Only in more recent years have calixarenes found applications in various biological areas. Their unique three dimensional structure along with their lack of toxicity and the relative ease of functionalisation has made this molecule the focus of many biological investiagtions in the.[91] Calixarenes have been widely used as a multivalent platform to anchor biological active molecules such as carbohydrates and peptides.[90] Such conjugates have been used by Ungaro *et al* for their lectin binding properties and protein surface recognition respectively. Calixarenes have also found important application in the field of the biotechnology as ion channels. Davis *et al* have reported the synthesis of calix[4]arenes functionalised with tetrabutylamide groups which are capable of binding to a lipid bilayer and transport ions effectively across the membrane. [92, 93, 94, 95] In recent years modified calixarenes have shown antiviral,[96] antibacterial,[97] antifungal,[98] antitubercolosis[99] and anticancer[100] activities.

In the past decade the interaction between cationic calixarenes and DNA has drawn the attention of several researchers. Since 2004 Ungaro *et al* have reported the synthesis of several calixarene derivatives bearing guanidinium functionality either on the upper or on the lower rim. These molecules were capable of binding nucleic acids and in some cases to transfect genes in to the cells.[101, 102, 103]

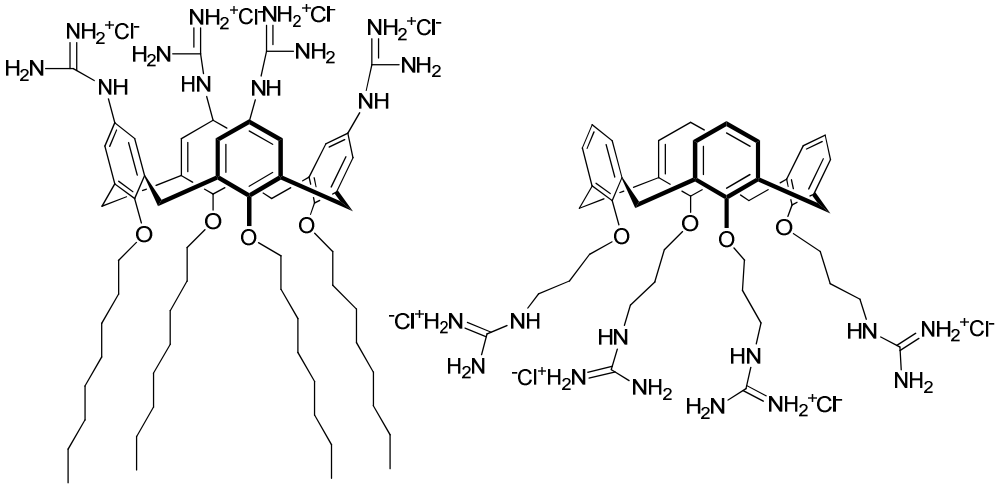


Figure 1.11: Guanidinium-functionalised Calix[4]arenes with Transfection properties

Schrader reported in 2006 a dimeric calix[4]arene structure with DNA recognition properties.[104] This molecule was composed of two calixarenes functionalised on the upper rim with amino groups linked through a hexanedioic bridge. The multicationic dimer exhibited selective binding

to the major groove of double stranded DNA without causing destabilisation or conformational change to the nucleic acid.

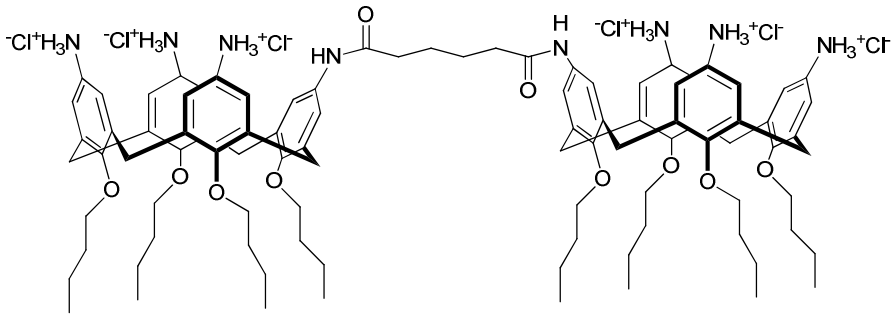
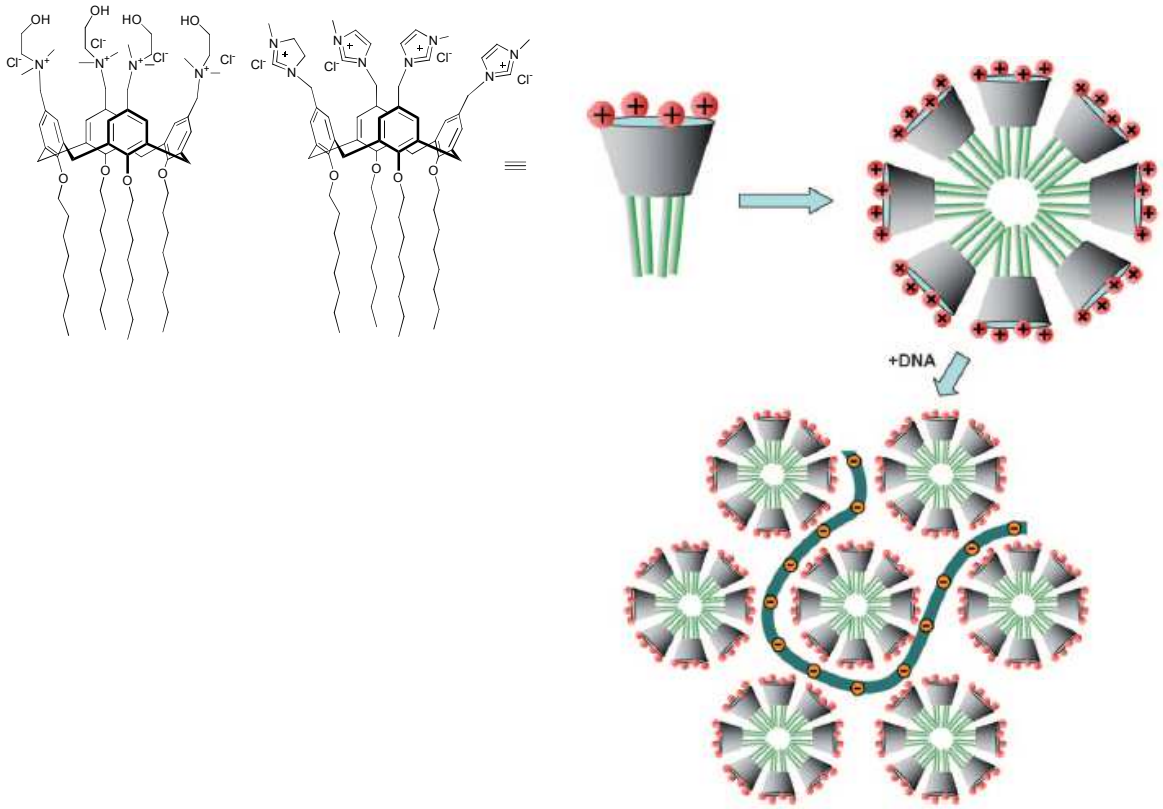


Figure 1.12: Schrader's cationic Bis-calix[4]arene

Recently Rodik *et al*. reported the synthesis of cationic amphiphilic calix[4]arenes in the cone conformation (scheme 1.6). These molecules are capable of self-assembling into micelles which on aggregating can complex DNA and transfect it into cells.



Scheme 1.7: Rodik's self-assembled micelles

1.2.4.Calixarene dendrimers as transfection agents

Previous work in the group has shown that calix[4]arene based dendrimers, functionalised with amino groups and therefore positively charged were able to bind to the negative back bone of nucleic acids.[105] The low toxicity of these molecules makes them suitable candidates for gene delivery in to cells. The central core for these multicalixarenes were synthesised by functionalizing all four available positions on the narrow rim with activated carboxylic acid groups. Three of the four hydroxyl positions on the narrow rim of the generation 1 calixarenes were alkylated with *n*-bromopropane to lock the conformation. The last position available was alkylated with *n*-bromopropyl phthalimide in order to introduce a protected amino group which could be deprotected and coupled to the activated carboxylic acid after the functionalisation of the wide rim with four amino groups either aromatic or aliphatic (figure 1.13).

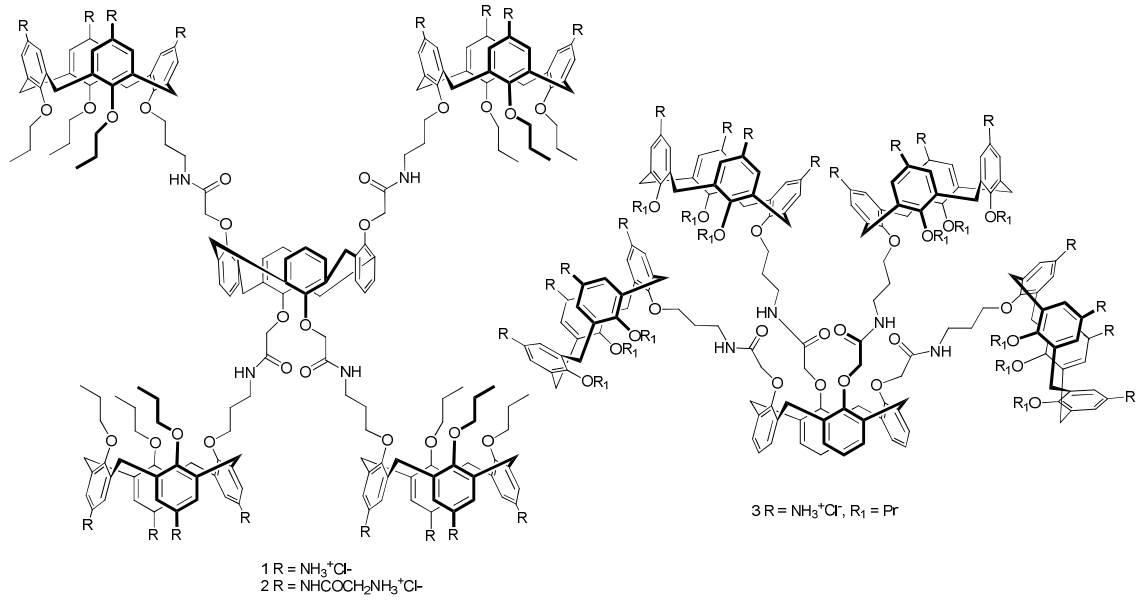


Figure 1.13: Cationic calix[4]arene based dendrimers.

The aim of our work is develop new strategies to improve the links between more calixarene scaffolds in order to obtain macromolecules with different shapes, functionalization and linkages to develop new analytical tools for the evaluation of novel DNA transfection vectors.

1.3. Click chemistry based multicalixarenes

Multicalixarenes can be described as multimeric structures formed by linking covalently two or more calixarenes scaffolds. By the selective functionalisation of the wide rim and/or the narrow rim of the central core (G0) it is possible to connect it to the lower rim of other calixarenes (G1). The formed multicalixarene can therefore have either a narrow rim-narrow rim or a wide rim-narrow rim linkage (figure 1.14).

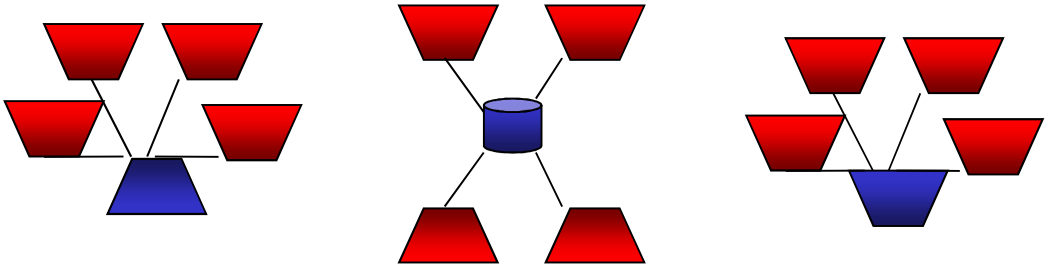
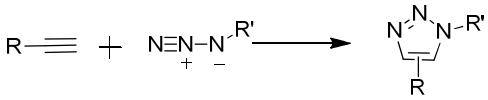


Figure 1.14: Schematic representation of multicalixarene linkages

To achieve the synthesis of the described dendrimeric multicalixarenes structures various linkers have been explored in literature and include: amide bonds [105, 106] aryl linkers,[107] ethers,[108] and alkyl chains.[109] These linkers can be obtained by functionalizing the central core and generation 1 with different groups which can then be coupled together. The most used, up till now, has been the amide bond in which one of the parts of the multicalixarene is functionalised with amino moieties and the other with an activated carboxylic acid.

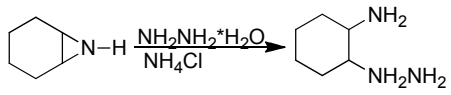
A different approach to the synthesis of multicalixarenes involves click chemistry as a method to create the links between calix[4]arene scaffolds. Click chemistry is a branch of chemistry which features the joining of small units together with heteroatom links.[110] In order to belong to this category the reaction must be modular, wide in scope, give very high yield, generate only inoffensive byproducts, and be stereospecific, moreover the process must employ simple reaction conditions, readily available starting materials and reagents, the use of no solvent or a solvent that is benign or easily removable, and simple product isolation.[111] These requirements have been introduced by K.B. Sharpless and coworkers, promoters of click chemistry.[112] A number of different reactions can be classified in this category:

a) cycloadditions of unsaturated species, especially 1,3-dipolar cycloaddition reactions, but also the Diels-Alder family of transformations;



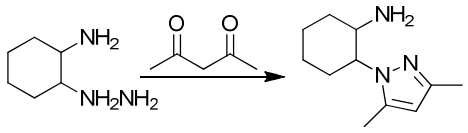
Scheme 1.8: Huisgen Cycloaddition

b) nucleophilic substitution chemistry, particularly ring-opening reactions of strained heterocyclic electrophiles such as epoxides, aziridines, aziridinium ions, and episulfonium ions;



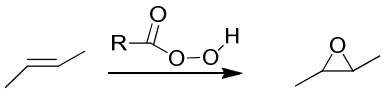
Scheme 1.9: Aziridine ring opening

c) carbonyl chemistry of the non-aldol type, such as formation of ureas, thioureas, aromatic heterocycles, oxime ethers, hydrazones, and amides;



Scheme 1.10: Heterocycle formation from carbonyl compound

d) additions to carbon-carbon multiple bonds, especially oxidative cases such as epoxidation, dihydroxylation, aziridination, and sulfenyl halide addition, but also Michael additions of Nu-H reactants.



Scheme 1.11: Epoxidation of olefins

One particularly popular reaction is the modified azide-alkyne Huisgen cycloaddition. This reaction is a 1,3 cycloaddition between an alkyne and an azide group in presence of Cu(I) with the formation of a triazole ring. The catalyst leads selectively to the formation of the 1-4 substituted product instead of a mixture of the 1-4 and 1-5 substituted.[113]

1.3.1.Previous examples

The first example of the use of triazole rings as linkers between calix[4]arene scaffolds was published in 2000 by Calvo-Flores *et al* (figure 1.15).[114] This was achieved by functionalising two of the hydroxyl groups on a calix[4]arene with azido groups. This molecule was then used as a central core and was reacted with two calix[4]arenes functionalised with a single alkyne moiety, introduced by alkylating the hydroxyl group with propargyl bromide. The reaction

yielded a mixture of two trimers. In one of the trimers the triazole rings were both 1,4 substituted. In the second trimer, one of the triazole rings was 1,4 substituted and the second one was 1,5 substituted. Interestingly a trimer in which both triazole rings were 1,5 substituted was not found in the mixture . These molecules were the first multicalixarenes linked with triazole rings to be described.

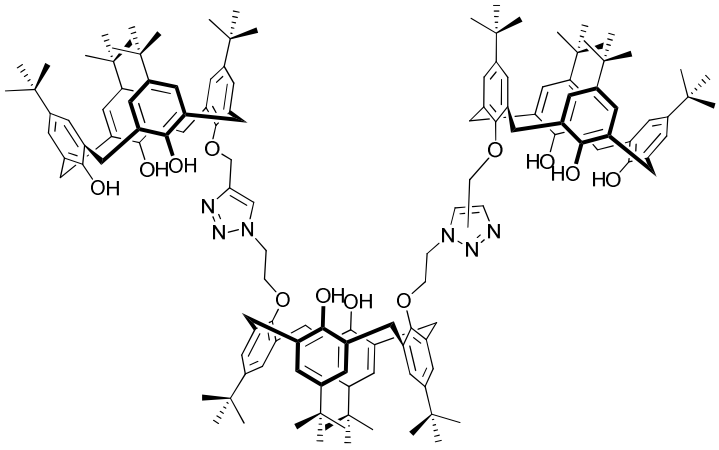
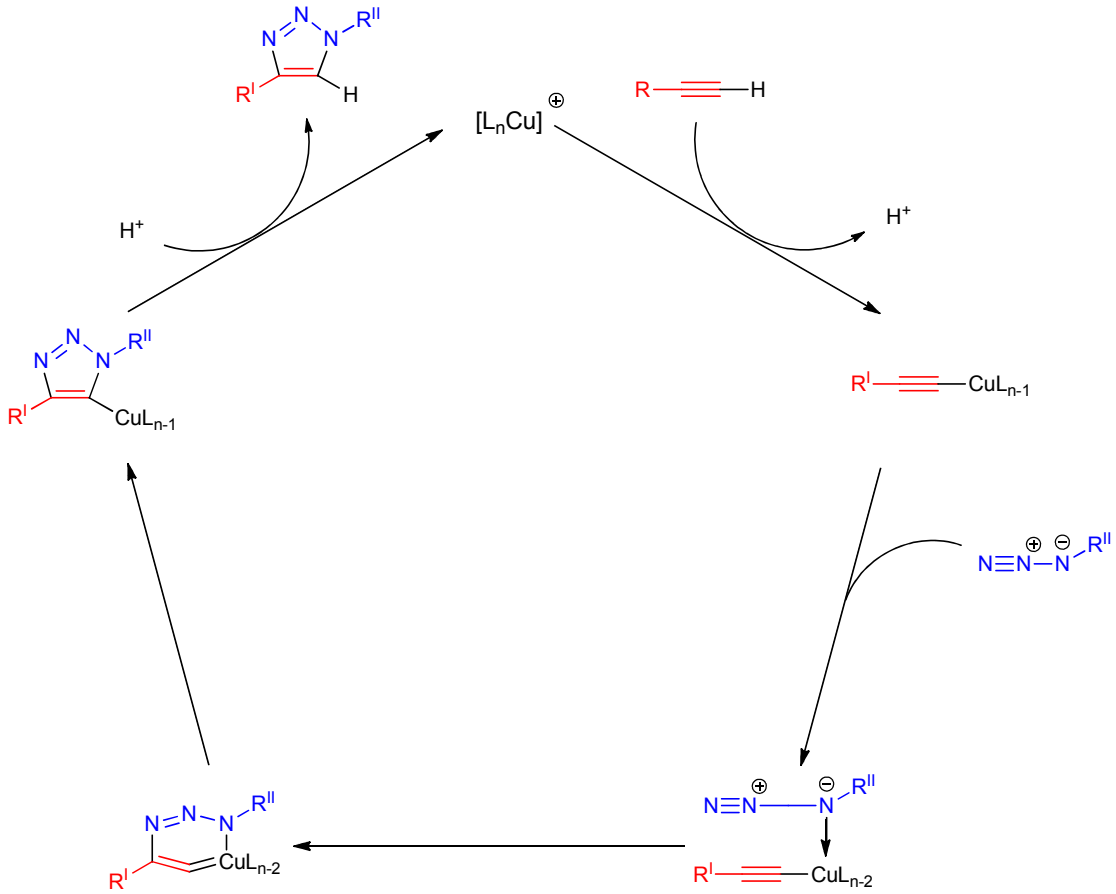


Figure 1.15: First example of a triazole linked multicalixarene

The mixture of products obtained by Calvo-Flores *et al*, can be explained considering that the Huisgen Cycloaddition can occur simply by thermal activation and requires a long time (up to few days) to yield the 1,4 and the 1,5 substituted products.[115] This is a disadvantage in the reaction if only one of the two products is required. The use of copper (I) catalysts was introduced for the first time in 2002 in two independent studies carried out by Sharpless' and Meldal's groups. This metal drives the reaction between azides and terminal alkynes toward the 1,4 substituted stereoisomer. Furthermore the reaction times are drastically reduced and thermal activation is not crucial.[113, 111]

The mechanism of the cycloaddition is still a matter of debate. Everyone agrees that the catalytic cycle begins with the insertion of the Cu(I) on the terminal alkyne through the formation of a π complex with the triple bond. This lowers the pKa for the terminal C-H in the alkyne resulting in the loss of the proton even in the lack of a base and forming the copper(I) acetylide.[111, 113, 116-117] The catalytic cycle continues with the reaction of the copper(I) acetylide with the terminal azide. The direct addition proposed in 2002 by Meldal *et al.* does not explain the increased rate of the reaction. The calculated activation barrier for this proposed mechanism is even higher than the one found without catalyst.[118] A series of intermediate steps have been proposed.[111, 116-119] The essential steps are summarised in scheme 1.12. The azide binds the copper atom through the lone pair of the nitrogen proximal to the carbon. Cyclization occurs with the formation of a bond between the distal nitrogen of the azide and the alkyne carbon. The six membered cycle which

includes also the metal rearranges, contracting to a five atom ring. Final protonation yields the triazole and the catalyst which can undergo another catalytic cycle.



Scheme 1.12: Copper catalysed Huisgen Cycloaddition catalytic cycle.

With a similar approach to the one used by Calvo-Flores, previous work in the group (unpublished results) has developed a route towards pentameric multicalixarenes (figure 1.16). These were obtained using as a central core a tetra azido functionalised calix[4]arene to which were then "clicked" in the presence of copper (I) catalyst, four generation 1 calix[4]arenes functionalised with an alkyne group each.

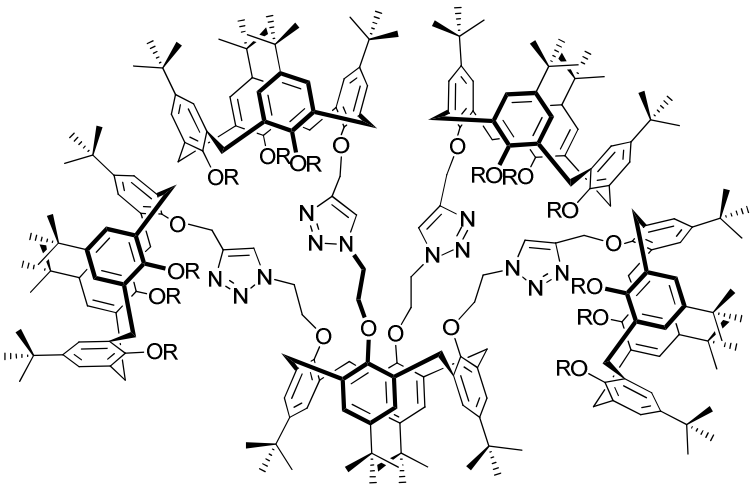


Figure 1.16: Triazole linked calix[4]arene based dendrimer

This first pentamer proved that the triazole ring was a valid linker to synthesise dendritic multicalixarenes. However, these molecules did not feature any functionalisation on the wide rim. In this study we have developed a strategy towards the synthesis of multicalixarene families carrying on the outer rim multiple positive charges with potential nucleic acid binding properties.

1.3.2.Synthesis of central cores

The aim of this work is to synthesise different families of amino functionalised multicalixarene able to bind nucleic acids and to act as transfection agents on a cellular level. This purpose has been achieved by functionalising the generation 1 of the multicalixarenes with either aromatic or aliphatic amines. Previous work in the Matthews' group has shown that the conformation of the central core, which affects the geometry of the dendrimer, can influence the tranfection properties.[105] This evidence suggested that it is worthwhile to synthesise central cores in both the cone and 1,3-alternate conformations to compare their behaviour in the biological systems.

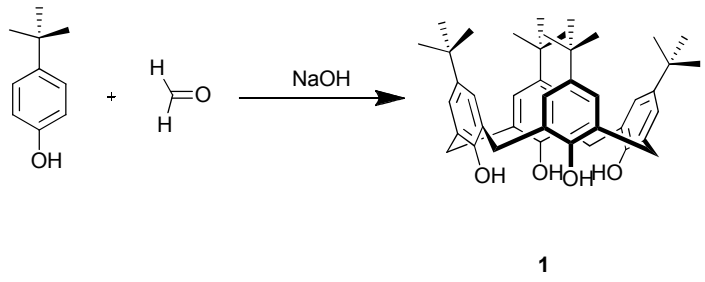
In order to investigate whether the rigidity of the system had an influence in the transfection activities the central cores were linked to the generation 1 through their narrow rims with 2, 4, and 6 carbon atom chain linkers. To gain more rigidity, a wide rim-narrow rim junction was also developed, in which the generation 1 is attached directly to the aromatic ring functionalised with azido groups.

1.3.2.1. Synthesis of central cores in the cone conformation

The central core can be alkylated at all four hydroxyls at the lower rim with functional groups which can be converted in to azides. This leads to the formation of a central core which has four reactive groups that can be coupled with an equal number of alkyne functionalised generation 1 calixarenes to prepare the desired dendrimers.

1.3.2.1.1. Tosyl method (C2)

p-Tert-butyl calix[4]arene (**1**) was the precursor of all the multicalixarene scaffolds described in this work. This can be synthesised *via* condensation of *p-tert*-butyl phenol and formaldehyde in the presence of a catalytic amount of sodium hydroxide.[120, 78] The reaction can be divided into two stages. The first stage was the formation of the linear polymer, a yellow mass which was obtained by heating and stirring the three reagents for about two hours. The second stage was the cyclization. After the dissolution of the solid in diphenyl ether, the water formed in the reaction was removed by distillation and the solution heated at reflux for a further two hours. The cyclic tetramer was precipitated from the resulting black solution by the addition of ethyl acetate (scheme 1.13).



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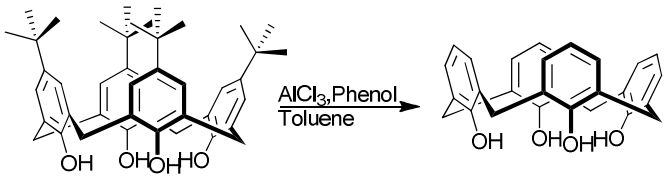
Scheme 1.13: Synthesis of *p-tert*-butyl calix[4]arene

The first two central cores were synthesised using as the starting compounds *p-tert*-butyl calix[4]arene and calix[4]arene. This latter compound was obtained by removing the *tert*-butyl group on the *para* position of *p-tert*-butyl calix[4]arene with aluminium trichloride in toluene in the presence of phenol (scheme 1.14).[121 82] This reaction, a Friedel-Crafts reverse alkylation is a common strategy frequently used to replace the *tert*-butyl group with a proton. The desired product was precipitated from methanol as an off white powder in excellent yield (80%)

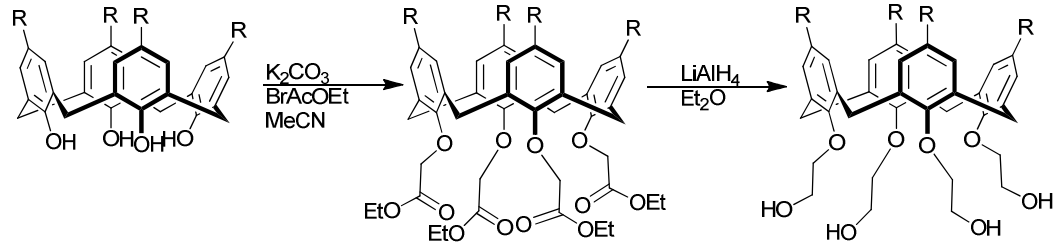


1 2

80%

Scheme 1.14: Friedel-Crafts reverse alkylation

The four hydroxyl groups of **1** and **2** were then alkylated using ethyl bromoacetate in the presence of an excess of potassium carbonate, a base known to lead the formation of the cone conformation (scheme1.15).[81] In this way the central core was functionalised with four carboxylic esters at the narrow rim and locked in the cone conformation as these moieties are too large to allow rotation through the annulus of the macrocycle and consequent change of conformation.[86] Recrystallisation at -20 ºC for several days from ethanol yielded the desired compounds **3** (79%) and **4** (66%) as colourless crystals. The ester groups were reduced in the following step using lithium aluminium hydride in diethyl ether to yield the respective tetra alcohol compounds as white powders **5** (84%) and **6** (53%).[122]



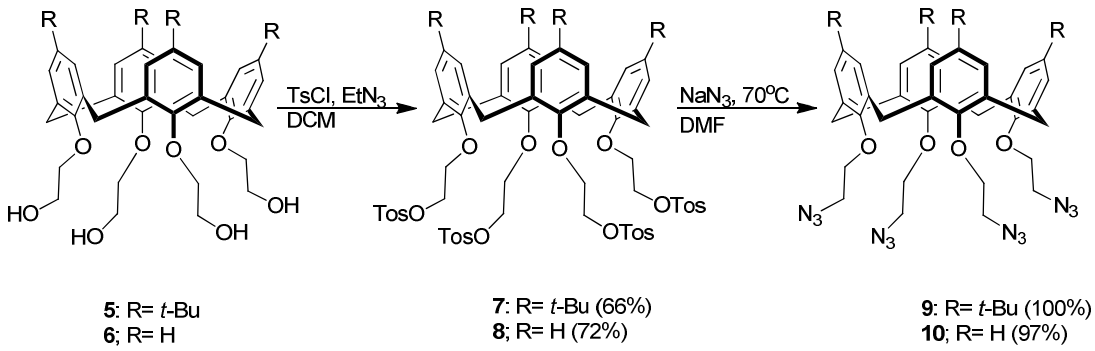
1: R= *t*-Bu
2; R= H

3: R= *t*-Bu (79%)
4; R= H (66%)

5: R= *t*-Bu (84%)
6; R= H (53%)

Scheme 1.15: Alkylation and reduction

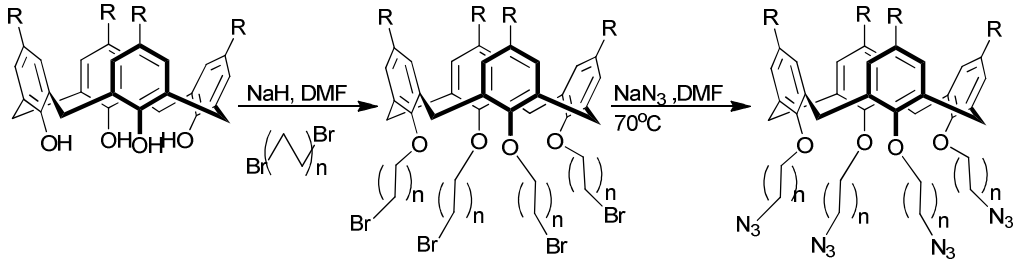
Hydroxyl groups are not good leaving groups in nucleophilic substitutions.[123] In order to be replaced they need therefore to be activated (scheme 1.16). The tetrahydroxy compounds **5** and **6** were activated by stirring with pyridine in the presence of tosyl chloride.[122] The active tosylated compounds were then stirred and heated at 70°C in dimethylformamide in the presence of sodium azide to yield the tetra azido functionalised central cores **9** and **10** (100% and 97%) (scheme 1.10).[124]



Scheme 1.16: Synthesis of tetra-azido functionalised calixarene *via* tosylate pathway

1.3.2.1.2. Direct alkylation (C4, C6)

The long synthetic pathway to obtain the tetra azido compounds led the way to an exploration of alternative synthetic strategies to functionalize the central cores with four azide groups. The new strategy involved the use of 1,4-dibromobutane[125, 126] and 1,6-dibromohexane as alkylating agents using sodium hydride as a base to deprotonate the hydroxyl groups of the starting materials **1** and **2** (scheme 1.17).[127] This base is known to lead the reaction to the formation of the cone conformation because of the sodium ion template effect.[81] The use of a strong base such as sodium hydride was required because of the poorer reactivity of the bromo-alkane compared to ethyl bromoacetate. The products were isolated by precipitation from dichloromethane/methanol as white powders.



Scheme 1.17: Alkylation with dibromo alkanes and substitution of the halogen with azido groups

The final step for the preparation of this series of central cores was the substitution of the bromine with sodium azide, a reaction which was carried out using the same conditions used for the formation of **9** and **10** to yield the tetra azido compounds with respectively four and six carbon atom chains (scheme 1.17).[124]

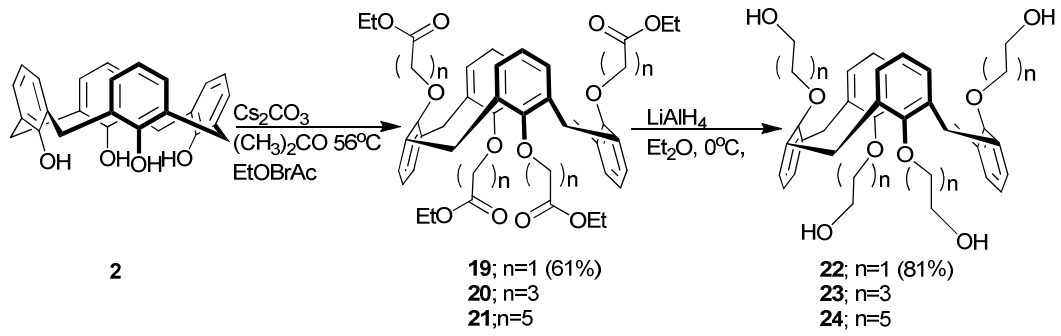
The same approach was attempted for the synthesis of the two carbon atom chain derivatives **9** and **10**. However, this approach failed which may be due to the very high reactivity of the 1,2-dibromo ethane compared to the analogues with longer chains .

1.3.3. Synthesis of central cores in the 1,3 alternate conformation

As discussed earlier in the chapter it would be interesting to determine whether different conformations of multicalixarenes act differently in biological systems and have different transfection efficiencies. Another family of central cores based on the 1,3 alternate conformation was required for this study. As previously explained it is possible to lock the conformation of a calix[4]arene in the 1,3-alternate conformation by performing the alkylation of the four hydroxyls groups in the presence of Cs_2CO_3.[128] In order to retain the conformation, the alkylating agent selected has to be bulky enough to prevent the rotation through the anulus.[65, 129] In this particular case the alkylating groups chosen had to be readily convertible to azides. To have the azido groups on different sides of the calixarene results in a less hindered position for the functional groups and a consequently larger space for the insertion of the generation 1 calixarenes which could mean better yields and improved DNA binding properties. Two different pathways have been explored to achieve the synthesis of these molecules.

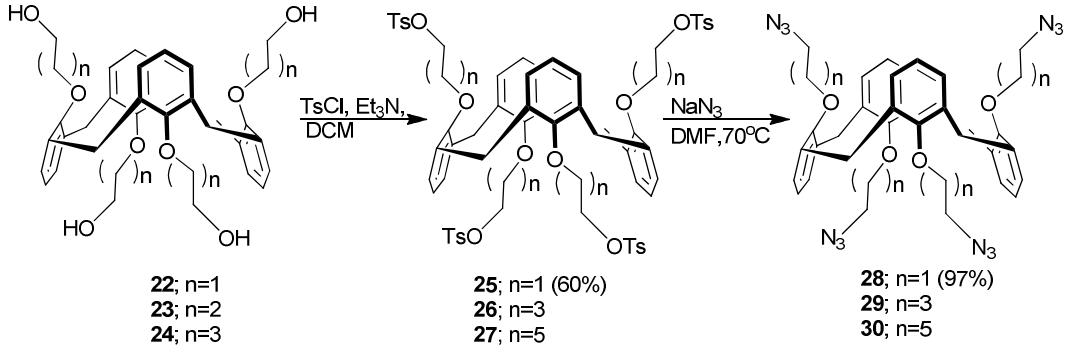
1.3.3.1. Tosylate pathway (C2, C4, C6)

The first approach towards the synthesis of central cores in the 1,3-alternate conformation was to follow the tosylate pathway which had given successful results in the synthesis of the central core in the cone conformation. Calix[4]arene **2** was heated at reflux in acetone, in the presence of Cs_2CO_3 as a base, and using ethyl-bromoacetate, ethylbromobutyrate or ethyl bromohexanoate as alkylating agent to yield after purification using column chromatography respectively **19**, **20** and **21** (scheme 1.18).



Scheme 1.18: Synthesis of tetra alcohol functionalised calixarene in 1,3-alternate conformation

The carboxylic acid derivatives obtained were processed using the same sequence of reactions described for the synthesis of **10**. In this fashion the three central cores **28**, **29** and **30** with a spacer respectively of two, four, and six carbon atoms were obtained after reduction, tosylation and conversion to the azide (scheme 1.13).

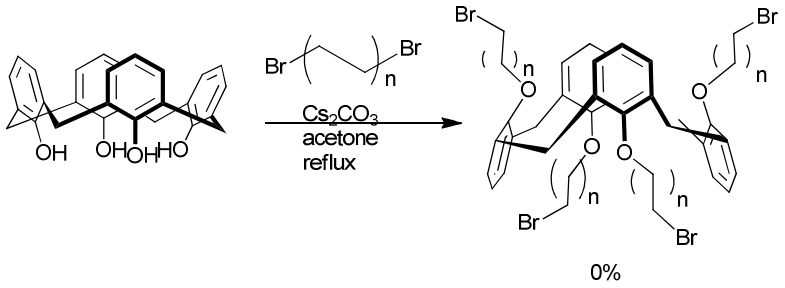


Scheme 1.19: Alcohol activation and substitution

The limiting step of this synthetic pathway was the tosylation reaction. The reason for the large difference in yields between the two carbon atoms derivative and the longer chains derivative is probably due to the very low solubility of their alcohol derivatives.

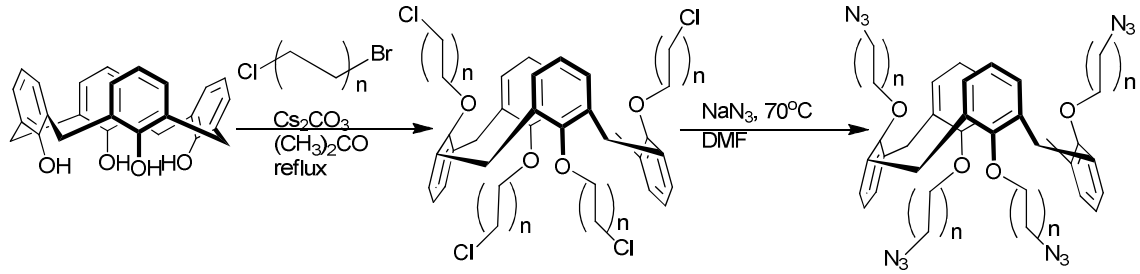
1.3.3.2. Direct alkylation

Although the central cores in the 1,3 alternate conformation were successfully synthesized, the low yields obtained in the tosylation step and the number of steps in the synthesis led the way towards the exploration of other strategies to alkylate the central core. In an alternative strategy di-bromoethane, di-bromobutane and di-bromohexane were investigated as the alkylating agent in the presence of Cs_2CO_3 (scheme 1.20).



Scheme 1.20: Attempted synthesis of central cores in the 1,3-alternate conformation *via* direct alkylation with di-bromo alkane derivatives.

An excess of alkylating agent was used to avoid the formation of dimers. Increasing the amount of alkylating agent was thought to decrease the likelihood of two calixarenes reacting with the same di-halo alkane molecule. Unfortunately none of these attempts yielded the desired compound. This may be due to the identical reactivity of the bromine atoms on the two termini of the alkyl chains. Following from this the same reactions have been repeated using as alkylating agents alkanes functionalised with bromine at one terminus and the less reactive chlorine at the other. The reaction was successful in the case of the four and six carbon atom chain demonstrating that the difference in reactivity leads the reaction only towards the substitution of the bromine leaving the chlorine on the aliphatic chains available for further substitution with the azide in the next step (scheme 1.21).



2

31 n=1 (0%)
32; n=2 (28%)
33; n=3 (50%)

34; n=2 (88%)
35; n=3 (91%)

Scheme 1.21: Synthesis of central cores in the 1,3-alternate conformation *via* direct alkylation with bromo-chloro-alkane derivatives.

On the other hand, the reaction of **2** with 1-bromo-2-chloroethane did not yield the desired compound. The result was a complex mixture of products which were not possible to assign with NMR spectroscopy. Probably the short distance between the halogens makes both the carbon atoms very electrophilic. The partial positive charge of the carbons, due to the electronegativity of the halogens directly attached to them, is increased because of the inductive effect of the electronegative halogen on the next carbon. This may result in an increased

reactivity which would drive the reaction toward the formation of complex structures therefore this method was not further investigated.

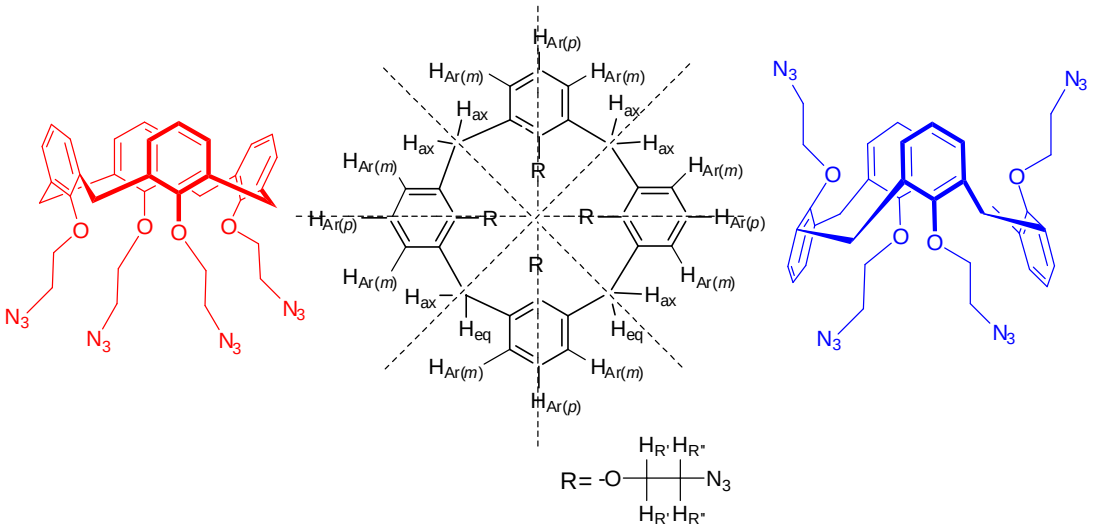


Figure 1.17: Symmetry of the central cores **10** and **28**

The central cores **10** and **28** are conformers (figure 1.17). They have two plans of symmetry. The differences in conformation can be detected by ^{1}H NMR analysis (figure 1.18). The symmetry of the central core in the cone conformation **10** (red) is shown by the two doublets at 4.46 ppm and at 3.72 ppm corresponding to the methylene bridge protons which are axial (H_{ax}) and equatorial (H_{eq}) respectively. Although H_{ax} and H_{eq} are chemically equivalent they are located in different magnetic environments. The first points down, and is therefore closer than the other to the oxygen which deshields it and falls at higher ppm than the other. In contrast, in the 1,3 alternate conformation (blue) the methylene bridges give only one signal: the singlet, integrating for 8 protons, at 3.84 ppm. This signal is typical of calixarenes in the 1,3-alternate conformation. In this case axial and equatorial protons are chemically and magnetically equivalent, each being at the same distance from the oxygen and the aromatic rings.

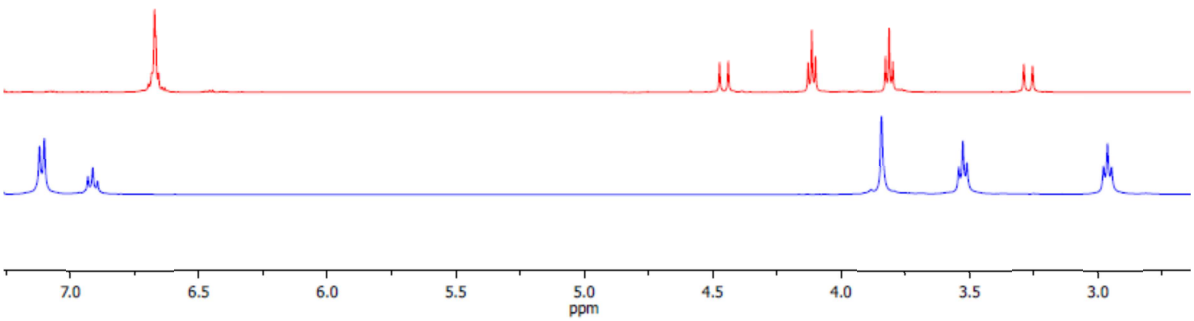
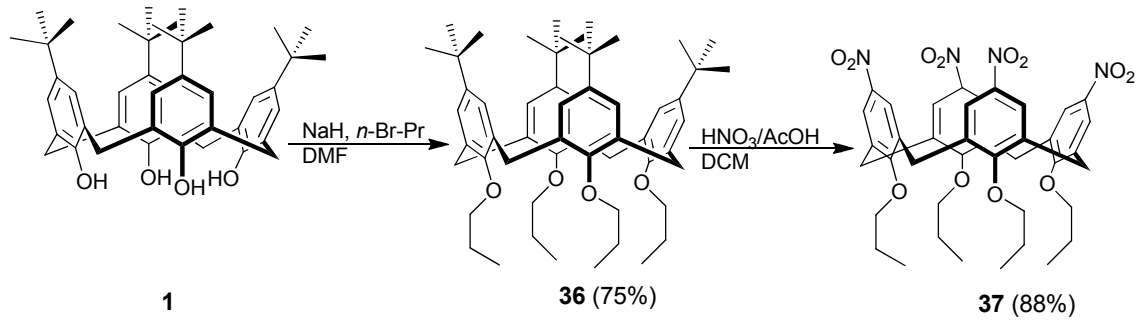


Figure 1.18: Comparison between the ^{1}H NMR spectra of the cone and the 1,3-alternate conformation central cores **10** and **28**

In the spectrum for the cone conformation (red), between the signals of the two methylene bridges, two triplets with a coupling constant (*J*) value of 6.0 Hz can be observed at 4.12 and 3.82 ppm. The most deshielded belongs to the protons on the carbon proximal to the oxygen in the ethoxy chain ($H_{R'}$) and the other, more shielded, belongs to the protons on the second carbon of the ethoxy chain ($H_{R''}$), the one proximal to the azido group. In the 1,3-alternate conformation (blue) it is possible to observe the same set of signals at 3.52 ppm and 2.96 ppm. The aromatic protons in the cone conformation give a multiplet which integrates for twelve protons (red). This is due to the overlap of the signals for the aromatic protons in *meta* ($H_{Ar(m)}$) and *para* position ($H_{Ar(p)}$). In the 1,3-alternate conformation the signals for the aromatic protons do not overlap and it is possible to detect a doublet for $H_{Ar(m)}$ and a triplet for $H_{Ar(p)}$, both with a coupling constant of 7.5 Hz.

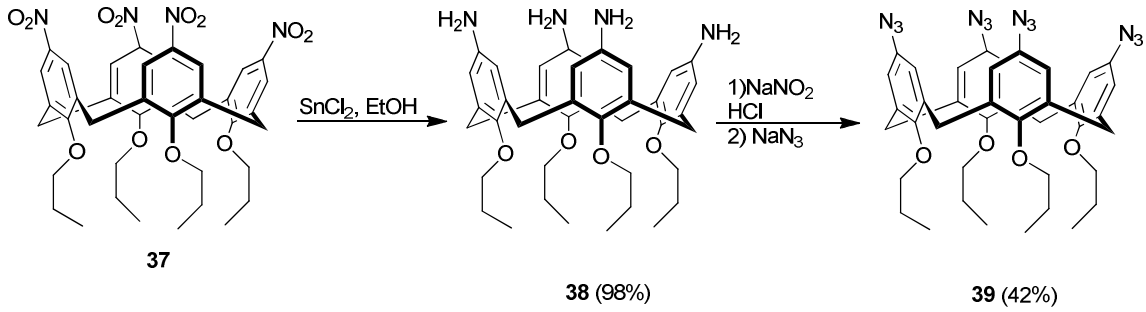
1.3.4.Synthesis of upper rim azido functionalised central core

As previously mentioned the generation 1 calixarenes can be connected to the central core with a narrow rim-wide rim linkage. Thus it was necessary to design a central core functionalised with four azido group on the wide rim. The first step of the synthesis was the locking of the calixarene conformation by alkylating the hydroxyl groups with *n*-bromopropane in the presence of sodium hydride (scheme 1.22). Using this base the calixarene was fixed in the cone conformation. The first reaction performed to modify the upper rim was an ipso-nitration. With this reaction it is possible to replace the *tert*-butyl groups in the *para* position with four nitro groups *via* electrophilic substitution. This reaction has been widely explored in literature and it is an effective route to insert a nitrogen atom at the *para* position of the aromatic ring. Different procedures use as nitrating agents, a mixture of nitric acid (HNO_3) either fuming or 65% with another acid: trifluoroacetic acid (TFA), sulphuric acid or glacial acetic acid.[130- 131] The starting material **36** was dissolved in dichloromethane and treated with a nitrating mixture composed of glacial acetic acid and fuming nitric acid mixed together in equal amounts.[130] The product was precipitated from dichloromethane/methanol as a pale yellow powder.



Scheme 1.22: Lock of the calixarene conformation and *ipso*-nitration

The nitro groups can be easily reduced to amino groups. Different methods have been described in literature. It is possible to perform the hydrogenation using hydrogen gas and catalysts such as palladium on activated carbon (Pd/C),[132 , 133] or platinum oxide (PtO_2).[134] Another possible widely used approached is the use of hydrazine as a source of hydrogen and Raney nickel as catalyst.[135, 131] Although these methods are all valid tools toward the synthesis of tetra amino calix[4]arenes, in our synthesis the nitro groups were reduced by heating at reflux temperature in ethanol in the presence of tin chloride as reducing agent (scheme 1.23).[136]



Scheme 1.23: Nitro-reduction and introduction of azido groups *via* diazonium salt.

The four amino groups on the wide rim were then transformed into diazionium salts. Substitution of these with azido groups yielded the target compound. This reaction has been described before on calix[6]arenes but not on calix[4]arenes.[137- 138,] To form the diazonium salt it was necessary to dissolve the free amine in 10% aqueous hydrochloric acid in order to have the protonated form of the amine. The solution was then allowed to react with sodium nitrite. Subsequently a solution of sodium azide in water was added drop wise. The crude compound was extracted with dichloromethane and purified by column chromatography to yield the desired compound.

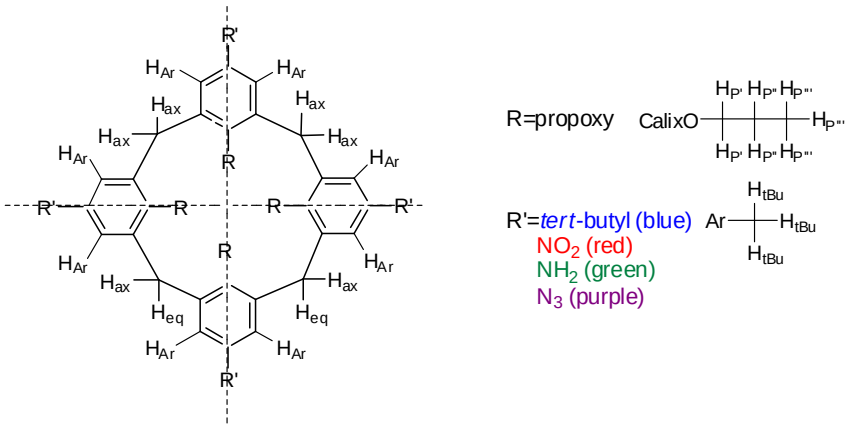


Figure 1.19: Symmetry of central core **39** and of its precursors.

Figure 1.20 shows the ^{1}H NMR analysis for each step in the synthetic pathway towards the synthesis of the central core carrying four azido groups on the upper rim. The conformation of the molecule is confirmed by the two doublets given by the methylene bridges H_{ax} and H_{eq} present in all the spectra. The propyl chains give three signals at around 4 ppm ($H_{P'}$), 2 ppm ($H_{P''}$) and 1 ppm ($H_{P'''}$), a triplet, a sextet and another triplet respectively. The singlet at 1.08 ppm in the spectrum of **36** (blue) integrates for 36 protons and belongs to the *tert*-butyl group. The replacement of this group by the nitro groups is shown by the disappearance of the signal in the spectra of **37** (red) and the shift of the aromatic protons signal. The electron withdrawing properties of the nitro group deshields the aromatic protons, shifting the singlet from 6.77 ppm for **36** to 7.57 ppm. Reduction of the nitro group to an amine, an electron donating group, shifts the signal upfield to 6.06 ppm (green). In the last spectrum (purple) the substitution of the amino groups with azides is shown by a further shift of the aromatic protons towards 6.30 ppm.

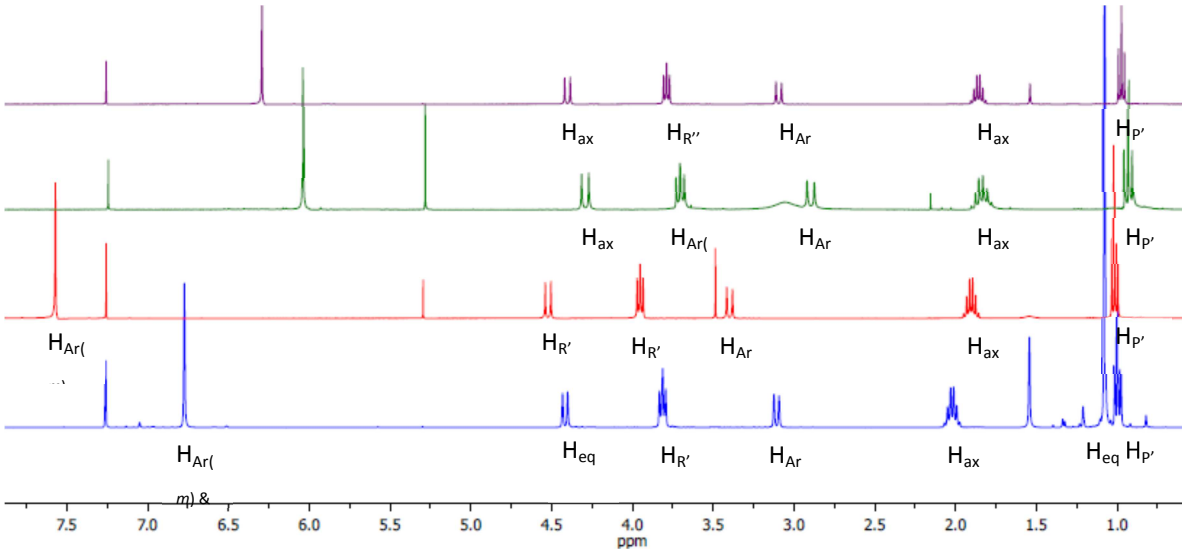
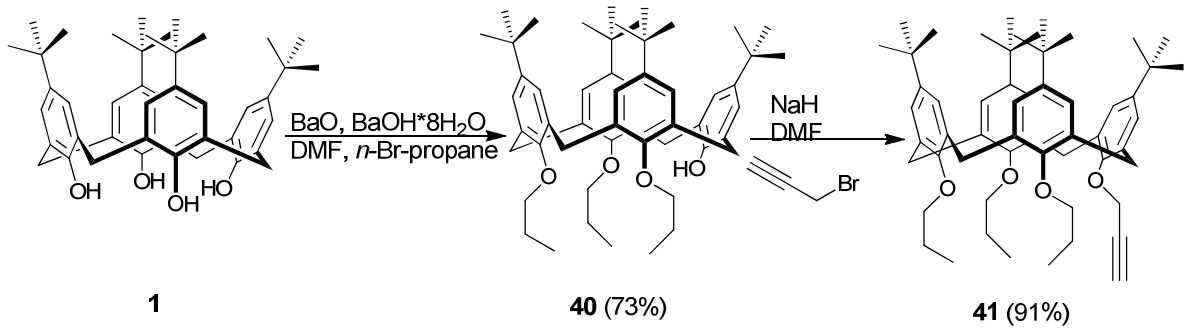


Figure 1.20: Comparison between the ^{1}H NMR spectra of each synthetic step of the synthesis of central core **39**

1.3.5.Synthesis of generation 1 calixarenes

1.3.5.1. Synthesis of aromatic amino functionalised generation 1 calixarenes

The generation 1 calixarenes were designed to have on the lower rim an alkyne moiety to be "clicked'' to one of the azides present on the central core and on the upper rim four protected amino groups. In the first step of the synthesis three of the four hydroxyl groups of p-tert-Butyl calixarene were alkylated with *n*-bromopropane. A mixture of bases composed of barium hydroxide octahydrate and barium oxide was used.[85] This mixture of bases has been widely used in the functionalisation of calix[4]arenes because of its peculiarity of allowing the alkylation only on three of the four position available. The barium ion coordinates very strongly with the fourth hydroxyl position on the calix restricting its reaction with the alkylating agent. The cone conformation is favoured in this reaction because of the interaction of the four oxygens with a single barium ion.[85] Crystallization from dichloromethane and methanol yielded the pure product as off white crystals (scheme 1.24).



1 **40** (73%) **41** (91%)

Scheme 1.24: Selective tri-O-alkylation and functionalisation with propargyl group.

By alkylating only three of the four positions available, the molecule gains more complex symmetry which results in three distinct regions indicated with the colours in figure 1.21. This variation is shown in the ^{1}H NMR spectra (figure 1.22) and explains the increased number of signals that can be observed compared with the spectra of the central cores previously described in figure 1.20 and 1.22.

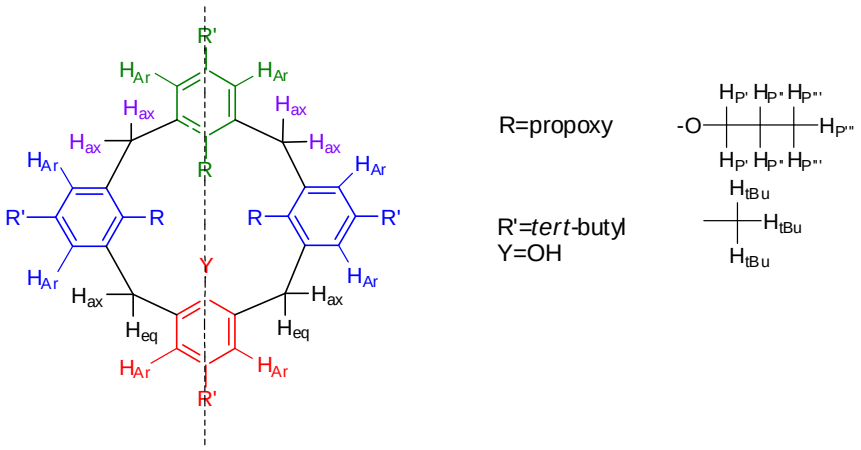


Figure 1.21: Symmetry of calixarene **40**

As it is possible to observe from the spectrum in figure 1.22, the methylene protons give four different doublets, two around 2.2 ppm for the equatorial protons and two around 4.3 ppm for the axial protons. It should be noted that in the case of the axial protons we observe an apparent triplet due to overlap. It is possible to observe two signals for each type of propyl proton, one of which integrates to the double of the other. The *tert*-butyl protons give three peaks, one of which integrates to the double of the others. The aromatic region shows three signals from 6.51 to 7.13 ppm. Also in this case there is overlap and the peak at 6.51 ppm is broad and integrates for four protons against the integral of two found for the other two aromatic signals. The multiplicity of the signals is due to the four different magnetic regions present in the aromatics.

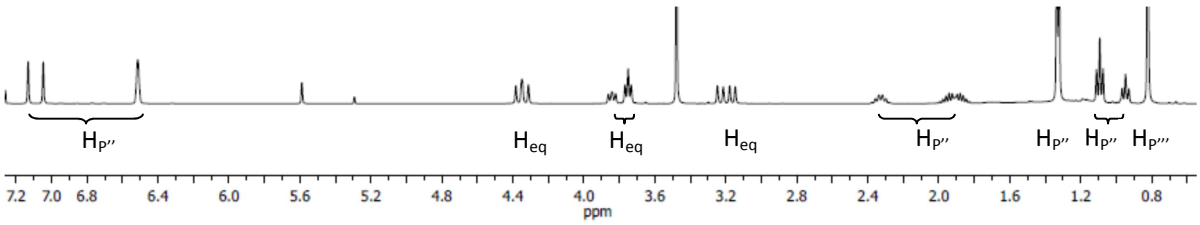
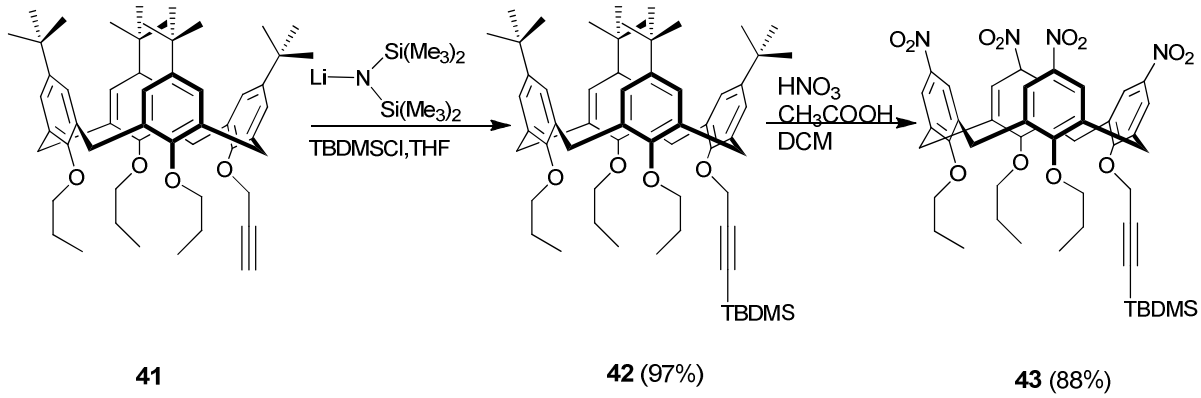


Figure 1.22: ^{1}H NMR spectrum of compound **40**

The alkyne was introduced on the last free phenolic position available. This was achieved by deprotonating the hydroxyl group with sodium hydride and alkylating it with propargyl bromide. The choice of the base allowed the retention of the cone conformation. After filtration over silica gel the product was collected as an off white powder.[139]

The synthesis was continued with the *ipso* nitration reaction described previously. Although this reaction gave the desired product the yield was not satisfactory and the product obtained after the precipitation was not pure enough to carry on with the synthesis. This is probably due to a side reaction between the electrophilic nitronium ion and the electron rich alkyne.[140] This

inconvenience was overcome by introducing a protecting group on the propargyl group. The selected protecting group to be used for this purpose was *tert*-butyl-dimethyl-silyl-chloride (scheme 1.25).[141]



41 **42** (97%) **43** (88%)

Scheme 1.25: Alkyne protection and *ipso*-nitration

This protecting group was selected over the other silanes because of its higher stability in acid and basic conditions.[142] Alkyne protons are basic and can be removed using bases such as lithium bis(trimethylsilyl)amide. The reaction was performed at -78^{o}C in THF. The starting alkyne was allowed to react with the base for about 20 minutes to allow the formation of the acetylide before the silylchloride derivative was added. The product was obtained in excellent yield after precipitation from dichloromethane and methanol.

The insertion of the propargyl group is shown in the ^{1}H NMR in figure 1.23. The characteristic signals of the terminal alkyne are the triplet at 2.37 ppm, which integrates for one proton (H_a), and the doublet at 4.97 ppm, which integrates for two protons (H_b). These two peaks have a *J* value of 2.5 Hz due to the long range coupling. In the spectrum of the protected compound **42** it is possible to notice the disappearance of the triplet, evidence of the removal of the proton H_a. A further proof of this is the loss of the splitting of the signal given by H_b. The singlet observed confirms that H_b lacks of any neighbouring proton to couple with. The two singlets shown at 0.00 and 0.8 ppm are typical of the newly introduced silyl protecting group and are given by H_c and H_d respectively.

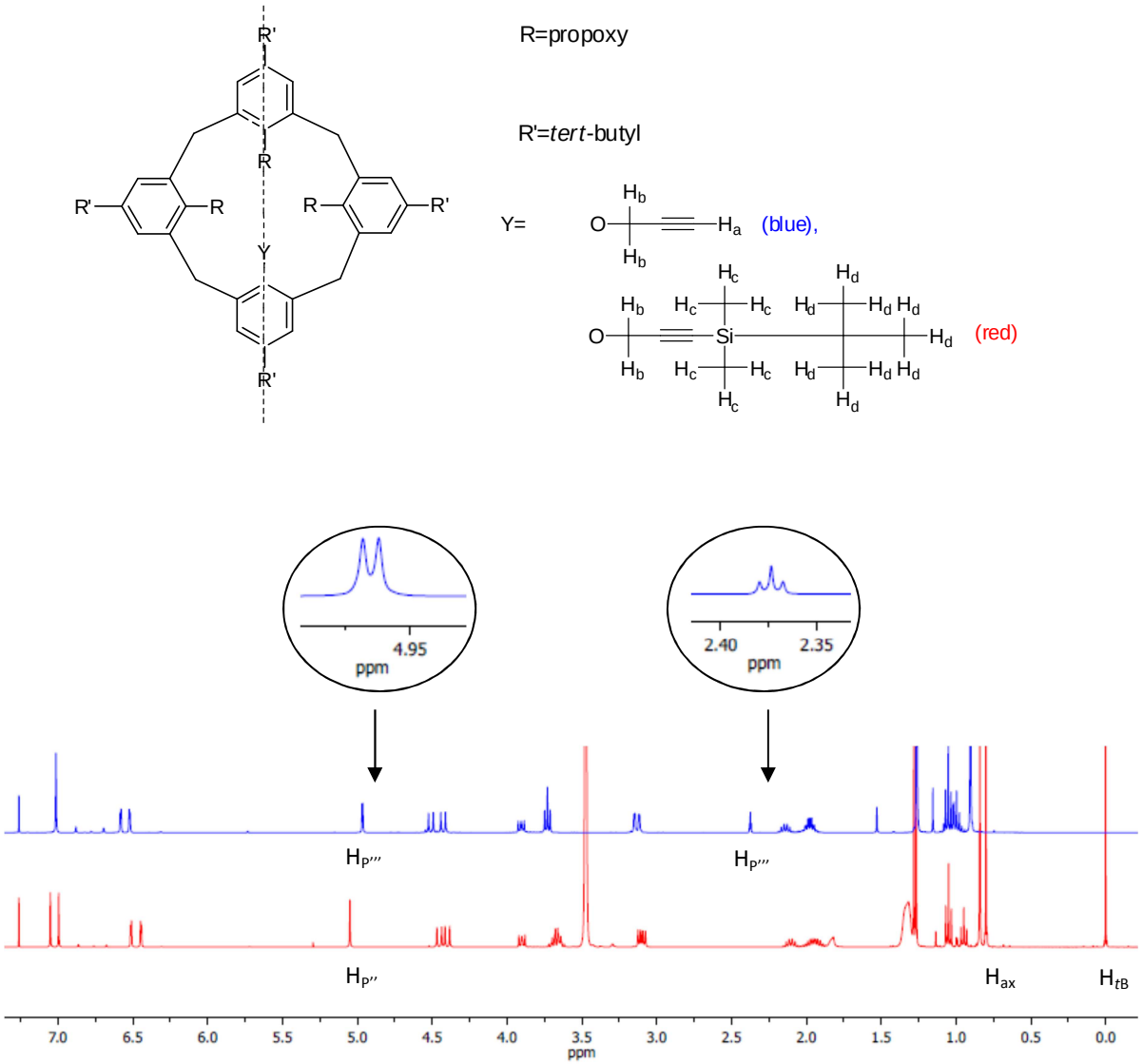
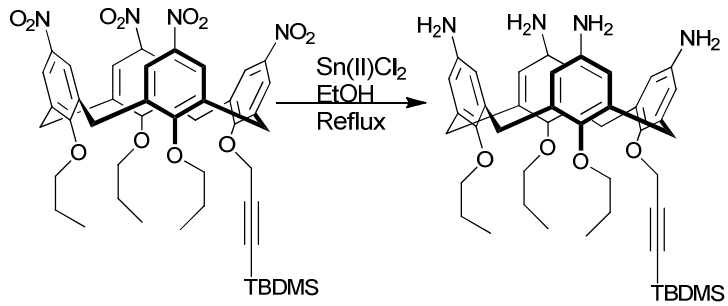


Figure 1.23: Comparison between ^{1}H NMR of **42** (red) and **41** (blue)

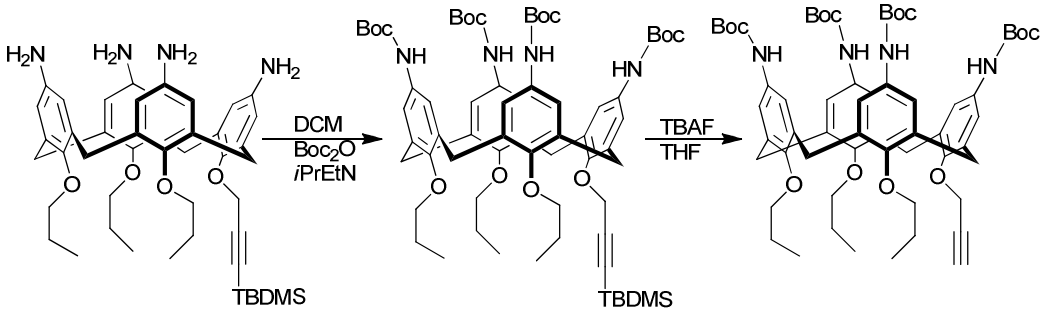
The protected compound was a much better material for the *ipso* nitration (scheme 1.19) than the unprotected analogue. The pure nitro derivative was achieved after precipitation from dichloromethane and methanol as a pale yellow powder. The tetra amino compound was obtained from the reduction of the nitro compound with tin chloride using the same procedure described for the synthesis of **38** (scheme 1.26).



43 **44** (95%)

Scheme 1.26: Reduction of the lower-rim protected nitro calixarene.

Di-*tert*-butyl dicarbonate (BOC) was selected as protecting group for the amino moieties on the wide rim because it is easily removable by treatment with HCl gas and the free product does not need to be purified because of the volatility of the side products formed in the reaction.[143] Although it can be removed by tetrabutylamonium fluoride (TBAF), the reagent used to deprotect the alkyne from the silane, its removal requires stronger conditions than the ones used to free the propargyl group.[144] The reaction was carried out at 0°C in the presence of the protecting group and of diisopropylamine (DIPEA) as a base (scheme 1.27).[145]



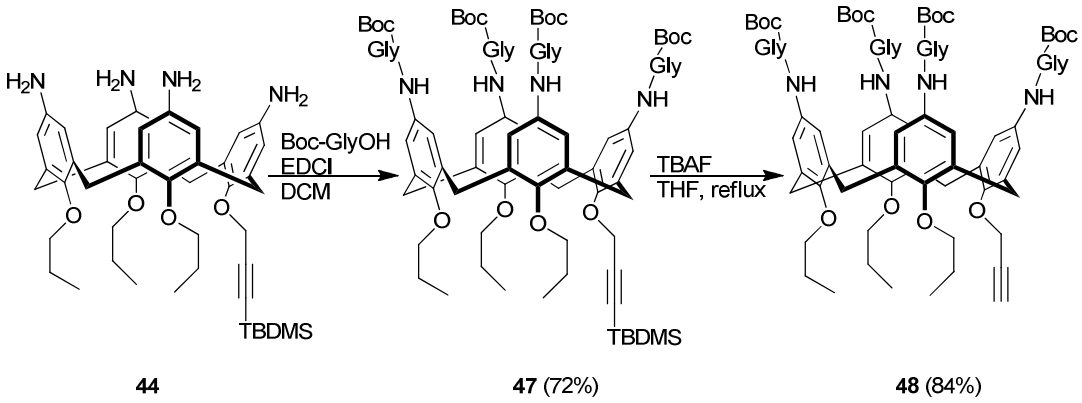
44 **45** (73%) **46** (62%)

Scheme 1.27: Boc protection of the aromatic amino groups and alkyne deprotection.

The compound was purified by column chromatography. The final step of the synthesis was the deprotection of the alkyne. The compound was stirred for 16 hours in THF in the presence of tetrabutylamonium fluoride, a reagent commonly used to remove silyl groups (scheme 1.27).[146] The reaction was performed at room temperature because at higher temperature the Boc-protecting group can also be removed from aromatic amines which are less stable than aliphatic amines.[144] Precipitation from dichloromethane and hexane yielded the desired compound as an off white powder.

1.3.5.2. Synthesis of aliphatic amino functionalised generation 1 calixarenes

The synthesis ofa suitable tetra amino substrate for the introduction of aliphatic amines on the wide rim of the generation 1 calixarene followed the same route described in the previous sections. The four amino groups were coupled with the BOC protected amino acid glycine (scheme 1.28). Different coupling reagents can be used to achieve this reaction. Previous work in the group has shown that is possible to insert this group using the succinimide activated ester of Boc-glycine with *N*,*N*-Diisopropylethylamine (DIPEA) and a catalytic amount of 4-dimethylaminopyridine (DMAP), in DCM, by stirring for 48 hours.[105] Another method described in literature uses the Boc-protected aminoacid, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBop) and DIPEA in dry DMF at room temperature.[143] Although these methods are both valid we have explored the use of carbodiimide coupling reagents. This family of reagents is widely used in peptide synthesis and could be used also to couple peptides on calixarenes.[147] The Boc-protected amino acid was stirred for half an hour in anhydrous DCM at 0°C in the presence of the coupling reagent to allow the formation of the adduct. The tetra-amino calix[4]arene, dissolved in the same solvent was then added. The reaction was conducted in anhydrous conditions under Argon to prevent the nucleophilic attack of water to the adduct formed by the amino acid and the coupling reagent. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was preferred to other coupling reagents such as dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (DIC) because of the water solubility of its urea derivative, the side product in the reaction.



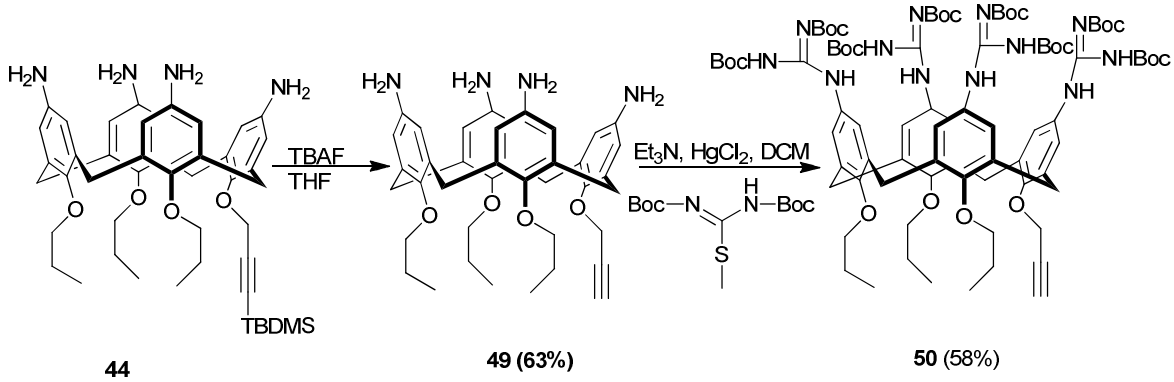
Scheme 1.28: Coupling of the aromatic amino groups with the Boc protected glycine and alkyne deprotection.

In the final step the TBDMS protecting group was removed by heating the compound at reflux in THF in the presence of TBAF to yield after precipitation from DCM and *n*-hexane the free alkyne as a pale yellow powder. This compound was not affected by the temperature, indicating the higher stability of the aliphatic amine derivatives.

1.3.5.3. Synthesis of guanidinium functionalised generation 1 calixarenes

Single calix[4]arenes functionalised on the wide or on the lower rim with guanidinium groups have been reported by Ungaro's group to bind and transfect nucleic acids.[103, 101 ,102, 148 ,148]It would be therefore interesting to explore their transfection potential when they are incorporated on a multicalixarene system. For this purpose we initially attempted to synthesise a calixarene carrying a guanidinium group protected with BOC on the upper rim and an alkyne function on the narrow rim. The tetra amino derivative **44** was dissolved in DMF and stirred in the presence of mercury chloride and N,N'-bis-Boc-2-methylthiourea. The resulting dark suspension was filtered and the resulting solution concentrated and purified by column chromatography.[101] Unfortunately the deprotection of the alkyne, performed by stirring the compound in THF in the presence TBAF, did not yield the desired compound. One of the reasons may be partial loss of the Boc protecting group on the guanidinium moiety.

To overcome this problem an alternative strategy was developed, the protecting group on the alkyne was removed before the insertion of the guanidinium, on the free amine. In this way we obtained the tetra amino derivative carring the free alkyne on the narrow rim (scheme 1.29).



Scheme 1.29: Deprotection of the alkyne group and functionalisation with Boc-protected Guanidinim groups.

1.3.6.Synthesis of multicalixarenes families

The multicalixarenes were assembled by reacting each central core carrying four azides with the mono alkyne functionalised generation 1 calix[4]arenes. Santoyo-Gonzales was the first to link calixarenes scaffolds using the the Azide-Alkyne Huisgen Cycloaddition.[114] As mentioned earlier in the chapter his investigation yielded a mixture of the 1,4 and 1,5 substituted products because the reaction was carried out without the copper(I) catalyst. To lead the reaction towards the formation of the 1,4 substituted product it is possible to use as a source of copper(I) catalyst either copper iodide or a mixture of copper sulphate and sodium ascorbate. In the first case the copper is already in the oxidation state required for the catalysis, whilst with the second approach it is reduced from copper(II) to copper(I) by the sodium ascorbate in the reaction mixture. Since Santoyo-Gonzales first example, a number of click reactions on calixarenes have been reported in literature using either the copper iodide[149] [150] [151] [139] or the copper sulphate method.[152] [139] In our investigation for the synthesis of the multicalixarenes carring the Boc-protected aromatic amino groups a mixture of copper(II) sulphate penta hydrate and sodium ascorbate was chosen as source of copper(I) ions. The conditions followed were developed during previous work in the group (unpublished results). A slight excess of the mono alkyne functionalised derivative **46** (1.2 equivalents for each azido group) was heated at 70 C^{o} in DMF for two hours in the presence of each azido functionalised central core, the copper sulphate pentahydrate (0.16 eq) and the sodium ascorbate (1.25 eq). Purification by column chromatography yielded the desired multicalixarenes. The slight excess of generation 1 calixarene was used to ensure that all of the azido groups in the central core would undergo reactions. Partial reaction of the azido groups of the central core could lead to the formation of dimers, trimers, and tetramers resulting in a mixture of products with difficult separations. However when the same conditions were reproduced using the Boc-gly calixarene derivative **48** as the generation 1 calixarene the reaction yielded a complex mixture of product. This failure led to the exploration of alternative conditions.

The second multicalixarene family, bearing Boc-gly functionalisations, was obtained by increasing the amount of catalyst (0.5 equivalents of $CuSO_4 \cdot 5\ H_2O$ and 2 equivalents of sodium ascorbate), the amount of alkyne functionalised generation 1 calixarene (1.25 equivalents) and the reaction temperature up to 110 ^{o}C.[139] In total twenty different multicalixarenes were synthesised. Ten different azido functionalised central cores were linked with two different types of generation 1 calix[4]arenes bearing either aromatic amines or aliphatic amines at the upper rim summarised in table1.1

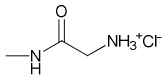
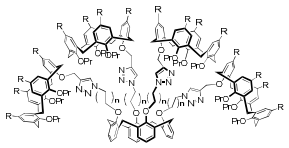
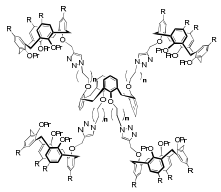
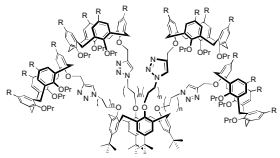
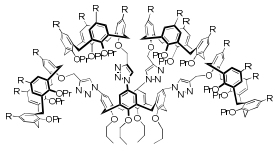
	—NHBoc		—$NH_3^+Cl^-$		
	**51** 55%	**61** 35%	**71** quantitative	**81** quantitative	n=1
	52 84%	**62** 41%	**72** quantitative	**82** quantitative	n=2
	53 61%	**63** 39%	**73** quantitative	**83** quantitative	n=3
	**54** 78%	**64** 42%	**74** quantitative	**84** quantitative	n=1
	55 73%	**65** 44%	**75** quantitative	**85** quantitative	n=2
	56 74%	**66** 53%	**76** quantitative	**86** quantitative	n=3
	**57** 75%	**67** 42%	**77** quantitative	**87** quantitative	n=1
	58 58%	**68** 46%	**78** quantitative	**88** quantitative	n=2
	59 93%	**69** 41%	**79** quantitative	**89** quantitative	n=3
	**60** 67%	**70** 43%	**80** quantitative	**90** quantitative	

Table 1.1: Multicalixarene families.

In figure 1.26 is displayed a superimposition of the ^{1}H NMR spectra for compounds **51** (blue), **54** (red), **57** (green) and **60** (purple). It is possible to notice how the spectra change according to the conformation of the central cores. The spectra can be divided into three main regions: the aromatic region, above 6 ppm, the methylene region between 6 ppm and 2.75 ppm and the aliphatic region below 2.75 ppm. In all the spectra the two signals of the "click" protons can be seen. The first of these signals falls in the aromatic region at the highest ppm. It integrates for four protons and can be attributed to the proton present on the triazole ring. The second peak falls in the methylene region. It integrates for eight protons and is due to the methylene located between the generation 1 calixarenes and the triazole rings. This last signal in some cases overlaps with the signal given by the methylene next to the oxygen in the central core The spectra for the multicalixarenes **51** (blue), **57** (green) and **60** (purple), having the central cores in the cone conformation, show between 4.75ppm and 2.75 ppm six doublets. Four of these signals are due to the asymmetric methylene bridge protons of the generation 1 calixarene. The other two doublets, integrating for four protons each, belong to the symmetrical methylene bridge protons in the central core.

As expected, the same region of the spectra for compound **54** (red), bearing the central core in the 1,3-alternate conformation, shows only four doublets corresponding to the asymmetric methylene bridge protons of the generation 1 calixarene. The signal for the methylene bridge protons of the central core, a singlet, falls around 4.8-4.9 ppm and overlaps with the signals of the methylene protons on the alkyl chain of the generation 1 close to the phenolic oxygens.

In compound **60** (purple) the central core in the cone conformation is linked to the generation 1 calixarene with a wide rim-narrow rim. Comparing the aliphatic region of the spectra of this compound with the others it is possible to notice an extra sextet and extra triplet belonging to the propyl chains on the central core.

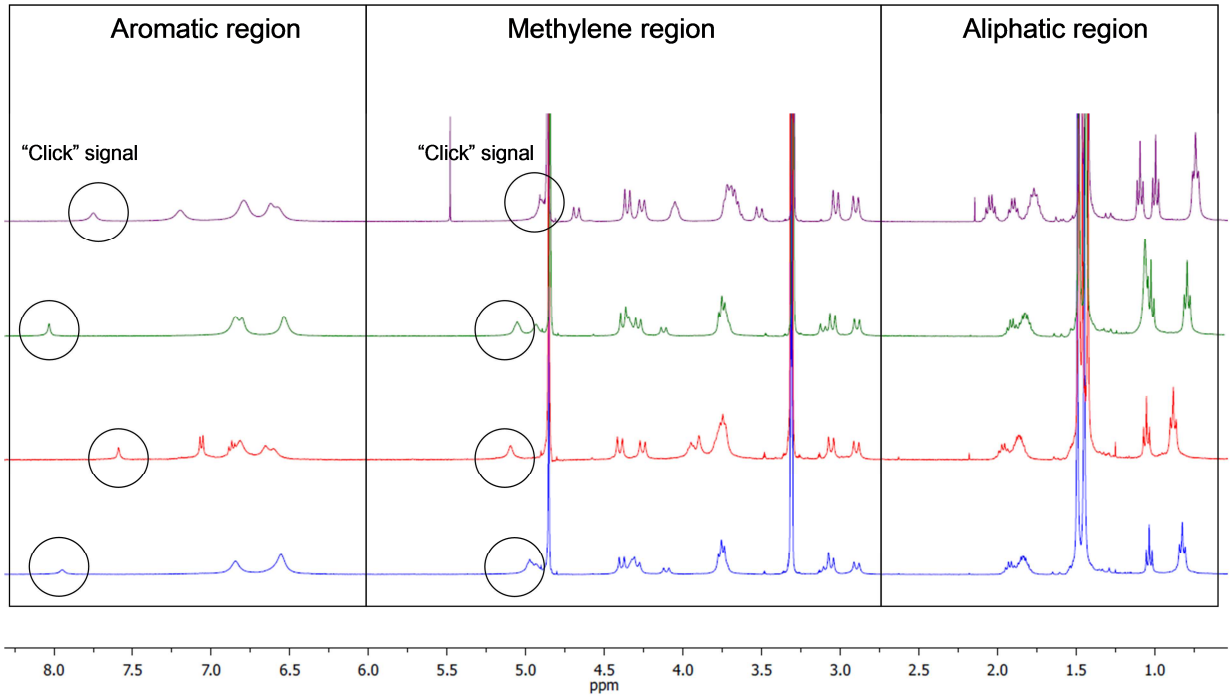


Figure 1.24: ^{1}H NMR Spectra for compound **51** (blue), **54** (red), **57** (green) and **60** (purple)

1.3.7. Removal of Boc-protecting groups from aliphatic and aromatic amino multicalixarenes.

In the final step of the synthesis the Boc protecting group was removed. As previously mentioned in the chapter this can be achieved by bubbling HCl gas through a solution of the protected multicalixarene. The multicalixarene bearing the masked amino moieties was dissolved in DCM and the gas was bubbled into the solution until the formation of a white

precipitate due to the formation of the chloride salt of the cationic multicalixarene, was observed. Removal of the solvent under reduced pressure yielded the desired compounds. This method was applied to all the multicalixrenes families and was successful in most cases. Four compounds in the cone conformation (**78**, **79**, **83** and **89**) showed an unexpected complexity in the ^{1}H NMR probably due to conformational issues. These compounds did not undergo biological evaluation although the MALDI TOF analysis confirmed the mass for compound **79** and **83**.

1.4. Biological evaluation

The two families of dendrimeric multicalixarenes bearing on the upper rim of their generation 1 free amino group were biologically tested. The fifteen compounds which underwent biological evaluation where functionalised with either aromatic amino groups (**71**, **72**, **73**, **74**, **75**, **76**, **77**, **78** and **80**) or aliphatic amines (**81**, **82**, **84**, **85**, **86**, **87** and **88**). The first test aimed to evaluate the toxicity of the compound on HL-60 cells through an MTS assay. HL-60 cells are commonly used in toxicity studies on calixarenes because their high sensitivity to toxins makes cell death easily detectable by colorimetric assay. [105, 153,154]

Once the lack of toxicity at therapeutically relevant concentrations was ascertained the ability to deliver nucleic acid into cells was tested with transfection studies.

1.4.1. Toxicity

Other studies have shown that compounds bearing amino groups could be toxic. It was therefore of major relevance to assess whether the amino multicalixarene families were compatible for biological applications in order to be used as a therapeutic tool for the transfection of DNA into the cells. Toxicity studies were carried out using an MTS assay.

The MTS assay is a colorimetric assay which uses a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethylphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, MTS) and an electron-coupling agent (phenazine methosulfate, PMS). This method allows determination of the cell viability and proliferation. The MTS reagent is converted by the dehydrogenase enzymes in metabolically active cells into a formazan that is soluble in the cell culture medium. The absorbance of the formazan in each well can be read using a microtiter plate reader, and is directly proportional to the number of living cells in culture.

The study was carried out incubating *Human promyelocytic leukemia* cells (HL-60) for 72 hours in the presence of a range of concentrations of multicalixarenes varying from 1mM to 100 nM. As control the cells were incubated in the media in the presence of the solvent used to solubilise the studied compounds. The multicalixarenes bearing aromatic amino functionalisation were dissolved in ethanol, whilst DMSO was used to solubilise the compounds bearing aliphatic amines. After 72 hours incubation, precipitation was observed for the two highest concentrations of all compounds, therefore the resulting data were not taken in to consideration. Before

performing the absorbance measurement 10µL of CellTiter 96® was added to each well and the plate was incubated for a further 4 hours. The asorbance was read at Absorbance was read at 495nm using BMG Labtech, Fluostar Galaxy 96 well plate reader. For concentrations between 10 µM and 100 nM none of the tested compounds showed signs of toxicity.

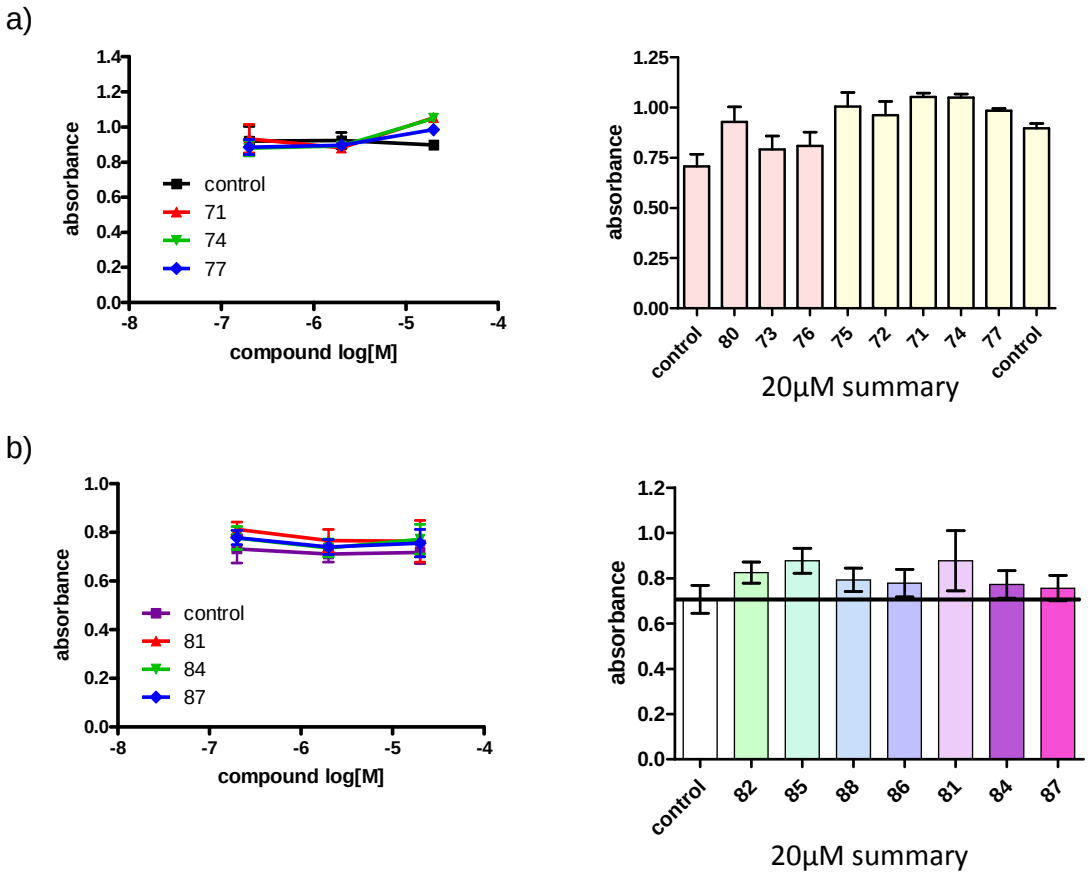


Figure 1.25: Graphic representation of cell viability for HL-60 cells after 72 hours incubation with a) aromatic amino multicalixarenes and b) aliphatic amino multicalixarenes

Figure 1.25 summarises the results of the toxicity studies carried out on the amino functionalised calixarenes. As it is possible to observe none of the tested compounds show toxicity in the range of concentrations evaluated. On the left hand side is shown the study at variable concentration whilst on the right hand side are summarised the results obtained at 20 µM concentration.

1.4.2. Transfection studies

The ability of the multicalixarenes to transfect genetic material in to the cell was investigated using Chinese hamster ovary cells. The experiment aimed to introduce into the cells plasmid DNA (pDs2-mito) which had been engineered to express MitoDsRed2, a fluorescent protein localised in the mitochondria? of the cells. In the case of successful transfection the fluorescence can be observed in the cell mitochondria. The commercially available transfection agent FuGENE® was used as positive control. Plasmid DNA and DMSO were used as negative controls.

The compounds under investigation, including the positive FuGENE®, in a concentration of 5 x 10^{-4} M were incubated with pDs2-mito for 15 minutes at room temperature. The mixture was then added to the cells and incubated for 24 hours. Following the incubation the cells were tested for mitocondria fluorescence to assess whether the compound had transfection properties.

Fluorescence was observed in all the cells treated with the multicalixarenes bearing aliphatic amines. The result proved that these compounds were capable of transfecting the nucleic acid into the cells. In particular the cells treated with compound **81** appeared to show the highest fluorescence.

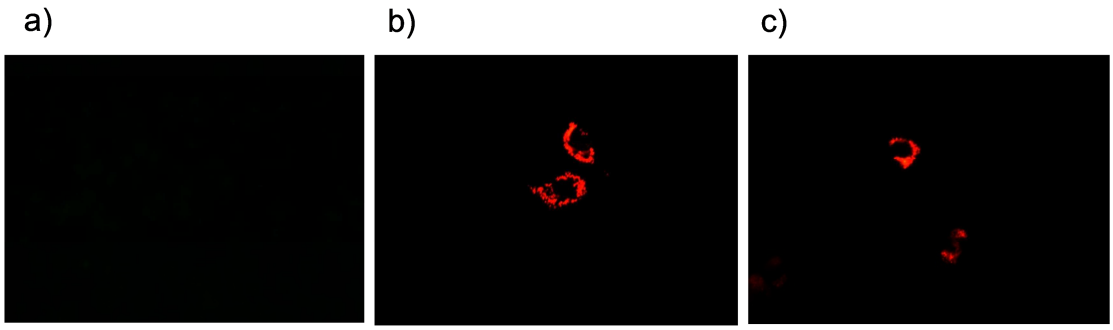


Figure 1.26: Transfection studies result a) negative control (DMSO and DNA); b) experiment (**81** and DNA); c) positive control (FuGENE® and DNA)

Figure 1.26 shows the results of the transfection studies carried out cells incubated with the DNA in the presence of multicalixarene **81**. The cells treated with this incubated with the positive control FuGENE® (figure 1.26b).

On the other hand among all the multicalixarenes bearing aromatic amines on their generation 1 only compound **80** was able to transfect the plasmid into the cells (figure 1.27).

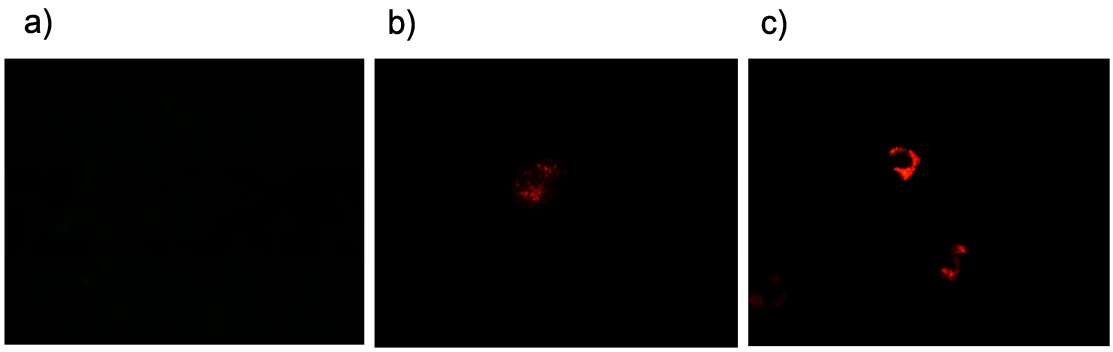


Figure 1.27: Transfection studies result a) negative control (DMSO and DNA); b) experiment (**80** and DNA); c) positive control (FuGENE® and DNA)

This result was unexpected because previous work in the group had shown that only the multicalixarene bearing aliphatic amines were able to transfect DNA. In this compound the generation 1 calixarenes are directly attached to the upper rim of the central core.

From the results obtained from these first transfection experiments it can be concluded that the presence of aliphatic amines is of major importance for the transfection efficiency of cationic multicalixarenes. The potency of the transfection seems to be higher with shorter linkers, having the maximum for multicalixarene **81** . The sum of these two results have led the study toward the synthesis of compound **90**, the analogue of compound **80**, bearing on the generation 1 aliphatic amino functionalities.

1.5. Conclusion and future work

In this chapter we have reported the synthesis of a library of cationic multicalixarene dendrimers which were investigated as transfection agents for the intracellular delivery of nucleic acid. The multicalixarenes were synthesised linking generation 1 calixarenes, prefunctionalised with protected either aromatic or aliphatic amines, with 10 different central cores through azide alkyne 1,3-dipolar cycloaddition. Central cores functionalised with four azido groups were reacted with mono-alkyne functionalised generation 1 calixarene in the presence of copper(I) catalyst. In this fashion 20 functionalised multicalixarenes with varing conformation and linker length were prepared. Once the dendrimers were formed the amino groups on the generation 1 calixarenes were revealed and the compounds underwent biological evaluation. Toxicity and transfection properties were evaluated. The toxicity was evaluated by performing an MTS assay

for 72 hours with HL-60 cells. None of the compound tested showed toxicity in a concentration between 10 μM and 100 nM. Transfection experiments were carried out using CHO cells and pDs2-mito, a plasmid DNA, which is translated in the cell into a fluorescent protein. All the cells treated with the plasmid in the presence of multicalixarenes functionalised with aliphatic amines were successfully transfected. In particular the cells treated with compound **81** showed the highest fluorescence. On the other hand, only one of the aromatic amino functionalised multicalixarenes, compound **80** was able to transfect the plasmid into the cells even if with less efficiency than the multicalixarene carrying the aliphatic amine. This result has led the way toward the rational design of compound **90**, analogue of **80** functionalised with aliphatic amines. The compound has been successfully synthesised and is now under biological investigation.

1.6. Experimental

5,11,17,23-*p*-*Tert*-butylcalix[4]arene (1)[78]

p-*Tert*-butyl phenol (200 g, 1,33 mmol), NaOH (1,20 g, 0.04 mol) and formaldehyde (128 mL, 4.43 mol) were heated to 120 °C over 2 h. The resultant yellow solid was dissolved in diphenyl ether (750 mL, 4.73 mol). Water of condensation (120 mL) was removed by distillation heating the solution at 100 ^{o}C under a stream of air, after which the solution was heated at reflux for 2 h. Following cooling, EtOAc (750 mL) was added to precipitate the crude product, which was collected by filtration, washed with EtOAc (2 x 150 mL), AcOH (2 x 150 mL), and H_2O (4 x 150 mL) to yield the desired compound as white crystals (106.42 g, 49%). **Mp** > 325 ° C (decomp); **^{1}H NMR** (400 MHz, $CDCl_3$); 10.33 (4H, s, ArO*H*), 7.07 (8H, s, Ar*H*), 4.24 (4H, br s, ArCH_2Ar), 3.49 (4H, br s, ArCH_2Ar), 1.10 (36H, s, C(CH_3)$_3$).

Calix[4]arene (2)[82]

$AlCl_3$ (11.27 g, 80.52 mmol) was added carefully to a stirred solution of p-t-butyl calixarene (**1)** (10 g, 15.34 mmol) and phenol (6.49 g, 80.52 mmol) in toluene (100 mL). After 2h stirring under air flow the mixture was poured in to 200 mL of 0.2 N HCl and stirred for a further 30 minutes. The separated organic layer was then washed twice with H_2O and the excess of solvent was removed under reduced pressure. The residue was precipitated from MeOH to give the pure compound (5.27g 80.3%). **Mp** > 250 ^{o}C (decomp); **^{1}H NMR** (400 MHz, $CDCl_3$); 10.23 (4H, s, ArO*H*), 7.08 (8 H, d, *J* =7.6 Hz, Ar*H*), 6.75 (4H, t, *J* =7.6 Hz, Ar*H*), 4.29 (4H, b s, ArCH_2Ar), 3.57 (4H, b s, ArCH_2Ar).

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-tetra(ethoxycarbonyl)methoxycalix[4]arene (3)[155]

A solution of **1** (16.00 g, 24.70 mmol) and potassium carbonate (10.00 g, 72.46 mmol) were heated at reflux in MeCN (250 mL) for 2 h before the addition of ethylbromoacetate (40 mL, 360.72 mmol), after which the solution was heated at reflux overnight. After cooling, the solvent was removed under reduced pressure and the residue taken up in DCM/H_2O. The organic layer was separated and washed with H_2O (3 x 100 mL), dried over $MgSO_4$ and the solvent removed *in vacuo*. Precipitation from DCM/MeOH yielded **3** (19.29 g, 79%) as white crystals. **Mp**; 121-123 ˚C; **^{1}H NMR** (400 MHz, $CDCl_3$); 6.77 (8H, s, Ar*H*), 4.85 (4H, d, *J* = 12.9 Hz, ArCH_2Ar), 4.80 (8H, s, OCH_2C(O)OEt) 4.20 (8H, q, *J* = 7.2 Hz, C(O)OCH_2CH$_3$), 3.19 (4H, d, *J* = 12.9 Hz, ArCH_2Ar), 1.29 (12H, t, *J* = 7.2 Hz, C(O)OCH$_2$CH_3), 1.07 (36H, s, C(CH_3)$_3$).

25,26,27,28-Tetra(ethoxycarbonyl)methoxycalix[4]arene (4)[81]

K_2CO_3 (5 g, 36.23 mmol) was added to a stirred solution of **2** (5 g, 11.79 mmol) in CH_3CN (165 mL). After 30 minutes ethylbromoacetate (12.5 ml, 112.72 mmol) was added and the reaction was heated at reflux for 18 hours. The suspension was cooled, filtered and the solvent removed from the filtrate under reduced pressure The residue was dissolved in DCM (150 mL) and washed with water (3x 100 mL). The organic layer was evaporated under reduced pressure to give a yellow oil, which was re-crystallized from EtOH at -20^oC to give the desired pure compound as white crystals (5.95 g, 65.7%). **Mp** 94-96 °C; **^{1}H NMR** (400 MHz, $CDCl_3$); 6.62 (12H, m, Ar*H*), 4.88 (4H, d, *J* =13.6 Hz, ArC*H*$_2$Ar), 4.74 (8H, s,OC*H*$_2$CO$_2$Et), 4.21 (8H, q, *J* = 7.8 Hz, C(O)OC*H*$_2$CH$_3$), 3.24 (4H, d, *J* = 13.6 Hz, ArC*H*$_2$CH$_2$Ar), 1.29 (12H, t, *J* =7.8 Hz, C(O)OCH$_2$C*H*$_3$).

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-hydroxyethoxycalix[4]arene (5)[124]

A solution of **3** (2.5 g, 2.67 mmol) in Et_2O (15 mL) was added dropwise to a stirred solution of $LiAlH_4$ (0.8 g, 21 mmol) in Et_2O (50 mL) at 0^oC. After 24 hours the reaction mixture was quenched carefully with 10% HCl (100 mL) and was diluted with DCM (100 mL). The organic layer was separated, washed with H_2O (2x100 mL), dried over $MgSO_4$ and the excess of solvent was evaporated under reduced pressure to give the title compound (2.0 g, 84%). **Mp** = 105-107 ^oC; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.86 (s, 8H, Ar*H*), 5.12 (s, 4H, OCH$_2$CH$_2$O*H*), 4.36 (d, *J* = 13.0 Hz, 4H, ArC*H*$_2$Ar), 4.16 – 3.88 (m, 16H, OC*H*$_2$CH$_2$OH, OCH$_2$C*H*$_2$OH), 3.23 (d, *J* = 13.0 Hz, 4H, ArC*H*$_2$Ar), 1.09 (s, 36H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.44, 145.83, 133.67, 125.69, 77.94, 61.83, 34.08, 31.52, 30.57.

25,26,27,28-Hydroxyethoxycalix[4]arene (6)[122]

A solution of **4** (1.5 g, 1.95 mmol) in Et_2O (10 mL) was added dropwise to a solution of $LiAlH_4$ (0.5 g, 13.2 mmol) in Et_2O (25 mL) and was stirred for 24 hours at 0^oC. The reaction mixture was quenched carefully with 10% HCl (100 mL) and was diluted with DCM (100 mL). The organic layer was separated, washed with H_2O (2x100), dried over $MgSO_4$ and the excess of solvent was evaporated to give the title compound (620 mg, 53%). **Mp** = 111-113 ^oC **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.72 – 6.62 (m, 12H, Ar*H*), 4.95 (bs, 4H, OCH$_2$CH$_2$O*H*), 4.39 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 4.06 – 3.99 (m, 8H, OCH$_2$C*H*$_2$OH), 3.98 – 3.93 (m, 8H, OC*H*$_2$CH$_2$OH), 3.26 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.18, 134.74, 128.92, 123.34, 77.99, 61.69, 30.44.

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-tosylethoxycalix[4]arene (7)[122]

Tosyl Chloride (6.38 g, 33.6 mmol) was added to a stirred solution of **5** (2.5 g, 2.8 mmol) in DCM (50 mL) in the presence of Et_3N (2.26 g, 22.4 mmol) at 0°C. After 24 hours the reaction was quenched with water (50 mL) and the organic layer was washed with 10% HCl (2 x 50 mL) and with brine (2 x 50 mL). The organic layer was separated, dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. Purification by column chromatography over silica gel (DCM:*n*-hexane:EtOAc=5:5:1) gave the desired compound as a white solid (2,66 g, 66%). **Mp** = 104-106 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 8H, CH_3ArHSO_3Ar), 7.33 (d, *J* = 8.0 Hz, 8H, CH_3ArHSO_3Ar), 6.70 (s, 8H, Ar*H*), 4.41 – 4.37 (m, 8H, OCH_2CH_2O), 4.22 (d, *J* = 13.0 Hz, 4H), 4.11 – 4.06 (m, 8H, OCH_2CH_2O), 3.00 (d, *J* = 13.0 Hz, 4H, $ArCH_2Ar$), 2.43 (s, 12H, CH_3ArHSO_3Ar), 1.05 (s, 36H, CH_3CAr). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.27, 145.36, 144.91, 133.57, 133.23, 130.09, 128.15, 125.32, 71.79, 69.67, 33.96, 31.50, 21.80.

25,26,27,28-Tosylethoxycalix[4]arene (8) [122]

Tosyl Chloride (1.27 g, 6.68 mmol) was added to a stirred solution of **6** (0.5 g, 0.83 mmol) in DCM (10 mL) in the presence of Et_3N (2.17 g, 5 mmol) at 0°C. After 24 hours the reaction was quenched with water (20 mL) and the organic layer was washed with 10% HCl (2 x 20 mL) and with brine (2 x 20 mL). The organic layer was separated, dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. Purification by column chromatography over silica gel (DCM:*n*-hexane:EtOAc=5:5:1) gave the desired compound as a white solid (0.73 g, 72%). **Mp** = 108-111 °C **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.5 Hz, 8H, CH_3ArHSO_3Ar), 7.32 (d, *J* = 8.4 Hz, 8H, CH_3ArHSO_3Ar), 6.56 (s, 12H, Ar*H*), 4.42 – 4.36 (m, 8H, OCH_2CH_2O), 4.30 (d, *J* = 13.5 Hz, 4H, $ArCH_2Ar$), 4.16 – 4.11 (m, 8H, OCH_2CH_2O), 3.05 (d, *J* = 13.5 Hz, 4H, $ArCH_2Ar$), 2.43 (s, 12H, CH_3ArHSO_3Ar).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.33, 144.97, 134.70, 133.09, 130.10, 128.57, 128.07, 122.89, 71.86, 69.86, 30.90, 21.79.

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-azaethoxycalix[4]arene (9)[124]

NaN_3 (641 mg, 15.84 mmol) was added to a stirred solution of **7** (590 mg, 0.41 mmol) in DMF (30 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound (0.38 g, 100%). **Mp** = 183-185 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.83 (s, 8H, Ar*H*), 4.38 (d, *J* = 12.5 Hz, 4H, $ArCH_2Ar$), 4.09 (t, *J* = 6.0 Hz, 8H. $OCH_2CH_2N_3$), 3.89 (t, *J* = 6.0 Hz, 8H, $OCH_2CH_2N_3$), 3.22 (d, *J* = 12.5 Hz, 4H, $ArCH_2Ar$), 1.10 (s, 36H, CH_3CAr). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.48, 145.53, 133.54, 125.42, 72.11, 51.08, 34.00, 31.48, 30.87

25,26,27,28-Azaethoxycalix[4]arene (10) [124]

NaN_3 (641 mg, 15.84 mmol) was added to a stirred solution of **8** (500 mg, 0.41 mmol) in DMF (30 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound (0.28 g, 97%). **Mp** = 188-191 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.84 – 6.44 (m, 12H, Ar*H*), 4.46 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 4.12 (t, *J* = 6.0 Hz, 8H, OC*H*$_2$CH$_2$N$_3$), 3.82 (t, *J* = 6.0 Hz, 8H, OCH$_2$C*H*$_2$N$_3$), 3.27 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.53, 134.63, 128.64, 122.99, 72.16, 51.25, 30.79

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-bromobutoxy-calix[4]arene (11)[125]

NaH (0.936 g, 36.8 mmol) was added to a stirred solution of **1** (3 g, 4.6 mmol) in DMF (100 mL). After 30 mintutes 1,4-dibromobutane (23.85 g, 110 mmol) was added and the reaction was stirred at room temperature for 24 hours. The mixture was carefully quenched with H_2O (100 mL) and extracted with DCM (3 x 100 mL). The combined organic layer was washed with 10% HCl (2 x 100 mL) and brine (2 x 100 mL), dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. The residue was precipitated from DCM:MeOH to give the product as a white solid (4.30 g, 78%). **Mp =** 141-143 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.79 (s, 8H, Ar*H*), 4.36 (d, *J* = 12.5 Hz, 4H, ArC*H*$_2$Ar), 3.91 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$Br), 3.53 (t, *J* = 6.5 Hz, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$Br), z, 4H, 3.15 (d, *J* = 12.5 H ArC*H*$_2$Ar), 2.23 – 2.11 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$Br), 2.06 – 1.97 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$Br), 1.09 (s, 36H, C*H*$_3$CAr). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.42, 143.77, 132.78, 124.18, 73.36, 33.03, 32.98, 30.57, 30.25, 28.71, 28.22.

25,26,27,28-Bromobutoxy-calix[4]arene (12)[156]

NaH (2.24 g, 93.6 mmol) was added to a stirred solution of **2** (5 g, 11.7 mmol) in DMF (150 mL). After 30 mintutes 1,4-dibromobutane (60.65 g, 280.8 mmol) was added and the reaction was stirred at room temperature for 24 hours. The mixture was then carefully quenched with H_2O (150 mL) and extracted with DCM (3 x 150 mL). The combined organic layer was washed with 10% HCl (2 x 150 mL) and brine (2 x 150 mL), dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. The residue was precipitated from DCM:MeOH to give the product as a white solid (4.98 g, 44%). **Mp** = 124-126 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.73 – 6.51 (m, 12H, Ar*H*), 4.40 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 3.94 (t, *J* = 7 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$Br), 3.51 (t, *J* = 6.5 Hz, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$Br), 3.20 (d, *J* = 13.4 Hz, 4H, ArC*H*$_2$Ar), 2.23 – 1.90 (m, 16H, OCH$_2$C*H*$_2$CH$_2$CH$_2$Br and OCH$_2$CH$_2$CH$_2$C*H*$_2$Br). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.26, 135.01, 128.44, 122.41, 74.10, 33.83, 31.13, 29.76, 29.11.

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-bromohexoxy-calix[4]arene (13)

NaH (0.936 g, 36.8 mmol) was added to a stirred solution of **1** (3 g, 4.6 mmol) in DMF (100 mL). After 30 mintutes 1,6-dibromohexane (26.84 g, 110 mmol) was added and the reaction was stirred at room temperature for 24 hours. The mixture was carefully quenched with H_2O (100 mL) and extracted with DCM (3 x 100 mL). The combined organic layer was washed with 10% HCl (2 x 100 mL) and brine (2 x 100 mL), dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. The residue was precipitated from DCM:MeOH to give the product as a white solid (3.23 g, 54%). **Mp** = 81–83 ^oC. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.78 (s, 8H, Ar*H*), 4.38 (d, *J* = 12.5 Hz, 4H, ArC*H*$_2$Ar), 3.86 (t, *J* = 8 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$Br), 3.45 (t, *J* = 7 Hz, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$Br), 3.13 (d, *J* = 12.5 Hz, 4H, ArC*H*$_2$Ar), 2.09 – 1.99 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$Br), 1.97 – 1.87 (m, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$Br), 1.62 – 1.52 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$Br), 1.49 – 1.39 (m, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$Br), 1.08 (s, 36H, C*H*$_3$CAr). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 153.56, 144.37, 133.75, 124.95, 75.08, 33.95, 33.83, 33.00, 31.47, 31.11, 30.22, 28.48, 25.53. **IR** *v* **=** 2950; 2857.10; 1738; 1581; 1480; 1462; 1437.37; 1390; 1359; 1297; 1245; 1198; 1122; 1108; 1047; 1025.

25,26,27,28-Bromohexoxy-calix[4]arene (14)

NaH (0.936 g, 36.8 mmol) was added to a stirred solution of **2** (2 g, 4.6 mmol)in DMF (100 mL). After 30 mintutes 1,6-dibromohexane (26.84 g, 110 mmol) was added and the reaction was stirred at room temperature for 24 hours. The mixture was carefully quenched with H_2O (100 mL) and extracted with DCM (3 x 100 mL). The combined organic layer was washed with 10% HCl (2 x 100 mL) and brine (2 x 100 mL), dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. The residue was precipitated from DCM:MeOH to give the product as a white solid (2.28 g, 46%) . **Mp** = 74-76 ^oC. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.75 – 6.60 (m, 12H, Ar*H*), 4.50 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 3.96 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$Br), 3.50 (t, *J* = 6.5 Hz, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$Br), 3.24 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 2.07 – 1.92 (m, 16H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$Br and OCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$Br), 1.69 – 1.57 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$Br), 1.57 – 1.42 (m, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$Br). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.42, 135.02, 128.19, 122.07, 74.81, 33.89, 32.93, 31.03, 30.15, 28.36, 25.52. **M/z (MALDI-TOF)** 1076.2 m/z $[M]^+$. **IR** *v* **=** 2930; 2855; 2358; 2342; 1584; 1453; 1429; 1381; 1290; 1244; 1207; 1088; 1027.

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-azabutoxycalix[4]arene (15)

NaN_3 (4 g, 61.54 mmol) was added to a stirred solution of **11** (3.03 g, 2.56 mmol) in DMF (100 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound (2.42 g, 92%). **Mp**= 118-120 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.79 (s, 8H, Ar*H*), 4.35 (d, *J* = 12.5 Hz, 4H, ArC*H*$_2$Ar), 3.89 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$N$_3$), 3.40 (t, *J* = 7.0 Hz, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$N$_3$), 3.15 (d, *J* = 12.5 Hz, 4H, ArC*H*$_2$Ar), 2.17 – 1.98 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$N$_3$), 1.77 – 1.67 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$N$_3$), 1.09 (s, 36H, C*H*$_3$CAr). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 153.07, 144.47, 133.43, 124.87, 74.31, 51.38, 33.66, 31.25, 30.93, 27.37, 25.52. **M/z (MALDI-TOF)** 1036.7 m/z $[M]^+$. **IR** *ν* = 2951; 2867; 2093; 1601; 1480; 1391; 1361; 1298; 1246; 1198; 1122; 1032.

25,26,27,28-Azabutoxycalix[4]arene (16)

NaN_3 (4 g, 61.54 mmol) was added to a stirred solution of **12** (2.47 g, 2.56 mmol) in DMF (100 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound (2.0 g, 84.5%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.66 – 6.55 (m, 12H, Ar*H*), 4.39 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 3.92 (d, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$Br), 3.38 (t, *J* = 7.0 Hz, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$Br), 3.19 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 2.08 – 1.89 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$Br), 1.83 – 1.63 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$Br). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.26, 135.01, 128.45, 122.43, 74.39, 51.62, 31.15, 27.63, 25.90. **M/z (MALDI-TOF)** 812.4m/z $[M]^+$. **IR** *ν* = 2916; 2865; 2090; 1583; 1452; 1382; 1349; 1290; 1243; 1211; 1188; 1087.

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-azahexoxycalix[4]arene (17)

NaN_3 (2.6 g, 40.08 mmol) was added to a stirred solution of **13** (2.17 g, 1.67 mmol) in DMF (100 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound as a colorless oil (1.9 g; 97 %). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.79 (s, 8H, Ar*H*), 4.39 (d, *J* = 12.4 Hz, 4H, ArC*H*$_2$Ar), 3.87 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$N$_3$), 3.32 (t, *J* = 7.0 Hz, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$N$_3$), 3.14 (d, *J* = 12.5 Hz, 4H, Ar*H*), 2.10 – 1.97 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$N$_3$), 1.72 – 1.61 (m, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$N$_3$), 1.58 – 1.38 (m, 16H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$N$_3$ and OCH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$N$_3$), 1.09 (s, 32H,

C*H*$_3$CAr). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 153.66, 144.49, 133.85, 125.07, 75.16, 51.56, 33.94, 31.57, 31.23, 30.34, 29.23, 27.15, 26.01. **M/z (MALDI-TOF)** 1148.8 m/z $[M]^+$. **IR** *ν* = 2948; 2861; 2091; 1739; 1601; 1480; 1390; 1360; 1297; 1247; 1198; 1122; 1014.

25,26,27,28-Azahexoxycalix[4]arene (18)

NaN_3 (2.6 g, 40.08 mmol) was added to a stirred solution of **14** (1.80 g, 1.67 mmol) in DMF (100 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound as a colorless oil (1.35 g, 88 %). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.69 – 6.54 (m, 12H, Ar*H*), 4.44 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 3.91 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$N$_3$), 3.32 (t, *J* = 7.0 Hz, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$N$_3$), 3.19 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 2.01 – 1.89 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$N$_3$), 1.71 – 1.61 (m, 8H, OCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$N$_3$), 1.54 – 1.40 (m, 16H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$N$_3$ and OCH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$N$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.49, 135.10, 128.27, 122.14, 74.85, 51.47, 31.10, 30.22, 29.11, 27.01, 25.94. **IR** *ν* = 2934; 2859; 2091; 1683; 1584; 1456; 1390; 1360; 1291; 1245; 1195; 1088; 1013.

25,26,27,28-Tetra(ethoxycarbonyl)methoxycalix[4]arene (1,3 alternate) (19)[157].

Cs_2CO_3 (38 g, 116.80 mmol) was added to a stirred solution of **2** (5 g, 11.68 mmol) in acetone (150 mL) at 56 °C. After 30 minutes ethylbromoacetate (25.84 ml, 233.64 mmol) was added and the reaction was heated at reflux for 24 hours. The excess of solvent was removed under reduced pressure. The residue was dissolved in DCM (150 mL) and washed with water (100 mL). The separated organic layer was washed with 10% HCl (2 x 100 mL) and with brine (2 x 100 mL). The excess of solvent was evaporated under reduced pressure to give an yellow oil, which was re-crystallized from EtOH at -20°C to give the desired pure compound as white crystals (5.50 g, 61%). **Mp** 95-97 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.71 (t, *J* = 7.5 Hz, 4H, Ar*H*), 4.25 (q, *J* = 7.0 Hz, 8H, OCH$_2$COOC*H*$_2$CH$_3$), 4.05 (s, 8H, OC*H*$_2$COOCH$_2$CH$_3$), 3.78 (s, 8H, ArC*H*$_2$Ar), 1.33 (t, *J* = 7.0 Hz, 12H, OCH$_2$COOCH$_2$C*H*$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.74, 155.61, 133.67, 130.50, 123.07, 69.84, 60.93, 35.65, 14.34.

25,26,27,28-Hydroxyethoxycalix[4]arene (1,3 alternate) (22) [158]

A solution of **19** (1.5 g, 1.95 mmol) in Et_2O (10 mL) was added dropwise to a solution of $LiAlH_4$ (0.5 g, 13.2 mmol) in Et_2O (25 mL) and was stirred for 24 hours at 0°C. The reaction mixture was quenched carefully with 10% HCl (100 mL) and was diluted with DCM (100 mL). The organic layer was separated, washed with H_2O (2x100), dried over $MgSO_4$ and the excess of solvent was evaporated under reduced pressure to give the title compound (950 mg, 81%). **Mp** >

263 (decompose). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.96 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.96 (s, 8H, ArCH_2Ar), 3.67 – 3.55 (m, 8H, OCH_2CH_2OH), 3.25 – 3.18 (m, 8H, OCH_2CH_2OH), 2.16 – 2.03 (m, 4H, OCH_2CH_2OH). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.30, 133.47, 129.67, 123.85, 71.60, 61.03, 38.23.

25,26,27,28-Tosylethoxycalix[4]arene (25)[158]

Tosyl Chloride (1.27 g, 6.68 mmol) was added to a stirred solution of **22** (0.5 g, 0.83 mmol) in DCM (10 mL) in the presence of Et_3N (2.17 g, 5 mmol) at 0°C. After 24 hours the reaction was quenched with water (20 mL) and the organic layer was washed with 10% HCl (2 x 20 mL) and with brine (2 x 20 mL). The organic layer was separated, dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure. Purification by column chromatography over silica gel (DCM:*n*-hexane:EtOAc=5:5:1) to give the desired compound as a white solid (0.61 g, 60%). **Mp** 193-195 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.0 Hz, 8H, CH_3ArHSO_3Ar), 7.39 (d, *J* = 8.0 Hz, 8H, CH_3ArHSO_3Ar), 6.91 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.60 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.68 – 3.62 (m, 16H, OCH_2CH_2OTos, ArCH_2Ar), 3.48 (t, *J* = 6.0 Hz, 8H, OCH_2CH_2OTos), 2.47 (s, 12H, CH_3ArHSO_3Ar). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.44, 145.15, 133.72, 133.09, 130.10, 129.75, 128.07, 123.27, 67.90, 67.67, 37.21, 21.80.

25,26,27,28-Azaethoxycalix[4]arene (1,3 alternate) (28)[159]

NaN_3 (641 mg, 15.84 mmol) was added to a stirred solution of **25** (500 mg, 0.41 mmol)in DMF (30 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound (0.28 g, 97%). **Mp** 103-105 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.91 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.84 (s, 8H, ArCH_2Ar), 3.53 (t, *J* = 6.5 Hz, 8H, $OCH_2CH_2N_3$), 2.96 (t, *J* = 6.5 Hz, 8H, $OCH_2CH_2N_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.90, 133.84, 129.62, 123.22, 68.15, 50.25, 37.54

25,26,27,28-Chlorobutoxy-calix[4]arene (32)

Cs_2CO_3 (30.5 g, 93.4 mmol) was added to a stirred solution of **2** (5 g, 11.68 mmol) in acetone (150 ml) at 56 °C. After 30 minutes 1-bromo-4-chlorobutane (10.82 mL, 93.4 mmol) was added and the reaction was heated at reflux for 12 hours. The solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with water. The organic layer was separated, washed with 10% HCl (2 x 100 mL) and with brine (2x100 mL), dried over dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to give a yellow oil. Purification by column chromatography over silica gel (DCM:*n*-hexane=1:1) and re-

crystallisation from DCM:EtOH gave the desired pure compound as white crystals (2.60g, 28%). **Mp** = 129-131 ^{o}C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.03 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.83 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.78 (s, 8H, ArCH_2Ar), 3.55 – 3.39 (m, 16H, OCH_2CH$_2$CH$_2$CH$_2$Cl and OCH$_2$CH$_2$CH$_2$CH_2Cl), 1.69 – 1.56 (m, 8H, OCH$_2$CH_2CH$_2$CH$_2$Cl), 1.50 – 1.39 (m, 8H, OCH$_2$CH$_2$CH_2CH$_2$Cl). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.80, 134.09, 129.69, 122.38, 69.86, 45.41, 38.08, 29.30, 27.26. **M/z (MALDI-TOF)** 804.3 m/z $[M + NH_4]^+$. **IR** *ν* **=** 2954; 2867; 1742; 1585; 1453; 1381; 1245; 1205; 1089; 1045; 1025.

25,26,27,28-Chlorohexoxy-calix[4]arene (33)

Cs_2CO_3 (12 g, 36.8 mmol) was added to a stirred solution of **2** (2 g, 4.6 mmol) in acetone (150 ml) at 56 ^{o}C. After 30 minutes 1-bromo-4-chlorobutane (5.47 mL, 36.8 mmol) was added and the reaction was heated at reflux for 12 hours. The solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with water. The organic layer was separated, washed with 10% HCl (2 x 100 mL) and with brine (2 x 100 ml), dried over dried over MgSO and the solvent was evaporated to give an yellow oil. Purification by column chromatography over silica gel (DCM:*n*-hexane=1:1) and re-crystallisation from DCM:EtOH gave the desired pure compound as white crystals (2.03g, 50 % yield). **Mp** = 111-113 ^{o}C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.01 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.72 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.68 (s, 8H, ArCH_2Ar), 3.64 – 3.50 (m, 16H, OCH_2CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$Cl and OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH_2Cl), 1.90 – 1.78 (m, 8H, OCH$_2$CH_2CH$_2$CH$_2$CH$_2$CH$_2$ Cl), 1.54 – 1.36 (m, 16H, OCH$_2$CH$_2$CH$_2$CH$_2$CH_2CH$_2$ Cl and OCH$_2$CH$_2$CH_2CH$_2$CH$_2$CH$_2$ Cl), 1.42 – 1.22 (m, 8H, OCH$_2$CH$_2$CH$_2$CH_2CH$_2$CH$_2$ Cl). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.66, 133.87, 129.70, 121.73, 71.41, 45.17, 37.02, 32.76, 29.95, 27.07, 25.42. **M/z (MALDI-TOF)** 916.4 m/z $[M + NH_4]^+$.

25,26,27,28-Azabutoxycalix[4]arene (1,3 alternate) (34)

NaN_3 (4.0 g, 61.54 mmol) was added to a stirred solution of **32** (2.0 g, 2.56 mmol) in DMF (100 mL) and heated for 24 hours at 70 ^{o}C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound as white solid (2.08 g, 88% yield). **Mp** = 120-122 ^{o}C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.03 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.81 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.77 (s, 8H, ArCH_2Ar), 3.49 (t, *J* = 6.9 Hz, 8H, OCH_2CH$_2$CH$_2$CH$_2$N$_3$), 3.21 (t, *J* = 6.6 Hz, 8H, OCH$_2$CH_2CH$_2$CH$_2$ N$_3$), 1.52 – 1.35 (m, 16H, OCH$_2$CH_2CH$_2$CH$_2$ N$_3$ and OCH$_2$CH$_2$CH_2CH$_2$ N$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.78, 134.05, 129.68, 122.23, 70.11, 51.51, 37.93, 27.09, 25.47. **M/z (MALDI-TOF)** 835.4 m/z $[M + Na]^+$. **IR** *ν* **=** 3064; 2934; 2864; 2097; 1582; 1445; 1430; 1385; 1373; 1244; 1182; 1086; 1076; 1044; 1025.

25,26,27,28-Azahexoxycalix[4]arene (35)

NaN_3 (2.6 g, 40.08 mmol) was added to a stirred solution of **33** (1.5 g, 1.67 mmol) in DMF (100 mL) and heated for 24 hours at 70 °C. The reaction was cooled, quenched with H_2O (50 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layer was dried over $MgSO_4$ and the excess of solvent was removed under reduced pressure to yield the pure compound as a colourless oil (1.4 g, 91%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.99 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.69 (t, *J* = 7.5 Hz, 4H, Ar*H*), 3.65 (s, 8H, ArCH_2Ar), 3.53 (t, *J* = 7.2 Hz, 8H, OC$H_2$$CH_2CH_2CH_2$-$CH_2CH_2N_3$), 3.31 (t, *J* = 6.9 Hz, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2N_3$), 1.73 – 1.58 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2N_3$), 1.57 – 1.45 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2N_3$), 1.41 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2N_3$), 1.36 – 1.20 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2N_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.67, 133.88, 129.77, 121.76, 71.63, 51.59, 36.91, 30.08, 29.07, 26.96, 25.77. **M/z (MALDI-TOF)** 947.5 m/z $[M + Na]^+$. **IR** *v* = 3063; 2935; 2856; 2090; 1582; 1446; 1428; 1379; 1348; 1242; 1191; 1183; 1087; 1077; 1037.

5, 11, 17, 23-*p*-*Tert*-butyl-25,26,27,28-tetrapropoxycalix[4]arene (36)[86]

NaH (7.36 g, 306.67 mmlol) was added to a stirred solution of **1** (25.00 g, 38.34 mmol) in DMF (400 mL). After 1 h *n*-PrBr (41.83 mL, 460.13 mmol) was added. The resulting solution was stirred for 5 days. H_2O (150 mL) was added and the resultant precipitate collected, dissolved in a minimum of DCM and precipitated by the addition of MeOH to yield (5, 11, 17, 23-*p*-*Tert*-butyl-25,26,27,28-tetrapropoxycalix[4]arene) (23.36 g, 75%) as a white powder. **Mp** 242-243 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.77 (s, 8H, Ar*H*), 4.42 (d, *J* = 12.5 Hz, 4H, ArCH_2Ar), 3.81 (t, *J* = 7.7 Hz, 8H, OC$H_2$$CH_2CH_3$), 3.11 (d, *J* = 12.5 Hz, 4H, ArCH_2Ar), 2.13 – 1.85 (m, 8H, $OCH_2CH_2CH_3$), 1.08 (s, 36H, $C(CH_3)_3$), 1.00 (t, *J* = 7.5 Hz, 12 H, $OCH_2CH_2CH_3$).

5,11,17,23-Tetranitro-25,26,27,28-tetrapropoxycalix[4]arene (37) [136, 160]

A mixture of 15 mL of glacial acetic acid and 15 mL of fuming nitric acid was added to a stirred solution of **37** (2.13 g, 2.81 mmol) in DCM (90 mL) The initially dark solution turned, after 30 minutes, to a bright yellow colour. The reaction mixture was washed several times with water until a neutral pH was achieved. The organic layer was dried over $MgSO_4$, and the solvent removed under reduced pressure. The resulting yellow solid was re-crystallized from DCM/MeOH giving the desired compound. (1.9 g, 88% yield). **Mp** 223-224 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.57 (s, 8H, Ar*H*), 4.53 (d, *J* = 14.0 Hz, 4H, ArCH_2Ar), 3.96 (t, *J* = 7.5 Hz, 8H, OC$H_2$$CH_2CH_3$), 3.40 (d, *J* = 14.0 Hz, 4H, ArCH_2Ar), 1.99 – 1.78 (m, 8H, $OCH_2CH_2CH_3$), 1.02 (t, *J* = 7.4 Hz, 12H, $OCH_2CH_2CH_3$).

5,11,17,23-Tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (38)[147]

Tin(II)chloride di-hydrate (10.5 g, 46.6 mmol) was added to a stirred solution of **37** (1.5 g, 1.94 mmol) in EtOH (100 mL). The mixture was heated at reflux temperature for 8 hours, cooled and the solvent removed under reduced pressure. The pale yellow solid was triturated and 10% $NaOH_{aq}$ (200 mL) was added. The resulting suspension was extracted with DCM (3 x 50 mL). The organic layer was separated, dried over $MgSO_4$ and the solvent removed under reduced pressure to give the desired compound as a brown vitreous solid (1.24 g, 98% yield). **Mp** > 250 °C (decomp.) **^{1}H NMR** (300 MHz, CDCl3) δ 6.06 (s, 8H, Ar*H*), 4.31 (d, *J* = 13.3 Hz, 4H, ArC*H*$_2$Ar), 3.72 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_3$), 2.91 (d, *J* = 13.3 Hz, 4H, ArC*H*$_2$Ar), 1.99 – 1.73 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 0.94 (t, *J* = 7.4 Hz, 12H, OCH$_2$CH$_2$C*H*$_3$).

5,11,17,23-Tetraazido-25,26,27,28-tetrapropoxycalix[4]arene (39)

$NaNO_2$ (441.6 mg 6.4 mmol) was added to a stirred solution of **38** in 10% HCl (100 mL) at 0^{o}C. After 20 minutes a solution of NaN_3 (325 mg, 5 mmol) in H_2O (5 mL) was added drop wise and stirred for a further 90 minutes. The formation of N_2 gas was observed. The reaction was extracted with DCM (3x50 mL). The organic layer was separated and washed with brine (2 x 50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by column chromatography over silica gel (DCM:*n*-hexane=1:1) gave the desired pure compound as off white solid (323 mg, 42 % yield). **Mp** 181-183. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.30 (s, 8H, Ar*H*), 4.41 (d, *J* = 13.6 Hz, 4H, ArC*H*$_2$Ar), 3.79 (t, *J* = 7.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_3$), 3.10 (d, *J* = 13.6 Hz, 4H, ArC*H*$_2$Ar), 1.95 – 1.79 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 0.98 (t, *J* = 7.4 Hz, 12H, OCH$_2$CH$_2$C*H*$_3$). **IR** *ν* = 2964; 2934; 2873; 2340; 2103; 1586; 1463; 1435; 1382; 1288; 1262; 1236; 1216; 1158; 1113; 1062.

5,11,17,23-*p*-*Tert*-butyl-25,26,27-tripropoxy-28-hydroxycalix[4]arene (40)[85]

BaO (17.15 g, 46.78 mmol) and $Ba(OH)_2$.8 H_2O (33.82 g, 107.35 mmol) were added to a stirred solution of **1** (20 g, 30.67 mmol) in DMF (500 mL) at 30 ^{o}C. After 15 minutes *n*-bromopropane (59.80 mL, 650 mmol) was added to the solution. After 48 hours the reaction was diluted with H_2O (300 mL) and DCM (300 mL). The separated organic layer was washed with H_2O (1 x 100 mL), 10% HCl (2 x 100 mL) and brine (1 x 100 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was precipitated from DCM:MeOH to give the desired product (17.4 g, 73%). **Mp** 114-115 °C; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.16 (2H, s, Ar*H*), 7.07 (2H, s, Ar*H*), 6.53 (4H, s, Ar*H*), 4.38 (2H, d, *J* =12.8 Hz, ArC*H*$_2$Ar), 4.35 (2H, d, *J* =12.8 Hz, ArC*H*$_2$Ar), 3.86 (2H, t, *J* = 7.4 Hz, OC*H*$_2$CH$_2$CH$_3$), 3.77 (4H, t, *J* = 7.4 Hz, OC*H*$_2$CH$_2$CH$_3$),3.25 (2H, d, *J* =12.8 Hz, ArC*H*$_2$Ar), 3.18 (2H, d, *J* =12.8 Hz, ArC*H*$_2$Ar), 2.35 (2H, m, OCH$_2$C*H*$_2$CH$_3$), 1.93 (4H, m, OCH$_2$C*H*$_2$CH$_3$), 1.36 (9H, s, (C*H*$_3$)$_3$Ar),

1.34 (9H, s, (C*H*$_3$)$_3$Ar), 1.11 (6H, t, *J* = 7.4 Hz, OCH$_2$CH$_2$C*H*$_3$), 0.97 (3H, t, *J* = 7.4 Hz, OCH$_2$CH$_2$C*H*$_3$), 0.84 (9H, s, (C*H*$_3$)$_3$Ar).

5,11,17,23-*p*-*Tert*-butyl-25,26,27-tripropoxy-28-propargyloxycalix[4]arene (41)[139]

NaH (95%, 2.58 g, 107.94 mmol) was added to a stirred solution of **40** (21 g, 26.94 mmol) in DMF (450 mL). After 30 minutes propargyl bromide (80%, 11.99, 107.94 mmol) was added drop wise and the resulting solution was stirred for 24 hours. DCM (500 mL) was added and the mixture was washed with H_2O (2 x 500 mL) and then with brine (2 x 500 mL). The organic layer was dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude material was dissolved in DCM and precipitated with MeOH to yield the compound as a off white powder (20 g, 91 %). **Mp** 138-139 °C; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.01 (4H, s, Ar*H*), 6.57 (2H, s, *J* =2.4 Hz, Ar*H*), 6.51 (2H, s, *J* =2.4 Hz, Ar*H*), 4.97 (2H, d, *J* =2.4 Hz, OC*H*$_2$CCH), 4.50 (2H, d, *J* =12.8 Hz ArC*H*$_2$Ar), 4.42 (2H, d, *J* =12.4 Hz, ArC*H*$_2$Ar), 3.90 (2H, t, *J* = 8.04 Hz, OC*H*$_2$CH$_2$CH$_3$), 3.73 (4H, t, *J* = 7.6 Hz, OC*H*$_2$CH$_2$CH$_3$),3.13 (2H, d, *J* =12.8 Hz, ArC*H*$_2$Ar), 3.12 (2H, d, *J* = 12.4 Hz, ArC*H*$_2$Ar), 2.37 (1H, , OCH$_2$CC*H*), 2.21 – 2.05 (2H, m, OCH$_2$C*H*$_2$CH$_3$), 2.05 – 1.87 (4H, m, OCH$_2$C*H*$_2$CH$_3$), 1.27 (9H, s, (C*H*$_3$)$_3$Ar), 1.26 (9H, s, (C*H*$_3$)$_3$Ar), 1.03 (6H, m, OCH$_2$CH$_2$C*H*$_3$), 0.96 (3H, t, *J* = 7.5 Hz, OCH$_2$CH$_2$C*H*$_3$), 0.89 (18H, s, (C*H*$_3$)$_3$Ar).

5,11,17,23-*p*-*Tert*-butyl-25,26,27-tripropoxy-28-(3-tert-Butyldimethylsilyl)-2-propynoxycalix[4]arene (42)

Lithium bis(trimethylsilyl)amide (1M in THF, 4.2 mL, 4.2 mmol) was added drop wise to a solution at -78 ^{o}C of **41** (3.2 g, 4 mmol) in THF (50 mL). After 30 minutes a solution of *tert*-butyldimethylsilyl chloride (0.90 g, 6 mmol in 5 mL of THF) was added and the reaction was stirred at room temperature for 16 hours. The reaction was diluted with EtOAc (20 mL) and washed with NH_4Cl_{sat} (2 x 30 mL) and with brine (2 x 30 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was dissolved in DCM (50 mL) and precipitated with MeOH to yield the title compound as white crystals (3.9 g, 97%). **Mp** 157-159 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.06 (s, 2H, Ar*H*), 7.01 (s, 2H, Ar*H*), 6.53 (d, *J* = 2.4 Hz, 2H, Ar*H*), 6.46 (d, *J* = 2.4 Hz, 2H, Ar*H*), 5.07 (s, 2H, OC*H*$_2$CCSi(CH$_3$)$_2$C(CH$_3$)$_3$), 4.47 (d, *J* = 13 Hz, 2H, ArC*H*$_2$Ar), 4.42 (d, *J* = 12 Hz, 2H, ArC*H*$_2$Ar), 3.99 – 3.87 (m, 2H, OC*H*$_2$CH$_2$CH$_3$), 3.68 (m, 4H, OC*H*$_2$CH$_2$CH$_3$), 3.12 (d, *J* = 12 Hz, 4H, ArC*H*$_2$Ar), 3.10 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 2.19 – 1.81 (m, 6H, OCH$_2$C*H*$_2$CH$_3$), 1.30 (s, 9H, C*H*$_3$CAr), 1.28 (s, 9H, C*H*$_3$CAr), 1.07 (t, *J* = 7.4 Hz, 6H, OCH$_2$CH$_2$C*H*$_3$), 0.96 (t, *J* = 7.5, 3.7 Hz, 3H, OCH$_2$CH$_2$C*H*$_3$), 0.86 (s, 18H, C*H*$_3$CAr), 0.82 (s, 9H, OCH$_2$CCSi(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.05 – -0.01 (m, 6H, OCH$_2$CCSi(C*H*$_3$)$_2$C(CH$_3$)$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 154.56, 153.22, 151.73, 145.34, 144.75, 144.13, 136.69, 135.58, 132.62, 132.27, 125.46, 125.10, 124.64, 124.46, 104.31, 89.73, 59.47, 34.16, 33.73, 32.37, 31.87, 31.81,

31.34, 31.11, 26.13, 23.77, 23.63, 16.56, 10.89, 10.21, -4.58. **M/z (MALDI-TOF)** 944.6936 m/z $[M + NH_4]^+$; **IR** *v* = 2957; 2874; 2175; 1582; 1469; 1248; 1239; 1195; 1120; 1105; 1070; 1044; 1008.

5,11,17,23-Tetra-nitro-25,26,27-tripropoxy-28-(3-tert-Butyldimethylsilyl)-2-propynoxycalix[4]arene (43)

A mixture of 30 mL of glacial acetic acid and 30 mL of fuming nitric acid was added to a stirred solution of **42** (5.2 g, 5.62 mmol) in DCM (180 mL) When the initial dark solution turned, after 30 minutes, to a bright yellow color the reaction was quenched with H_2O (200 mL). The separated organic layer was washed several times with water until a neutral pH was achieved. The organic layer was dried over $MgSO_4$, and the solvent removed under reduced pressure. The resulting crude material was re-crystallized from DCM/MeOH giving the desired compound as a yellow solid (4.9 g, 88%). **Mp** 243-246 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.07 (s, 2H, Ar*H*), 8.06 (s, 2H, Ar*H*), 7.15 (s, 4H, Ar*H*), 5.00 (s, 2H, OCH_2CCSi$(CH_3)_2$C$(CH_3)_3$), 4.57 (d, *J* = 14 Hz, 2H, ArCH_2Ar), 4.54 (d, *J* = 14 Hz, 2H, ArCH_2Ar), 4.16 – 4.08 (m, 2H, OC$H_2$$CH_2CH_3$), 3.86 – 3.78 (m, 4H, OC$H_2$$CH_2CH_3$), 3.41 (d, *J* = 14.0, 2H, ArCH_2Ar), 3.40 (d, *J* = 14.0, 2H, ArCH_2Ar), 2.01 – 1.83 (m, 6H, $OCH_2CH_2CH_3$), 1.10 (t, *J* = 7.4 Hz, 6H, $OCH_2CH_2CH_3$), 0.97 (t, *J* = 7.4 Hz, 3H, $OCH_2CH_2CH_3$), 0.80 (s, *J* = 2.9 Hz, 9H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.02 (s, 6H, $OCH_2CCSi(CH_3)_2C(CH_3)$). **^{13}C NMR** (101 MHz, CDCl3) δ 162.80, 160.81, 159.81, 143.82, 143.17, 138.35, 136.62, 134.62, 134.06, 124.91, 124.52, 123.50, 123.40, 99.93, 93.51, 78.19, 78.03, 60.80, 31.68, 31.11, 25.87, 23.57, 23.36, 16.40, 10.66, 10.05, -4.78. **M/z (MALDI-TOF)** 883.3586m/z $[M + H]^+$; **IR** *v* = 3077; 2958; 2175; 2925; 2877; 2175; 1585; 1523; 1447; 1343; 1262; 1201; 1095.

5,11,17,23-Tetra-amino-25,26,27-tripropoxy-28-(3-tert-Butyldimethylsilyl)-2-propynoxycalix[4]arene (44)

Tin(II)chloride di-hydrate (28 g, 124.6 mmol) was added to a stirred solution of **43** (4 g, 4.45 mmol) in EtOH (200 mL). The mixture was heated at reflux for 8 hours, cooled and the solvent removed under reduced pressure. The pale yellow solid was triturated with 10% NaOH and extracted with DCM. After separation the organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to give the desired compound as a brown vitreous solid (3.23 g, 95%). **Mp** 157-159 °C; **^{1}H NMR** (400 MHz, MeOD) δ 6.56 (s, 2H, Ar*H*), 6.53 (s, 2H, Ar*H*), 6.00 (s, 4H, Ar*H*), 4.76 (s, 2H, OCH_2CCSi$(CH_3)_2$C$(CH_3)_3$), 4.45 (d, *J* = 13.5 Hz, 2H, ArCH_2Ar), 4.39 (d, *J* = 13.5 Hz, 2H, ArCH_2Ar), 3.88 (t, *J* = 7.7, 2H, OC$H_2$$CH_2CH_3$), 3.66 (t, *J* = 6.6 Hz, 4H, OC$H_2$$CH_2CH_3$), 3.01 (d, *J* = 13.5 Hz, 4H, ArCH_2Ar), 1.94 – 1.83 (m, 6H, $OCH_2CH_2CH_3$), 1.10 (t, *J* = 7.4 Hz, 6H, $OCH_2CH_2CH_3$), 0.94 (t, *J* = 7.5 Hz, 2H, $OCH_2CH_2CH_3$), 0.88 (s, 9H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.06 (s, 6H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$). **^{13}C NMR** (101 MHz,

MeOD) δ 153.71, 152.85, 149.45, 142.04, 141.96, 139.51, 138.29, 137.02, 136.51, 134.43, 134.39, 119.59, 119.53, 118.55, 117.92, 104.71, 90.43, 78.16, 77.98, 61.03, 32.62, 31.95, 26.55, 24.61, 24.40, 17.28, 11.44, 10.61, -4.40. **M/z (MALDI-TOF)** 763.4613 m/z $[M + H]^+$; **IR** *ν* **=** 3347; 2958; 2360; 1735; 1607; 1465; 1384; 1247; 1214; 1005.

5,11,17,23-Tetra-Boc-amino-25,26,27-tripropoxy-28-(3-tert-Butyldimethylsilyl)-2-propynoxycalix[4]arene (45)

Di-*tert*-butyl dicarbonate (14.6 g, 67.1 mmol) and diisopropylethylamine (11.66 mL, 67.10 mmol) were added to a stirred solution at 0 ^oC of **44** (6.4 g, 8.3 mmol) in DCM (150 mL). The reaction was stirred for 8 h under argon atmosphere. The solution was quenched with H_2O and the separated organic layer was washed with 10% HCl (2 x 50 mL) and with brine (50 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by column chromatography over silica gel (DCM:MeOH=15:1) yielded the pure product as a pale yellow solid (7.04 mg, 73%). **^{1}H NMR** (400 MHz, MeOD) δ 7.09 (s, 4H, Ar*H*), 6.42 (s, 4H, Ar*H*), 5.09 (s, 2H, OC*H*$_2$CCSi(CH$_3$)$_2$C(CH$_3$)$_3$), 4.47 (d, *J* = 13.0 Hz, 2H, ArC*H*$_2$Ar), 4.43 (d, *J* = 13.0 Hz, 2H, ArC*H*$_2$Ar), 4.01 – 3.93 (m, 2H, OC*H*$_2$CH$_2$CH$_3$), 3.72 – 3.56 (m, 4H, OC*H*$_2$CH$_2$CH$_3$), 3.08 (d, *J* = 13.0 Hz, 4H, ArC*H*$_2$Ar), 2.14 – 1.80 (m, 6H, OCH$_2$C*H*$_2$CH$_3$), 1.54 (s, 18H, OC(C*H*$_3$)$_3$), 1.41 (s, 18H, OC(C*H*$_3$)$_3$), 1.11 (t, *J* = 7.5 Hz, 6H, OCH$_2$CH$_2$C*H*$_3$), 0.97 (t, *J* = 7.5 Hz, 3H, OCH$_2$CH$_2$C*H*$_3$), 0.85 (s, 9H, OCH$_2$CCSi(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.03 (s, 6H, OCH$_2$CCSi(C*H*$_3$)$_2$C(CH$_3$)$_3$). **^{13}C NMR** (101 MHz, MeOD) δ 155.99, 155.73, 155.70, 154.28, 152.91, 151.36, 138.86, 137.46, 135.16, 134.71, 134.35, 134.27, 133.92, 121.54, 121.10, 120.90, 120.78, 104.74, 90.97, 80.65, 80.59, 80.49, 78.47, 78.14, 60.57, 33.04, 31.89, 28.86, 28.85, 28.82, 26.54, 24.69, 24.48, 17.22, 11.38, 10.54, -4.40. **M/z (MALDI-TOF)** 1180.6 m/z $[M + NH_4]^+$; **IR** *ν* **=** 3318; 2961; 2928; 1703; 1601; 1519; 1470; 1366; 1245; 1151; 1063; 1033; 1003.

5,11,17,23- Tetra-Boc-amino -25,26,27-tripropoxy-28-propargyloxycalix[4]arene (46)

Tetra-*n*-butylammonium fluoride solution in THF (1 M, 30.6 mL, 30.6 mmol) was added to a stirred solution of **45** (7.12 g, 6.12 mmol) in THF (60 mL). After 8 h the reaction was diluted with EtOAc (50 mL) and quenched with NH_4Cl saturated solution (100 mL). The separated organic layer was washed with NH_4Cl saturated solution (50 mL) and brine (2 x 50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by column chromatography over silica gel (*n*-hexane:DCM:MeOH=10:10:0.5) yielded the pure product as a pale yellow solid (4 g, 62%). **^{1}H NMR** (400 MHz, MeOD) δ 7.06 (s, 4H, Ar*H*), 6.43 (s, 4H, Ar*H*), 4.98 (d, *J* = 2 Hz, 2H, OC*H*$_2$CCH), 4.51 (d, *J* = 13 Hz, 2H, ArC*H*$_2$Ar), 4.44 (d, *J* = 13 Hz, 2H, ArC*H*$_2$Ar), 3.97 (t, *J* = 8 Hz, 2H, OC*H*$_2$CH$_2$CH$_3$), 3.69 (t, *J* = 7 Hz, 4H, OC*H*$_2$CH$_2$CH$_3$), 3.09 (d, *J* = 13 Hz, 2H, ArC*H*$_2$Ar), 3.09 (d, *J* = 13 Hz, 2H, ArC*H*$_2$Ar), 2.72 (t, *J* = 2 Hz, 1H, OCH$_2$CC*H*), 2.10 – 1.86 (m, 6H, OCH$_2$C*H*$_2$CH$_3$), 1.53 (s, 9H, OC(C*H*$_3$)$_3$), 1.53 (s, 9H,

$OC(CH_3)_3$), 1.42 (s, 18H, $OC(CH_3)_3$), 1.10 (t, *J* = 7.4 Hz, 6H, $OCH_2CH_2CH_3$), 0.98 (t, *J* = 7.5 Hz, 3H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 156.00, 155.83, 155.75, 154.25, 153.02, 151.83, 138.50, 137.37, 135.07, 134.75, 134.52, 134.30, 133.91, 121.55, 121.09, 121.00, 81.73, 80.66, 80.51, 78.44, 78.10, 76.32, 60.59, 32.74, 31.93, 28.82, 24.60, 24.45, 11.27, 10.53. **M/z (MALDI-TOF)** 1180.6 m/z $[M + NH_4]^+$; **IR** *ν* = 3308; 2974; 2930; 2360; 1699; 1600; 1519; 1471; 1415; 1390; 1366; 1213; 1151; 1064; 1004.

5,11,17,23-Tetra-Boc-Glycine-25,26,27-tripropoxy-28-(3-tert-Butyldimethylsilyl)-2-propynoxycalix[4]arene (47)

A solution of Boc glycine (2.1 g, 12 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (2.48 g, 16 mmol) in DCM (100 mL) was stirred at 0°C for 40 minutes before the addition of a solution of **44** (1.53 g, 2 mmol) in DCM (50 mL). After 24 hours the reaction mixture was quenched with H_2O (100 mL), the separated organic layer was washed with 10% HCl (2 x 100 mL) and with brine (1 x 100 mL), dried over $MgSO_4$ and the excess of solvent was evaporated under reduced pressure. Purification by column chromatography over silica gel (EtOAc:*n*Hexane:MeOH=1:1:0.1) yielded the desired compound as an off white solid (2.02 g, 72%). **^{1}H NMR** (400 MHz, MeOD) δ 7.29 (s, 2H, Ar*H*), 7.28 (s, 2H, Ar*H*), 6.57 (s, 2H, Ar*H*), 6.53 (s, 2H, Ar*H*), 5.09 (s, 2H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 4.50 (d, *J* = 13 Hz, 2H, $ArCH_2Ar$), 4.47 (d, *J* = 13 Hz, 2H, $ArCH_2Ar$), 4.01 (t, *J* = 8.0 Hz, 2H, $OCH_2CH_2CH_3$), 3.87 (s, 4H, $ArNHCOCH_2NHCOOC(CH_3)_3$), 3.75 – 3.66 (m, 4H, $OCH_2CH_2CH_3$), 3.64 (s, 4H, $ArNHCOCH_2NHCOOC(CH_3)_3$), 3.12 (d, *J* = 13.0 Hz, 4H, $ArCH_2Ar$), 2.09 – 1.84 (m, 6H, $OCH_2CH_2CH_3$), 1.49 (s, 18H, $OC(CH_3)_3$), 1.44 (s, 18H, $OC(CH_3)_3$), 1.12 (t, *J* = 7.5 Hz, 6H, $OCH_2CH_2CH_3$), 0.97 (t, *J* = 7.5 Hz, 3H, $OCH_2CH_2CH_3$), 0.84 (s, 9H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.02 (s, 6H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$). **^{13}C NMR** (101 MHz, MeOD) δ 170.17, 170.04, 169.71, 158.50, 153.77, 152.27, 139.11, 137.61, 134.94, 134.58, 134.47, 133.23, 122.20, 121.58, 121.48, 104.37, 91.28, 80.70, 78.50, 78.28, 60.63, 45.03, 44.73, 32.95, 31.91, 28.77, 26.49, 24.69, 24.47, 17.21, 11.33, 10.46, -4.46. . **M/z (MALDI-TOF)** 1413.7 m/z $[M + Na]^+$; **IR** *ν* = 3307; 2930; 1667; 1600; 1470; 1418; 1391; 1366; 1248; 1213; 1163; 1028.

5,11,17,23-Tetra-Boc-Glycine-25,26,27-tripropoxy-28- propargyloxycalix[4]arene (48)

Tetra-*n*-butylammonium fluoride solution in THF (1 M, 8.36 mL, 8.36 mmol) was added to a stirred solution of **44** (2.35 g, 1.67 mmol) in THF (16 mL) at reflux temperature. After 8 h the reaction was diluted with EtOAc (25 mL) and quenched with NH_4Cl saturated solution (50 mL). The separated organic layer was washed with NH_4Cl saturated solution (25 mL) and brine (2 x 25 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by column chromatography over silica gel (EtOAc:*n*Hexane:MeOH=1:1:0.1) yielded the pure

product as a pale yellow solid (1.78 g, 83.5%). **Mp**>186 (decompose) ^{o}C; **^{1}H NMR** (400 MHz, MeOD) δ 7.25 (s, 2H, Ar*H*), 7.24 (s, 2H, Ar*H*), 6.58 (s, 2H, Ar*H*), 6.56 (s, 2H, Ar*H*), 4.96 (s, 2H, OCH_2CCH), 4.53 (d, *J* = 13 Hz, 2H, ArCH_2Ar), 4.47 (d, *J* = 13 Hz, 2H, ArCH_2Ar), 4.00 (t, *J* = 8.0 Hz, 2H, OCH_2CH$_2$CH$_3$), 3.85 (s, 4H, ArNHCOCH_2NHCOOC(CH$_3$)$_3$), 3.71 (t, *J* = 6.7 Hz, 4H, OCH_2CH$_2$CH$_3$), 3.65 (s, 4H, ArNHCOCH_2NHCOOC(CH$_3$)$_3$), 3.12 (bd, *J* = 13.0 Hz, 4H, ArCH_2Ar), 2.75 (t, *J* = 2.3 Hz, 1H, OCH$_2$CC*H*), 2.08 – 1.86 (m, 6H, OCH$_2$CH_2CH$_3$), 1.49 (s, 18H, OC(CH_3)$_3$), 1.45 (s, 18H, OC(CH_3)$_3$), 1.11 (t, *J* = 7.4 Hz, 6H, OCH$_2$CH$_2$CH_3), 0.97 (t, *J* = 7.5 Hz, 3H, OCH$_2$CH$_2$CH_3). **^{13}C NMR** (101 MHz, MeOD) δ 170.11, 169.70, 158.44, 155.16, 153.84, 152.75, 138.64, 137.45, 134.93, 134.69, 134.49, 133.67, 133.20, 122.14, 121.72, 121.60, 81.44, 80.70, 78.45, 78.17, 76.57, 60.72, 45.02, 44.75, 32.65, 31.95, 28.78, 24.58, 24.42, 11.26, 10.49. **M/z (MALDI-TOF)** 1294.7 m/z [M + NH$_4$]$^+$; **IR** *v* **=** 3307; 2963; 2919; 2849; 2363; 1670; 1600; 1516; 1471; 1418; 1365; 1278; 1247; 1214; 1162; 1052, 1007.

5,11,17,23- Tetra-amino-25,26,27-tripropoxy-28-propargyloxycalix[4]arene (49)

Tetra-*n*-butylammonium fluoride solution in THF (1 M, 5 mL, 5 mmol) was added to a stirred solution of **44** (763 mg, 1 mmol) in THF (10 mL). After 8 h the reaction was diluted with EtOAc (20 mL) and quenched with NH$_4$Cl saturated solution (50 mL). The separated organic layer was washed with NH$_4$Cl saturated solution (25 mL) and brine (2 x 25 mL), dried over MgSO$_4$ and the solvent removed under reduced pressure to yield the desired product (408 mg, 63%), which was used in the following step without further purification. **Mp** > 160 ^{o}C (decompose); **^{1}H NMR** (300 MHz, CDCl$_3$) δ 6.42 (s, 2H), 6.41 (s, 2H), 5.81 (s, 2H), 5.80 (s, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 4.38 (d, *J* = 13.5 Hz, 2H), 4.31 (d, *J* = 13.1 Hz, 2H), 3.86 (t, *J* = 7.9 Hz, 2H), 3.60 (d, *J* = 13.8 Hz, 4H), 3.22 (s, 8H), 2.94 (d, *J* = 13.8 Hz, 2H), 2.92 (d, *J* = 13.1 Hz, 2H), 2.31 (t, *J* = 2.4 Hz, 1H), 1.97 – 1.74 (m, 6H), 1.03 (t, *J* = 7.4 Hz, 6H), 0.89 (t, *J* = 7.5 Hz, 3H). **M/z (MALDI-TOF** 649.3745 m/z [M + NH$_4$]$^+$; **IR** *v* **=** 3290; 2966; 2878; 2160; 1584; 1515; 1452; 1340; 1303; 1262; 1207; 1091; 1059.

5,11,17,23-Tetra-bis-boc-guanidinium-25,26,27-tripropoxy-28- propargyloxycalix[4]arene (50)

1,3-Bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (643 mg, 2.22 mmol), HgCl$_2$ (601 mg, 2.22 mmol) and Et$_3$N (0.768 mL, 5.52 mmol) were added to a stirred solution at 0 ^{o}C of **49** (300 mg, 0.46 mmol) in DMF (7 mL). After 8 h the black suspension was diluted with DCM (10 mL) and the solid was filtered off under reduced pressure. The excess of solvent was removed under reduced pressure. Purification by column chromatography over silica gel (*n*Hexane:Et$_2$O in gradient from 4:1 to 1:4) yielded the desired compound as an off white solid (431 mg, 58%). **^{1}H NMR** (300 MHz, CDCl$_3$) δ 11.69 (s, 1H, N*H*), 11.67 (s, 1H, N*H*), 11.52 (s, 2H, N*H*), 10.30 (s, 1H, N*H*), 10.21 (s, 1H, N*H*), 9.50 (s, 2H, N*H*), 7.33 (s, 2H, Ar*H*), 7.25 (s, 2H, Ar*H*), 6.71 (s, *J* =

2.3 Hz, 1H, Ar*H*), 6.71 (s, 1H, Ar*H*), 6.64 (s, 1H, Ar*H*), 6.63 (s, 1H, Ar*H*), 5.00 (d, *J* = 2.4 Hz, 2H, OCH_2CCH), 4.50 (d, *J* = 13.3 Hz, 2H, ArCH_2Ar), 4.42 (d, *J* = 12.5 Hz, 2H, ArCH_2Ar), 3.98 (t, *J* = 8.2, 2H, OCH_2CH_2CH_3), 3.66 (t, *J* = 7.1 Hz, 4H, OCH_2CH_2CH_3), 3.18 (d, *J* = 13.3 Hz, 2H, ArCH_2Ar), 3.17 (d, *J* = 12.5 Hz, 2H, ArCH_2Ar), 2.33 (t, *J* = 2.4 Hz, 1H, OCH_2CC*H*), 2.03 – 1.84 (m, 6H, OCH_2CH_2CH_3), 1.52 (s, 18H, OC(CH_3)$_3$), 1.50 (s, 9H, OC(CH_3)$_3$), 1.49 (s, 9H, OC(CH_3)$_3$), 1.45 (s, 18H, OC(CH_3)$_3$), 1.42 (s, 18H, OC(CH_3)$_3$), 1.03 (t, *J* = 7.4 Hz, 6H, OCH_2CH_2CH_3), 0.88 (t, *J* = 7.3 Hz, 3H, OCH_2CH_2CH_3). **M/z (MALDI-TOF** 1634.9 m/z [M + NH_4]$^+$; **IR** *v* **=** 3263; 2972; 2878; 1716; 1621; 1455; 1409; 1393; 1366; 1249; 1214, 1142; 1102; 1057; 1003.

General method for the synthesis of Boc-amino-Multicalixarenes

Generation 1 calixarene (4.5 eq) was added to a solution of central core (1 eq) in DMF (15 mL) in the presence of $CuSO_4 \cdot 5H_2O$ (26.1 mg, 0.105 mmol, 0.66 eq) and sodium ascorbate (208 mg, 1.05 mmol, 5 eq). The reaction was stirred for 2 hours at 110^{o}C. The solution was diluted with DCM (50 mL) and washed with H_2O (2 x 50 mL) and brine (2 x 50 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel, (DCM:EtOAc in gradient from 20:1 to 5:1) and precipitation from DCM/*n*-hexane yielded the desired compounds as white solids.

Multicalixarene (51)

Generation 1 = 46 (1 g, 0.95 mmol), Central core = **10** (140 mg, 0.2 mmol) Product = **51** (770 mg, 78.5%) **Mp** >213 ^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.95 (s, 4H, Az*H*), 6.85 (s, 16H, Ar*H*), 6.56 (s, 28H, Ar*H*), 5.02 – 4.91 (m, 16H, OCH_2CCAz and OCH_2CH_2Az), 4.51 – 4.24 (m, 24H, ArCH_2Ar and OCH_2CH_2Az), 4.11 (d, *J* = 13.5 Hz, 4H, ArCH_2Ar), 3.85 – 3.63 (m, 24H, OCH_2CH_2CH_3), 3.13 – 3.02 (m, 12H, ArCH_2Ar), 2.90 (d, *J* = 13 Hz, 8H, ArCH_2Ar), 2.04 – 1.71 (m, 24H, OCH_2CH_2CH_3), 1.49 (s, 72H, OC(CH_3)$_3$), 1.45 (s, 72H, OC(CH_3)$_3$), 1.04 (t, *J* = 7.5 Hz, 12H, OCH_2CH_2CH_3), 0.83 (t, *J* = 7.3 Hz, 24H, OCH_2CH_2CH_3). **^{13}C NMR** (101 MHz, MeOD) δ 155.91, 155.56, 154.14, 153.28, 151.56, 145.59, 136.67, 136.61, 134.55, 134.05, 133.85, 129.82, 126.13, 124.05, 121.50, 121.39, 120.94, 80.62, 78.22, 78.04, 51.67, 32.54, 32.11, 31.46, 24.59, 24.26, 11.28, 10.91. **M/z (MALDI-TOF)** 4920.7 m/z [M+Na]$^+$; **IR** *v* **=** 3330; 2973; 2930; 2874; 2360; 1706; 1596; 1520; 1476; 1365; 1214; 1152; 1043; 1001.

Multicalixarene (52)

Generation 1 = **46** (1 g, 0.95 mmol), **16** (170 mg, 0.2 mmol) Product = **52** (881 mg, 88%) **Mp** >180^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.81 (s, 4H, Az*H*), 6.80 (s, 16H, Ar*H*), 6.66 – 6.47 (m, 28H, Ar*H*), 4.99 (s, 8H, OCH_2CCAz), 4.51 (s, 8H,

$OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 4.42 – 4.34 (m, 12H, $ArCH_2Ar$), 4.26 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 3.94 (s, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 3.80 – 3.69 (m, 24H, $OCH_2CH_2CH_3$), 3.13 (d, *J* = 14.0 Hz, 4H, $ArCH_2Ar$), 3.05 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 2.90 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 2.14 – 1.73 (m, 48H, $OCH_2CH_2CH_3$, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$ and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.51 – 1.42 (m, 152H, $OC(CH_3)_3$, and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.04 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.85 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 157.51, 155.86, 155.57, 153.94, 153.38, 151.63, 145.31, 136.06, 134.59, 134.07, 133.92, 129.53, 125.91, 123.34, 121.34, 120.99, 80.59, 78.14, 78.06, 75.37, 54.80, 51.37, 32.50, 32.11, 28.95, 28.91, 28.85, 24.59, 24.27, 11.22, 10.93. **M/z (MALDI-TOF)** 5032.7 m/z $[M+Na]^+$; $]^+$; **IR** *ν* **=** 3325; 2972; 2931; 2873; 2363; 1712; 1596; 1519; 1474; 1366; 1215; 1153; 1062; 1001.

Multicalixarene (53)

Generation 1 = **46** (1 g, 0.95 mmol), **18** (194 mg, 0.2 mmol) Product **53** (626 mg, 61.4%) **Mp** >178^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.78 (s, 4H, Az*H*), 6.93 (d, *J* = 7.6 Hz, 8H, Ar*H*), 6.80 (bs, 16H, Ar*H*), 6.71 – 6.44 (m, 20H, Ar*H*), 5.07 (s, 8H, OCH_2CCAz), 4.47 – 4.35 (m, 16H, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$ and $ArCH_2Ar$), 4.30 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 3.77 (bs, 24H, $OCH_2CH_2CH_3$), 3.65 (bs, 8H, $ArCH_2Ar$), 3.42 – 3.35 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 3.05 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 2.96 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 2.05 – 1.79 (m, 32H, $OCH_2CH_2CH_3$ and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.47 (s, 72H, $OC(CH_3)_3$), 1.46 (s, 36H, $OC(CH_3)_3$), 1.45 (s, 36H, $OC(CH_3)_3$), 1.39 – 1.34 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.31 – 1.25 (m, 16H, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$ and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.02 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.91 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 158.07, 155.81, 155.65, 153.91, 153.30, 151.62, 145.27, 136.28, 135.72, 135.21, 134.63, 134.13, 133.97, 130.83, 125.73, 122.87, 121.26, 121.07, 80.58, 78.06, 72.04, 67.56, 54.80, 51.34, 38.39, 32.55, 32.13, 31.76, 30.94, 28.92, 28.89, 27.73, 26.65, 24.59, 24.29, 11.23, 10.98. **M/z (MALDI-TOF)** 5144.7 m/z $[M+Na]^+$; **IR** *ν* **=** 3324; 2973; 2930; 2873; 1716; 1595; 1519; 1474; 1366; 1291; 1214; 1153; 1063; 1002.

Multicalixarene (54)

Generation 1 = **46** (1 g, 0.95 mmol), Central core = **38** Product = **54** (770 mg, 78.5%) **Mp** >215 ^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.59 (s, 4H, Az*H*), 7.06 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.87 (t, *J* = 7.5 Hz, 4H, Ar*H*), 6.82 (bs, 16H, Ar*H*), 6.65 (bs, 8H, Ar*H*), 6.60 (bs, 8H, Ar*H*), 5.10 (s, 8H, OCH_2CCAz), 4.40 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 4.26 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 3.99 – 3.86 (m, 16H, OCH_2CH_2Az and $ArCH_2Ar$), 3.83 – 3.67 (m, 32H, OCH_2CH_2Az and $OCH_2CH_2CH_3$), 3.06 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 2.90 (d, *J* = 13.0 Hz, 8H, $ArCH_2Ar$), 2.00 – 1.82 (m, 24H, $OCH_2CH_2CH_3$), 1.49 (s, 72H, $OC(CH_3)_3$), 1.46 (s, 36H,

OC(CH_3)$_3$), 1.42 (s, 36H, OC(CH_3)$_3$), 1.05 (t, *J* = 7.4 Hz, 12H, OCH$_2$CH$_2$CH_3), 0.88 (t, *J* = 7.3 Hz, 24H, OCH$_2$CH$_2$CH_3); **^{13}C NMR** (101 MHz, MeOD) δ 156.97, 155.86, 155.66, 153.96, 153.31, 151.35, 145.23, 136.48, 136.30, 135.54, 134.71, 134.10, 133.93, 130.80, 126.20, 121.31, 121.00, 80.59, 78.18, 78.02, 50.19, 38.55, 32.60, 32.09, 28.91, 24.60, 24.26, 11.22, 10.91; **M/z (MALDI-TOF)** 4920.7 m/z [M+Na]$^+$; **IR** *v* = 3326; 2973; 2930; 2874; 2358; 1704; 1596; 1520; 1470; 1416; 1366; 1242; 1214; 1153; 1061; 1001.

Multicalixarene (55)

Generation 1 = **46** (1 g, 0.95 mmol), Central core = **34**(170 mg, 0.2 mmol) Product = **55** (714 mg, 71.4%) **Mp** >180^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.78 (s, 4H, Az*H*), 6.96 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.77 (s, 16H, Ar*H*), 6.72 – 6.58 (m, 20H, Ar*H*), 5.16 (s, 8H, OCH_2CCAz), 4.41 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 4.35 – 4.26 (m, 16H, ArCH_2Ar and OCH$_2$CH$_2$CH$_2$CH_2Az), 3.85 – 3.72 (m, 32H, OCH_2CH$_2$CH$_3$ and ArCH_2Ar), 3.45 – 3.36 (m, 8H, OCH_2CH$_2$CH$_2$CH$_2$Az), 3.06 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.95 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.02 – 1.85 (m, 24H, OCH$_2$CH_2CH$_3$), 1.73 – 1.60 (m, 8H, OCH$_2$CH$_2$CH_2CH$_2$Az), 1.47 (s, 108H, OC(CH_3)$_3$), 1.43 (s, 36H, OC(CH_3)$_3$), 1.30 – 1.19 (m, 8H, OCH$_2$CH_2CH$_2$CH$_2$Az), 1.03 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$CH_3), 0.92 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$CH_3); **^{13}C NMR** (101 MHz, MeOD) δ 158.17, 155.85, 155.62, 153.91, 153.42, 151.45, 145.26, 136.35, 135.75, 135.43, 134.66, 134.07, 133.97, 130.83, 125.77, 123.38, 121.34, 120.96, 80.58, 78.10, 70.67, 67.34, 51.39, 39.09, 32.62, 32.12, 28.91, 28.06, 27.89, 24.59, 24.33, 11.18, 10.97. **M/z (MALDI-TOF)** 5032.7 m/z [M+Na]$^+$; **IR** *v* = 3329; 2973; 2930; 2873; 1705; 1595; 1515; 1475; 1415; 1366; 1243; 1214; 1153; 1061; 1000.

Multicalixarene (56)

Generation 1 = **46** (1 g, 0.95 mmol), Central core = **35** (194 mg, 0.2 mmol) Product = **56** (759 mg, 74.4%) **Mp** >179^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.78 (s, 4H, Az*H*), 6.93 (d, *J* = 7.6 Hz, 8H, Ar*H*), 6.80 (bs, 16H, Ar*H*), 6.71 – 6.44 (m, 20H, Ar*H*), 5.07 (s, 8H, OCH_2CCAz), 4.47 – 4.35 (m, 16H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH_2Az and ArCH_2Ar), 4.30 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 3.77 (bs, 24H, OCH_2CH$_2$CH$_3$), 3.65 (bs, 8H, ArCH_2Ar), 3.42 – 3.35 (m, 8H, OCH_2CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$Az), 3.05 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.96 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.05 – 1.79 (m, 32H, OCH$_2$CH_2CH$_3$ and OCH$_2$CH$_2$CH$_2$CH$_2$CH_2CH$_2$Az), 1.47 (s, 72H, OC(CH_3)$_3$), 1.46 (s, 36H, OC(CH_3)$_3$), 1.45 (s, 36H, OC(CH_3)$_3$), 1.39 – 1.34 (m, 8H, OCH$_2$CH_2CH$_2$CH$_2$CH$_2$CH$_2$Az), 1.31 – 1.25 (m, 16H, OCH$_2$CH$_2$CH_2CH$_2$CH$_2$CH$_2$Az and OCH$_2$CH$_2$CH$_2$CH_2CH$_2$CH$_2$Az), 1.02 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$CH_3), 0.91 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$CH_3); **^{13}C NMR** (101 MHz, MeOD) δ 158.07, 155.81, 155.65, 153.91, 153.30, 151.62, 145.27, 136.28, 135.72, 135.21, 134.63, 134.13, 133.97, 130.83, 125.73, 122.87, 121.26, 121.07, 80.58, 78.06, 72.04, 67.56, 54.80, 51.34, 38.39, 32.55, 32.13, 31.76, 30.94, 28.92, 28.89,

27.73, 26.65, 24.59, 24.29, 11.23, 10.98. **M/z (MALDI-TOF)** 5145.0 m/z $[M+Na]^+$; **IR** *v* = 3331; 2972; 2932; 1710; 1596; 1521; 1476; 1415; 1365; 1243; 1215; 1153; 1062; 1001.

Multicalixarene (57)

Generation 1 = **46** (1 g, 0.95 mmol), Central core = **9** (195 mg, 0.2) Product = **57** (775 mg, 75.70%).**Mp** >219 °C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.04 (s, 4H, Az*H*), 6.85 (s, 16H, Ar*H*), 6.81 (s, 8H, Ar*H*), 6.55 (s, 16H, Ar*H*), 5.06 (s, 8H, OCH_2CCAz), 4.94 (s, 8H, OCH$_2$CH_2Az), 4.43 – 4.25 (m, 24H, OCH_2CH$_2$Az and ArCH_2Ar), 4.13 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 3.82 – 3.68 (m, 24H, OCH_2CH$_2$CH$_3$), 3.15 – 3.02 (m, 12H, ArCH_2Ar), 2.90 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 1.96 – 1.75 (m, 24H, OCH$_2$CH_2CH$_3$), 1.49 (s, 72H, OC(CH_3)$_3$), 1.45 (s, 72H, OC(CH_3)$_3$), 1.07 (s, 36H, CH_3CAr), 1.03 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$CH_3), 0.80 (t, *J* = 7.4 Hz, 24H, OCH$_2$CH$_2$CH_3). **^{13}C NMR** (101 MHz, MeOD) δ 155.86, 155.56, 154.12, 153.75, 153.23, 151.63, 146.71, 145.67, 136.66, 135.91, 135.38, 134.51, 134.07, 133.85, 126.69, 126.18, 121.45, 121.35, 120.97, 80.57, 78.18, 78.01, 74.17, 67.72, 54.80, 51.52, 34.86, 32.58, 32.04, 31.73, 28.98, 28.93, 28.85, 24.59, 24.24, 11.32, 10.91. **M/z (MALDI-TOF)** 5145.0 m/z $[M+Na]^+$; **IR** *v* = 3329; 2973; 2930; 2873; 1712; 1595; 1524; 1475; 1415; 1366; 1243; 1214; 1153; 1061; 1000.

Multicalixarene (58)

Generation 1 = **46** (1 g, 0.95 mmol), Central core = **15** (140 mg, 0.2 mmol) Product = **58** (614 mg, 58%) **Mp** >186°C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 7.88 (s, 4H, Az*H*), 6.92 – 6.70 (m, 24H, Ar*H*), 6.60 (s, 16H, Ar*H*), 5.00 (s, 8H, OCH_2CCAz), 4.57 (bs, 8H, OCH$_2$CH$_2$CH$_2$CH_2Az), 4.39 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 4.33 (d, *J* = 12.0 Hz, 4H, ArCH_2Ar), 4.26 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 3.90 (s, 8H, OCH_2CH$_2$CH$_2$CH$_2$Az), 3.74 (s, 24H, OCH_2CH$_2$CH$_3$), 3.11 – 3.00 (m, 12H, ArCH_2Ar), 2.91 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.08 (s, 16H, OCH$_2$CH_2CH$_2$CH$_2$Az and OCH$_2$CH$_2$CH_2CH$_2$Az), 1.99 – 1.90 (m, 8H, OCH$_2$CH_2CH$_3$), 1.90 – 1.77 (m, 16H, OCH$_2$CH_2CH$_3$), 1.48 (s, 72H, OC(CH_3)$_3$), 1.46 (s, *J* = 2.9 Hz, 36H, OC(CH_3)$_3$), 1.44 (s, 36H, OC(CH_3)$_3$), 1.13 – 1.00 (m, 48H, (CH_3)$_3$CAr and OCH$_2$CH$_2$CH_3), 0.84 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$CH_3). **^{13}C NMR** (101 MHz, MeOD) δ 155.82, 155.58, 154.72, 153.95, 153.32, 151.64, 145.66, 145.30, 136.33, 135.67, 134.92, 134.54, 134.07, 133.91, 126.29, 125.91, 121.31, 121.00, 80.56, 78.14, 78.05, 75.67, 67.62, 54.80, 51.47, 34.77, 32.52, 32.12, 28.96, 28.92, 28.86, 24.58, 24.27, 11.24, 10.93. **M/z (MALDI-TOF)** 5256.9 m/z $[M+Na]^+$; **IR** *v* = 3326; 2965; 2930; 2870; 1699; 1596; 1516; 1475; 1415; 1365; 1240; 1214; 1152; 1057; 1001.

Multicalixarene (59)

Generation 1 = **46** (1 g, 0.95 mmol), **17** (230 mg, 0.2 mmol) Product = **17** (1.05 g, 93%), **Mp** >176^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 7.61 (s, 4H, Az*H*), 6.75 – 6.50 (m, 24H, Ar*H*), 6.41 (s, 16H, Ar*H*), 4.79 (s, 8H, OCH_2CCAz), 4.29 – 4.12 (m, 20H, ArCH_2Ar, and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 4.08 (d, *J* = 12.5 Hz, 8H, ArCH_2Ar), 3.69 – 3.45 (m, 32H, OC$H_2CH_2CH_3$, OC$H_2CH_2CH_2CH_2$Az), 2.83 (d, *J* = 12.5 Hz, 12H, ArCH_2Ar), 2.74 (d, *J* = 13 Hz, 8H, ArCH_2Ar), 1.87 – 1.54 (m, 40H, $OCH_2CH_2CH_3$, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az, and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 1.32 – 1.17 (m, 156H, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and $OC(CH_3)_3$), 1.14 – 1.04 (m, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 0.90 – 0.77 (m, 48H, CH_3CAr and $OCH_2CH_2CH_3$), 0.67 – 0.61 (m, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 155.85, 155.81, 155.63, 154.91, 153.94, 153.29, 151.68, 145.41, 145.27, 136.34, 135.57, 135.05, 134.60, 134.11, 133.93, 126.16, 125.75, 121.39, 121.30, 121.07, 80.57, 78.15, 78.05, 76.19, 67.70, 51.33, 34.76, 32.53, 32.14, 31.94, 31.53, 28.95, 27.96, 27.14, 24.60, 24.26, 11.24, 10.96. **M/z (MALDI-TOF)** 5369.1 m/z $[M+Na]^+$; **IR** *v* = 3330; 2962; 1698; 2869; 1698; 1596; 1518; 1478; 1414; 1365; 1241; 1214; 1152; 1061; 1002.

Multicalixarene (60)

Generation 1 = **46** (1 g, 0.95 mmol), **39** (151 mg, 0.2 mmol) Product = **60** (664 mg, 67%), **Mp** > 216 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 7.76 (s, 4H, Az*H*), 7.21 (s, 8H, Ar*H*), 6.80 (s, 16H, Ar*H*), 6.70 – 6.49 (m, 16H, Ar*H*), 4.98 – 4.89 (m, 8H, OCH_2CCAz), 4.69 (d, *J* = 13.5 Hz, 4H, ArCH_2Ar), 4.36 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 4.27 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 4.12 – 3.99 (m, 8H, OC$H_2CH_2CH_3$), 3.77 – 3.62 (m, 24H, OC$H_2CH_2CH_3$), 3.53 (d, *J* = 13.5 Hz, 4H, ArCH_2Ar), 3.04 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.91 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 2.11 – 2.00 (m, 8H, $OCH_2CH_2CH_3$), 1.96 – 1.85 (m, 8H, $OCH_2CH_2CH_3$), 1.83 – 1.71 (m, 16H, $OCH_2CH_2CH_3$), 1.48 (s, 72H, $OC(CH_3)_3$), 1.45 (s, 36H, $OC(CH_3)_3$), 1.44 (s, 36H, $OC(CH_3)_3$), 1.10 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 1.00 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.75 (t, *J* = 7.0 Hz, 24H, $OCH_2CH_2CH_3$).**^{13}C NMR** (101 MHz, MeOD) δ 158.28, 155.83, 155.80, 155.66, 153.96, 153.22, 151.81, 145.70, 137.60, 136.49, 135.56, 134.56, 134.02, 133.87, 133.11, 123.43, 121.65, 121.30, 80.58, 78.63, 78.02, 67.85, 32.45, 32.14, 28.96, 28.93, 28.87, 24.52, 24.46, 24.18, 11.31, 10.85; **M/z (MALDI-TOF)** 4976.7 m/z $[M+Na]^+$; **IR** *v* = 3328; 2971; 2930; 2873; 1698; 1595; 1519; 1475; 1415; 1365; 1292; 1215; 1153; 1062; 999.

General method for the synthesis of Boc-Gly Multicalixarenes

Generation 1 calixarene (5 eq) was added to a solution of central core (1 eq) in DMF (15 mL) in the presence of $CuSO_4 \cdot 5H_2O$ (100 mg, 0.4 mmol, 2 eq) and sodium ascorbate (316 mg, 1.6 mmol, 8 eq). The reaction was stirred for 2 hours at 110°C. The solution was diluted with DCM (50 mL) and washed with H_2O (2 x 50 mL) and brine (2 x 50 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel, (*n*-hexane:EtOAc:MeOH = 1:1:0.1) and precipitation from DCM/*n*-hexane yielded the desired compound as white solid.

Multicalixarene (61)

Generation 1 = **48** (1.05 g, 0.784 mmol), Central core = **10** (109mg, 0.156 mmol) Product = **61** (400 mg, 44%). **Mp** > 250 °C (decompose) **^{1}H NMR** (400 MHz, MeOD) δ 8.10 (s, 4H, Az*H*), 7.16 (s, 10H, Ar*H*), 6.97 (s, 10H, Ar*H*), 6.62 (s, 24H, Ar*H*), 4.89 (s, 8H, OCH_2CCAz), 4.35 (m, 28H, ArCH_2Ar and OCH_2CH_2Az), 3.94 – 3.59 (m, 56H, ArNHCOCH_2NHCOOC$(CH_3)_3$ and OC$H_2CH_2CH_3$), 3.20 – 2.97 (m, 20H, ArCH_2Ar), 2.85 (s, 8H, OCH_2CH_2Az), 2.02 – 1.63 (m, 24H, $OCH_2CH_2CH_3$), 1.47 (s, 72H, OC(C$H_3)_3$), 1.45 (s, 36H, OC(C$H_3)_3$), 1.45 (s, 36H, OC(C$H_3)_3$), 1.03 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.87 – 0.66 (bs, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 170.12, 169.79, 158.44, 155.16, 153.90, 152.43, 145.39, 136.88, 135.68, 135.25, 133.80, 133.44, 133.12, 129.84, 126.26, 124.13, 121.84, 80.76, 78.28, 78.00, 73.68, 68.17, 51.46, 45.11, 32.43, 32.08, 30.72, 28.90, 24.56, 24.18, 11.32, 10.75. **M/z (MALDI-TOF)** 5832.9 m/z $[M+Na]^+$; **IR** *v* = 3298; 2974; 2933; 2871; 1686; 1600; 1504; 1470; 1418; 1391; 1366; 1245; 1220; 1163; 1049; 1003.

Multicalixarene (62)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **16** (162 mg, 0.2 mmol) Product = **Multicalixarene 12, d-t-bu-C4-BocGly (331)** (490 mg, 41% yield).**Mp** >226 °C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 8.00 (s, 4H, Az*H*), 7.03 (s, 8H, Ar*H*), 7.00 (s, 8H, Ar*H*), 6.78 (s, 8H, Ar*H*), 6.68 (s, 16H, Ar*H*), 4.94 (s, 8H, OCH_2CCAz), 4.56 (bs, 8H, $OCH_2CH_2CH_2CH_2$Az), 4.47 – 4.21 (m, 20H, ArCH_2Ar), 3.95 – 3.63 (m, 64H, OC$H_2CH_2CH_2CH_2$Az, ArNHCOCH_2NHCOOC$(CH_3)_3$, and OC$H_2CH_2CH_3$), 3.23 – 2.77 (m, 20H, ArCH_2Ar), 2.22 – 2.00 (m, 16H $OCH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_2CH_2$Az), 2.01 – 1.67 (m, 24H, $OCH_2CH_2CH_3$), 1.46 (s, 108H, OC(C$H_3)_3$), 1.44 (s, 36H, OC(C$H_3)_3$), 1.11 – 0.96 (m, 48H, CH_3CAr and $OCH_2CH_2CH_3$), 0.80 (t, *J* = 7.3 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 170.01, 169.80, 158.46, 155.00, 154.72, 154.08, 152.62, 145.74, 145.19, 136.81, 136.65, 136.13, 135.58, 134.93, 133.86, 133.45, 133.22, 126.33, 126.00, 121.99, 121.75, 80.76, 78.24, 78.10, 75.70, 67.98, 54.83, 51.51, 45.09, 44.99, 44.92, 34.79, 32.49, 32.25, 32.10, 28.89,

24.59, 24.25, 11.28, 10.84. **M/z (MALDI-TOF)** 5946.0 m/z $[M+Na]^+$; ; **IR** *v* = 3295; 2970; 2934; 2876; 1682; 1598; 1504; 1470; 1418; 1391; 1365; 1247; 1218; 1163; 1048; 1003.

Multicalixarene (63)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **18** (185 mg, 0.2 mmol) Product = **63** (467 mg, 39%); **Mp** >231 ^oC (decompose) **^{1}H NMR** (400 MHz, MeOD) δ 7.94 (s, 4H, Az*H*), 7.01 (s, 8H, Ar*H*), 6.99 (s, 8H, Ar*H*), 6.73 (s, 8H, Ar*H*), 6.71 (s, 8H, Ar*H*), 6.57 – 6.45 (m, 12H, Ar*H*), 4.97 (s, 8H, OCH_2CCAz), 4.37 (m, 28H, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and Ar$CH_2$Ar), 4.00 – 3.60 (m, 64H, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az, ArNHCO$CH_2$NHCOO$C(CH_3)_3$ and $OCH_2CH_2CH_3$), 3.12 – 2.90 (m, 20H, ArCH_2Ar), 2.01 – 1.87 (m, 24H, $OCH_2CH_2CH_3$, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 1.87 – 1.72 (m, 16H, $OCH_2CH_2CH_3$), 1.46 (m, 160H, 1.57 – 1.35, $OC(CH_3)_3$, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 1.03 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.82 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 169.97, 169.85, 158.42, 157.68, 154.90, 154.06, 152.70, 145.12, 136.78, 136.58, 136.16, 136.10, 135.53, 133.82, 133.47, 133.22, 129.38, 125.81, 123.14, 121.86, 80.74, 78.18, 78.04, 75.91, 68.11, 51.27, 45.03, 32.49, 32.15, 31.86, 31.41, 28.87, 27.86, 27.13, 24.57, 24.19, 11.31, 10.87. **M/z (MALDI-TOF)** 6058.3 m/z $[M+Na]^+$; **IR** *v* = 3298; 2975; 2934; 2870; 1673; 1598; 1505; 1466; 1418; 1390; 1366; 1245; 1216; 1161; 1049; 1001.

Multicalixarene (64)

Generation 1 = **48** (1 g, 0.784 mmol), Central core = **28** (109mg, 0.156 mmol) Product = **64** (385 mg, 42.5%).**Mp** > 248 ^oC (decompose) **^{1}H NMR** (400 MHz, MeOD) δ 7.76 (s, 4H, Az*H*), 7.01 (d, *J* = 6.5 Hz, 8H, Ar*H*), 6.93 (s, 16H, Ar*H*), 6.82 (s, 12H, Ar*H*), 6.79 (s, 8H, Ar*H*), 5.14 (s, 8H, OCH_2CCAz), 4.42 (d, *J* = 12.5 Hz, 8H, ArCH_2Ar), 4.33 (d, *J* = 12.0 Hz, 8H, ArCH_2Ar), 3.98 (s, 8H, OCH_2CH_2Az), 3.88 – 3.66 (m, 72H, ArNHCOCH_2NHCOO$C(CH_3)_3$, OCH_2CH_2Az and $OCH_2CH_2CH_3$), 3.08 (d, *J* = 12.5 Hz, 8H, ArCH_2Ar), 2.96 (d, *J* = 12.0 Hz, 8H, ArCH_2Ar), 2.00 – 1.79 (m, 24H, $OCH_2CH_2CH_3$), 1.45 (s, 108H, $OC(CH_3)_3$), 1.43 (s, 36H, $OC(CH_3)_3$), 1.03 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.88 (t, *J* = 6.5 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 169.94, 158.41, 156.99, 154.80, 154.20, 152.51, 145.18, 136.49, 135.87, 135.47, 133.97, 133.39, 130.83, 126.24, 124.86, 122.04, 81.50, 80.71, 78.08, 69.00, 67.51, 45.02, 38.52, 32.57, 32.14, 28.86, 24.56, 24.28, 11.22, 10.96. **M/z (MALDI-TOF)** 5833.0 m/z $[M+Na]^+$; **IR** *v* = 3301; 2974; 2932; 2871; 1673; 1600; 1504; 1470; 1417; 1392; 1366; 1246; 1220; 1163; 1050; 1003.

Multicalixarene (65)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **34** (162 mg, 0.2 mmol) Product = **65** (519 mg, 43.9%).**Mp** > 230 ^{o}C (decompose). **^{1}H NMR** (400 MHz, MeOD) δ 7.94 (s, 4H, Az*H*), 6.95 (s, 12H, Ar*H*), 6.93 (s, 12H, Ar*H*), 6.80 (s, 8H, Ar*H*), 6.78 (s, 8H, Ar*H*), 6.66 (t, *J* = 7 Hz, 4H, Ar*H*), 5.15 (s, 8H, OCH_2CCAz), 4.42 (d, *J* = 12.5 Hz, 8H, ArCH_2Ar), 4.36 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 4.28 (s, 8H, $OCH_2CH_2CH_2CH_2Az$), 3.84 – 3.69 (m, 64H, ArNHCOCH_2NHCOOC$(CH_3)_3$, ArCH_2Ar and OC$H_2CH_2CH_3$), 3.41 – 3.34 (m, 8H, OC$H_2CH_2CH_2CH_2Az$), 3.08 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 2.96 (d, *J* = 12.5 Hz, 8H, ArCH_2Ar), 2.00 – 1.81 (m, 24H, $OCH_2CH_2CH_3$), 1.67 (s, 8H, $OCH_2CH_2CH_2CH_2Az$), 1.46 (s, 108H, OC$(CH_3)_3$), 1.45 (s, 36H, OC$(CH_3)_3$), 1.22 (s, 8H, $OCH_2CH_2CH_2CH_2Az$), 1.00 (t, *J* = 7.0 Hz, 12H, $OCH_2CH_2CH_3$), 0.89 (t, *J* = 7.0 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 169.94, 169.85, 163.08, 158.44, 158.16, 154.81, 154.25, 152.52, 145.15, 136.52, 136.38, 135.86, 135.43, 133.42, 130.74, 125.83, 123.40, 121.93, 121.87, 80.73, 78.23, 78.14, 70.71, 51.34, 45.01, 38.93, 32.54, 32.11, 28.85, 28.08, 27.85, 24.57, 24.32, 11.15, 10.94. **M/z (MALDI-TOF)** 5945.1 m/z $[M+Na]^+$; **IR** *ν* **=** 3308; 2974; 2931; 2876; 1675; 1599; 1503; 1474; 1419; 1391; 1366; 1246; 1220; 1162; 1048; 1002.

Multicalixarene (66)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **35** (185 mg, 0.2 mmol) Product = **66** (639 mg, 53.0 %) .**Mp** > 232 ^{o}C (decompose) **^{1}H NMR** (400 MHz, MeOD) δ 7.90 (s, 4H, Az*H*), 6.98 (s, 16H, Ar*H*), 6.93 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.79 (s, 8H, Ar*H*), 6.74 (s, 8H, Ar*H*), 6.62 (t, *J* = 7.5 Hz, 4H, Ar*H*), 5.04 (s, 8H, OCH_2CCAz), 4.46 – 4.29 (m, 24H, ArCH_2Ar and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 3.84 – 3.60 (m, 64H, ArCH_2Ar, OC$H_2CH_2CH_3$ and ArNHCOCH_2NHCOOC$(CH_3)_3$), 3.40 – 3.32 (m, 8H, OC$H_2CH_2CH_2CH_2CH_2CH_2Az$), 3.10 – 2.94 (m, 16H, ArCH_2Ar), 1.96 – 1.79 (m, 32H, $OCH_2CH_2CH_3$ and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.46 (s, 144H, OC$(CH_3)_3$), 1.29 (m, 24H, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$ and $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 1.01 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.87 (t, *J* = 7.0 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 169.94, 158.44, 158.08, 154.83, 154.16, 152.63, 145.12, 136.63, 136.53, 136.29, 136.24, 135.70, 135.23, 133.93, 133.49, 133.29, 130.80, 125.78, 121.87, 121.80, 80.72, 78.11, 72.00, 67.68, 51.32, 44.99, 32.52, 32.13, 31.75, 30.94, 27.73, 26.69, 24.59, 24.26, 11.28, 10.97. **M/z (MALDI-TOF)** 6057.3 m/z $[M+Na]^+$; **IR** *ν* **=** 3299; 2973; 2931; 2872; 1683; 1598; 1506; 1464; 1418; 1390; 1366; 1245; 1220; 1163; 1048; 1002.

Multicalixarene (67)

Generation 1 = **48** (1 g, 0.784 mmol), Central core = **9** (145 mg, 0.156 mmol)Product = **67** (500 mg, 53.1%); **Mp** >252 °C (decompose) **^{1}H NMR** (400 MHz, MeOD) δ 8.15 (s, 4H, Az*H*), 7.14 (s, 8H, Ar*H*), 6.99 (s, 8H, Ar*H*), 6.84 (s, 8H, Ar*H*), 6.63 (s, 16H, Ar*H*), 4.96 (s, 8H, OCH_2CCAz), 4.50 – 4.27 (m, 24H, ArCH_2Ar and OCH_2CH_2Az), 4.18 (d, *J* = 11 Hz, 4H, ArCH_2Ar), 3.91 – 3.66 (m, 56H, $ArNHCOCH_2NHCOOC(CH_3)_3$ and $OCH_2CH_2CH_3$), 3.22 – 3.01 (m, 20H, ArCH_2Ar and $OCH_2CH_2CH_3$,), 2.89 (s, 8H, ArCH_2Ar) 1.98 – 1.69 (m, 24H, $OCH_2CH_2CH_3$), 1.47 (s, 72H, $OC(CH_3)_3$), 1.45 (s, 36H, $OC(CH_3)_3$), 1.45 – 1.42 (m, 36H), 1.08 (s, 36H, $OC(CH_3)_3$), 1.02 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.75 (t, *J* = 6.5 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 170.08, 169.83, 158.45, 155.12, 153.92, 152.52, 146.77, 145.46, 137.05, 136.86, 135.77, 135.30, 133.81, 133.46, 133.12, 126.72, 126.25, 122.21, 121.96, 121.84, 80.75, 78.26, 78.02, 51.41, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 45.10, 44.96, 34.86, 32.41, 32.00, 28.88, 24.56, 24.17, 11.32, 10.76. **M/z (MALDI-TOF)** 6058.4 m/z $[M+Na]^+$; **IR** *ν* = 3311; 2965; 2928; 2870; 1682; 1599; 1504; 1470; 1418; 1391; 1365; 1245; 1216; 1164; 1049; 1003.

Multicalixarene (68)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **15** (204 mg, 0.2 mmol) Product = **68** (559 mg, 46%); **Mp** > 229 °C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.00 (s, 4H, Az*H*), 7.03 (s, 8H, Ar*H*), 7.00 (s, 8H, Ar*H*), 6.78 (s, 8H, Ar*H*), 6.68 (s, 16H, Ar*H*), 4.94 (s, 8H, OCH_2CCAz), 4.56 (bs, 8H, $OCH_2CH_2CH_2CH_2Az$), 4.47 – 4.21 (m, 20H, ArCH_2Ar), 3.95 – 3.63 (m, 64H, $OCH_2CH_2CH_2CH_2Az$, $ArNHCOCH_2NHCOOC(CH_3)_3$, and $OCH_2CH_2CH_3$), 3.23 – 2.77 (m, 20H, ArCH_2Ar), 2.22 – 2.00 (m, 16H $OCH_2CH_2CH_2CH_2Az$ and $OCH_2CH_2CH_2CH_2Az$), 2.01 – 1.67 (m, 24H, $OCH_2CH_2CH_3$), 1.46 (s, 108H, $OC(CH_3)_3$), 1.44 (s, 36H, $OC(CH_3)_3$), 1.11 – 0.96 (m, 48H, CH_3CAr and $OCH_2CH_2CH_3$), 0.80 (t, *J* = 7.3 Hz, 24H, $OCH_2CH_2CH_3$). **^{13}C NMR** (101 MHz, MeOD) δ 170.01, 169.80, 158.46, 155.00, 154.72, 154.08, 152.62, 145.74, 145.19, 136.81, 136.65, 136.13, 135.58, 134.93, 133.86, 133.45, 133.22, 126.33, 126.00, 121.99, 121.75, 80.76, 78.24, 78.10, 75.70, 67.98, 54.83, 51.51, 45.09, 44.99, 44.92, 34.79, 32.49, 32.25, 32.10, 28.89, 24.59, 24.25, 11.28, 10.84. **M/z (MALDI-TOF)** 6170.3 m/z $[M+Na]^+$; **IR** *ν* = 3299; 2965; 2932; 2873; 1673; 1598; 1504; 1470; 1418; 1391; 1365; 1245; 1216; 1163; 1050; 1002.

Multicalixarene (69)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **17** (230 mg, 0.2 mmol) Product = **69** (467 mg, 38.7 %); **Mp** >240 °C (decompose) **^{1}H NMR** (400 MHz, MeOD) δ 7.98 (s, 4H, Az*H*), 7.03 (s, 16H, Ar*H*), 6.80 (s, 8H, Ar*H*), 6.75 (s, 8H, Ar*H*), 6.71 (s, 8H, Ar*H*), 4.98 (s, 8H, OCH_2CCAz), 4.57 – 4.23 (m, 28H, ArCH_2Ar and, $OCH_2CH_2CH_2CH_2CH_2CH_2Az$), 3.95 – 3.66

(m, 64H, ArNHCOCH_2NHCOOC$(CH_3)_3$, OCH_2CH_2CH_3, and OCH_2CH_2CH_2CH_2Az), 3.04 (m, 20H, ArCH_2Ar), 2.10 – 1.75 (m, 40H, OCH_2CH_2CH_2CH_2CH_2CH_2Az, OCH_2CH_2CH_2CH_2CH_2CH_2Az and OCH_2CH_2CH_3), 1.48 (s, 160H, OCH_2CH_2CH_2CH_2CH_2CH_2Az, OCH_2CH_2CH_2CH_2CH_2CH_2Az and OC$(CH_3)_3$), 1.09 (s, 36H, CH_3CAr), 1.05 (t, *J* = 7.5 Hz, 12H, OCH_2CH_2CH_3), 0.83 (t, *J* = 6.9 Hz, 24H, OCH_2CH_2CH_3). **^{13}C NMR** (101 MHz, MeOD) δ 169.95, 169.82, 158.42, 154.92, 154.00, 152.65, 145.47, 145.13, 136.80, 136.62, 136.03, 135.52, 135.03, 133.88, 133.49, 133.22, 133.20, 131.22, 130.38, 127.77, 127.35, 126.20, 125.81, 125.79, 122.02, 121.92, 121.76, 121.71, 80.71, 78.20, 78.05, 76.19, 68.03, 54.80, 51.32, 45.04, 34.76, 32.45, 32.12, 31.95, 31.53, 28.86, 28.00, 27.17, 24.58, 24.20, 11.29, 10.86. **M/z (MALDI-TOF)** 6281.3 m/z $[M+Na]^+$; **IR** *ν* **=** 3299; 2965; 2933; 2868; 1673; 1599; 1504; 1463; 1418; 1391; 1366; 1245; 1213; 1163; 1049; 1003.

Multicalixarene (70)

Generation 1 = **48** (1.28 g, 1 mmol), Central core = **10** (151 mg, 0.2 mmol) Product = **70** (400 mg, 43%). **M/z (MALDI-TOF)** = 5889.3 m/z $[M+Na]^+$.

General method for the removal of boc protecting group from multicalixarenes

HCl gas was bubbled through a solution of the Boc-protected multicalixarene (0.03 mmol) in DCM (5 ml) until the formation of precipitate was observed, after which time it was left fo react for a further 10 minutes to ensure the reaction went to completion. Argon was bubbled in to the suspension for 10 minutes to de gas the solution from the HCl. Removal of the solvent under reduced pressure yielded the desired compounds.

Multicalixarene (71)

Starting material = **51** (148 mg; 0.03 mmol). Product = **71** (quantitative). **Mp** > 260 ^oC (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.04 (s, 4H, Az*H*), 6.98 (s, 16H, Ar*H*), 6.73 (s, 8H, Ar*H*), 6.71 (s, 8H, Ar*H*), 6.60 – 6.46 (m, 12H, Ar*H*), 5.16 – 5.01 (m, 16H, OCH_2CCAz and OCH_2CH_2Az), 4.62 – 4.47 (m, 16H, OCH_2CH_2Az and ArCH_2Ar), 4.43 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 4.15 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 3.95 – 3.82 (m, 24H), 3.38 – 3.32 (m, 8H, ArCH_2Ar), 3.25 (d, *J* =13.5 Hz, 8H, ArCH_2Ar), 3.06 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 2.02 – 1.79 (m, 24H, OCH_2CH_2CH_3), 1.07 (t, *J* = 7.5 Hz, 12H, OCH_2CH_2CH_3), 0.89 (t, *J* = 7.5 Hz, 24H, OCH_2CH_2CH_3); **^{13}C NMR** (101 MHz, MeOD) δ 158.27, 157.52, 156.68, 155.87, 144.66, 138.01, 137.92, 137.68, 137.16, 135.49, 129.79, 126.50, 126.21, 126.02, 125.90, 124.61, 124.35, 124.19, 124.03, 78.62, 78.51, 74.03, 67.78, 51.96, 31.97, 31.56, 24.52, 24.29, 11.06, 10.73. **M/z (MALDI-TOF)** = 3318.9 m/z $[M-16HCl+Na]^+$. **IR** *ν* **=** 3392; 2873; 2593; 1583; 1525; 1465; 1386; 1213; 1147; 1091; 1038; 1000.

Multicalixarene (72)

Starting material = **52** (150 mg; 0.03 mmol). Product = **72** (quantitative). **Mp** > 245 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.09 (s, 4H, Az*H*), 6.98 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.87 (s, 16H, Ar*H*), 6.68 (t, *J* = 7.5 Hz, 4H, Ar*H*), 6.64 (s, 8H, Ar*H*), 6.62 (s, 8H, Ar*H*), 5.17 (s, 8H, OCH_2CCAz), 4.51 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 4.47 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 4.38 (t, *J* = 7.0Hz, 8H, $OCH_2CH_2CH_2CH_2$Az), 3.89 (t, *J* = 7.5 Hz, 24H, OC$H_2CH_2CH_3$), 3.76 (s, 8H, ArCH_2Ar), 3.42 (t, *J* = 6.5 Hz, 8H, OC$H_2CH_2CH_2CH_2$Az), 3.24 (d, *J* = 13.5 Hz, 8H, ArCH_2Ar), 2.00 – 1.84 (m, 24H, $OCH_2CH_2CH_3$), 1.82 – 1.74 (m, 8H, $OCH_2CH_2CH_2CH_2$Az), 1.36 – 1.26 (m, 8H, $OCH_2CH_2CH_2CH_2$Az), 1.05 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.92 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$).**^{13}C NMR** (101 MHz, MeOD) δ 158.27, 158.07, 157.67, 155.95, 144.42, 137.92, 137.31, 135.45, 130.84, 126.43, 126.09, 125.96, 125.88, 124.56, 124.36, 124.25, 123.31, 78.66, 78.58, 70.91, 67.61, 51.45, 38.68, 32.01, 31.59, 28.02, 27.88, 24.55, 24.30, 10.95, 10.68; **M/z (MALDI-TOF)** = 3430.9m/z $[M-16HCl+Na]^+$. **IR** *ν* **=** 3393; 2872; 2598; 1583; 1524; 1463; 1386; 1214; 1147; 1093; 1038; 1000.

Multicalixarene (73)

Starting material = **53** (153 mg; 0.03 mmol). Product = **73** (quantitative). **Mp** > 239 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.11 (s, 4H, Az*H*), 7.00 (s, 16H, Ar*H*), 6.73 (s, 8H, Ar*H*), 6.69 (s, 8H, Ar*H*), 6.60 – 6.41 (m, 12H, Ar*H*), 5.09 (s, 8H, OCH_2CCAz), 4.57 – 4.43 (m, 22H, ArCH_2Ar and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 4.39 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 4.02 – 3.74 (m, 32H, OC$H_2CH_2CH_3$ and OC$H_2CH_2CH_2CH_2CH_2CH_2$Az), 3.41 – 3.22 (m, 16H, ArCH_2Ar (detected by COSY)), 3.09 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 2.07 – 1.79 (m, 40H, $OCH_2CH_2CH_3$, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 1.59 – 1.38 (m, 16H, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 1.08 (t, *J* = 7.50 Hz, 12H, $OCH_2CH_2CH_3$), 0.88 (bs, 24H, $OCH_2CH_2CH_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.35, 157.64, 157.53, 156.05, 138.05, 137.98, 137.68, 137.12, 136.13, 129.33, 126.34, 125.94, 125.78, 124.73, 124.47, 124.22, 123.08, 78.68, 75.90, 68.24, 32.14, 31.60, 27.93, 27.16, 24.60, 24.36, 11.17, 10.88.**M/z (MALDI-TOF)** = 3543.0 m/z $[M-16HCl+Na]^+$; **IR** *ν* **=** 3390; 2870; 2600; 1583; 1524; 1464; 1386; 1215; 1147; 1089; 1038; 1000.

Multicalixarene (74)

Starting material = **54** (148 mg; 0.03 mmol). Product = **74** (quantitative). **Mp** > 248 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.13 (s, 4H, Az*H*), 7.08 (d, *J* = 7.2 Hz, 8H, Ar*H*), 6.97 (s, 16H, Ar*H*), 6.89 (t, *J* = 7.0 Hz, 4H, Ar*H*), 6.73 (s, 8H, Ar*H*), 6.70 (s, 8H, Ar*H*), 5.17 (s, 8H, OCH_2CCAz), 4.53 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 4.50 (d, *J* = 13.0 Hz, 8H, ArCH_2Ar), 4.18 (t, *J* = 6.0 Hz, 8H, OCH_2CH_2Az), 3.98 – 3.83 (m, 32H, ArCH_2Ar and OC$H_2CH_2CH_3$), 3.74

(t, *J* = 6.0 Hz, 8H, OC*H*$_2$CH$_2$Az), 3.40 – 3.25 (m, 16H, ArC*H*$_2$Ar), 2.01 – 1.83 (m, 24H, OCH$_2$C*H*$_2$CH$_3$), 1.07 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$C*H*$_3$), 0.88 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$C*H*$_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.33, 157.58, 156.99, 155.96, 144.57, 138.03, 137.98, 137.74, 137.19, 135.72, 131.11, 126.58, 126.47, 126.00, 125.87, 124.61, 124.36, 124.20, 78.67, 78.53, 69.45, 67.69, 50.57, 38.31, 32.00, 31.58, 24.55, 24.26, 10.99, 10.63..**M/z (MALDI-TOF)** = 1099.6 m/z [M-16HCl+4H]$^{4+}$. **IR** *v* = 3405; 2873; 2602; 1584; 1530; 1466; 1386; 1217; 1147; 1096; 1041; 1000.

Multicalixarene (75)

Starting material = **55** (148 mg; 0.03 mmol). Product = **75** (quantitative). **Mp** > 245 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.09 (s, 4H, Az*H*), 6.98 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.87 (s, 16H, Ar*H*), 6.68 (t, *J* = 7.5 Hz, 4H, Ar*H*), 6.64 (s, 8H, Ar*H*), 6.62 (s, 8H, Ar*H*), 5.17 (s, 8H, OC*H*$_2$CCAz), 4.51 (d, *J* = 13.5 Hz, 8H, ArC*H*$_2$Ar), 4.47 (d, *J* = 13.5 Hz, 8H, ArC*H*$_2$Ar), 4.38 (t, *J* = 7.0Hz, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$Az), 3.89 (t, *J* = 7.5 Hz, 24H, OC*H*$_2$CH$_2$CH$_3$), 3.76 (s, 8H, ArC*H*$_2$Ar), 3.42 (t, *J* = 6.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$Az), 3.24 (d, *J* = 13.5 Hz, 8H, ArC*H*$_2$Ar), 2.00 – 1.84 (m, 24H, OCH$_2$C*H*$_2$CH$_3$), 1.82 – 1.74 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$Az), 1.36 – 1.26 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$Az), 1.05 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$C*H*$_3$), 0.92 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$C*H*$_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.27, 158.07, 157.67, 155.95, 144.42, 137.92, 137.31, 135.45, 130.84, 126.43, 126.09, 125.96, 125.88, 124.56, 124.36, 124.25, 123.31, 78.66, 78.58, 70.91, 67.61, 51.45, 38.68, 32.01, 31.59, 28.02, 27.88, 24.55, 24.30, 10.95, 10.68.**M/z (MALDI-TOF)** = 3430.9 m/z [M-16HCl+Na]$^{+}$. **IR** *v* = 3393; 2872; 2598; 1583; 1524; 1463; 1386; 1214; 1147; 1093; 1038; 1000.

Multicalixarene (76)

Starting material = **56** (148 mg; 0.03 mmol). Product = **76** (quantitative). **Mp** > 245 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.09 (s, 4H, Az*H*), 6.98 (d, *J* = 7.5 Hz, 8H, Ar*H*), 6.87 (s, 16H, Ar*H*), 6.68 (t, *J* = 7.5 Hz, 4H, Ar*H*), 6.64 (s, 8H, Ar*H*), 6.62 (s, 8H, Ar*H*), 5.17 (s, 8H, OC*H*$_2$CCAz), 4.51 (d, *J* = 13.5 Hz, 8H, ArC*H*$_2$Ar), 4.47 (d, *J* = 13.5 Hz, 8H, ArC*H*$_2$Ar), 4.38 (t, *J* = 7.0Hz, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$Az), 3.89 (t, *J* = 7.5 Hz, 24H, OC*H*$_2$CH$_2$CH$_3$), 3.76 (s, 8H, ArC*H*$_2$Ar), 3.42 (t, *J* = 6.5 Hz, 8H, OC*H*$_2$CH$_2$CH$_2$CH$_2$Az), 3.24 (d, *J* = 13.5 Hz, 8H, ArC*H*$_2$Ar), 2.00 – 1.84 (m, 24H, OCH$_2$C*H*$_2$CH$_3$), 1.82 – 1.74 (m, 8H, OCH$_2$CH$_2$C*H*$_2$CH$_2$Az), 1.36 – 1.26 (m, 8H, OCH$_2$C*H*$_2$CH$_2$CH$_2$Az), 1.05 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$C*H*$_3$), 0.92 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$C*H*$_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.27, 158.07, 157.67, 155.95, 144.42, 137.92, 137.31, 135.45, 130.84, 126.43, 126.09, 125.96, 125.88, 124.56, 124.36, 124.25, 123.31, 78.66, 78.58, 70.91, 67.61, 51.45, 38.68, 32.01, 31.59, 28.02, 27.88, 24.55, 24.30, 10.95, 10.68.**M/z (MALDI-TOF)** = 3430.9 m/z [M-16HCl+Na]$^{+}$. **IR** *v* = 3393; 2872; 2598; 1583; 1524; 1463; 1386; 1214; 1147; 1093; 1038; 1000.

Multicalixarene (77)

Starting material = **57** (153 mg; 0.03 mmol). Product = **77** (quantitative). **Mp** > 258 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.16 (s, 4H, Az*H*), 6.95 (s, 16H, Ar*H*), 6.81 (s, 8H, Ar*H*), 6.70 (s, 8H, Ar*H*), 6.69 (s, 8H, Ar*H*), 5.16 (t, *J* = 6.0 Hz, 8H, OCH_2CH_2Az), 5.11 (s, 8H, OCH_2CCAz), 4.54 – 4.47 (m, 16H, OCH_2CH_2Az and $ArCH_2Ar$), 4.47 – 4.40 (m, *J* = 13.5 Hz, 8H, $ArCH_2Ar$), 4.20 – 4.13 (m, *J* = 13.0 Hz, 4H, $ArCH_2Ar$), 3.93 – 3.82 (m, 24H, $OCH_2CH_2CH_3$), 3.37 – 3.29 (m, 8H $ArCH_2Ar$), 3.27 – 3.20 (m, *J* = 13.5 Hz, 8H, $ArCH_2Ar$), 3.12 – 3.05 (d, *J* = 13.0 Hz, 4H, $ArCH_2Ar$), 2.00 – 1.91 (m, 8H, $OCH_2CH_2CH_3$), 1.88 – 1.79 (m, 16H, $OCH_2CH_2CH_3$), 1.10 – 1.01 (m, 48H, CH_3CAr and $OCH_2CH_2CH_3$), 0.89 – 0.81 (m, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.27, 157.53, 155.86, 153.81, 146.82, 137.99, 137.86, 137.70, 137.17, 134.50, 126.67, 126.00, 125.88, 124.65, 124.42, 124.22, 78.61, 78.53, 74.13, 67.85, 51.79, 34.83, 31.94, 31.56, 24.53, 24.29, 11.10, 10.77;.**M/z (MALDI-TOF)** = 881.0136 m/z $[M\text{-}16HCl+4H]^{4+}$. **IR** *v* **=** 3388; 2871; 2596; 1582; 1519; 1463; 1385; 1217; 1146; 1125; 1039; 999.

Multicalixarene (80)

Starting material = **60** (148 mg; 0.03 mmol). Product = **80** (quantitative). **Mp** > 226 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.21 (s, 4H, Az*H*), 7.30 (s, 8H, Ar*H*), 7.02 – 6.94 (m, 16H, Ar*H*), 6.74 (s, 8H, Ar*H*), 6.68 (s, 8H, Ar*H*), 5.08 (s, 8H, OCH_2CCAz), 4.68 (d, *J* = 13.5 Hz, 4H, $ArCH_2Ar$), 4.51 (d, *J* = 13.5 Hz, 8H, $ArCH_2Ar$), 4.42 (d, *J* = 13.5 Hz, 8H, $ArCH_2Ar$), 4.05 (t, *J* = 7.0 Hz, 8H, $OCH_2CH_2CH_3$), 3.90 – 3.79 (m, 24H, $OCH_2CH_2CH_3$), 3.53 (d, *J* = 13.5 Hz, 4H, $ArCH_2Ar$), 3.37 – 3.28 (m, 8H, $ArCH_2Ar$), 3.24 (d, *J* = 13.5 Hz, 8H, $ArCH_2Ar$), 2.06 – 1.91 (m, 16H, $OCH_2CH_2CH_3$), 1.85 – 1.76 (m, 16H, $OCH_2CH_2CH_3$), 1.12 – 1.04 (m, 24H, $OCH_2CH_2CH_3$), 0.79 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.38, 158.28, 157.46, 156.00, 144.88, 138.04, 137.89, 137.76, 137.62, 137.12, 132.87, 126.36, 125.93, 125.76, 124.65, 124.46, 124.19, 123.92, 121.19, 78.65, 78.51, 68.10, 32.10, 31.91, 31.55, 26.12, 24.49, 24.44, 24.20, 11.08, 10.75, 10.49;.**M/z (MALDI-TOF)** = 838.9640 m/z $[M\text{-}16HCl+4H]^{4+}$.

Multicalixarene (81)

Starting material = **61** (174 mg; 0.03 mmol). Product = **81** (quantitative). **Mp** > 250 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.16 (s, 4H, Az*H*), 6.95 (s, 16H, Ar*H*), 6.81 (s, 8H, Ar*H*), 6.70 (s, 8H, Ar*H*), 6.69 (s, 8H, Ar*H*), 5.16 (t, *J* = 6.0 Hz, 8H, OCH_2CH_2Az), 5.11 (s, 8H, OCH_2CCAz), 4.54 – 4.47 (m, 16H, OCH_2CH_2Az and $ArCH_2Ar$), 4.47 – 4.40 (m, *J* = 13.5 Hz, 8H, $ArCH_2Ar$), 4.20 – 4.13 (m, *J* = 13.0 Hz, 4H, $ArCH_2Ar$), 3.93 – 3.82 (m, 24H, $OCH_2CH_2CH_3$), 3.37 – 3.29 (m, 8H $ArCH_2Ar$), 3.27 – 3.20 (m, *J* = 13.5 Hz, 8H, $ArCH_2Ar$),

3.12 – 3.05 (d, *J* = 13.0 Hz, 4H, ArC*H*$_2$Ar), 2.00 – 1.91 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 1.88 – 1.79 (m, 16H, OCH$_2$C*H*$_2$CH$_3$), 1.10 – 1.01 (m, 48H, C*H*$_3$CAr and OCH$_2$CH$_2$C*H*$_3$), 0.89 – 0.81 (m, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$C*H*$_3$); **^{13}C NMR** (101 MHz, MeOD) δ 158.27, 157.53, 155.86, 153.81, 146.82, 137.99, 137.86, 137.70, 137.17, 134.50, 126.67, 126.00, 125.88, 124.65, 124.42, 124.22, 78.61, 78.53, 74.13, 67.85, 51.79, 34.83, 31.94, 31.56, 24.53, 24.29, 11.10, 10.77;.**M/z (MALDI-TOF)** = 4209.2 m/z [M-16HCl+1H]$^+$. **IR** *ν* = 3410; 2956; 2865; 1686; 1601; 1562; 1474; 1385; 1216; 1147; 1125; 1042; 1002.

Multicalixarene (82)

Starting material = **62** (178 mg; 0.03 mmol). Product = **82** (quantitative). **Mp** > 250 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.17 (s, 4H, Az*H*), 7.17 (s, 8H, Ar*H*), 7.08 (s, 8H, Ar*H*), 6.77 (s, 16H, Ar*H*), 6.71 (s, 8H, Ar*H*), 4.85 (s, 8H, OC*H*$_2$CCAz (detected by HSQC)), 4.61 (s, 8H, OCH$_2$CH$_2$CH$_2$C*H*$_2$Az), 4.45 – 4.27 (m, 20H, ArC*H*$_2$Ar), 3.97 – 3.71 (m, 64H, OC*H*$_2$CH$_2$CH$_3$, ArNHCOC*H*$_2$NH$_3^+$Cl$^-$and OC*H*$_2$CH$_2$CH$_2$CH$_2$Az), 3.16 – 3.03 (m, 20H, ArC*H*$_2$Ar), 2.13 (s, 16H, OCH$_2$C*H*$_2$CH$_2$CH$_2$Az and OCH$_2$CH$_2$C*H*$_2$CH$_2$Az), 1.95 – 1.77 (m, 24H, OCH$_2$C*H*$_2$CH$_3$), 1.14 – 0.96 (m, 48H, C*H*$_3$CAr and OCH$_2$CH$_2$C*H*$_3$), 0.77 (t, *J* = 7.4 Hz, 24H, OCH$_2$CH$_2$C*H*$_3$); **^{13}C NMR** (101 MHz, DMSO) δ 163.93, 163.85, 152.98, 152.07, 151.14, 142.99, 135.07, 135.03, 134.04, 133.61, 132.39, 132.31, 131.82, 119.46, 76.59, 76.12, 40.71, 33.45, 31.11, 30.71, 27.01, 26.88, 22.79, 22.27, 10.40, 9.64; **M/z (MALDI-TOF)** = 1137.3785 m/z [M-15HCl+3H]$^{3+}$. **IR** *ν* = 3403; 2960; 2878; 1683; 1603; 1559; 1468; 1381; 1220; 1147; 1123; 1044; 1003.

Multicalixarene (83)

Starting material = **63** (178 mg; 0.03 mmol). Product = **83** (quantitative). **Mp** > 215 ^{o}C (decompose); **M/z (MALDI-TOF)** = 1109.0994 m/z [M-16HCl+4H]$^{4+}$. **IR** *ν* = 3403; 2958; 2872; 1683; 1603; 1559; 1468; 1381; 1220; 1147; 1123; 1044; 1003. **IR** *ν* = 3410; 2960; 2878; 1682; 1601; 1561; 1470; 1385; 1218; 1147; 1123; 1045; 1003.

Multicalixarene 34 (84)

Starting material = **64** (174 mg; 0.03 mmol). Product = **84** (quantitative). **Mp** > 220 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 7.96 (s, 4H, Az*H*), 7.09 – 6.98 (m, 24H, Ar*H*), 6.90 – 6.79 (m, 20H, Ar*H*), 5.11 (s, Hz, 8H, OC*H*$_2$CCAz), 4.49 – 4.41 (m, 16H, ArC*H*$_2$Ar), 4.07 (s, 8H, OCH$_2$C*H*$_2$Az), 3.90 – 3.75 (m, 72H, ArC*H*$_2$Ar, OC*H*$_2$CH$_2$CH$_3$, ArNHCOC*H*$_2$NH$_3^+$Cl$^-$ and OC*H*$_2$CH$_2$Az), 3.16 – 3.09 (m, 16H, ArC*H*$_2$Ar), 2.10 – 1.74 (m, 24H, OCH$_2$C*H*$_2$CH$_3$), 1.05 (t, *J* = 7.3 Hz, 12H, OCH$_2$CH$_2$C*H*$_3$), 0.87 (t, *J* = 7.2 Hz, 24H), 0.87 (t, *J* = 7.2 Hz, 24H, OCH$_2$CH$_2$C*H*$_3$).**^{13}C NMR** (101 MHz, DMSO) δ 163.94, 163.87, 155.27, 152.91, 152.23, 151.11,

143.09, 134.84, 134.37, 133.75, 132.39, 132.31, 131.99, 129.28, 124.73, 122.61, 119.48, 76.24, 40.69, 40.58, 31.11, 30.83, 22.79, 22.36, 22.36, 10.35, 10.35, 9.79. **M/z (MALDI-TOF)** = 4209.0 m/z [M-16HCl+H] $^{+}$. **IR** ν = 3332; 2961; 2943; 2865; 1683; 1602; 1557; 1466; 1386; 1215; 1147; 1042; 1002.

Multicalixarene (85)

Starting material = **66** (178 mg; 0.03 mmol). Product = **86** (quantitative). **Mp** > 262 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.05 (s, 4H, Az*H*), 7.05 (s, 8H, Ar*H*), 7.03 (s, 8H, Ar*H*), 6.96 (d, *J* = 7.0 Hz, 8H, Ar*H*), 6.85 (s, 8H, Ar*H*), 6.82 (s, 8H, Ar*H*), 6.69 (t, *J* = 7.0 Hz, 4H, Ar*H*), 5.13 (s, 8H, OCH_2CCAz), 4.50 – 4.39 (m, 16H, ArCH_2Ar), 4.35 (s, 8H, $OCH_2CH_2CH_2CH_2$Az), 3.90 – 3.71 (m, 64H, $ArNHCOCH_2NH_3^+Cl^-$, $OCH_2CH_2CH_3$ and ArCH_2Ar), 3.39 (s, 8H, $OCH_2CH_2CH_2CH_2$Az), 3.14 (d, *J* = 12.5 Hz, 8H, ArCH_2Ar), 3.10 (d, *J* = 12.5 Hz, 8H ArCH_2Ar), 1.99 – 1.83 (m, 24H, $OCH_2CH_2CH_3$), 1.79 – 1.70 (m, 8H, $OCH_2CH_2CH_2CH_2$Az), 1.32 – 1.26 (m, 8H, $OCH_2CH_2CH_2CH_2$Az), 1.03 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.89 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$); **^{13}C NMR** (101 MHz, DMSO) δ 163.92, 156.37, 152.83, 152.30, 151.03, 142.93, 134.71, 134.58, 134.03, 133.63, 132.41, 132.26, 132.01, 129.34, 124.30, 119.48, 76.52, 76.28, 69.44, 66.29, 49.15, 40.66, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 31.20, 30.80, 26.28, 26.14, 22.78, 22.41, 10.29, 9.92, 9.92; **M/z (MALDI-TOF)** = 4321.4 m/z [M-16HCl+H] $^{+}$. **IR** ν = 3338; 3975; 2929; 2872; 1681; 1601; 1465; 1385; 1220; 1147; 1044; 1002.

Multicalixarene (86)

Starting material = **65** (181 mg; 0.03 mmol). Product = **85** (quantitative). **Mp** > 260 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.04 (s, 4H, Az*H*), 7.09 (s, 8H, Ar*H*), 7.07 (s, 8H, Ar*H*), 6.97 (d, *J* = 7.00 Hz, 8H, Ar*H*), 6.90 – 6.80 (m, 8H, Ar*H*), 6.77 (s, 8H, Ar*H*), 6.66 (t, *J* = 7.0 Hz, 4H, Ar*H*), 5.04 (s, 8H, OCH_2CCAz), 4.62 – 4.36 (m, 24H, ArCH_2Ar and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 3.96 – 3.71 (m, 56H, $OCH_2CH_2CH_3$ and $ArNHCOCH_2NH_3^+Cl^-$), 3.68 (s, 8H, ArCH_2Ar), 3.40 (s, 8H, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 3.13 (d, *J* = 12.0 Hz, 8H, ArCH_2Ar), 3.10 (d, *J* = 12.0 Hz, 8H, ArCH_2Ar), 2.04 – 1.73 (m, 32H, $OCH_2CH_2CH_2CH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_3$), 1.46 – 1.19 (m, 32H, $OCH_2CH_2CH_2CH_2CH_2CH_2CH_2$Az, $OCH_2CH_2CH_2CH_2CH_2CH_2$Az and $OCH_2CH_2CH_2CH_2CH_2CH_2$Az), 1.04 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_3$), 0.88 (t, *J* = 7.5 Hz, 24H, $OCH_2CH_2CH_3$); **^{13}C NMR** (101 MHz, DMSO) δ 163.93, 163.86, 156.38, 152.91, 152.19, 151.10, 142.90, 134.89, 134.34, 133.82, 133.52, 132.31, 131.92, 129.29, 124.26, 119.46, 76.53, 76.19, 49.24, 40.70, 40.59, 30.82, 30.06, 29.03, 25.94, 24.80, 22.79, 22.34, 10.34, 9.85; **M/z (MALDI-TOF)** = 4433.5 m/z [M-16HCl+H] $^{+}$. **IR** ν = 3423; 3059; 2930; 2872; 1673; 1601; 1556; 1470; 1385; 1215; 1145; 1043; 1002.

Multicalixarene (87)

Starting material = **67** (181 mg; 0.03 mmol). Product = **87** (quantitative). **Mp** > 268 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.38 (s, 4H, Az*H*), 7.26 (s, 8H, Ar*H*), 7.12 (s, 8H, Ar*H*), 6.88 (s, 8H, Ar*H*), 6.71 (s, 8H, Ar*H*), 6.67 (s, 8H, Ar*H*), 5.07 (s, 8H, OCH_2CCAz), 4.51 – 4.33 (m, 24H, ArCH_2Ar and OCH$_2$CH_2Az), 4.16 (d, *J* = 12.7 Hz, 4H, ArCH_2Ar), 4.00 – 3.70 (m, 64H, OCH_2CH$_2$CH$_3$, ArNHCOCH_2NH$_3^+$Cl$^-$, OCH_2CH$_2$Az), 3.22 – 3.04 (m, 20H, ArCH_2Ar), 1.92 – 1.74 (m, 24H, OCH$_2$CH_2CH$_3$), 1.11 (s, 36H, CH_3CAr), 1.05 (t, *J* = 7.5 Hz, 12H, OCH$_2$CH$_2$CH_3), 0.74 (t, *J* = 7.5 Hz, 24H, OCH$_2$CH$_2$CH_3); **^{13}C NMR** (101 MHz, DMSO) δ 163.95, 163.88, 152.97, 152.07, 151.06, 144.64, 143.36, 135.05, 134.04, 133.62, 132.40, 131.82, 125.06, 124.47, 119.55, 76.58, 76.13, 54.94, 40.75, 33.52, 31.02, 22.78, 22.28, 10.41, 9.62; **M/z (MALDI-TOF)** = 4433.8 m/z [M-16HCl+H] $^{+}$. **IR** *v* **=** 3338; 3975; 2929; 2872; 1681; 1601; 1465; 1385; 1220; 1147; 1044; 1002.

Multicalixarene (88)

Starting material = **68** (184 mg; 0.03 mmol). Product = **86** (quantitative). **Mp** > 248 ^{o}C (decompose); **^{1}H NMR** (400 MHz, MeOD) δ 8.17 (s, 4H, Az*H*), 7.17 (s, 8H, Ar*H*), 7.08 (s, 8H, Ar*H*), 6.77 (s, 16H, Ar*H*), 6.71 (s, 8H, Ar*H*), 4.85 (s, 8H, OCH_2CCAz (detected by HSQC)), 4.61 (s, 8H, OCH$_2$CH$_2$CH$_2$CH_2Az), 4.45 – 4.27 (m, 20H, ArCH_2Ar), 3.97 – 3.71 (m, 64H, OCH_2CH$_2$CH$_3$, ArNHCOCH_2NH$_3^+$Cl$^-$and OCH_2CH$_2$CH$_2$CH$_2$Az), 3.16 – 3.03 (m, 20H, ArCH_2Ar), 2.13 (s, 16H, OCH$_2$CH_2CH$_2$CH$_2$Az and OCH$_2$CH$_2$CH_2CH$_2$Az), 1.95 – 1.77 (m, 24H, OCH$_2$CH_2CH$_3$), 1.14 – 0.96 (m, 48H, CH_3CAr and OCH$_2$CH$_2$CH_3), 0.77 (t, *J* = 7.4 Hz, 24H, OCH$_2$CH$_2$CH_3): **^{13}C NMR** (101 MHz, DMSO) δ 163.93, 163.85, 152.98, 152.07, 151.14, 142.99, 135.07, 135.03, 134.04, 133.61, 132.39, 132.31, 131.82, 119.46, 76.59, 76.12, 40.71, 33.45, 31.11, 30.71, 27.01, 26.88, 22.79, 22.27, 10.40, 9.64; **M/z (MALDI-TOF)** = 1515.8352 m/z [M-15HCl+3H] $^{3+}$. **IR** *v* **=** 3403; 2960; 2872; 1683; 1603; 1559; 1468; 1247; 1220; 1147; 1044; 1003.

Multicalixarene (90)

Calixarene **48** (1.27 g, 1 mmol) was added to a solution of **39** (151 mg, o.2 mmol) in DMF (15 mL) in the presence of $CuSO_4 \cdot 5H_2O$ (100 mg, 0.4 mmol,) and sodium ascorbate (316 mg, 1.6 mmol). The reaction was stirred for 2 hours at 110^{o}C. The solution was diluted with DCM (50 mL) and washed with H_2O (2 x 50 mL) and brine (2 x 50 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel, (*n*-hexane:EtOAc:MeOH = 1:1:0.1) and precipitation from DCM/*n*-hexane yielded compound **70** as a white solid (**M/z (MALDI-TOF) =** 5889.3 m/z [M+H] $^{+}$).

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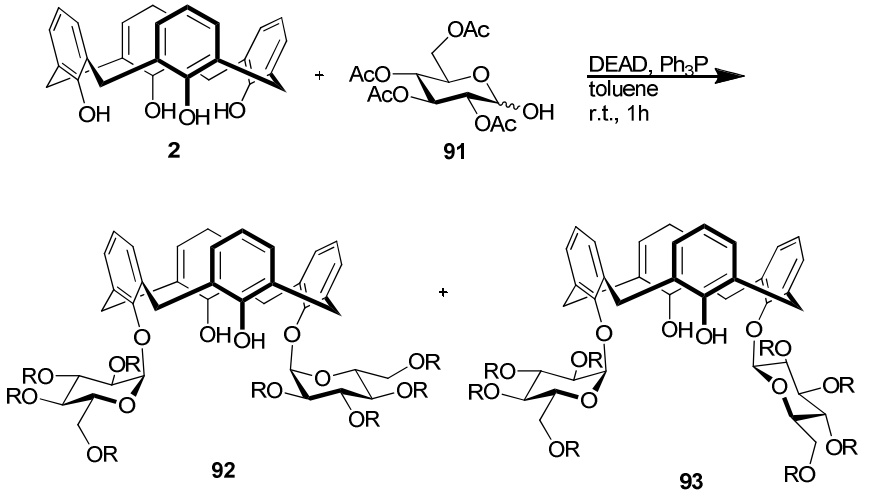
Chapter 2: Calixarene Glycocomjugates

Chapter 2. Calixarene glycoconjugates

2.1 Introduction

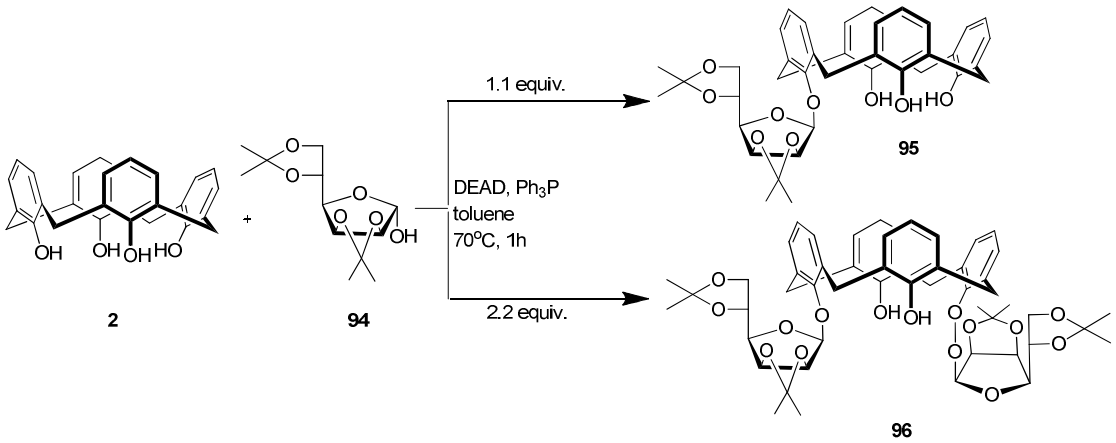
Interactions between proteins on the cell surface and carbohydrates are involved in a multitude of biological processes such as various intercellular communications, transduction events and adhesion of bacteria and viruses to cell surface.[1] These interactions are normally very weak, showing association constants (Kd) around 10^6 M^{-1} for monosaccharides. Nevertheless these interactions can be strong and specific when a set of multivalent saccharides are joined together with the appropriate spatial disposition. The enhancement interaction is higher than explainable with an increased concentration of monovalent saccharides and is called the "cluster effect".[2] This phenomenon makes glyco-clusters important biological tools to target proteins and their functions. The synthesis of multivalent saccharides has been the focus of many studies in recent years. Rigid molecules such as cyclodextrins, resorcinarenes and calixarenes have been used as backbone to anchor a number of sugar molecules.[3]

The first example of calix[4]arenes glycoconjugates was described in 1994 by Dondoni *et al*. Calixarene **2** was reacted with tetra-*O*-acetyl-α,β-D-glucopyranose **91** under Mitsunobu conditions. This first attempt gave a mixture of both the α,β and the α,α-biglucoside (**92** and **93**) which were isolated in modest yields (scheme 2.1).[4]



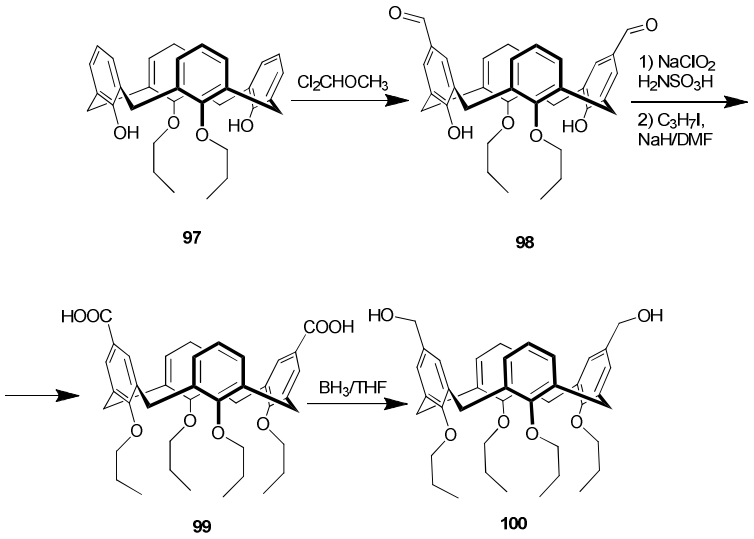
Scheme 2.1 First example of glycoconjugates.

In later work[5] the conditions were improved by the authors and the narrow rim of calixarene **2** was successfully embroidered with the configurationally stable α-D-mannofuranose diacetonide **94** to yield the the calixarene mannosides **95** and **96** (scheme 2.2).



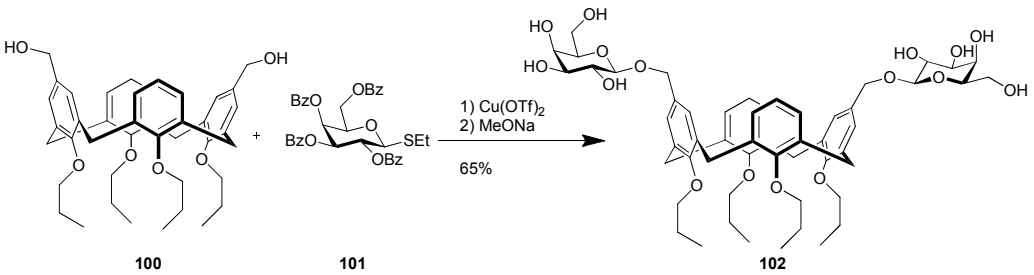
Scheme 2.2: Synthesis of calixarene mannosides

In the same publication the authors described also the synthesis of calixarenes glycosylated on the wide rim[5]. A bishydroxymethyl-substituted calix[4]arene **100** was obtained through selective formylation of the diametrical positions of 1,3-dipropoxycalixarene **97**.[6] The compound was oxidised to the diacid analogue **99** before alkylation of the last two free phenolic oxygens and consequent lock in the cone conformation. Reduction with borane (BH_3) in THF gave the desired bis-alcohol . (scheme 2.3).



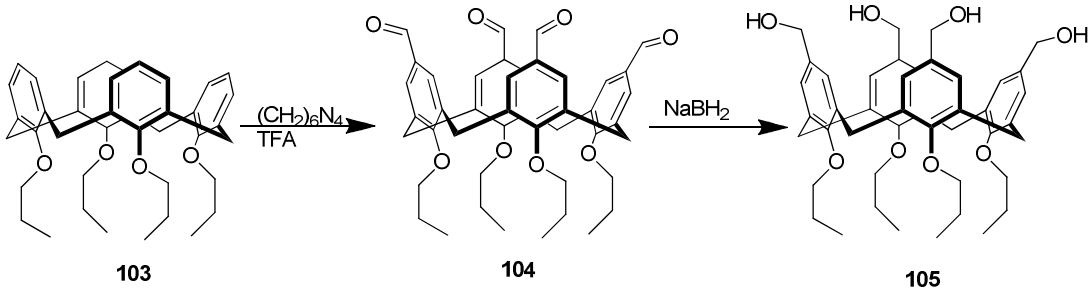
Scheme 2.3: Synthesis of bis alcohol functionalised calixarene.

The oxygens on the upper rim were then successfully coupled with 2.6 equivalents of ethyl tetra-*O*-benzoyl-1-thio-*β*-D-galactoside **101** in the presence of copper (II) triflate ($Cu(OTf)_2$) in acetonitrile at room temperature to yield after deprotection the di-glycoside **102** in 65% yield (scheme 2.4) .[5]



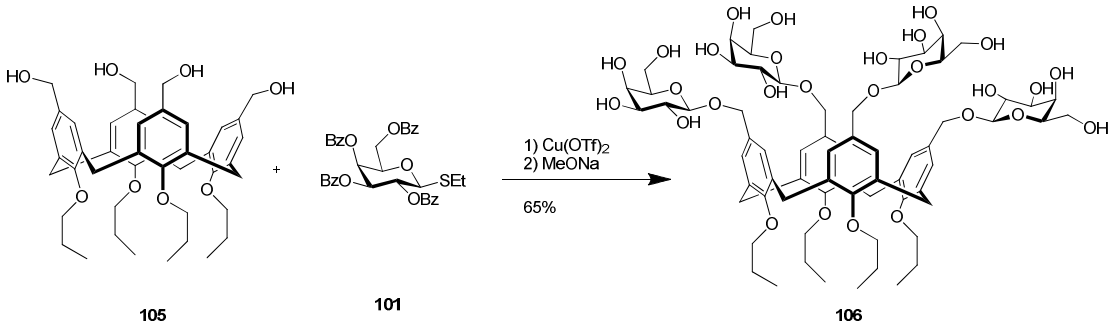
Scheme 2.4: Synthesis of Bis-Galactose Glycoconjugate

With a similar strategy they synthesised the first tetra-*O*-galactosyl calix[4]arene **106**, using as a building block the tetrahydroxymethyl substituted calix[4]arene **105**. This compound was obtained *via* Duff formylation of tetrapropoxycalix[4]arene and subsequent reduction to the tetra-alcohol (scheme 2.5).



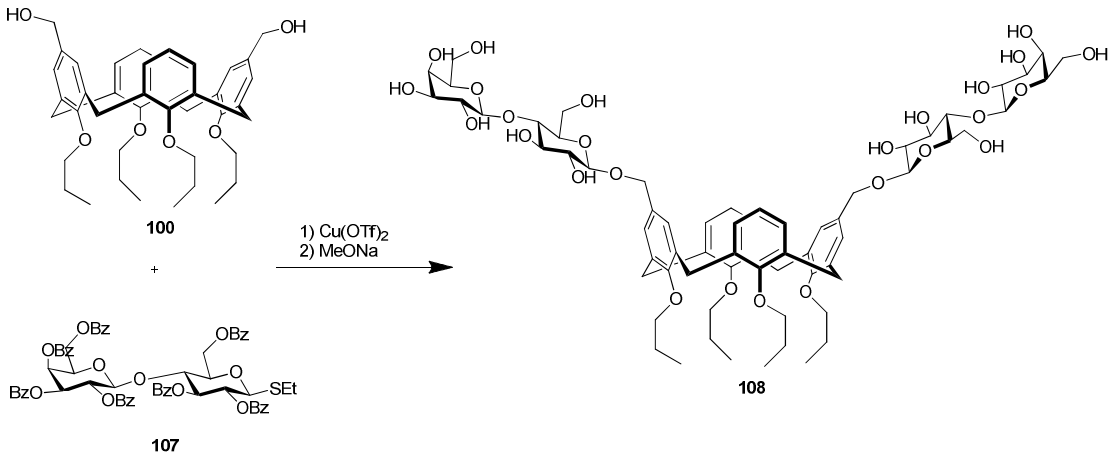
Scheme 2.5: Tetra hydroxymethyl functionalisation of the upper rim of Calixarene

Compound **105** was then coupled with six equivalents of the galactoside **101** to yield the desired compound (65%) (Scheme 2.6).



Scheme 2.6 Synthesis of the first tetravalent calix[4]arene glycoconjugate

The good results obtained with this approach encouraged the authors towards the use of a di-glycoside thioethyl heptabenzoyl-β-D-lactoside **107**. The reaction afforded the desired compound **108** in low yield when the bishydroxymethyl-substituted calix[4]arene **100** was used as a starting material (scheme 2.7).



Scheme 2.7: Di-lactoside calixarene glycoconjugate

The tetra-substituted compound was not isolated when **105** was reacted with six equivalents of the di-glycoside **107**. Interestingly the only product of this reaction was **11**, the ether-bridged calixarene deriving from the acid-catalyzed intramolecular coupling of two distal hydroxymethyl groups (figure 2.1). Similar ether bridged structures such as **109** and **110** where isolated as side products also in the synthesis of **102** and **106**.

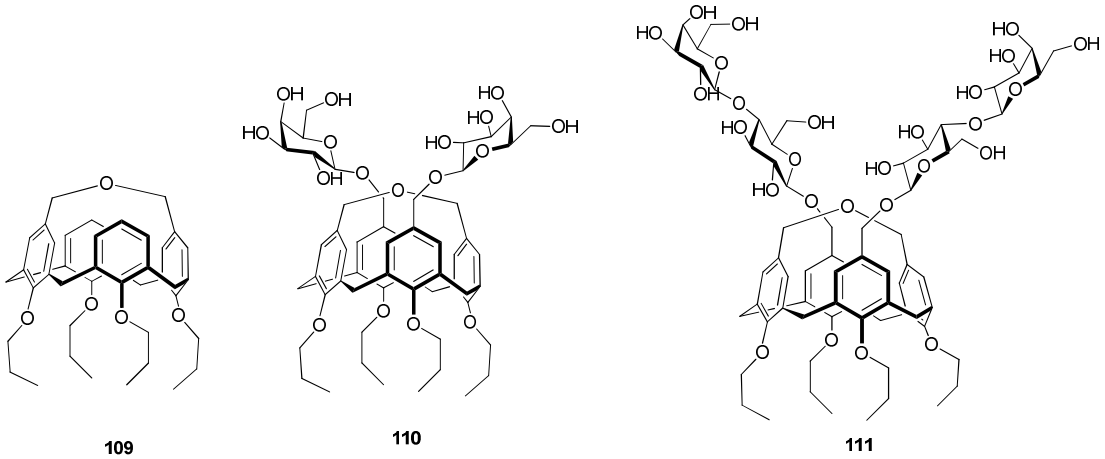
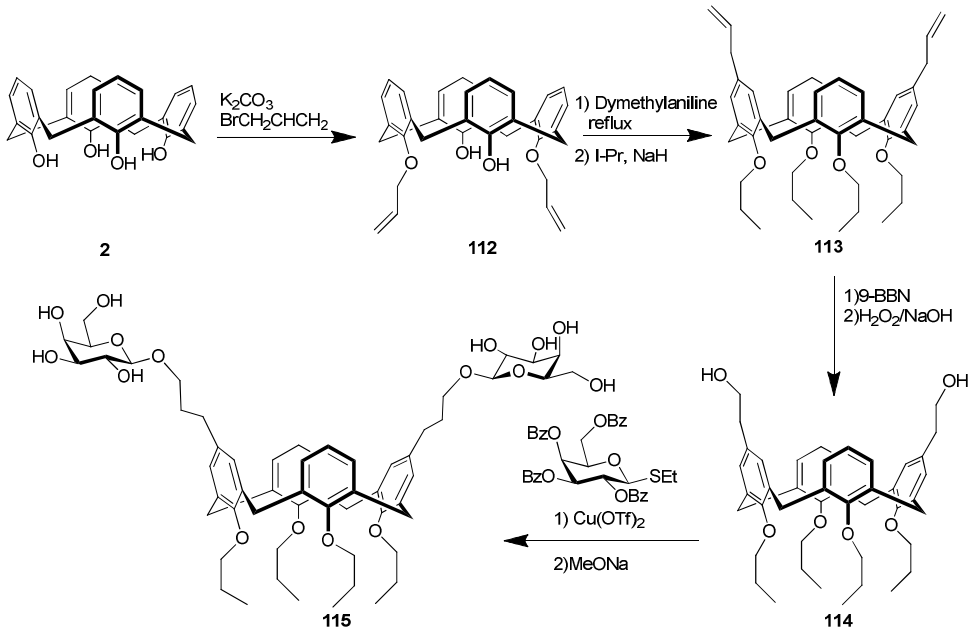


Figure 2.1: Ether bridged side product obtained by Dondoni *et al*.

The formation of the ether-bridged side products was overcome a few years later in another paper from the same authors. The introduction of a longer spacer chain between the alcoholic oxygens and the phenyl rings on the wide rim, allowed the synthesis, in good yields, of the bis- and tetra- calixarene glycoconjugates.

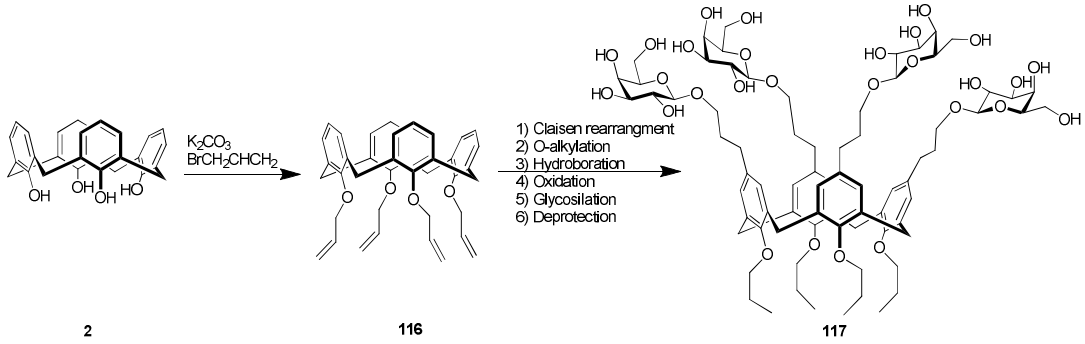
The bis-alcohol derivative was synthesised starting from calix[4]arene **2**, which can be alkylated with two equivalents of allyl bromide in the presence of K_2CO_3 to give **112**. This compound can undergo Claisen rearrangement to yield a calixarene with the two allyl groups on the wide rim.[7] At this stage the conformation was locked by alkylating the phenolic oxygens with iodopropane in the presence of NaH to give compound **113**. The allyl groups were converted to alcohols

through hydroboration/oxidation to give the bis-alcohol derivative **114**, which was glycosylated to give, after deprotection of the sugar moieties, compound **115** (scheme 2.8).



Scheme 2.8: Synthesis of bis-glycoconjugate with three carbon atom spacer.

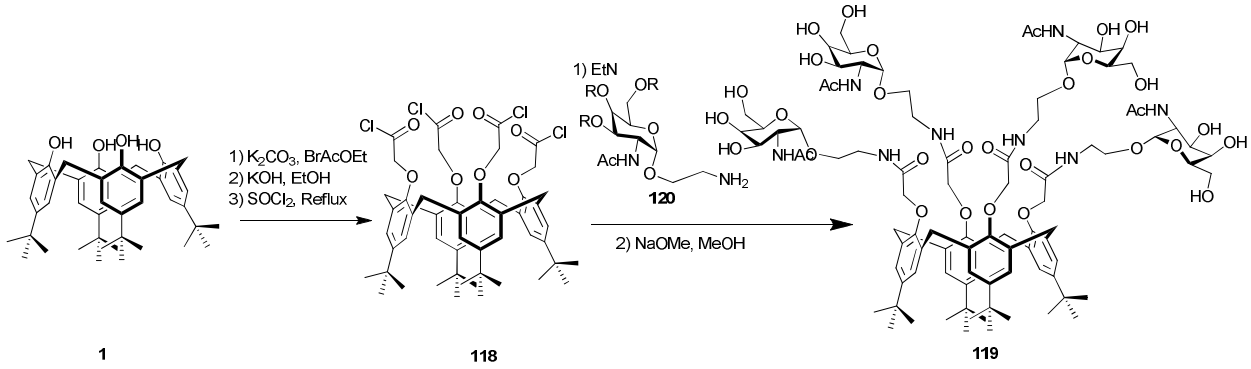
The use of NaH as a base and an excess of alkylating agent in the first synthetic step led to the formation of the tetra-allyl derivative **116**,[8] which can undergo the same set of reactions described above to give the tetra-alcohol derivative and was successfully coupled with the glycoside to yield the glucoconjugate **117** (scheme 2.9).



Scheme 2.9: Synthesis of tetra glycoconjugate with three carbon atom spacer

Linkage of sugars and calix[4]arenes through amide bond formation was first explored by Roy and Kim.[9] Their approach was successful toward the synthesis of water soluble glyco-calix[4]arene dendrimers. The synthethic strategy followed was to attach α-Galacto-acetyl-amine (α -GalNAc) derivative **120** to a tetra acyl chloride calix[4]arene central core **118** through an amide bond. The central core was synthesised starting from *p-tert*-butyl calix[4]arene **1**. In the first step **1** was O-alkylated with ethyl bromoacetate in the presence of K_2CO_3. Subsequent base hydrolysis yielded the tetra-acid derivative which could be transformed to the acyl chloride

derivative **118** upon treatment with thionyl chloride. Coupling of **118** with the acetylgalactosamino derivative **120** yielded the glycoconjugate **119** (scheme 2.10).



Scheme 2.10: Amide linked glycoconjugate

The synthesis of glycocalixarenes with higher valencies were obtained by incorporating the galactosamine derivative in multi-branched structures (figure 2.2) before coupling with the calix[4]arene central core **118**. In this fashion they obtained dendrimers bearing up to sixteen sugar moieties.

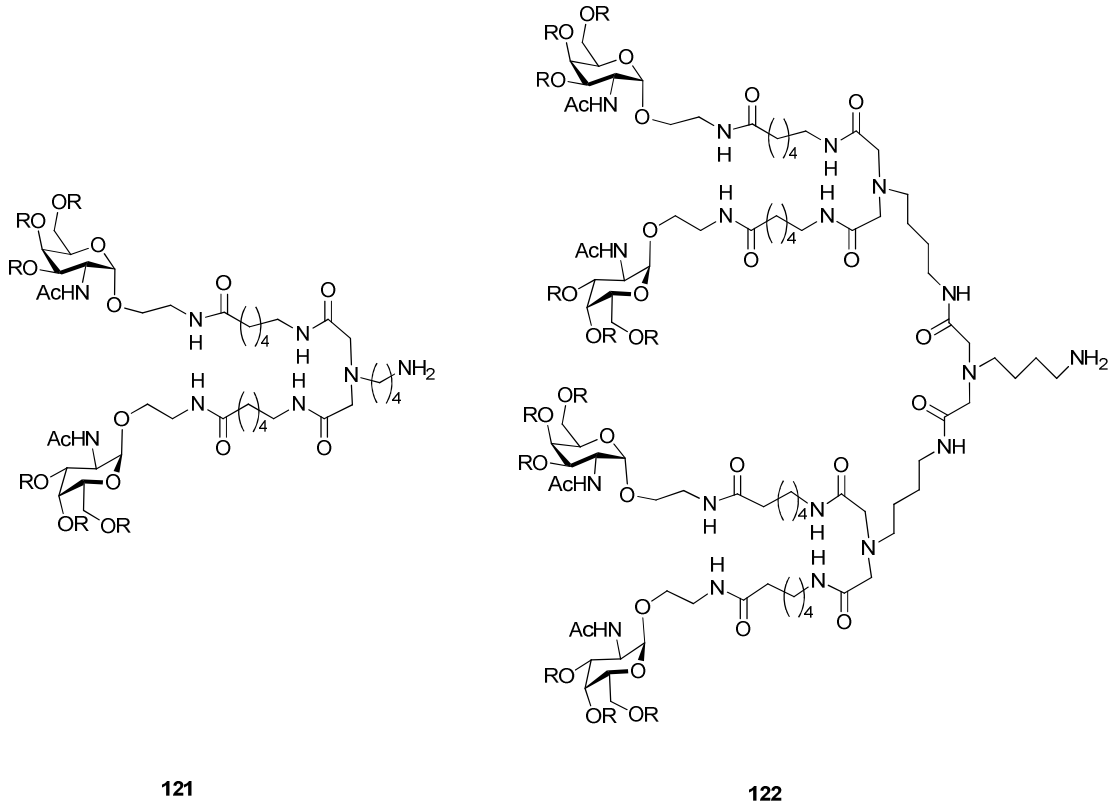
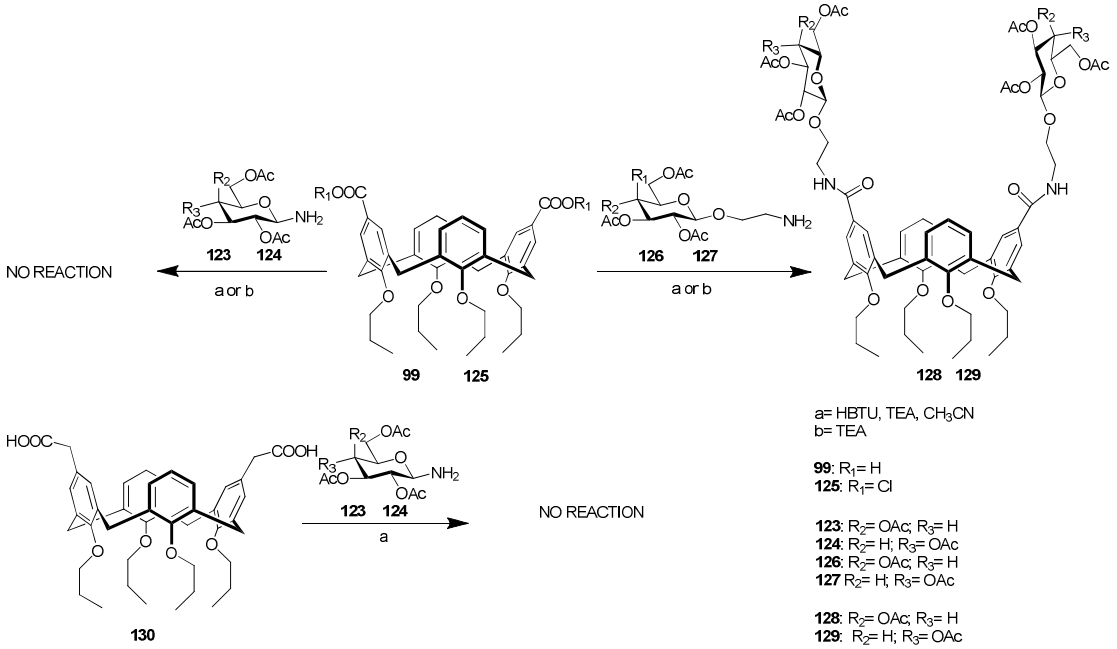


Figure 2.2: Multi-branched sugars.

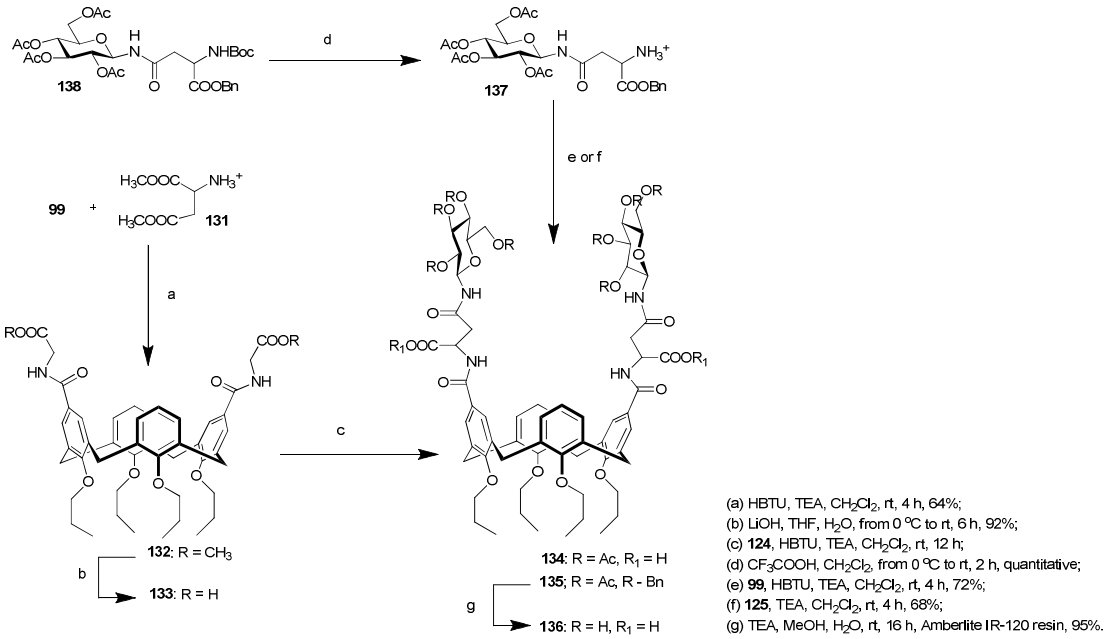
Ungaro and co-workers have explored the possibility of using amide bonds to link carbohydrates to the wide rim of calix[4]arenes.[10] In a first attempt they investigated linking galactosamine derivatives **123** and **124** to a calix[4]arene functionalised with two carboxylic acid groups[11] **99** in

the presence of O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and triethylamine (TEA) at room temperature (scheme 2.11) Unfortunately the reaction did not yield the expected compound but the benzotriazole ester instead.[10] The di-carboxylic acid was converted to the chloride derivative **125**,[12] but it failed again to react with the galactosamine derivatives **123** and **124** in the presence of TEA as base.[10] The unsuccessful approach led the way towards the introduction of a spacer either on the calixarene or on the sugar. Di-*p*-carboxymethylcalix[4]arene **130**, synthesised via hydrolysis of a cyano precursor according to Gutche's method,[13] failed to couple with the galactosamines **123** and **124**.[10] Modified galactosamines **126** and **127** with a two carbon atoms spacer were successfully coupled with the Di-carboxycalix[4]arene **99** and with the acyl chloride derivative **125** to give the glycoconjugates **128** and **129**.



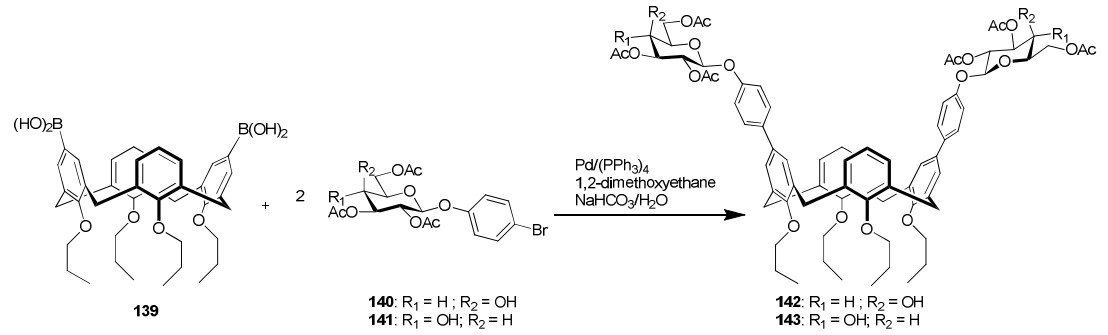
Scheme 2.11: Attempts towards the synthesis of Glucosamine glycoconjugates

In the same paper, the authors describe also the synthesis of a glycoconjugates using aspartic acid residues as spacers (scheme 2.12). In the first approach the amino acid methyl ester **131** was coupled with the di-carboxylic acid calixarene **99** to give **132**. Demethylation and reaction with the glucosamine **124** yielded a complex mix of product in which the sugar-peptidocalix[4]arene conjugate **134** could be detected by mass spectrophotomtry but could not be isolated. Significantly better results were obtained when the galactosamine **124** was coupled with the protected aminoacid first. Deprotection of the N-terminus and coupling with either **99** or **125** yielded the desired sugar–peptidocalix[4]arene conjugates **135** which was then fully deprotected to give **136**.



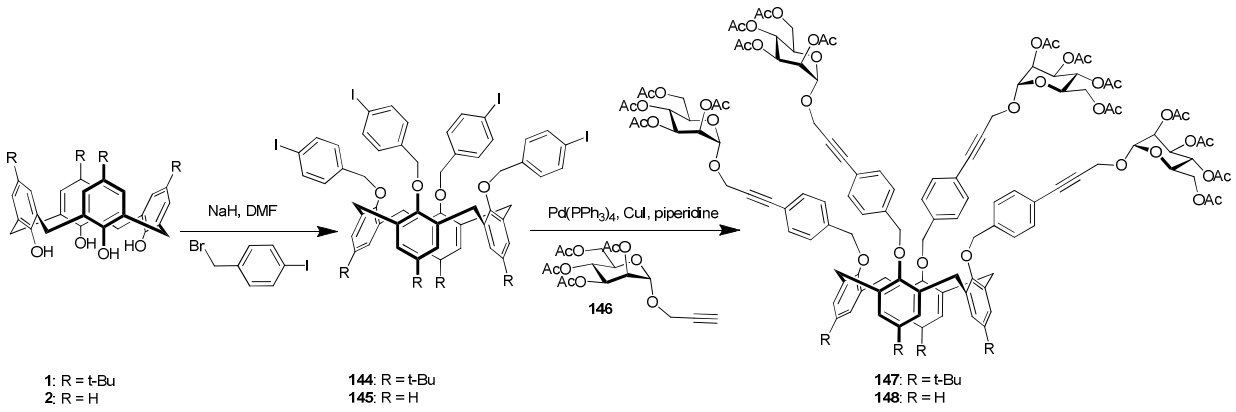
Scheme 2.12: Sugar-peptido-calixarene.

In 1998 Felix *et al.* proposed another interesting route toward the synthesis of calix[4]arene glycoconjugates. They performed a palladium Pd(0) catalysed Suzuki cross coupling between a *p*-bromophenyl glycoside and the boronic acid derivative of a calix[4]arene **139** (scheme 2.13).[14] **139** was obtained from a di-bromo precursor[15] and coupled with **140** and **141** in the presence of tris(dibenzylideneacetone)dipalladium(0), triphenyl phosphine (PPh_3) and hydrogen carbonate solution in 1,2-dimethoxyethane to obtain the glycoconjugates **142** and **143**.



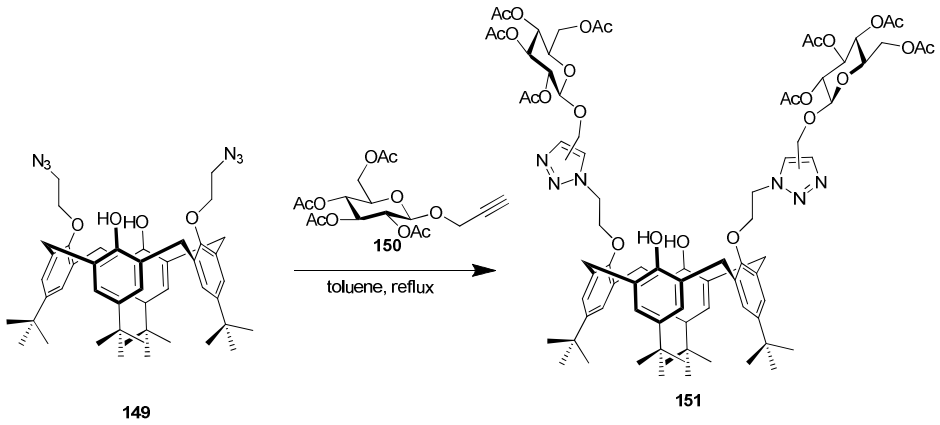
Scheme 2.13: Glycoconjugation *via* Suzuki cross coupling.

Glycosylation of calix[4]arenes at the lower rim was achieved *via* Sonogashira coupling reaction by Pérez-Balderas and Santoyo-González.[16] *p-Tert*-butylcalix[4]arene **1** and calix[4]arene **2** were alkylated with *p*-iodobenzyl bromide to give the 4-iodophenylcalixarenes **144** and **145** (scheme 2.14). Propargyl mannoside **146** was coupled with the calixarene derivatives **144** and **145** in the presence of $Pd(PPh_3)_4$ and CuI in anhydrous piperidine to yield the glycoconjugates **147** and **148**.



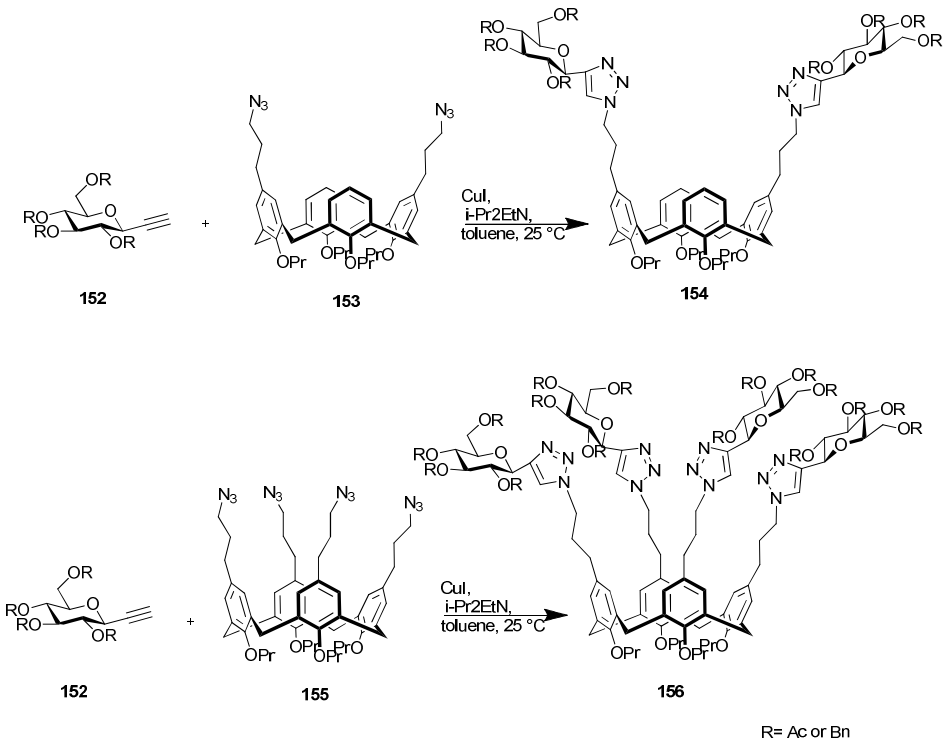
Scheme 2.14: Glycoconjugation *via* Sonogashira coupling.

Another valuable route towards the synthesis of calix[4]arene glycoconjugates was first reported by Santoyo-González and co-workers in 2000.[17] In their approach azido functionalised calix[4]arene **149** was linked to propargylated glucoside **150** through a thermally activated 1,3 dipolar cycloaddition (scheme 2.16). The experiment yielded a mixture of 1,4 and 1,5 substituted products **151** because of the lack of regioselectivity of the reaction.



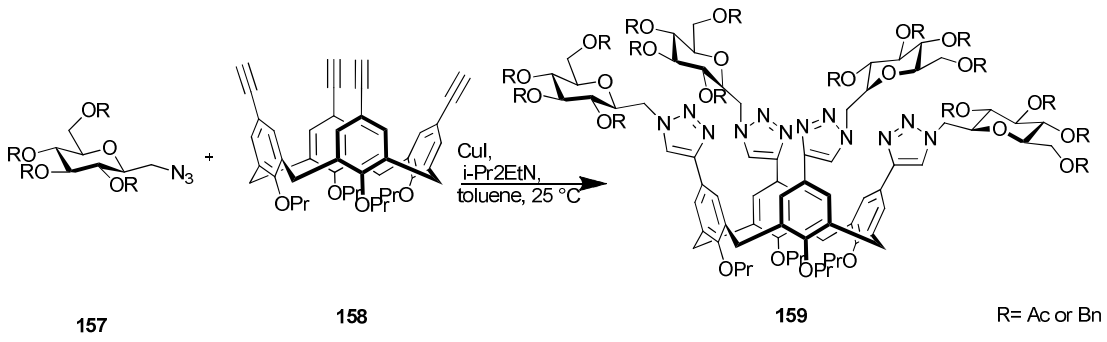
Scheme 2.15: First glycoconjugate formed by cycloaddition.

The stereoselectivity gained by the use of copper (I) as catalyst,[18, 19] made this reaction a useful tool to tether carbohydrates to calixarene scaffolds. In 2006 Dondoni and Marra reported the synthesis of calix[4]arene glycoconjugates linked through 1,4 substituted triazole rings.[20] In their work they proposed two strategies, both using copper iodide as the source of copper (I). In the first approach the C-glycoside (scheme 2.16), functionalised on the anomeric carbon with an ethynyl group **152**,[21] was linked to calix[4]arenes **153** and **155** functionalised on the wide rim respectively with two and four propylazido groups obtained from polyalcohol precursors[22]. The reaction yielded the glycoconjugates **154** and **156**.



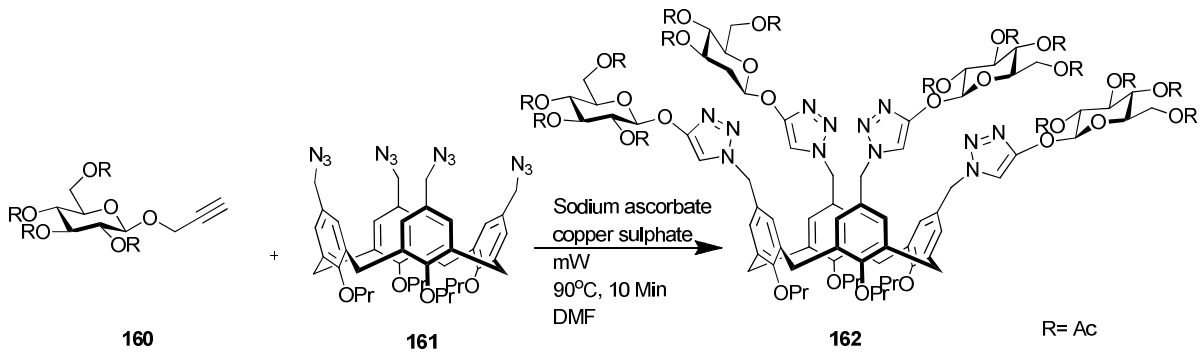
Scheme 2.16: Dondoni's first approach to click chemistry linked C-glycoconjugates

In the second approach they prepared a C-glucoside bearing an azide moiety **157**, which was linked to a calix[4]arene bearing four ethynyl groups on the wide rim **158**, obtained from the tetra-iodo precursor,[23] to yield the glycoconjugate **159** (scheme 2.18).



Scheme 2.17: Dondoni's second approach to click chemistry linked C-glycoconjugates

In 2007, Bew's group explored the potential of the "click" reaction as a tool to embroider calixarene scaffolds with sugar functions. In his work the propargylated O-glucoside **160** was clicked with an azido calixarene core synthesised from the chloromethyl precursor.[24] The reaction took place under microwave irradiation, using copper sulphate and sodium ascorbate as a source of copper (I) to yield compound **162** (scheme 2.18).



160 161 162

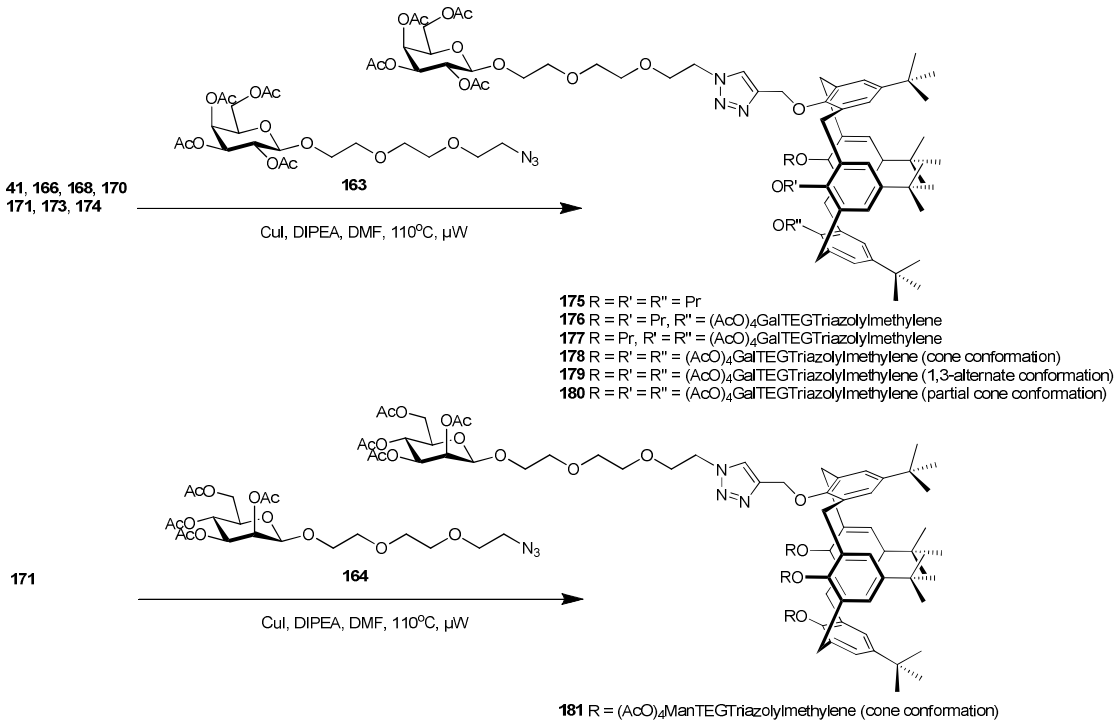
Scheme 2.18: Bew's Glycoconjugate

A different approach towards the synthesis of calix[4]arene glycoconjugates was reported in a joint paper by Matthews and Vidal in 2009.[25] The molecules proposed carried from one up to four glycoside units at the narrow rim of the calixarene scaffold. The tetravalent glycoconjugates were synthesised in the cone, 1,3-alternate and partial cone conformation. Peracetylated galactose and mannose were functionalised with an azido group incorporating a triethylene glycol chain spacer **163** and **164** (Scheme 2.19 and 2.20). The calixarene scaffold was locked in one of the desired conformations and functionalised with one or more propargyl groups as shown in the scheme 2.20. The copper catalysed cycloaddition reaction was performed with different copper (I) sources, at different temperatures, in different solvent and with or without microwave irradiation. The optimum conditions were found to be either:

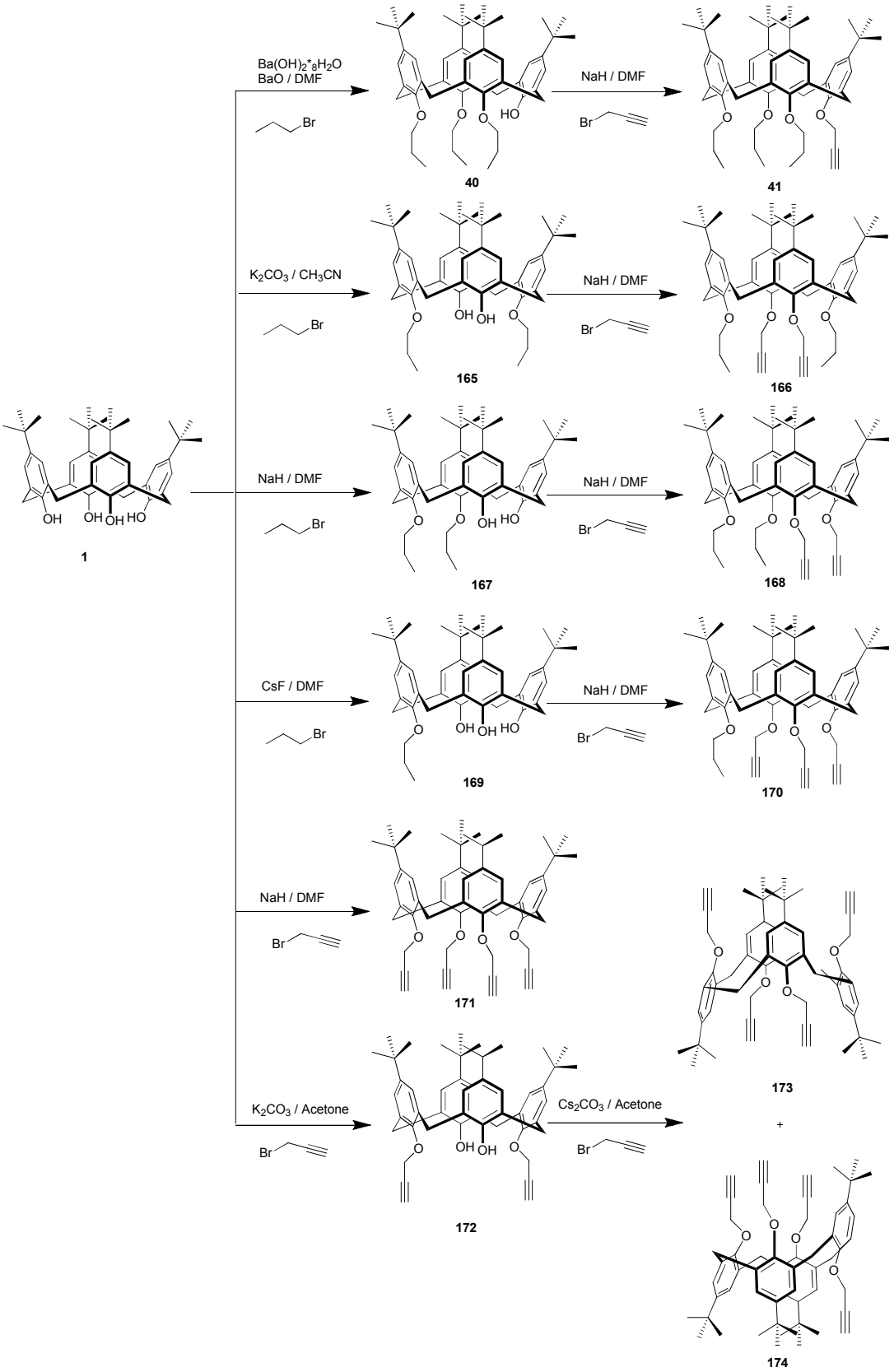
- 0.5 equivalents of CuI and 5 equivalents of di-*iso*-propylethylamine (iPr_2Net) in DMF at 110^{o}C under microwave irradiation or,

- 0.5 equivalents of $CuSO_4 \cdot 5\ H_2O$ and 2 equivalents of sodium ascorbate in DMF at 110 ^{o}C under microwave irradiation

The aims and outcomes of their work will be discussed in details in the next section (2.2).



Scheme 2.19: Matthews' and Vidal's Glycoconjugates.



Scheme 2.20: Synthesis of mono-, bis-, tris-, and tetra-alkyne calixarenes in various conformations.

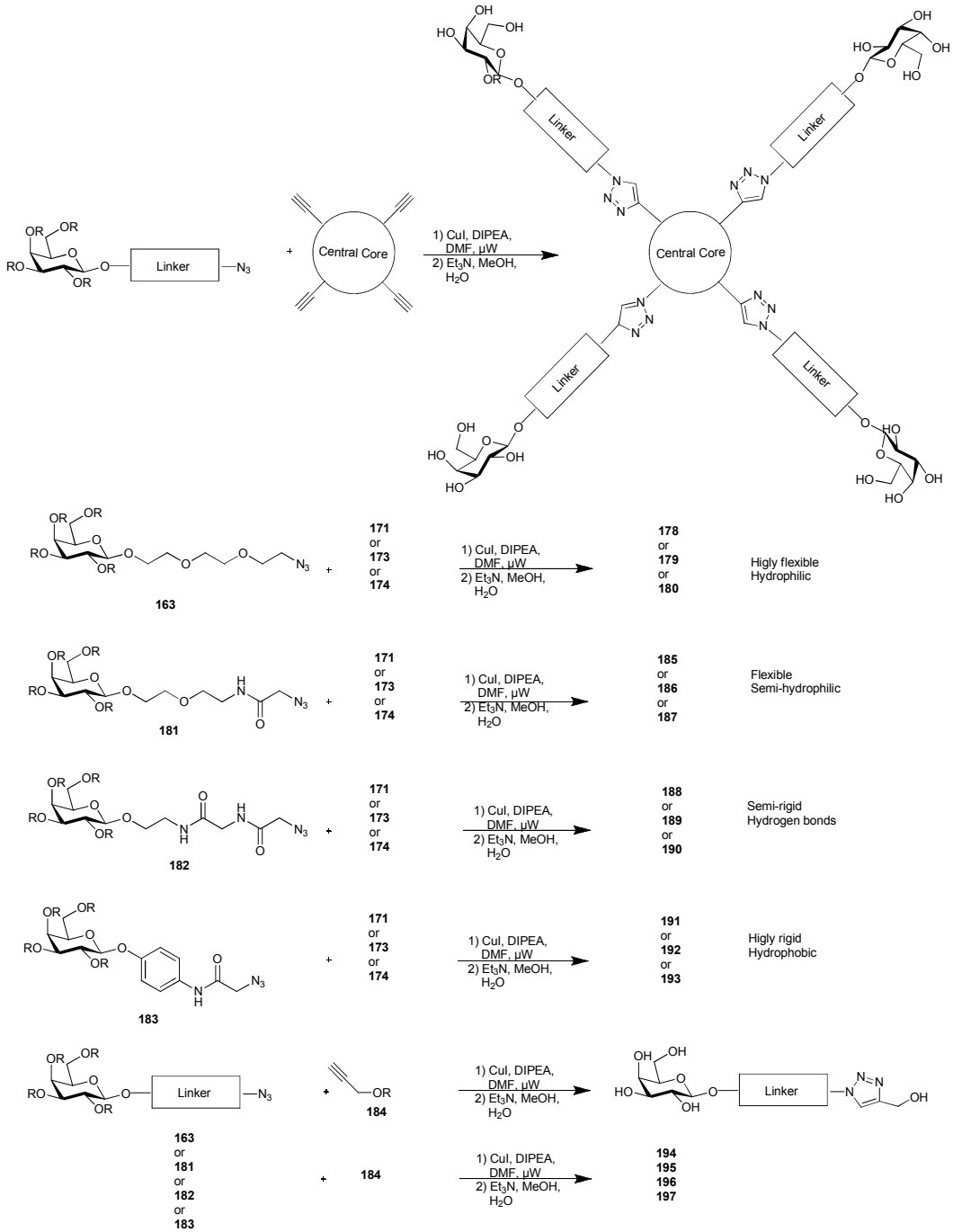
2.2 Preliminary results and rationale

Matthews and Vidal aimed to target the galactose-binding lectin PA-IL from the opportunistic bacterium *Pseudomonas aeruginosa*. This bacterium is a major causative agent of lung infections in cystic fibrosis patients.[25] The virulence of *Pseudomonas aeruginosa* is related to its cell adhesion property which is mediated by the bacterial lectins.[26] Lectins are proteins which bind mono- and oligosaccharides reversibly with high specificity.[27] They do not have catalytic activity and unlike antibodies they are not products of an immune response. Each lectin has two or more carbohydrate binding sites, therefore they do not only interact with the sugar on the cell surface but they can also cross-link the cells causing precipitation. This phenomenon is called cell agglutination. Formation of cross-links and consequent precipitation was observed also when lectins reacted with oligosaccharides and/or glycoproteins. Agglutination and precipitation can be inhibited by binding with the sugar specific for the lectins. This type of protein has been found in most organisms in nature such as viruses, bacteria, plants and animals and play a major role in cell recognitions and cell adhesion. Two different types of lectin have been isolated from the bacteria: PA-IL which binds D-galactose and its derivatives and PA-IIL L-fucose, D-mannose, L-galactose and d-fructose.[28] PA-IL binds selectively D-galactose with an association constant (K_a) of 3.4×10^4 M^{-1}. The binding is stronger when the sugar bears a lipophylic group on the anomeric position. PA-IL can also bind disaccharides having a terminal galactose molecule. It is a tetrameric protein and each monomer contains a sugar binding site. Occupation of such glycoside binding sites could prevent the cell adhesion properties of the lectin and therefore the associated bacterium virulence. Matthews and Vidal's glycoconjugates were used to bind this type of lectin.

In their study they synthesised seven calix[4]arene glycoconjugates in different conformations and with different galactose loads.[25] Their work showed that multivalency plays a role in binding PA-IL, the tetravalent molecules are the best ligands, in agreement with the "cluster effect" theory. Enhanced binding was found with the molecules in the 1,3-alternate and in the partial cone conformation, showing that also the spatial disposition of the galactose residues has an effect on the binding.

Glycoconjugates in the 1,3 alternate conformation are able to bind the carbohydrate binding sites located on different lectins forming branched filaments. This theory was confirmed in 2011 by atomic force microscopy studies, which showed images of the filaments formed by the lectins in the presence of the glycoclusters **179**.[29] In latter work the triethylene glycol linker which connected the sugar moieties to the calixarene scaffold was compared to newly designed analogues. These new linkers were the same length of the one previously used but differed in both flexibility and hydrophilicity (scheme 2.21).

The azido functionalised glycosides with the different linkers were reacted with the tetra-alkyne calix[4] arene in the cone, 1,3-alternate and partial cone conformation. To asses the binding properties as a monomer the azido-linkers were also reacted with propargyl acetate yielding after deacetylation the alcohol derivatives.



Scheme 2.21: Development of new linkers for improved binding.

The biological investigation of the PA-IL binding properties confirmed that the glycoconjugate in the 1,3-alternate conformation was the most effective in binding the lectin. Analysis of the monomers showed comparable binding properties for all the structures. The compound bearing

the aromatic linker showed the highest affinity for PA-IL unfortunately the low solubility in the assay media did not allow the full collection of the experimental data for the glycoconjugates bearing such galactoside.

A water soluble calix[4]arene glycoconjugate, carrying the phenyl spacer, is thus the first target compound of this chapter.

The results obtained by Matthews and Vidal in their work suggested also the opportunity to introduce sugars units into a multicalixarene structure. Polycationic multialixarenes are able to bind and transfect nucleic acids in to the cells.[30] On the other hand, as shown by Matthews and Vidal' experience, calixarenes embroidered with appropriate sugar moieties are able to bind selectively lectins.[25] Therefore joining these two properties together it may be possible to synthesise a molecule able to transfect the nucleic acids selectively into the cells carrying a target lectin.

Another topic which will be discussed in the chapter is the synthesis of octavalent calix[4]arenes able to bear two different sugar species (eg. flucose and galactose) on the upper and lower rim. This approach may lead to the synthesis of molecules which are able to bind at the same time both PA-IL and PA-IIL.

2.3 Increasing solubility through functionalisation

The approach chosen towards the synthesis of water soluble calix[4]arene glycoconjugates was the introduction of hydrophilic groups on the calixarene scaffold. Previous work in the group showed that the amino group in its hydrochloride form gives water solubility to single calix[4]arenes.[30] Therefore we aim to synthesise analogues of glycoconjugate **191** and **178** functionalised with four amino groups on the upper rim of the calixarene.

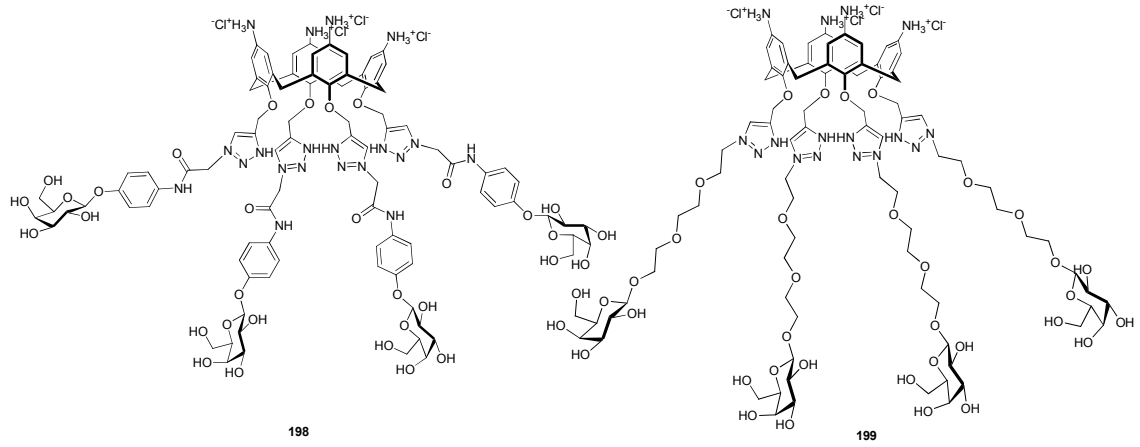
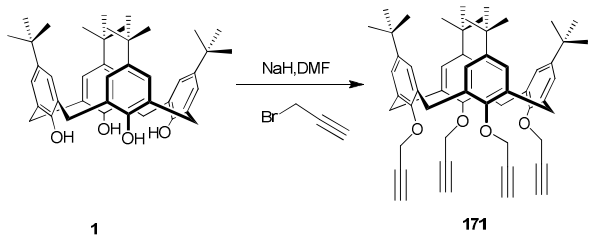


Figure 2.3: Target compounds

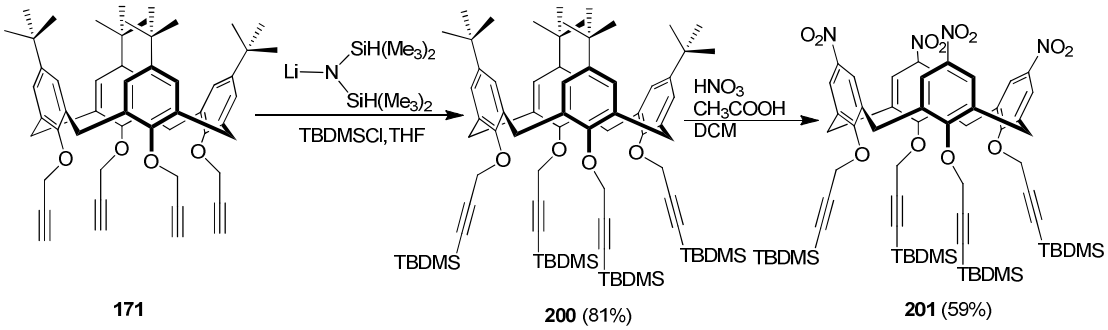
Following the Ryu and Zhao procedure, *p-tert*-butyl calix[4]arene was alkylated with propargyl bromide in the presence of NaH, this reaction allowed the introduction on the narrow rim of four alkyne functional groups and locks the scaffold in the cone conformation (scheme 2.22).[31]



1 171

Scheme 2.22: Synthesis of tetra alkyne functionalised calixarene in the cone conformation.

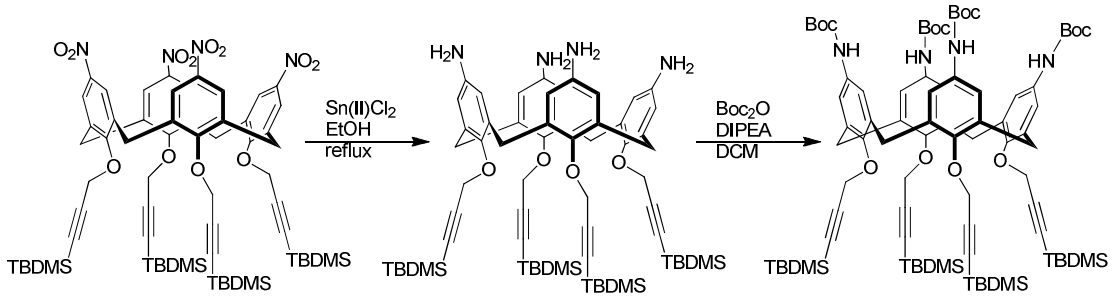
The synthetic route to introduce Boc protected amino groups on the wide rim of the calixarene scaffold was the same designed for the synthesis of the generation 1 calixarene **46**. Initially *ipso*-nitration was attempted by treating a solution of **171** in DCM with a mixture of fuming nitric acid and glacial acetic acid in equal volumes.[32] The reaction yielded a yellow precipitate insoluble in both organic and inorganic solvents which was not the desired product. The failure of the experiment was probably due to the reaction between the electron rich alkynes and the electrophile nitronium ion. Acetylenes and nitronium ions are able to react in gas phase as reported in 2000 by Bernardi *et al* .[33] Milder conditions, using less equivalents of the nitrating mixture and/or lesser reaction time did not yield the expected product. To overcome this problem an alkyne protecting group was required. Among the silyl protecting group *tert*-butyldimethylsilyl chloride (TBDMSCl) was chosen for this purpose, the reason for this choice will be explained in detail in paragraph 2.4.1. The four acidic alkyne protons were removed in the presence of the base LiHMDS. The compound was allowed to react at -78^{o}C for twenty minutes before the addition of TBDMSCl. Precipitation from DCM/MeOH yielded the pure compound in excellent yield (81%). After the introduction of the protecting group the ipsonitration could be performed. The pure product was precipitated from DCM/MeOH as a pale yellow solid (59%) (scheme 2.23).



171 **200** (81%) **201** (59%)

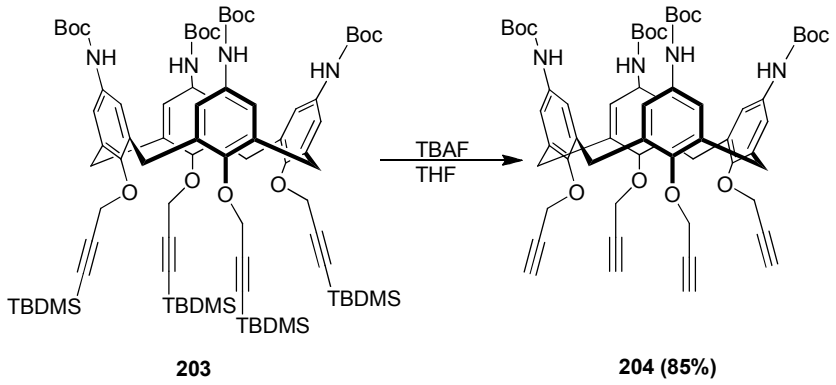
Scheme 2.23: TBDMS protection and *ipso* nitration

The synthesis continued with the reduction of the nitro groups to amino groups using $SnCl_2$ dihydrate.[34] The product was recovered after basic work up and removal of the solvent as a dark vitreous solid (97%). The subsequent Boc protection of the four amino groups followed the same anhydrous conditions used for the synthesis of compound **45** already discussed in chapter 1. Purification of the crude product over column chromatography yielded **203** (79%) (scheme 2.24).



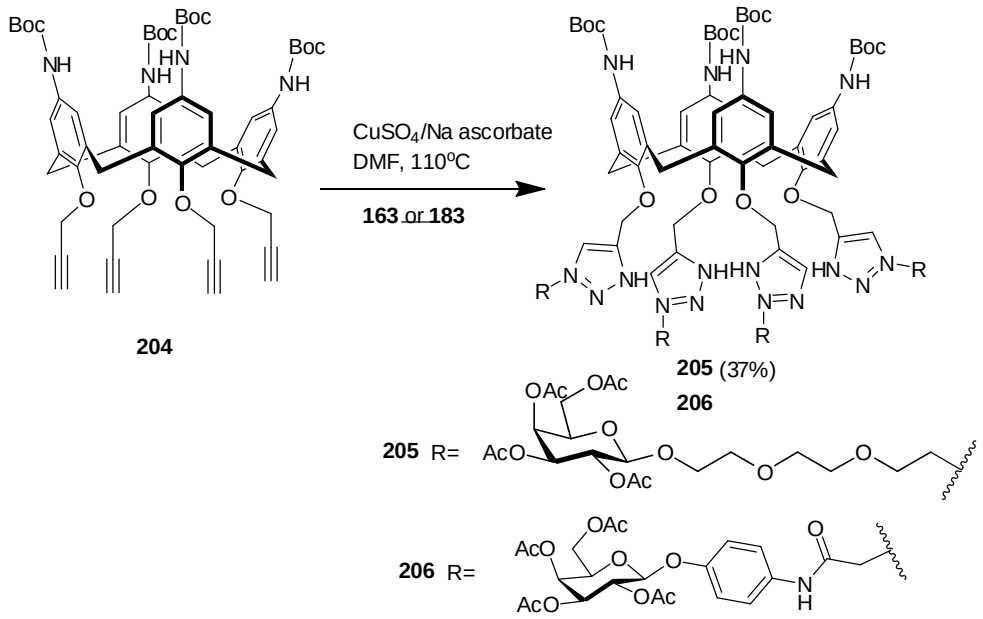
Scheme 2.24: Nitro reduction and Boc protection of the amino derivative.

At this stage the silyl protection was removed from the propargyl groups. As mentioned in chapter 1, sixteen hours stirring at room temperature in the presence of five equivalent of TBAF deprotected the alkynes without removing the Boc protection on the amino groups. The pure compound **204** was obtained after precipitation from DCM/*n*-Hexane (85%) (scheme 2.25).



Scheme 2.25: Alkyne deprotection

Compound **204** functionalised with four alkyne groups at the lower rim and with four Boc protected amino groups on the wide rim was "clicked" with the azido galactose derivatives **163** and **183**. The method chosen for the cycloaddition was the one optimised by Vidal's group which used as a source of Cu(I) the a mixture of $CuSO_4$ and sodium ascorbate. The reaction was performed in DMF at 110°C without microwave irradiation. The products were purified by column chromatography to yield the pure compounds **205** and **206** (scheme 2.26).



Scheme 2.26: Glycoconjugation.

Figure 2.4 is a comparison between the spectra of **205** (red) and of **206** (blue). In both spectra it easy to identify the click proton, with a chemical shift around 8 ppm. At 6.75 ppm both spectra show a broad singlet which is due to the calixarene aromatic protons. The two doublets, having a coupling constant of 9Hz, in the aromatic region of the spectra for compound **207** (blue) belong to the aromatic linker. The central area of the spectra between 5.5ppm and 3.5 ppm is not readily interpreted because of the overlap of galactose peaks with the signals of the linker and of the calixarene. Nevertheless all the multiplets could be assigned with two dimensional NMR experiments (COSY, HMBS and HSQC). Around 3 ppm both spectra clearly show the methylene bridge of the calixarenes. In the upper field region it is possible to identify the signals of the acetate protecting the sugar. The last peak, at 1.5 ppm, integrating for 36 protons, belongs to the Boc protecting group.

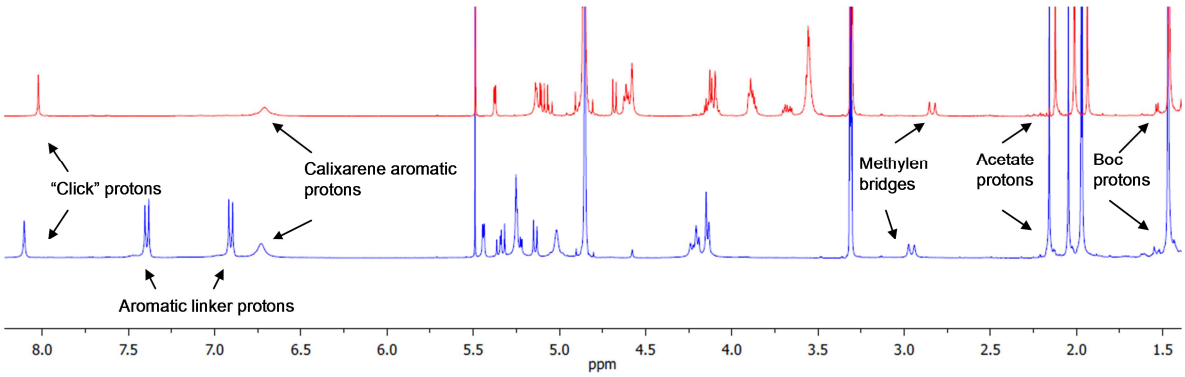


Figure 2.4: ^{1}H NMR spectra comparison of **205** (red) and of **206** (blue).

The glycoconjugates **205** and **206** were sent to the University of Lyon 1 to be deprotected and biologically tested on PA-IL. The removal of the Boc group can be achieved by bubbling gaseous HCl in to a solution in DCM of the compound, whilst the acetates can be hydrolysed from the sugars residue by stirring the compound with a mixture of triethylamine, methanol and water.

2.4 Design of multicalixarene glycoconjugates

Different carbohydrates have been shown to bind different lectins. Incorporating a carbohydrate selective for a specific lectin on a tranfection agent, could lead to molecules able to transfect nucleic acids in to the cells carrying such a lectin. As was shown from previous work in the group, cationic multicalixarenes bind and transfect nucleic acids in to the cells. In our work we have designed a route which would allow us to introduce sugars moieties on multicalixarenes with cell transfection properties. In order to be able to achieve such a result it was necessary to design central cores able to carry four amino functionalised generation 1 calixarenes and the sugars (figure 2.5).

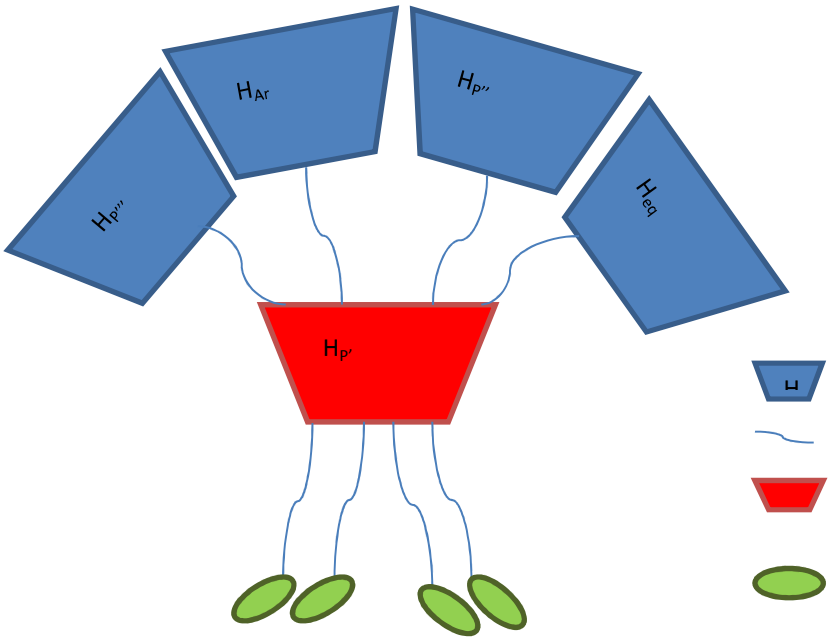
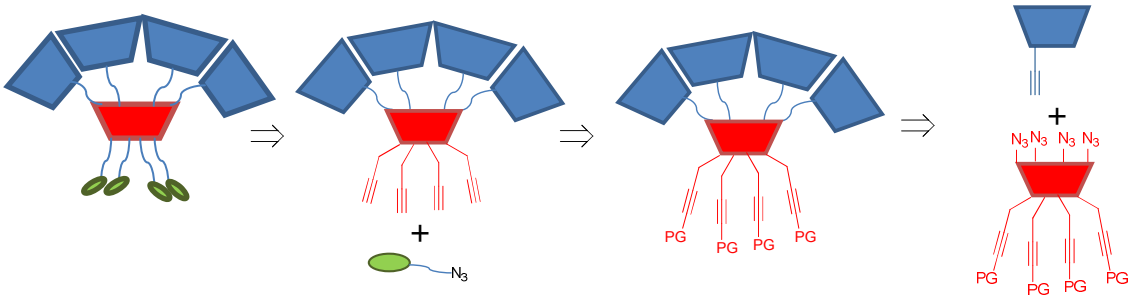


Figure 2.5: Design of Multicalixarene Glycoconjugate

As was described on chapter 1 it is possible to synthesize multicalixarenes through narrow rim-wide rim junctions. The generation 1 calixarenes can be functionalised at the narrow rim with an alkyne fuction which can be “clicked” to the wide rim of a central core functionalised with four azido groups. This approach opened the opportunity to introduce further functionalizations on the narrow rim of the central core. A retro synthetic scheme in which a central core

functionalised with four azido groups and four alkyne groups was reacted respectively to alkyne generation 1 calixarenes and azido sugars was designed (scheme 2.27).

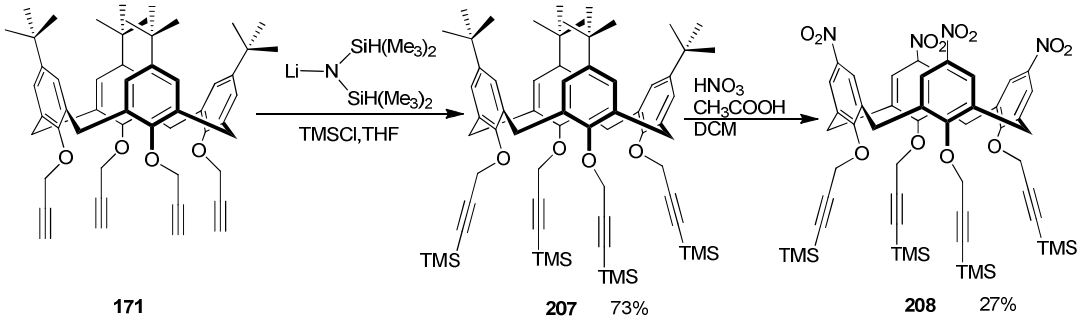


Scheme 2.27: Retro-synthetic strategy for Multicalixarene Glycoconjugates

According to this retro-synthetic scheme it was necessary to synthesise a central core carrying four azido groups on the wide rim, which can be reacted with the monoalkyne functionalised generation 1 calixarenes, and four protected alkyne groups on the narrow rim which after the formation of the multicalixarene could be deprotected and "clicked" with the azido functionalised sugar.

2.4.1 Synthesis of azido-alkyne functionalised central core

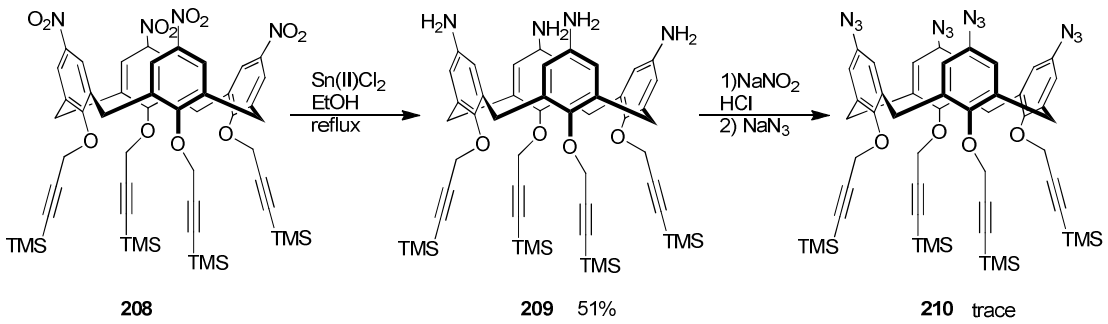
As discussed in paragraph 2.3, a protecting group is required before nitration of alkyne modified calixarene. Whilst we came to the use of TBDMSCl, previously we considered the use of the trimethylsilane analogue (TMSCl). Initially the four alkyne groups were protected with trimethylsilyl-chloride (TMSCl)[35] before performing the *ipso*-nitration[32].



Scheme 2.28: TMS protection and *ipso* nitration

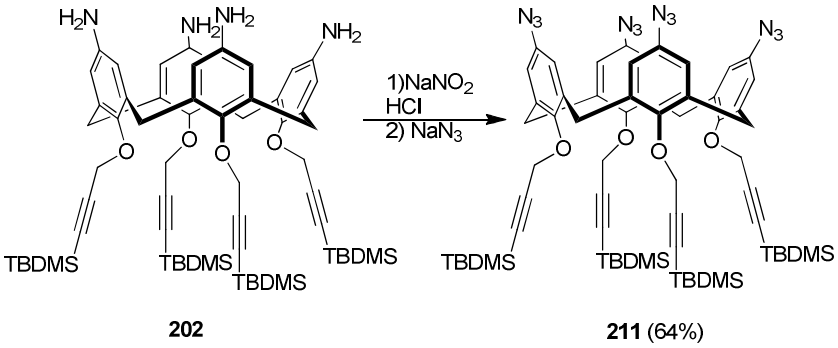
The tetranitro derivative in this case was obtained in low yield after a complicated purification. The tetranitro derivative was then reduced following the tin chloride method[34] and the resulting amino derivative underwent substitution with the azide *via* the diazonium salt.[36] The poor stability of the TMS groups in the acidic conditions necessary to form the diazonium salt gave as a result of the reaction a mixture of the tetra-protected, tri-protected, di-protected, mono-

protected and unprotected compounds. This result was confirmed once the five spot identifiable by TLC were purified over silica gel and analysed by ^{1}H NMR.



Scheme 2.29: Reduction of nitro groups and substitution of amino groups *via* diazonium salt

The same series of reactions were repeated again using, as the protecting group for the alkynes, *tert*-butyl-dimethylsilylchloride (TBDMSCl) which is known to be more stable[37]. The only product obtained in the synthesis was this time the tetrazazido compound with four TBDMS protected alkyne groups **211**.



Scheme 2.30: Synthesis of tetraazido functionalised central core.

Figure 2.6 shows the spectra of all the synthetic steps of the synthesis of **211**. The alkyne peaks, a doublet and a triplet at 4.80 ppm and at 2.47ppm respectively, in the spectrum of **171** (blue), disappear upon the introduction of the protecting group, to be replaced by three singlets at 4.80 ppm, 0.88 ppm and 0.74 ppm in the spectrum of **200** (red). Comparing this spectrum with the spectrum of compound **201** (green) the removal of the *tert*-butyl group *via ipso*-nitration can be confirmed by the disappearance of the peak at 1.04 ppm and by the downfield shift of the signal for the aromatic protons due to the high deshilding effect of the electron withdrawing group. Reduction of the nitro groups in to amines, electro donating groups, produces in the spectra compound **202** (purple) a further upfield shift of the aromatic protons. In the last step once the

amino groups have been replaced by azido functions, the aromatic signal shifts again from 6.10 ppm in **202** to 6.36 ppm in **211** (dark blue).

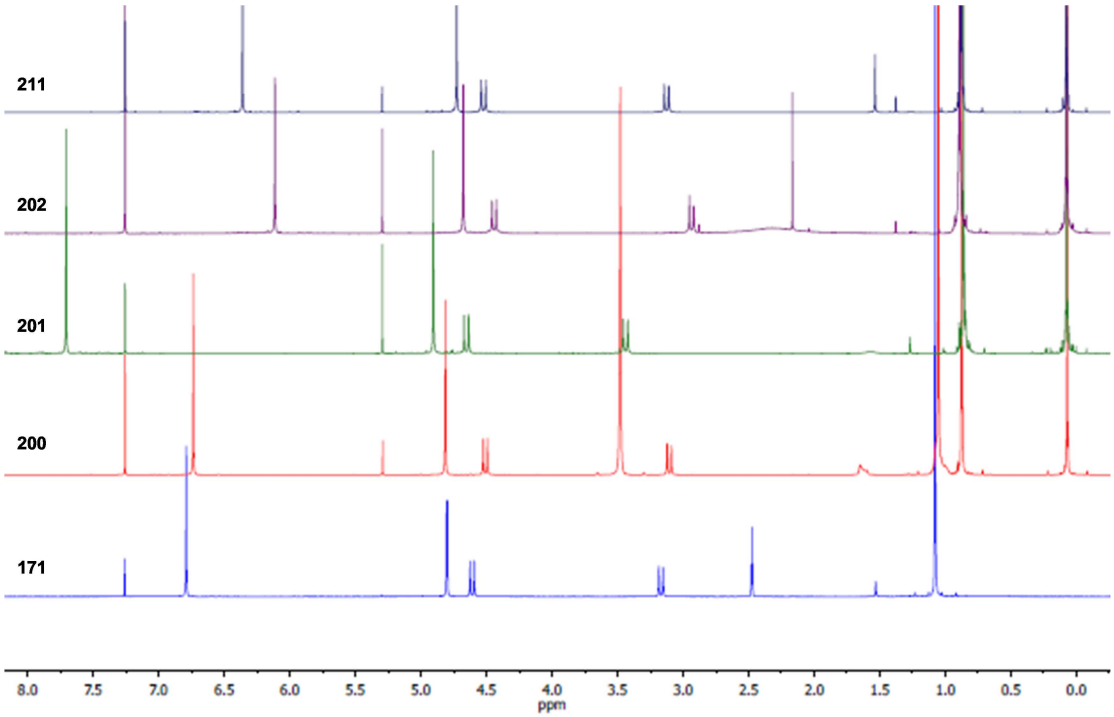
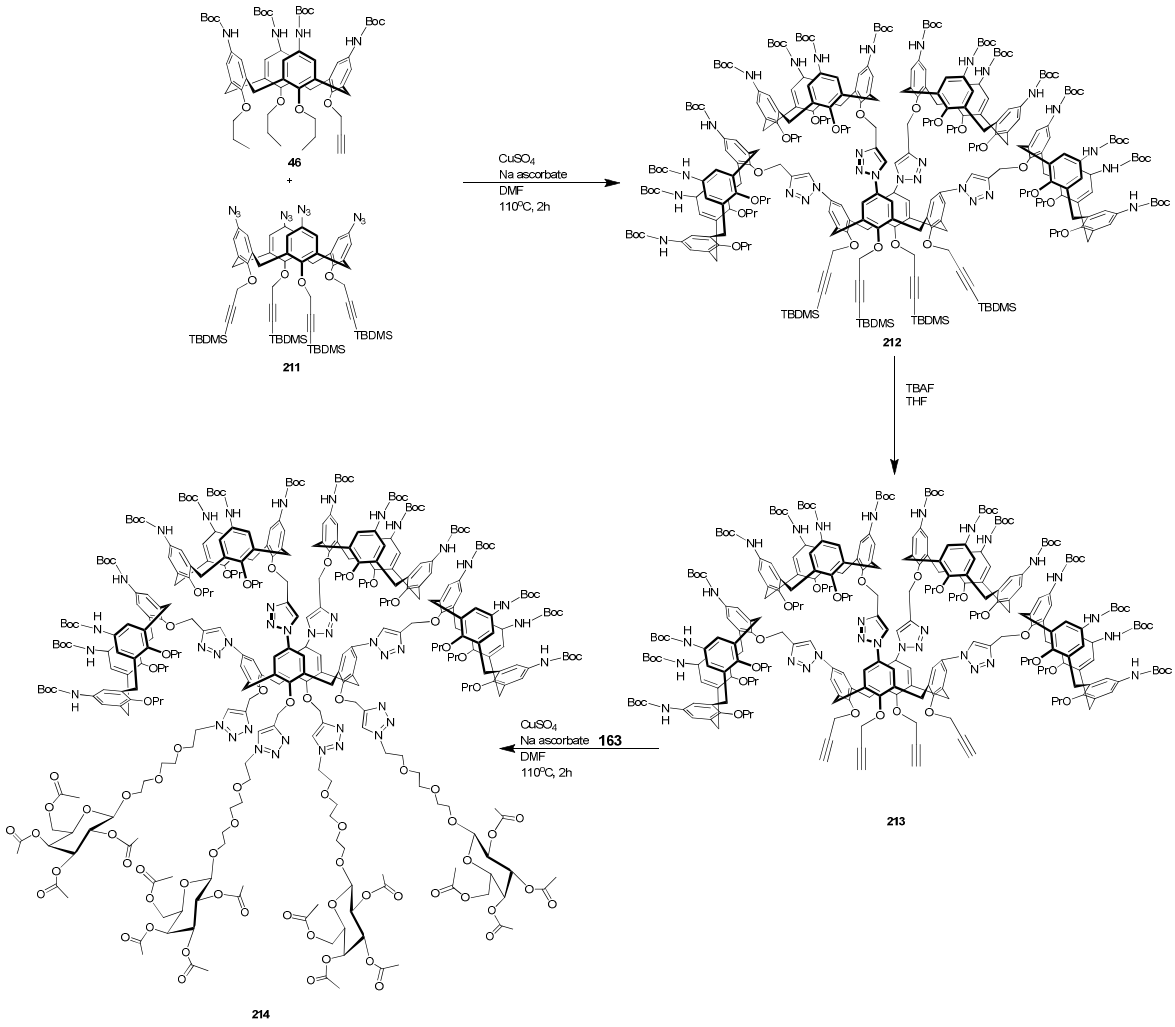


Figure 2.6: ^{1}H NMR Spectra of all the steps of the synthesis of **211**

2.4.2 Assembly of the multicalixarene glycoconjugate

The first step towards the multicalixarene glycoconjugate was the assembly of the multicalixarene (scheme 2.27). The four azido functions of the central core **211** were clicked with the alkyne group of the Boc-amino functionalised generation 1 calixarene **46**. The reaction was performed using as a source of Cu(I) the combination of copper sulphate and sodium ascorbate. The product was purified by column chromatography to yield the multicalixarene **212**. At this point the alkyne protecting groups could be removed by stirring the product with TBAF for 16 hours at room temperature. The revealed alkynes groups of **213** were "clicked" with the azido-galactose derivative to obtain the multicalixarene glycoconjugate **214**.

Compound **214**, the first multicalixarene glycoconjugate synthesised, once fully deprotected will be tested to investigate the binding towards bacterial lectins and its gene trasfection properties.

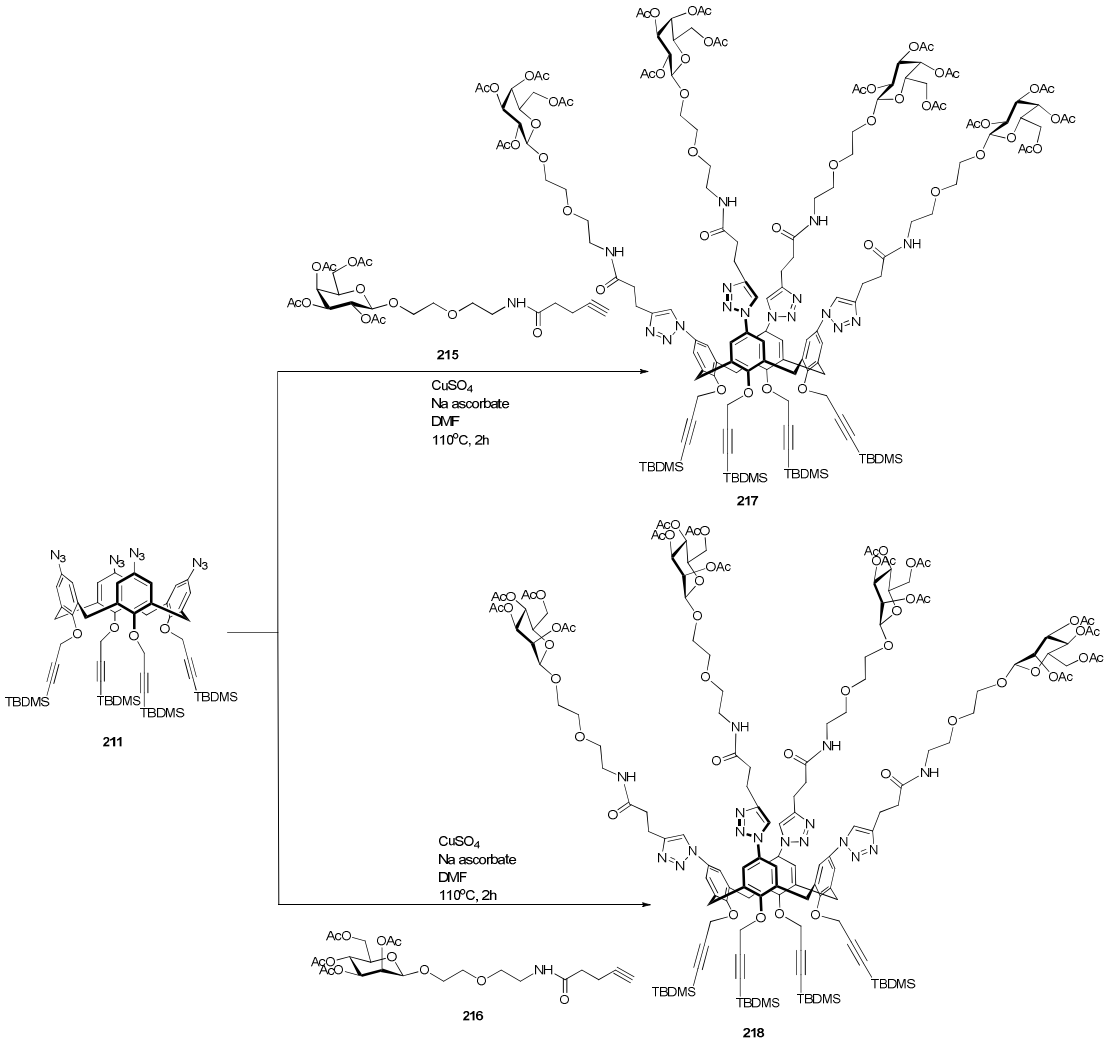


Scheme 2.31: Assembly of Multicalixarene Glycoconjugate

2.5 Synthesis of octavalent glycoconjugates

The azido functionalisation on the wide rim and the protected alkyne groups on the lower rim would allow the introduction of two different molecules on **211** *via* sequential CuAAC Reactions. This property would enable the synthesis of an octavalent calix[4]arene glycoconjugate bearing two different sugars on the upper and lower rim. A calixarene glycoconjugate with these features, could have interesting biological activity towards the binding of the lectins of *Pseudomonas aeruginosa*. For example, one bearing fucose and galactose, or mannose and galactose, or mannose and fucose could be prepared. Mannose and galactose derivatives (figure 2.7) with an alkyne functionalisation were available from Vidal's laboratory. These glycosides were successfully conjugated with the central core **211**, using

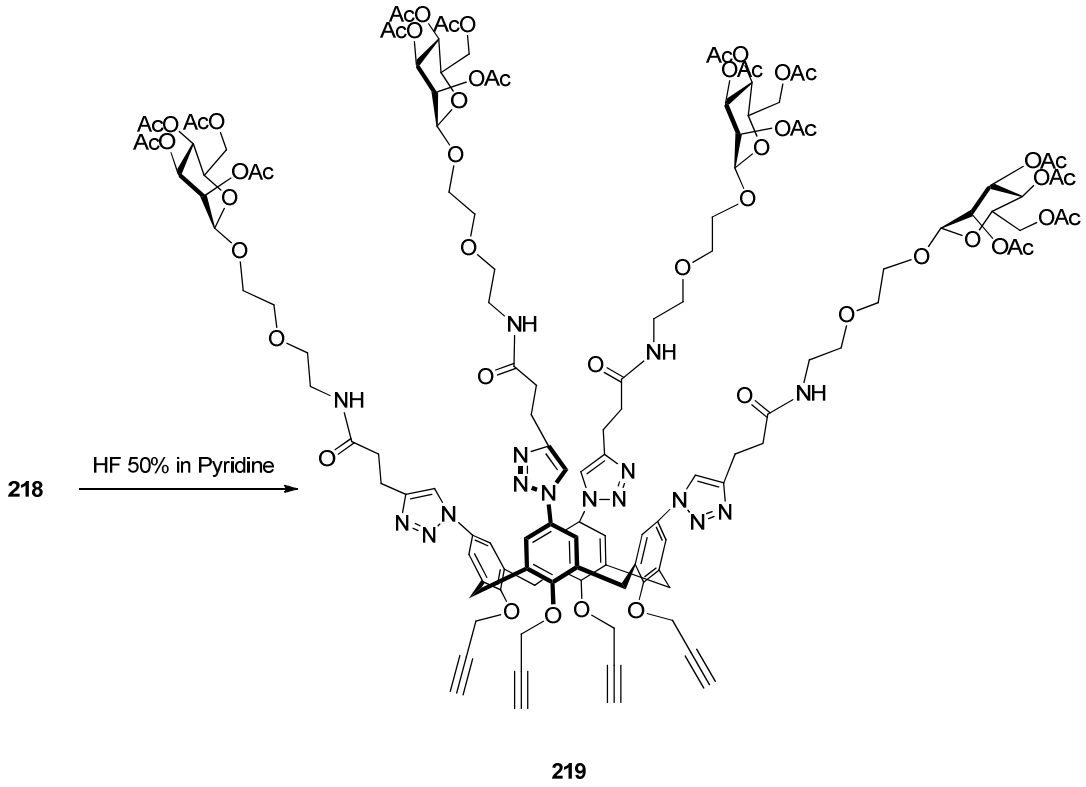
copper sulphate and sodium ascorbate as a source of copper (I) catalyst, to give respectively **217** and **218**.



Scheme 2.32: Upper rim functionalisation of **211** with mannose and galactose derivatives

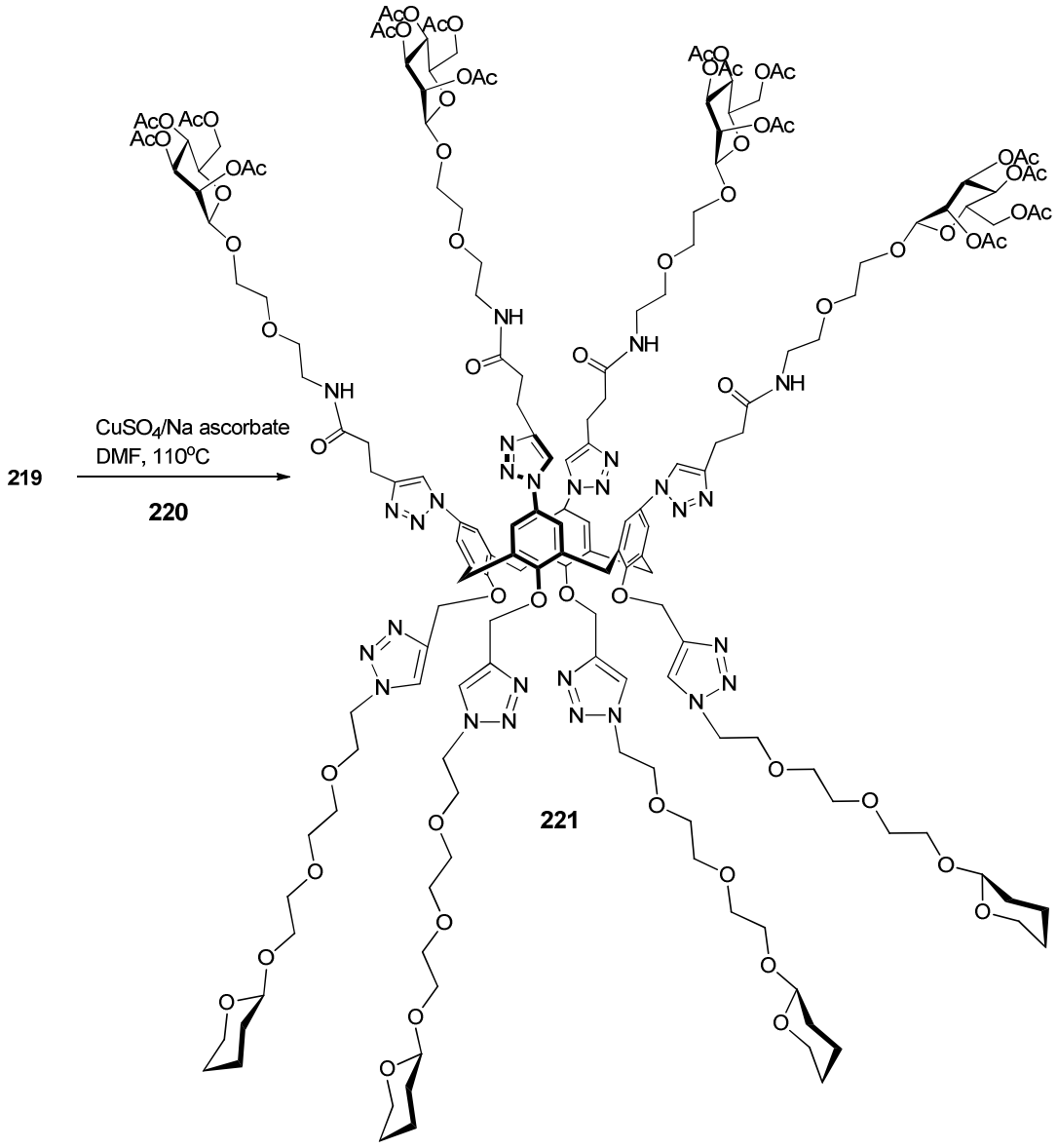
To enable reaction with either azido-functionalised fucose or galactose the masked alkyne moieties needed to be revealed.

The method of first choice was the treatment of the molecules with TBAF as this route had previously been used successfully for preparation of multicalixarenes. Unexpectedly this method did not yield the desired compound. The silyl protecting group was successfully removed from compound **218** using the alternative of HF in pyridine.



Scheme 2.33: Deprotection of the alkyne groups

Although the desired compound was detected by mass spectrometry, the ^{1}HNMR analysis was unexpectedly complex which could be due either to the partial loss of the protecting groups on the sugar or to a change of conformation of the calixarene. Dondoni *et al.* have shown how calixarene glycoconjugates bearing galactose unit on the upper rim favoured an open or flattened conformation.[22]. However, compound **219** was reacted in the presence of copper sulphate and sodium ascorbate with the azido flucose derivative **220** (scheme 2.34). Unfortunately the ^{1}H NMR after a first purification by column chromatography was inconclusive to determinate the structure of the product. Compound **221** was sent for mass analysis to confirm mass before further purification was attempted.



Scheme 2.34: Attempted synthesis of Octavalent Glycoconjugate

2.6 Conclusion and future work

This chapter describes our approach to calixarene glycoconjugates. Previous studies carried out in collaboration with Dr. Sebastien Vidal from the University of Lyon 1, had developed triazole linked galactose glycoconjugates that showed good binding properties toward the lectin PA-IL from the bacteria *Pseudomonas aeruginosa.* For some of these compounds solubility in aqueous media was an issue which had limited the biological evaluation. Part of our work was focused on the generation of calixarene glycoconjugates functionalised with amino groups. We have successfully synthesised compound **205** and **206**, which are now ready to undergo biological evaluation. The success of the synthetic route developed may allow in the near future the synthesis of analogues carrying on the upper rim aliphatic amines or guanidinim functions as

described in chapter 1. The second part of our work aimed to develop a route towards the synthesis of multicalixarene glycoconjugates. These molecules have potential to combine the cationic multicalixarenes DNA transfection properties with the specific binding of glycoconjugate to lectins, leading to selective delivery of genetic material to target cells. The achievement of this goal was possible thanks to the design of central core **211**. This molecule has been developed to introduce in separate steps through “click” chemistry four alkyne and four azido functionalised molecules, in our case monoproargylated calixarene and azido sugars. With this approach we have also investigated the synthesis of octavalent glycoconjugates. The introduction into the upper rim of mannose and galactose derivative was straightforward. However the deprotection of the propargyl group in the presence of the sugars was troublesome. The problems were overcome using HF in pyridine and the synthesis of the octavalent glycoconjugate is waiting to be confirmed by mass spectrometry analysis.

2.7 Experimental

5,11,17,23-*p*-*Tert*-butyl-25,26,27,28-tetra-(3-tert-Butyldimethylsilyl)-2-propynoxycalix[4]arene (200)

Lithium bis(trimethylsilyl)amide (1M in THF, 72 mL, 72 mmol) was added drop wise to a solution of **2** (7.20 g, 8.82 mM) in THF (80 mL) pre-cooled to -78^{o}C. After 30 minutes a solution of TBDMSCl (11.39 g, 72 mmol in 20 mL of THF) was added to the reaction and stirred at room temperature for 16 hours. The reaction was diluted with EtOAc (40 mL) and washed with NH_4Cl_{sat} (2 x 30 mL) water (30 mL and brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated. Re-crystallisation from DCM/MeOH yielded the title compound as a white solid (9.04g, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.77 (s, 8H, Ar*H*), 4.84 (s, 8H, OC*H*$_2$CCSi(CH$_3$)$_2$C(CH$_3$)$_3$), 4.54 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 3.13 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 1.08 (s, 36H C*H*$_3$CAr), 0.90 (s, 36H, OCH$_2$CCSi(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.10 (s, 24H, OCH$_2$CCSi(C*H*$_3$)$_2$C(CH$_3$)$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 151.9, 145.1, 134.6, 124.7, 103.4, 89.4, 61.2, 33.8, 23.6, 31.4, 26.1, 16.4, -4.6. **IR** *v* = 2953, 2928, 2856, 1480, 1471, 1463, 1412, 1391, 1361, 1301, 1248, 1197. **Mpt** 214-216°C.

5,11,17,23-tetra-nitro-25,26,27,28-tetra-(3-tert-butyldimethylsilyl)-2-propynoxycalix[4]arene (201)

A mixture of 10 ml of glacial acetic acid and 10 ml of fuming nitric acid was added to a stirred solution of **3** (2.00 g, 1.57 mmol) in DCM (50 ml) and stirred for 18 hours. The reaction mixture was washed several times with water. The organic layer was dried over $MgSO_4$, and the solvent removed under reduced pressure. The resulting yellow solid was re-crystallized from DCM/MeOH to give the title compound as a cream solid (1.13 mg, 59% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.72 (s, 8H, Ar*H*), 4.92 (s, 8H, OC*H*$_2$CCSi(CH$_3$)$_2$C(CH$_3$)$_3$), 4.66 (d, *J* = 14 Hz, 4H, ArC*H*$_2$Ar), 3.45 (d, *J* = 14 Hz, 4H, ArC*H*$_2$Ar), 0.87 (s, 36H, OCH$_2$CCSi(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.09 (s, 24H, OCH$_2$CCSi(C*H*$_3$)$_2$C(CH$_3$)$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 159.5, 143.8, 136.1, 124.1, 99.7, 93.2, 62.5, 32.2, 30.8, 25.6, 16.3, -4.8. **IR** *v* = 2953, 2929, 2857, 1587, 1519, 1463, 1344, 1305, 1288, 1249, 1205. **Mpt** 219-221°C.

5,11,17,23-tetra-amino-25,26,27,28-tetra-(3-tert-butyldimethylsilyl)-2-propynoxycalix[4]arene (202)

Tin(II)chloride di-hydrate (5.07 g, 22.2 mmol) was added to a solution of **4** (1.00 g, 0.81 mmol) in EtOH (50 ml). The mixture was heated at reflux temperature for 18 hours, cooled and the solvent removed under reduced pressure. The pale yellow solid was triturated with 10% $NaOH_{aq}$ and extracted with DCM. After separation the organic layer was dried over $MgSO_4$ and the

solvent removed under reduced pressure to give the desired compound as a brown vitreous solid (1.36 g, 97% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.12 (s, 8H, Ar*H*), 4.70 (s, 8H, OC*H*$_2$CCSi$(CH_3)_2$C$(CH_3)_3$), 4.45 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 2.94 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 0.91 (s, 36H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.09 (s, 24H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 148.1, 141.2, 136.0, 115.4, 103.6, 89.2, 61.6, 32.4, 25.9, 16.4, -4.8. **IR** *v* = 3350, 2928, 2951, 2856, 1615, 1471, 1361, 1248, 1207, 1131. **Mpt** 150-152°C.

5,11,17,23-tetra-Boc-amino-25,26,27,28-tetra-(3-tert-butyldimethylsilyl)-2-propynoxycalix[4]arene (203)

Di-*t*-butyl- dicarbonate (2.00 g, 9.2 mmol in 10 mL of anhydrous $CHCl_3$) was added to a stirring solution of **5** (1.25 g, 1.15 mmol in 40 mL of anhydrous $CHCl_3$) at 0^{o}C in the presence of DiPEA (1.7 mL, 9.2 mL) as a base and stirred for 18 h under an argon atmosphere. The reaction was then diluted with CH_2Cl_2 (50 mL) and washed with water (2 x 100 mL) and brine (2 x 100 mL). The organic layer was dried over $MgSO_4$ and concentrated. Purification by column chromatography (DCM: EtOAc 30:1 yielded the desired pure product (1.36 g, 79%).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.75 (s, 8H, Ar*H*), 6.19 (s, 4H, BocN*H*Ar), 4.76 (s, 8H, OC*H*$_2$CCSi$(CH_3)_2$C$(CH_3)_3$), 4.52 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 3.12 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 1.48 (s, 36H, $(CH_3)_3CO$), 0.90 (s, 36H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.09 (s, 24H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 153.0, 150.9, 135.6, 133.0, 119.4, 89.9, 61.7, 32.3, 28.40, 26.1, 16.4, -4.6. **IR** *v* = 3301, 2951, 2929, 2856, 1695, 1600, 1546, 1526, 1472, 1416, 1362, 1298, 1247, 1211, 1156. **Mpt** 215-217°C.

5,11,17,23-Tetra-Boc-amino-25,26,27,28-tetra propargyloxycalix[4]arene (204)

TBAF solution (1M in THF, 24 ml, 24 mmol) was added to a solution of **6** (1.8 g, 1.2 mmol in 50 ml of THF) and stirred for 18 h at room temperature. The reaction was diluted with EtOAc (150 ml) and stirred for a further 10 minutes in the presence of NH_4Cl_{sat}(150 mL). The organic layer was washed with NH_4Cl_{sat} (1 x 150 mL) and brine (2 x 150 mL), dried over $MgSO_4$ and concentrated. The crude compound was dissolved in DCM and precipitated with hexane to yield the title compound (1.05 g, 85%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.77 (s, 8H, Ar*H*), 6.24 (s, 4H, NH), 4.73 (d, *J* = 2.4 Hz, 8H, C*H*$_2$CH), 4.58 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 3.18 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 2.46 (t, *J* = 2.4 Hz, 4H, CH_2CH), 1.49 (s, Hz, 36H, $(CH_3)_3CO$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 153.1, 151.0, 135.5, 133.2, 119.5, 80.4, 80.1, 74.8, 61.2, 32.2, 28.40. **IR** *v* = 3292, 2977, 2922, 1699, 1600, 1524, 1475, 1416, 1392, 1366, 1294, 1244, 1208, 1149. **Mpt** 150-152°C.

Compound 205

GalPhNAz (302.29 mg, 0.58 mmol) was added to a solution of **7** (100 mg, 0.096 mmol) in DMF (10 mL) in the presence of $CuSO_4 \cdot 5H_2O$ (47.9 mg, 0.19 mmol) and sodium ascorbate (152 mg, 0.77 mmol). The reaction was stirred for 2 hours at 110°C. The solution was diluted with EtOAc (40 mL) and washed with H_2O (2 x 50 mL) and brine (2 x 50 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel, (EtOAc/*n*-Hex/MeOH=1/1/0.2) yielded the desired compound as a off white solid (93.4 mg, 37%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82 (s, 4H, Az*H*), 6.62 (s, 8H, Ar*H*), 6.32 (s, 4H, ArN*H*C$(CH_3)_3$), 5.38 (dd, *J* = 3.4, 0.9 Hz, 4H, *H*-4'''), 5.20 (dd, *J* = 10.5, 7.9 Hz, 4H, *H*-2'''), 5.06 – 4.95 (m, 12H, *H*-3''' and OC*H*$_2$CCAz), 4.57 – 4.50 (m, 12H, *H*-1'''and OCH_2CH_2Az), 4.19 – 4.08 (m, 12H, *H*-6'''and ArC*H*$_2$Ar), 3.96 – 3.90 (m, 8H, *H*-5'''and OC*H*$_2CH_2O$), 3.84 (t, *J* = 5.3 Hz, 8H, OC*H*$_2CH_2Az$), 3.73 – 3.67 (m, 4H, OC*H*$_2CH_2O$), 3.62 – 3.58 (m, 8H, OC*H*$_2CH_2O$), 3.55 (s, 16H, OC*H*$_2CH_2O$), 2.89 (d, *J* = 13.5 Hz, 4H, ArC*H*$_2$Ar), 2.14 (s, 12H, OCOC*H*$_3$), 2.03 (s, 12H, OCOC*H*$_3$), 2.02 (s, 12H, OCOC*H*$_3$), 1.98 (s, 12H, OCOC*H*$_3$), 1.47 (s, 36H, C(C*H*$_3)_3$).

Compound 206

Gal-TEG-Az (292.1 mg, 0.58 mmol) was added to a solution of **7** (100 mg, 0.096 mmol) in DMF (10 mL) in the presence of $CuSO_4 \cdot 5H_2O$ (47.9 mg, 0.19 mmol) and sodium ascorbate (152 mg, 0.77 mmol). The reaction was stirred for 2 hours at 110°C. The dark solution was diluted with EtOAc (40 mL) and washed with H_2O (2 x 50 mL) and brine (2 x 50 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel (EtOAc/*n*-Hex/MeOH=1/1/0.2) yielded the desired compound as an off white solid (143 mg, 49%)

5,11,17,23-Tetra-azido-25,26,27,28- tetra-(3-tert-butyldimethylsilyl)-2-propynoxycalix[4]-arene (211)

$NaNO_2$ (303 mg, 4.39 mmol) was added to a solution of **5** (750 mg, 0.69 mmol) in 10% HCl_{aq} (50 ml) and EtOH (25 ml). The reaction was stirred for 20 minutes at 0°C before the addition of a solution of NaN_3 (223 mg, 3.34 mmol) in H_2O (20 ml). The reaction was stirred for further 2 hours and the formation of gas was observed. DCM 50 ml was added to the reaction mixture and the separated organic layer was then washed with brine (3 x 20 ml), dried over $MgSO_4$ and concentrated. Purification by column chromatography over silica gel (DCM/*n*Hexane=1/1) yielded the desired compound as a white foam (64%).^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (s, 8H, Ar*H*), 4.72 (s, 8H, OC*H*$_2$CCSi$(CH_3)_2$C$(CH_3)_3$), 4.51 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 3.12 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 0.87 (s, 36H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.07 (s, 24H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.35 (s, 8H, Ar*H*), 4.72 (s, 8H, OC*H*$_2$CCSi$(CH_3)_2$C$(CH_3)_3$), 4.51 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 3.12 (d, *J* = 13 Hz, 4H, ArC*H*$_2$Ar), 0.87 (s, 36H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$), 0.07 (s, 24H, $OCH_2CCSi(CH_3)_2C(CH_3)_3$).

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Chapter 3: Multicalixarenes

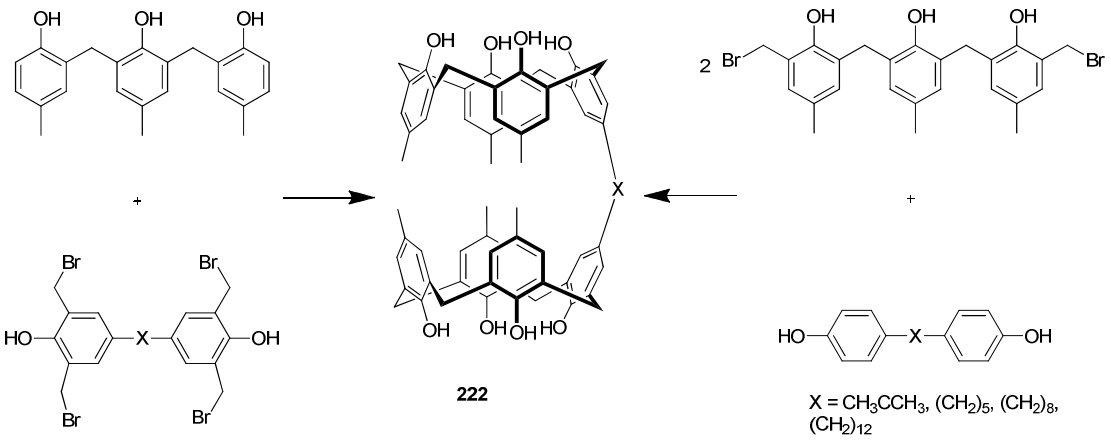
Chapter 3: Multicalixarenes

3.1 Introduction

Multicalixarenes can be described as multimeric structures formed by linking covalently two or more calixarenes scaffolds. Considerable effort has been placed in this area and literature shows many examples of these macromolecular structures, with great differences in size, shape and architecture. Calixarenes macrocycles linked together can form chains, tubes or dendrimers.

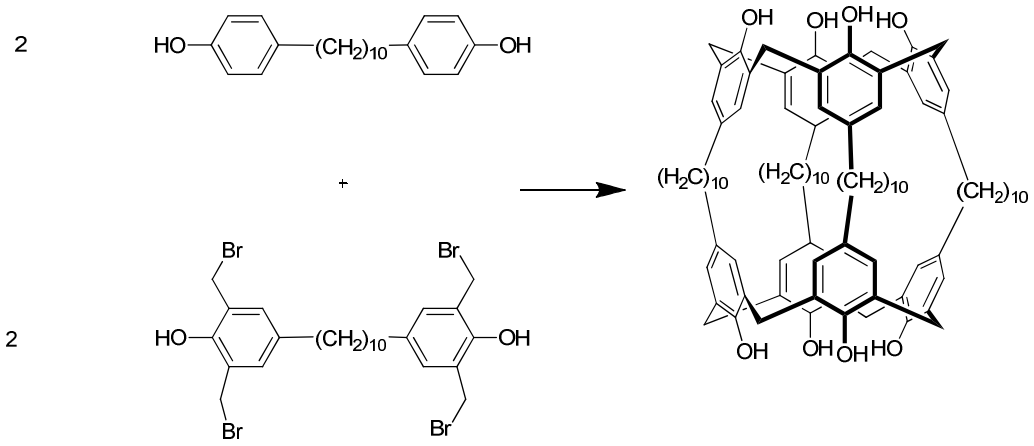
3.1.1 Upper rim-upper rim linked multicalixarenes

The first example of a double calix[4]arene appeared in the literature in 1989 from the group of Böhmer.[1] This head to head dimer **222** was achieved by condensing either tetra-bromomethylated diphenols with a linear trimer or a bis-bromomethylated trimer with diphenols in refluxing dioxane in the presence of titanium tetrachloride ($TiC1_4$) (scheme 3.1).



Scheme 3.1: First example of a calixarene dimer by Böhmer *et al.*

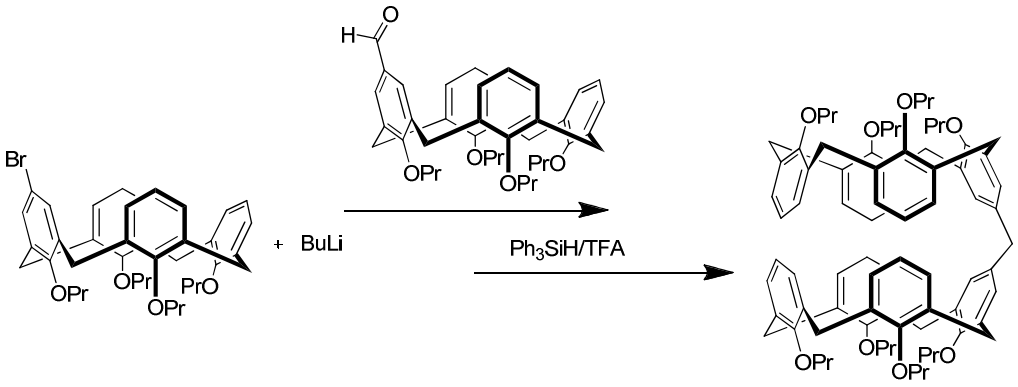
Using the same principle Böhmer and co-workers succeeded in the synthesis of the first calixarene tube **223** (scheme 3.2). By reacting together, tetra-bromomethylated diphenols and diphenols in the same conditions in a 1:1 ratio, they obtained a bis-calixarene linked with four alkyl bridges.[1]



223

Scheme 3.2: First example of calixarene tube.

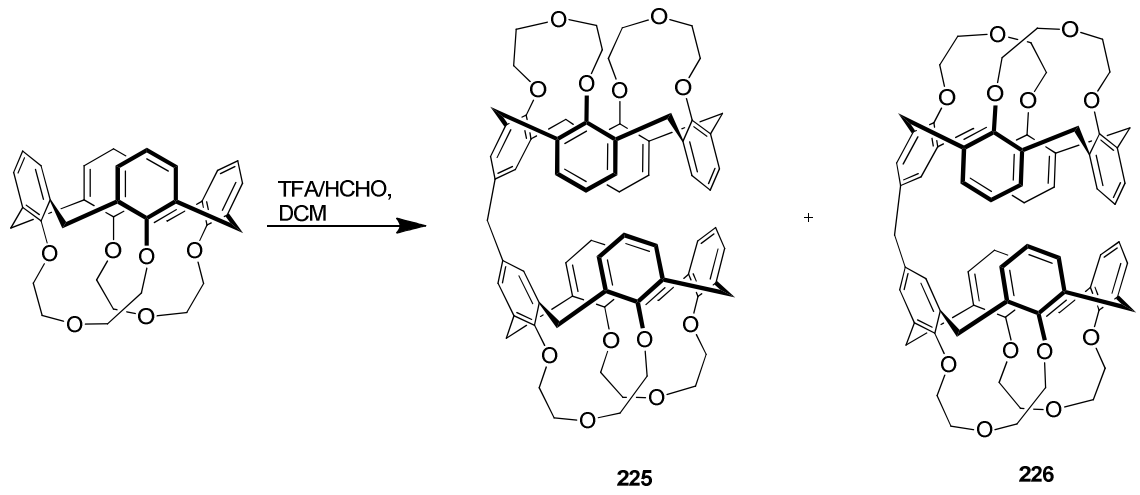
Similar molecules were obtained a few years later by Shinkai's group **(**scheme 3.3) and by Arduini *et al*. (scheme 3.4) using two different approaches. Both studies yielded bis-calixarenes linked at their wide rims through a methylene bridge. Shinkai's method involved two steps, in the first the calixarene bromoderivative was treated with butyl lithium before reacting with a mono aldehyde functionalised calixarene. Reduction in the second step yielded the final product **224**.[2]



224

Scheme 3.3: Head to head dimer: Shinkai's approach.

In Arduini's approach the same link was obtained by reacting a more rigid calixarene, bearing two crown links, with para-formaldehyde, in the presence of CF_3COOH as catalyst (scheme 3.4).[3] The reaction afforded a mixture of the monobridged isomers **225** and **226** which could not be separated.



Scheme 3.3: Head to head dimer by Arduini *et al*.

A completely different approach towards the synthesis of bis-calixarenes linked *via* the wide rim was described by Neri *et al*. The bis-calixarene was obtained by joining the two units "head-to-head" with a direct biphenyl-like *para-para* linkage (figure 3.1).[4] The double calixarene **227** was obtained simply by heating *p*-H-calix[4]arene in CH_3CN at reflux in the presence of $FeCl_3 \cdot 6\,H_2O$. Later work by Neri described the same reaction with calixarenes locked in the 1,3-alternate and partial cone conformations.[5]

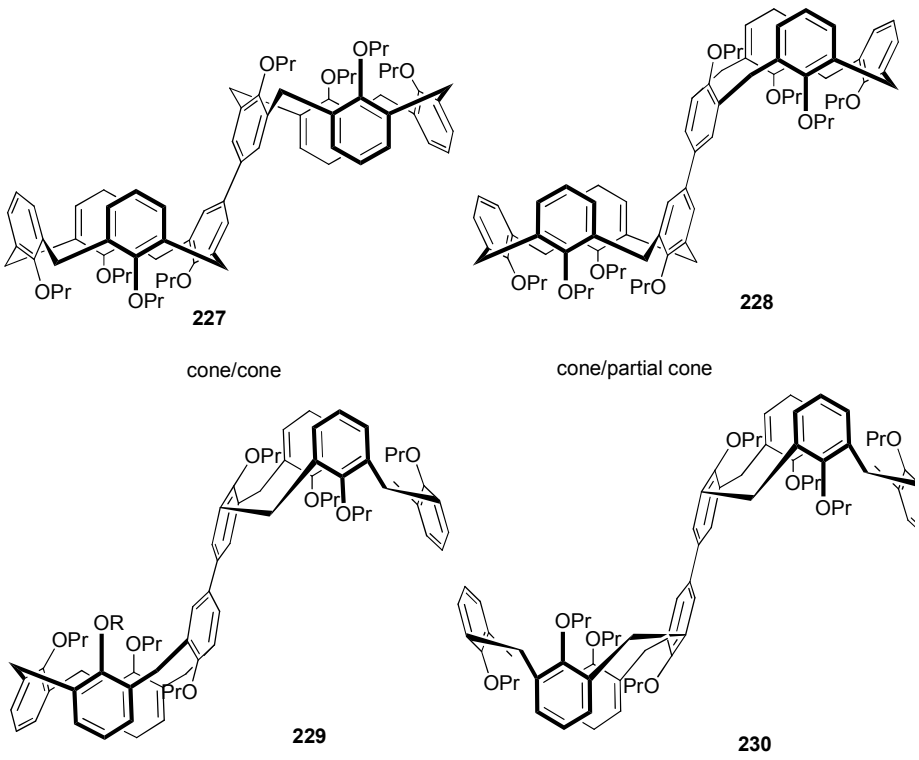


Figure 3.1: Calixarene dimers in different conformations by Neri *et al*.

In the same year Mogck *et al.* published the synthesis of covalently linked dimers **231**.[6] A monoamino calixarene was prepared through mono *ipso*-nitration and subsequent reduction of *p*-tert-butyl calixarene tetraesters. Reaction with various di-acid chlorides yielded the respective double calixarenes linked through an amide bond. (figure 3.2).

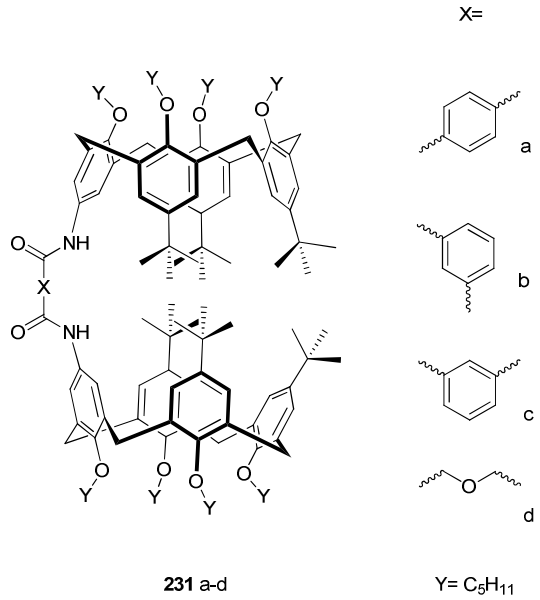
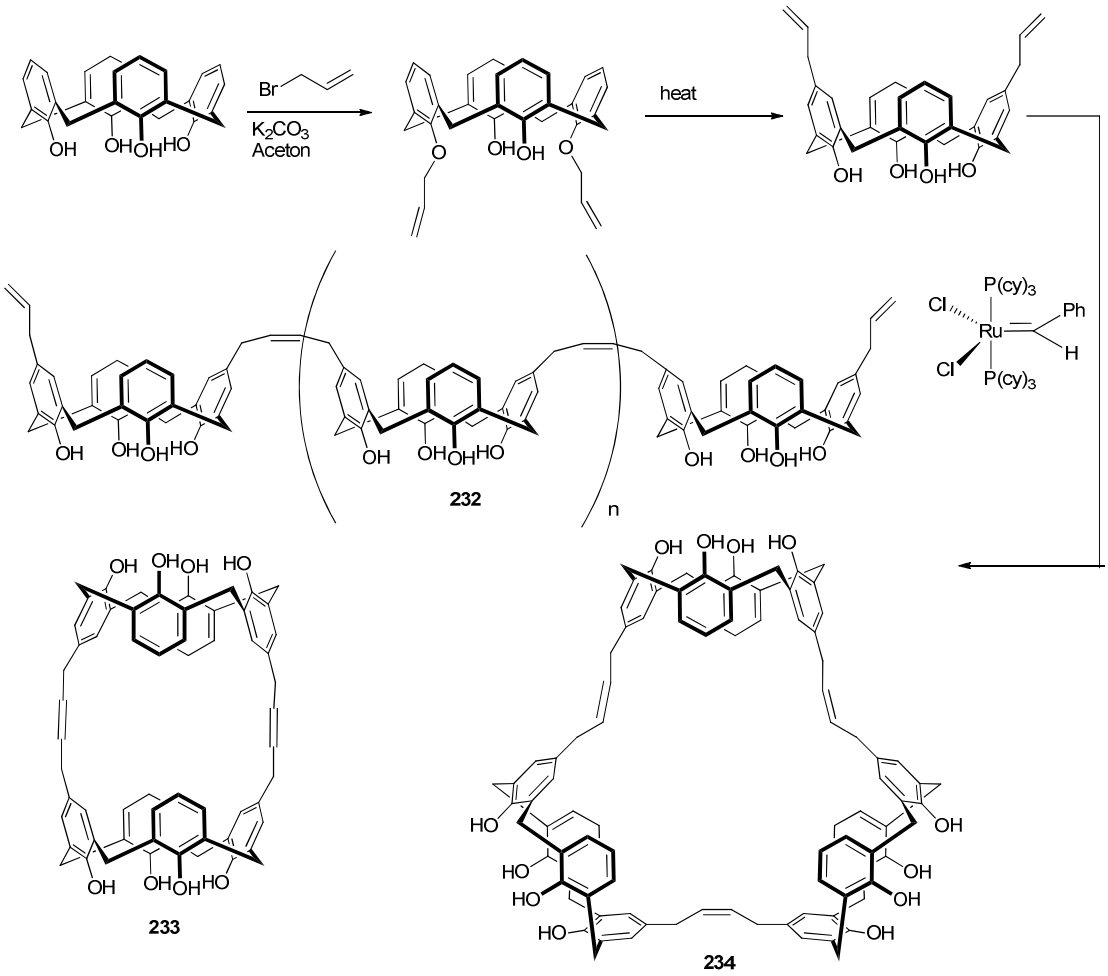


Figure 3.2: Di- and tri-calixarenes

McKervey and Pitarch reported in 1996 the synthesis of macrocyclic structures obtained by functionalising two distal hydroxyl groups of calix[4]arenes with allyl bromide.[7, 8] Due to the lack of a substituent on the *para* position of the aromatic ring, upon heating, the molecule can undergo a Claisen rearrangement to give a calixarene unit carrying the allyl functionalisation on the upper rim. Such molecules can react in the presence of an alkylidene ruthenium catalyst to form chains **232**, cyclic dimers **233** and cyclic trimmers **234** (scheme 3.5).



Scheme 3.5: Calixarene chain and cyclic dimer and trimer *via* allyl functionalisation of the upper rim.

3.1.2 Lower rim-lower rim linked multicalixarenes

Böhmer has also been a pioneer of the narrow rim-narrow rim linkage. In a paper with McKervey they reported the first example **235** in 1990.[9] They demonstrated two types of linkages: amide and ester (figure 3.2). They were obtained by reacting two identical calixarenes locked in the cone conformation and mono functionalised with an acid chloride with either 1,2-diaminoethane or ethylene glycol.

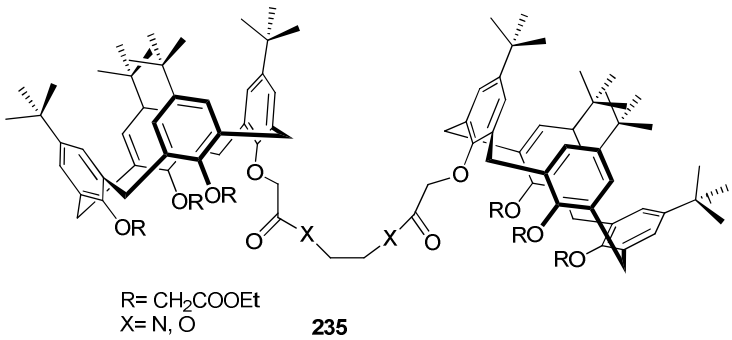


Figure 3.3: First example of narrow rim-narrow rim linkage

In another paper the same authors reported the synthesis of macrocyclic assemblies in which two or three calix[4]arene subunits were connected *via* two bridges between the oxygen atoms at the l- and 3-positions (figure 3.3).[10] The result was achieved by using difunctional reagents such as a diacid dichloride, which was too rigid to allow intramolecular bridging of the calix[4]arene units.

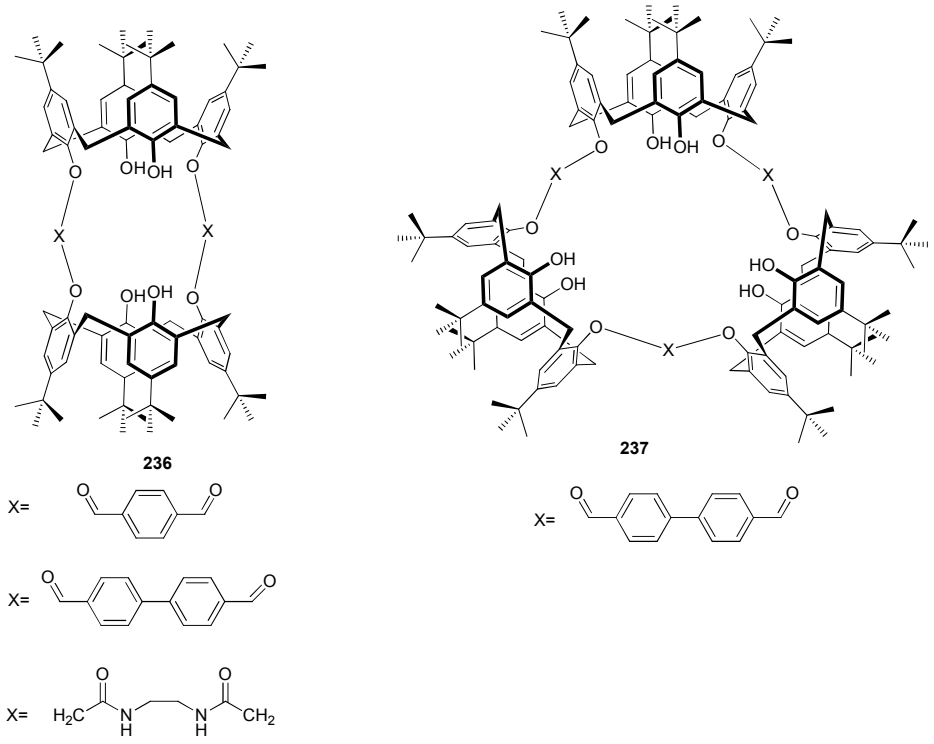
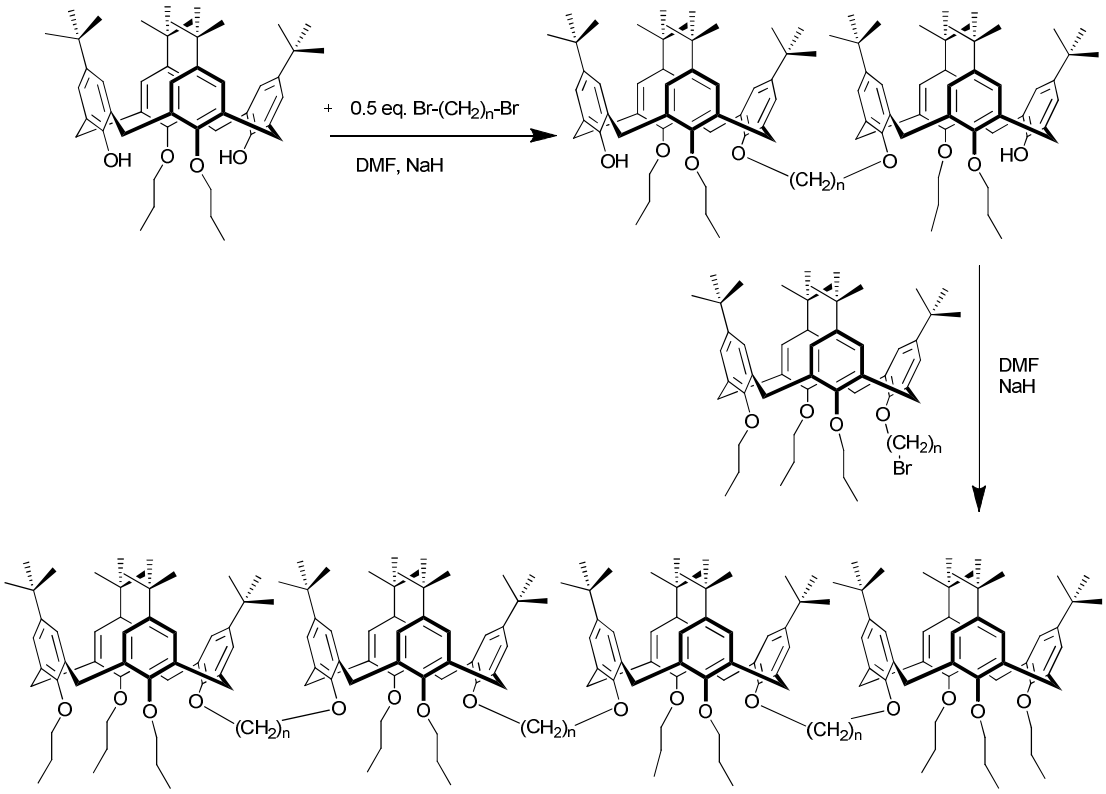


Figure 3.4: Double and triple calixarene macrocycles.

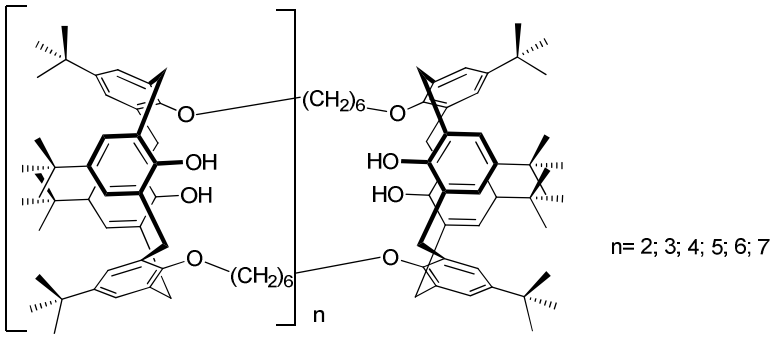
Further examples of a narrow rim narrow rim linkage were described by Kolbe and Shinkai in 1995. In their work they connected calixarene units using dibromoalkane linkers (scheme 3.6). The reaction was carried out in dimethyformamide using sodium hydride as base. This allowed them to synthesise dimers, trimers and tetramers connected linearly **238**.[11]



238

Scheme 3.6: Shinkai's linear tetramer.

More work following the same strategy allowed them to extend the linear array and to form macrocyclic molecules composed of up to eight calixarene units (figure 3.4).[12]



239

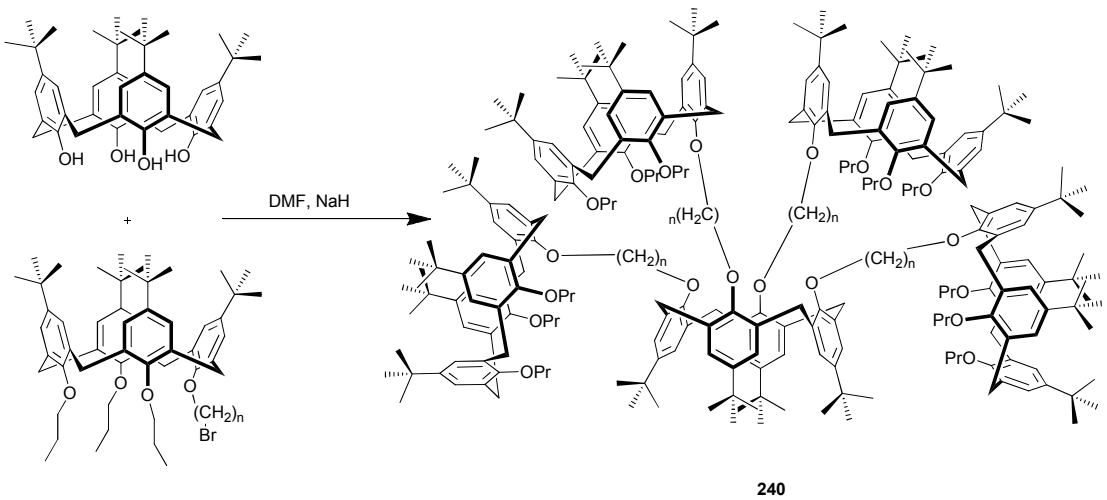
Figure 3.5: Shinkai's "macrocycle of macrocycles".

3.1.3 Calixarene based dendrimers

Multiple calixarene units organized in dendritic arrays have been described in literature since the mid 1990's. These molecules can have either a non-calixarene central core or a calixarene central core. Since their discovery calixarene based dendrimers have evolved into large macromolecules bearing in their structures up to twenty-one calixarene units organised in multiple generations.

3.1.3.1 Dendrimers with a calixarene central core

The synthesis of the first multicalixarene dendrimers was reported in 1995 by Lhotak and Shinkai. The lower rim-lower rim approach used for the synthesis of multicalixarene chains and "macrocycle of macrocycles" enabled also the preparation of a multicalixarene with a dendritic structure. This molecule was a pentamer **240** obtained by reacting *p-tert*-butyl calix[4]arene with 8 equivalents of the bromide derivative (scheme 3.7).[11]



Scheme 3.7: First example of a calix[4]arene based dendrimer.

Since this first example, many other calixarene based dendrimers have been described in the literature. In 1998 Mogck *et al.* published the synthesis of covalently linked multicalixarene pentamers.[6] A monoamino calixarene was prepared through mono *ipso*-nitration and subsequent reduction of *p*-tert-butyl calixarene tetraesters. Reaction with a central core locked either in the cone or in the 1,3-alternate conformation by four acetyl chloride functions yielded the pentamers shown in figure 3.5. (Figure 3.6)

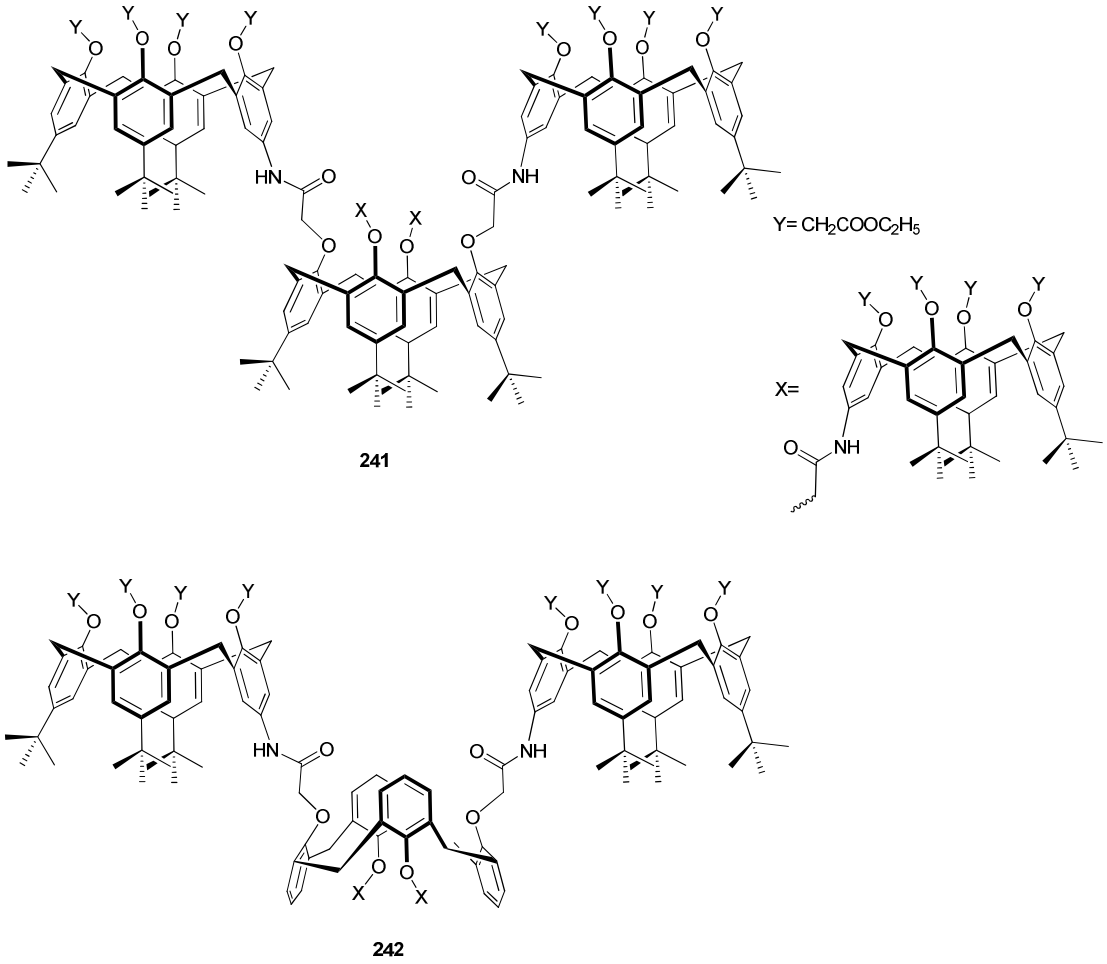


Figure 3.6: Mogck's Dendrimers

Interesting dendrimeric structures were reported by Prados and de Mendoza in 2010. Their pentamers were synthesised by connecting four calix[6]arenes to a calix[4]arene central core (figure 3.7). The dendrimers were obtained by connecting the building blocks with either ureido-phenyl[13] or aza-phenyl linkers.[14]

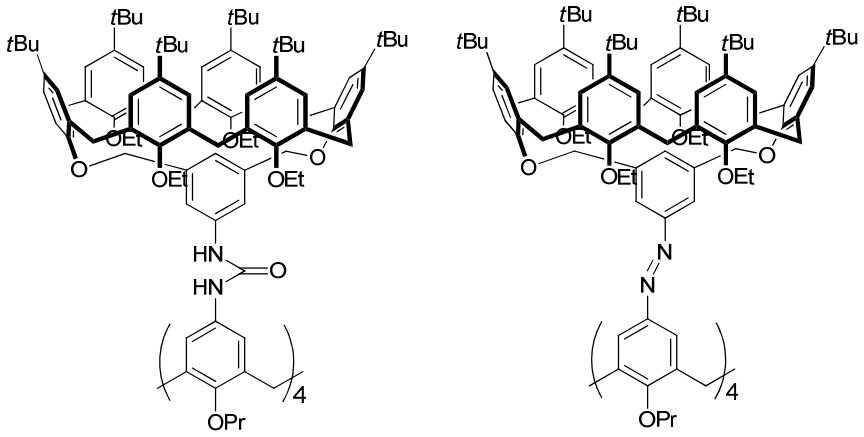


Figure 3.7: Prados and de Mendoza's dendrimers.

3.1.3.2 Dendrimers with a non calixarene central core

Mogck also explored the possibility of linking mono amino functionalised generation 1 calixarenes to a non calixarene central core. The same approach described for the synthesis of dendrimeric pentamers yielded a trimer when 1,3,5-benzenetricarbonyl chloride was used as a central core (figure 3.8).

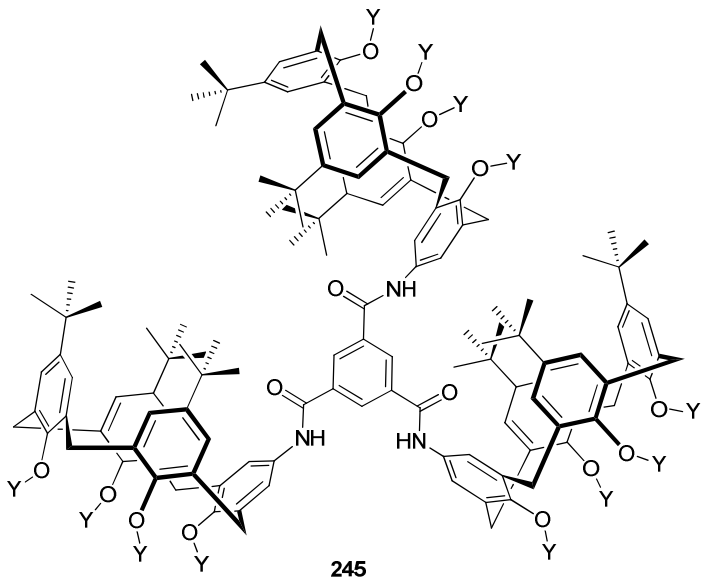
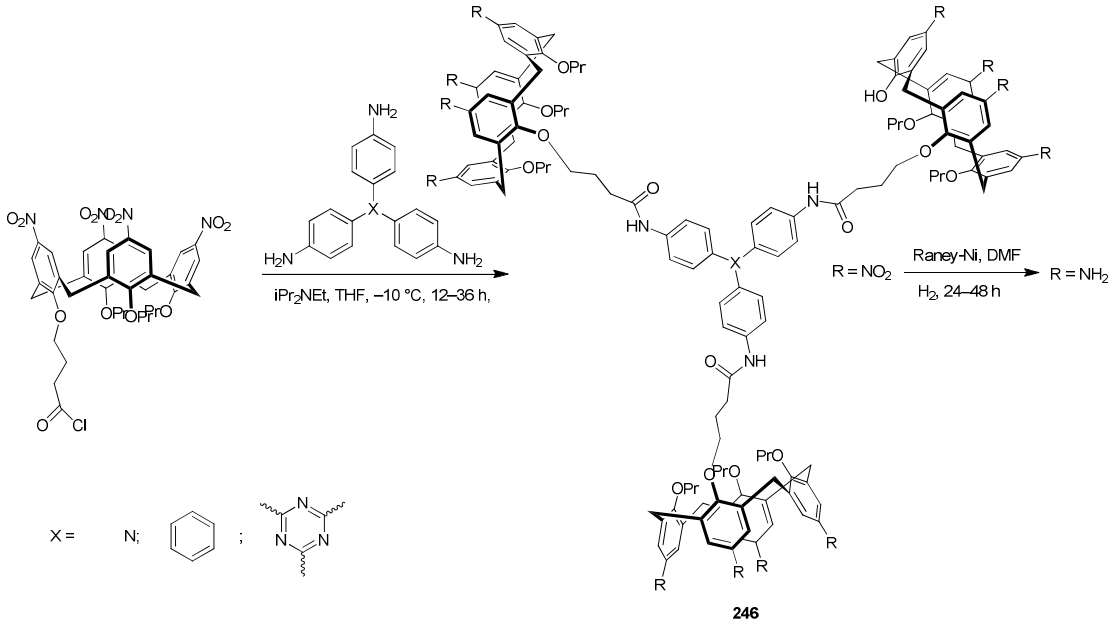


Figure 3.8: Mogck's Trimers

More examples of non-calixarene central core trimers have been reported in recent years by Pappalardo *et al.*[15] Their approach, similar to the one pioneered by Mogck, used amide bonds to assemble dendritic structures. Nitrocalixarenes, functionalised at the lower rim with an acyl chloride, were linked to trivalent amino central cores. The nitro groups were subsequently reduced to obtain amino functionalised oligomers (scheme 3.8).



Scheme 3.8: Pappalardo's Trimers

Another example of non-calixarene central core dendrimers is described in the work of Cheriaa, Abidi and Vicens (figure 3.9). The early stages of their work involved the synthesis of tri-calixarenes using mono methoxycarbonylcalix[4]arenes linked together through tris(2-aminoethyl)amine or "tren" bridges.[16]

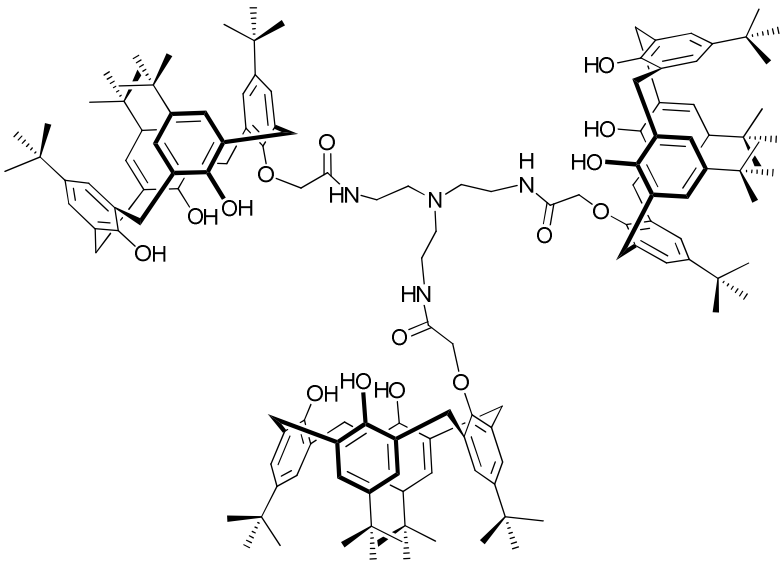


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Figure 3.9: "Tren" Dendrimer

3.1.3.3 Large calixarene based dendrimers

In the past decade a few examples of dendritic calixarenes bearing more than five calix[4]arene units in their structures have been reported. The first example was reported in 2002 by Szemes *et al.* They synthesised a dendritic structure bearing seven calix[4]arenes units linked through amide bonds, using a divergent strategy.[17] A central core functionalised with two amino groups on the upper rim was reacted with two equivalents of generation 1 calixarene bearing an acyl chloride function at the lower rim and two nitro groups on the upper rim. The trimer obtained was treated with hydrogen in the presence of palladium on carbon to reduce the nitro groups to aromatic amines. Four generation 2 calixarenes functionalised with an acyl chloride group were coupled to the amino functionalised trimer obtaining a second generation calixarene dendrimer **248** (figure 3.10).

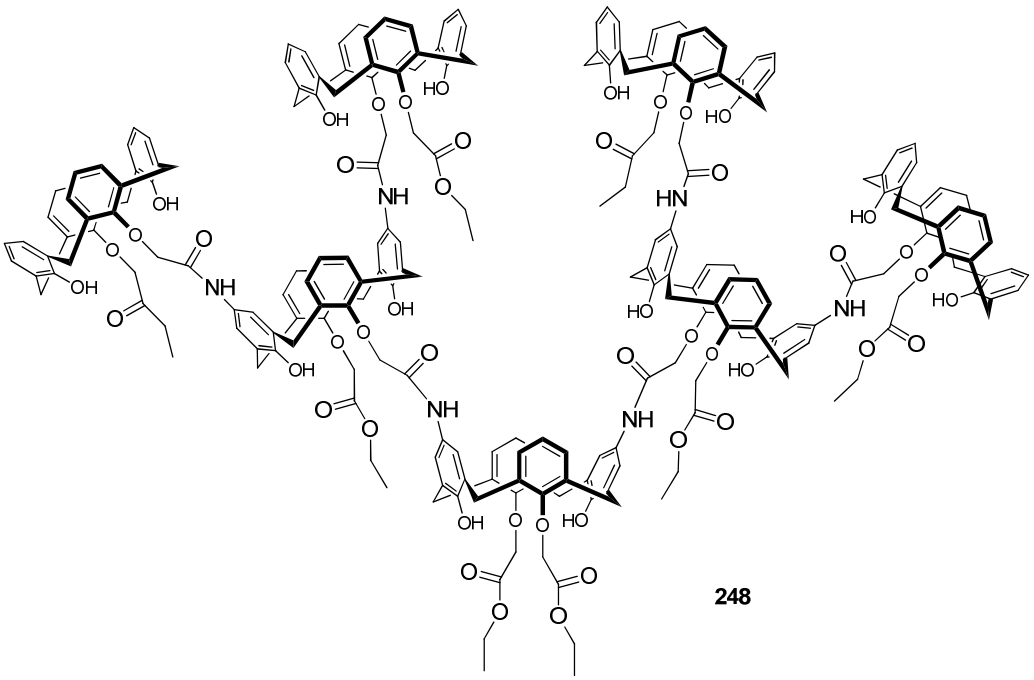
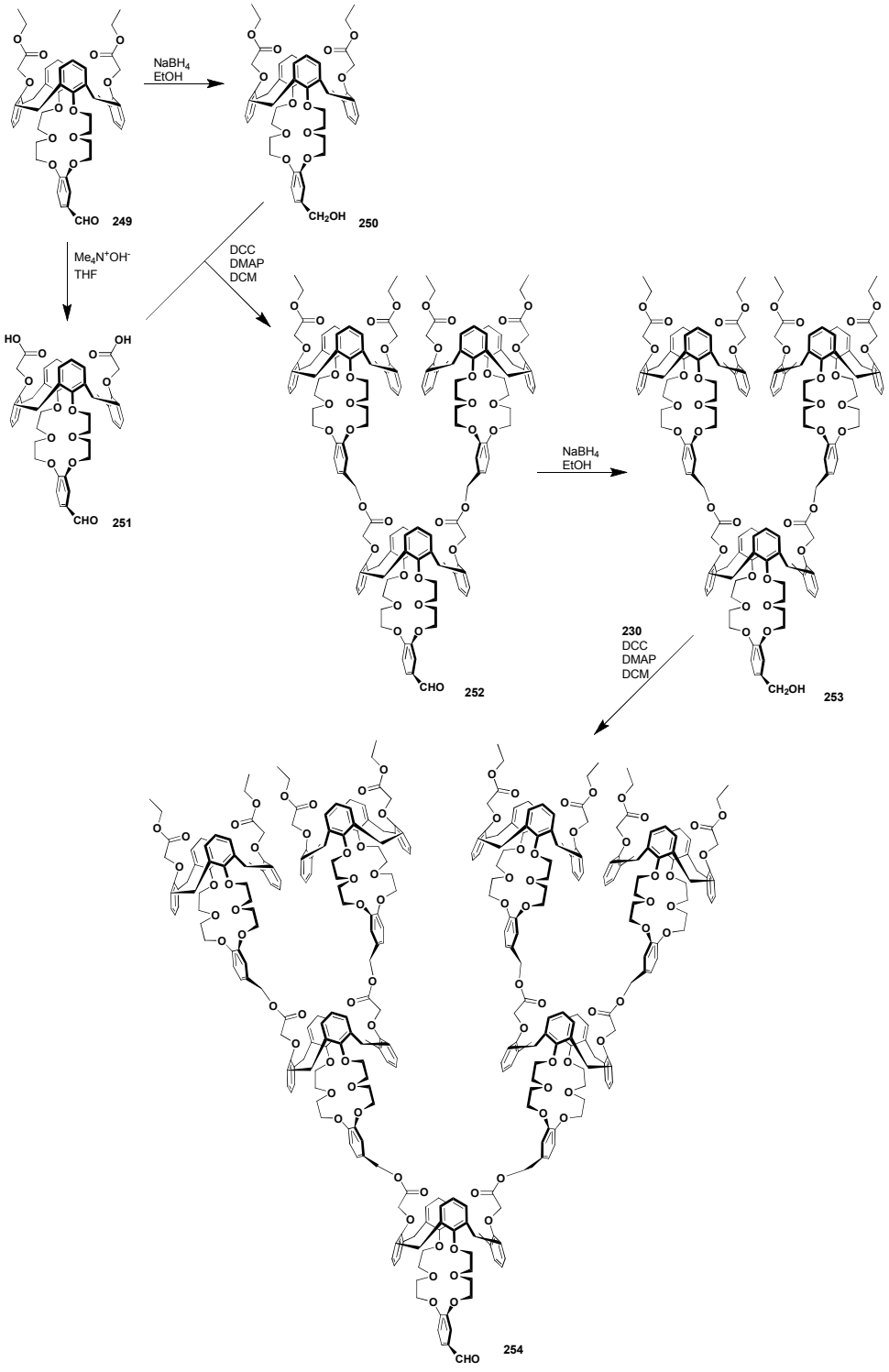


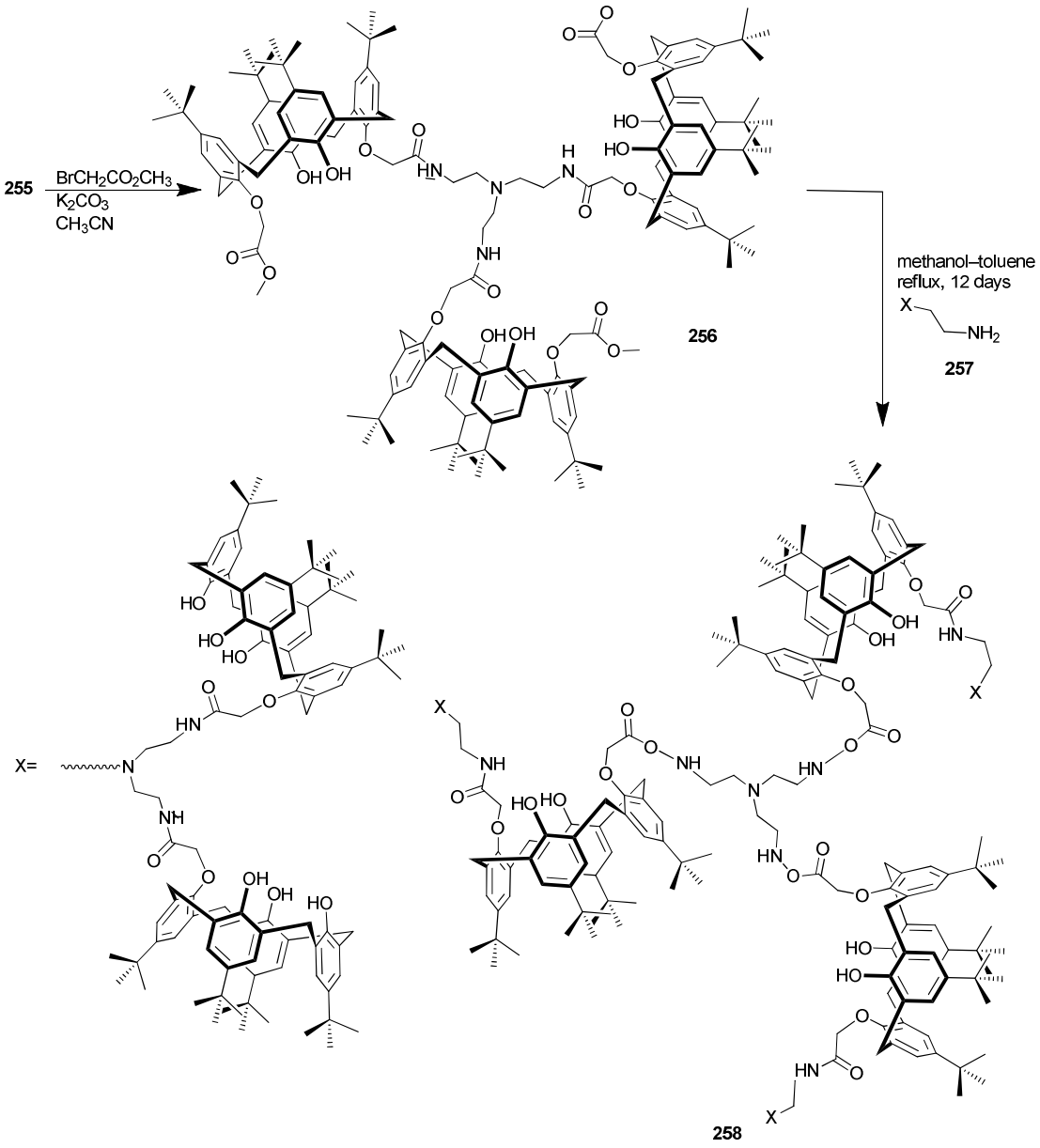
Figure 3.10: Calixarene Septamer

Three years after Szemes's paper, Bu *et al* reported the synthesis of a similar dendritic structure, also bearing seven calix[4]arene units.[18] Bu used a 1,3-alternate calix[4]arene crown derivative **249** as a building block (scheme 3.9). Using a convergent strategy the generation 2 calixarenes were first linked to the generation 1 calixarenes and the resulting trimer linked to a central core. The calix[4]arene crown **249** was reduced with sodium borohydride ($NaBH_4$) in THF to give **250**. The ester functionality of **249** was hydrolysed to give the free di-carboxylic acid which was used as generation 1 calixarene **251**. Generation 1 and 2 were coupled together in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and Dimethylaminopyridine (DMAP) in DCM. The aldehyde present on the resulting trimer **252** was then reduced using $NaBH_4$ and coupled with further **230** to give the final dendrimer **254**.



Scheme 3.9: Bu's dendrimer

In 2005 Cheriaa reported an extension of their work on "tren" dendrimers in the formation of hyper branched dendrimers (scheme 3.10)[19] **255** was transformed into **256** *via* selective monoalkylation. Then, **256** was reacted with 6 equiv. of di-calixarene **257** to obtain a dendrimer consisting of nine calixarenes.

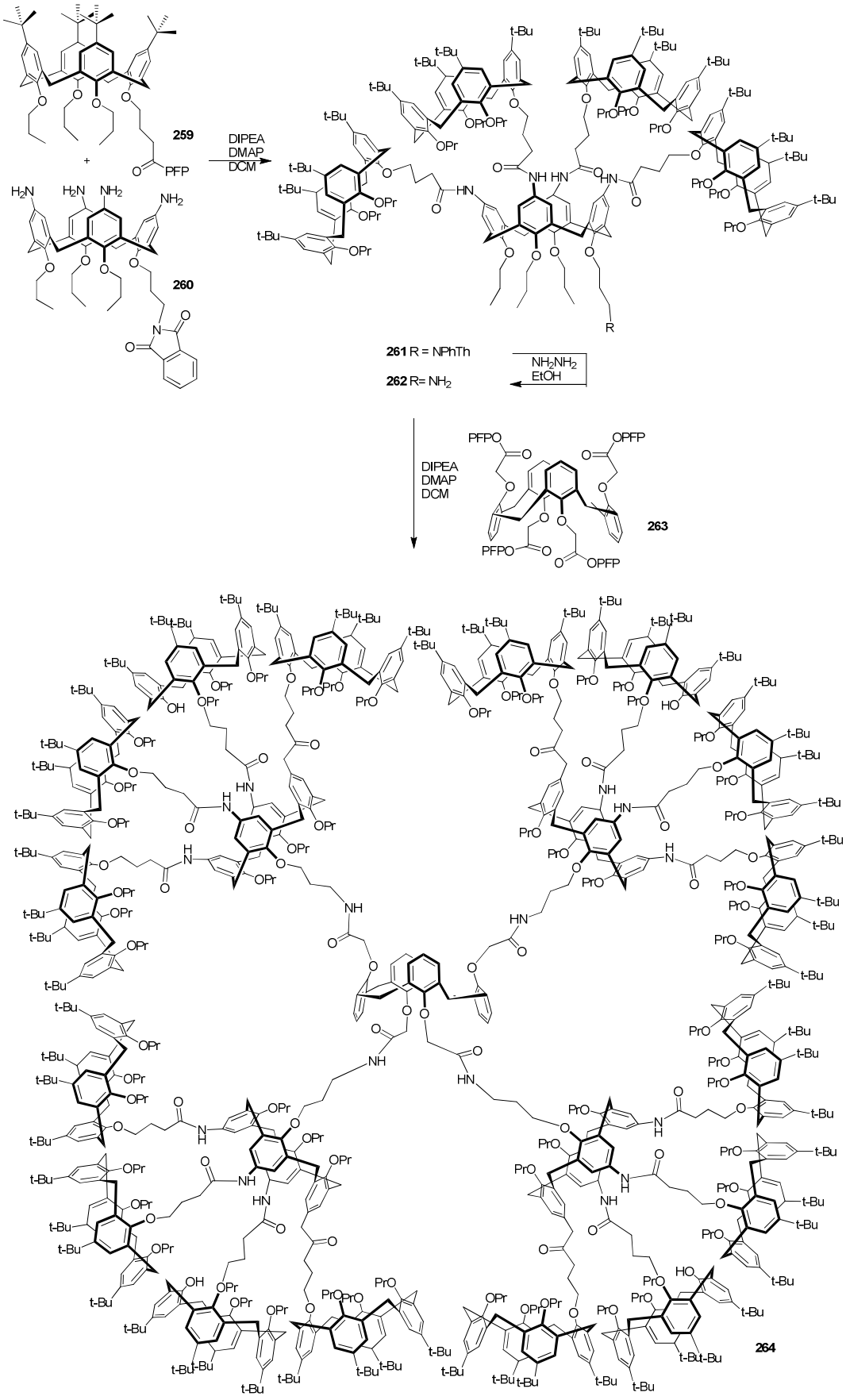


Scheme 3.10: Hyper branched "Tren" dendrimer

The largest multicalixarene known, was reported by the Matthews' group in 2010.[20] The structure was composed of twenty-one calix[4]arene units linked with amide bonds. Similarly to Bu's approach, Lalor's dendrimer was synthesised using a convergent strategy. *p-Tert*-butyl calixarene was locked in the cone conformation with three propyl chains and an ester. Following the ester hydrolysis, the free carboxylic acid was reacted with pentafluorophenol (PFP) in the presence of DCC to yield the generation 2 calixarene **234** (scheme 3.11). The generation 1 calixarene was also locked in the cone conformation by three propyl chains and by a propyl phthalamide group. The wide rim was functionalised with four amino groups to give the generation 1 calixarene **260**. The amino groups of **260** were coupled with four equivalent of **259**

in the presence of di *iso*-propylethylamine (DIPEA) and DMAP in DCM to yield the pentamer **261**.

The amino group, masked by the phthalamide, was revealed through treatment with hydrazine in ethanol. The pentamer with the primary amino function **262** was coupled with central core **263**, locked in the 1,3-alternate conformation with four active esters. The coupling performed in the presence of DIPEA and DMAP yielded the largest dendrimeric multicalixarene synthesised to date **264**.



Scheme 3.11: Lalor's dendrimer

3.2 Aims of the chapter

In the previous chapters we have reported the synthesis of several calixarene based dendrimers. The structures described were all prepared by reacting mono alkyne functionalised generation 1 calixarenes with a tetra azido functionalised central core to form triazole linked multicalixarenes. The central cores investigated were either in the cone or in the 1,3-alternate conformation. The azido functionalities were introduced by two different strategies. In the first case the conformation of the calix[4]arene was locked in the desired conformation by reacting the four hydroxyl positions with either di-haloalkanes or ethyl bromoacetate, which could be converted into azides. In this way we succeeded in the synthesis of the structures shown in figure 3.11.

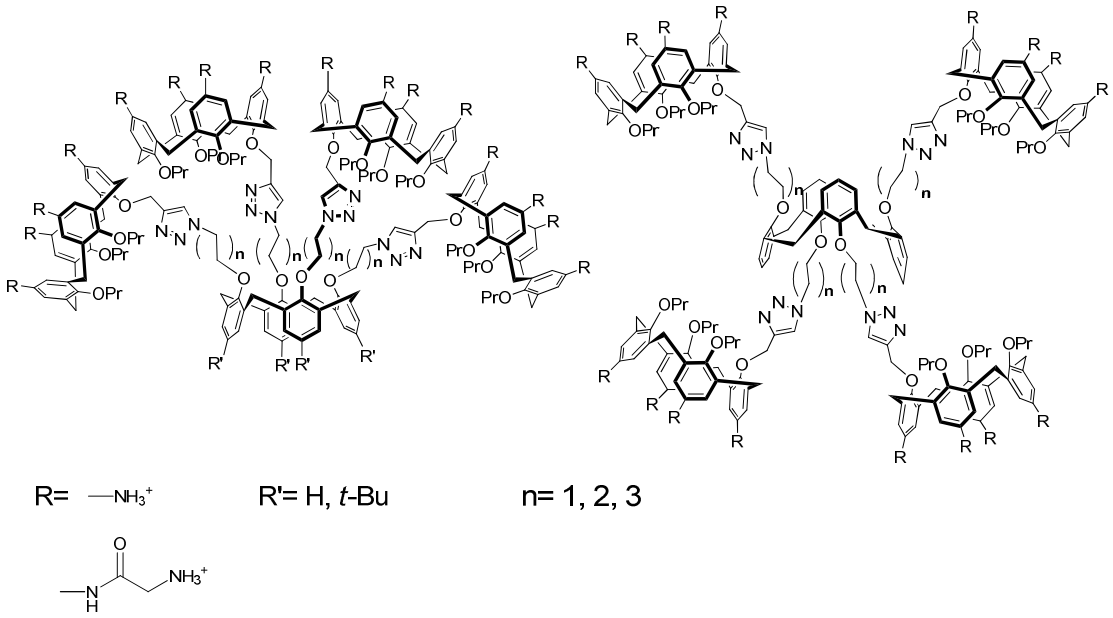
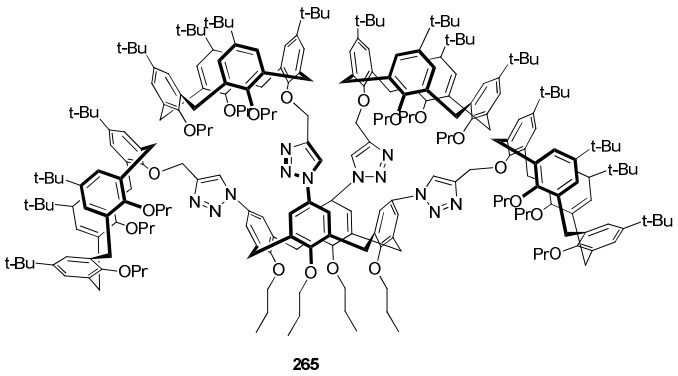


Figure 3.11: Examples of multicalixarenes obtained *via* O-alkyl-azide functionalisation of central cores.

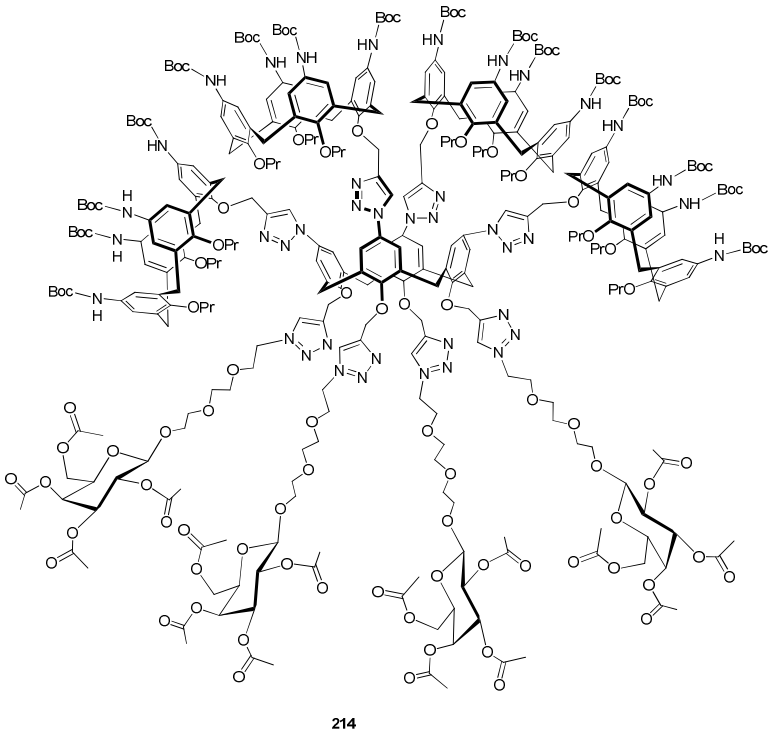
In the second case the azido functionalities were introduced on the aromatic rings of calixarenes locked in the cone conformation by propyl groups. Functionalisation of the upper rim with azido groups was possible in three synthetic steps: *ipso* nitration, reduction, substitution *via* diazonium salt formation, on the *para* position of the tetra alkylated *p-tert*butylcalix[4]arenes. The pentamers obtained in this fashion featured narrow rim-upper rim linkages. Multicalixarene **265** (figure 3.12) was the first of the series to be synthesised. This prototype molecule featured *tert*-butyl groups at the upper rim of the generation 1 calixarenes. However analogues of this molecule carrying aliphatic or aromatic amines have been successfully synthesised and their syntheses have been explained in details in the first chapter of this thesis.



265

Figure 3.12: Example of narrow rim-upper rim linked multicalixarenes

This strategy allowed the introduction, at the lower rim of the calixarene, of functionalities which can subsequently be activated for a second “click” reaction. An example of this potential has been explained in chapter 2. Functionalisation of the lower rim of the central core with four alkyne groups in the first step of the synthesis and subsequent protection with TBDMS, allowed a second “click” reaction to be performed after assembly of the multicalixarene and removal of the protecting group. The process yielded the first multicalixarene glycoconjugate shown in figure 3.13.



214

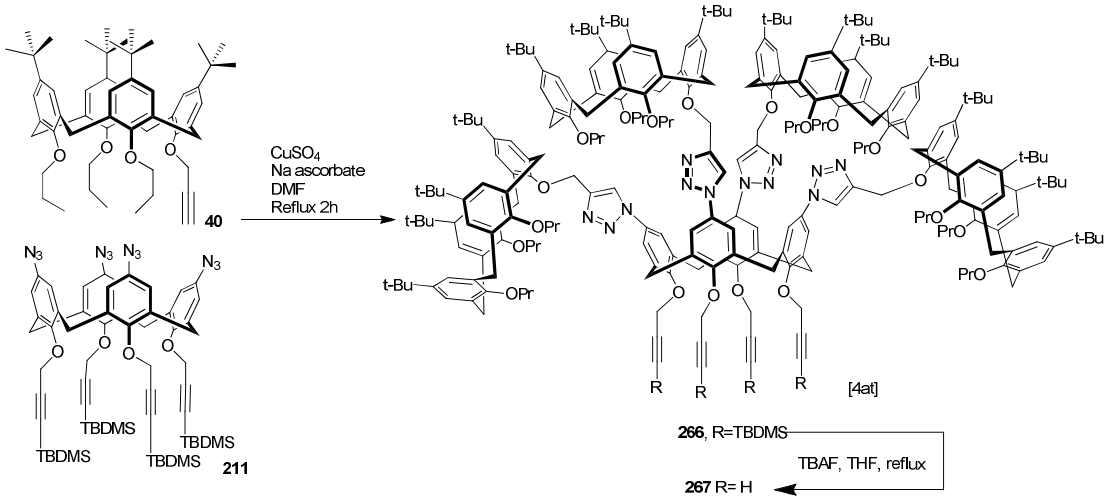
Figure 3.13: Structure of Multicalixarene Glycoconjugate **214**

In this chapter we aim to further investigate the potential of this strategy towards the assembly of dendritic molecules featuring a higher number of calixarene units in their structures.

3.3 Synthesis of nonamers

The central core **211,** described in chapter 2 and successfully used for the synthesis of multicalixarenes glycoconjugates, could be used also to synthesise multicalixarenes bearing eight generation 1 calixarenes.

The tetra azido compound was reacted with four equivalent of mono alkyne functionalised calixarene **40** (scheme 3.12), in the presence of catalytic CuI, to yield the first example of a pentameric multicalixarene 2**66** featuring functionality at the lower rim.



Scheme 3.12: Synthesis of Multicalixarene **267**

As previously, **266** was treated with TBAF to remove the silyl protecting group. Figure 3.15 shows a comparison between the spectra of the multicalixarene before (blue) and after (red) deprotection. In the red spectra the peaks for the TBDMS peaks around 1.25 and 0 ppm disappeared upon removal of the protecting group. The presence of the alkyne terminal proton is confirmed by the triplet at 2.75 ppm and the doublet at 4.8 ppm with coupling constant around 2 Hz.

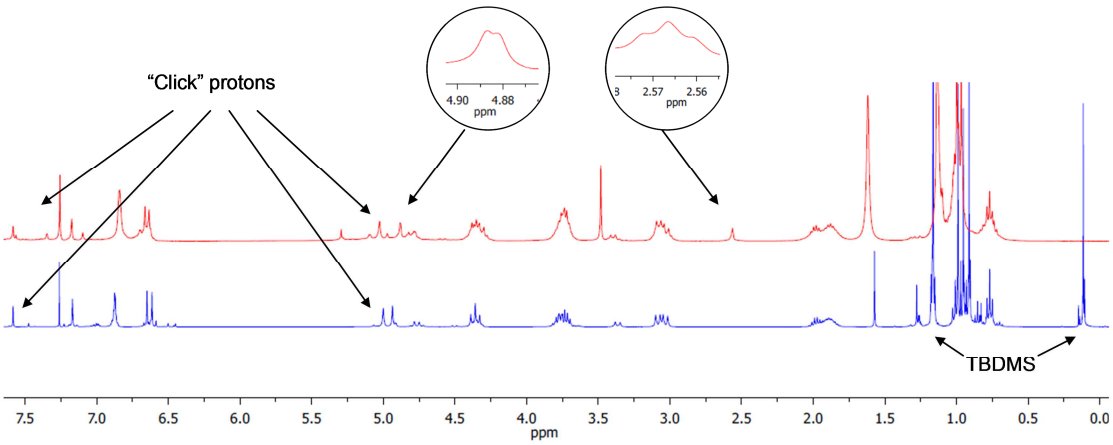
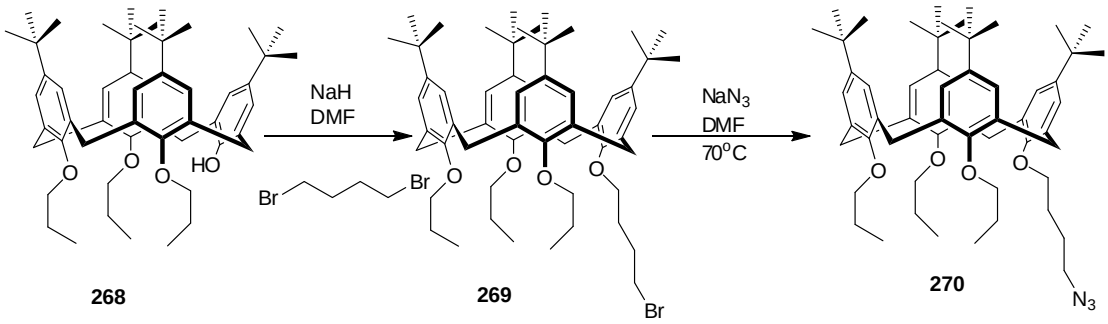


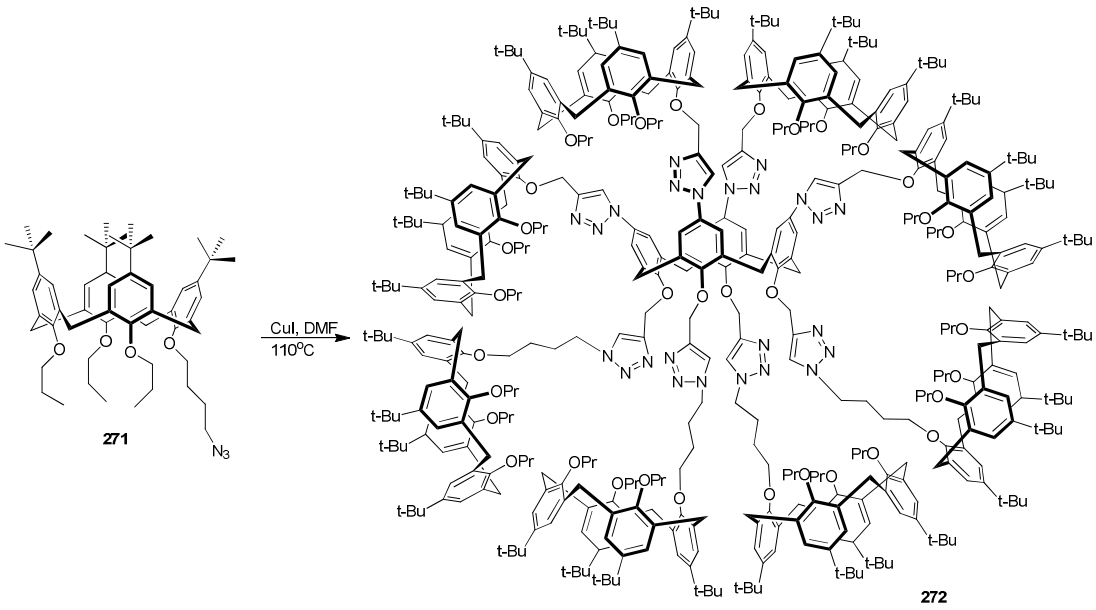
Figure 3.14: Comparison of the ^{1}H NMR spectra of compound **266** (blue) and compound **267** (red)

The four unmasked alkyne groups were then reacted with four equivalents of a calixarene functionalised at the lower rim with an azide. A suitable calixarene molecule for the second cycloaddition was prepared by alkylating the free hydroxyl group of *p-tert*-butyl tripropoxy calix[4]arene **268** with dibromo butane in the presence of NaH. In the next step the halogen was readily replaced by heating the compound at 70^{o}C in DMF in the presence of sodium azide.



Scheme 3.13: Synthesis of mono azido functionalised generation 1 calixarene

The mono azido functionalised calixarene obtained was "clicked" to the tetra alkyne functionalised multicalixarene **267**. The cycloaddition was carried out using CuI as a source of copper (I) catalyst and yielded a multicalixarene consisting of a central core surrounded by eight generation 1 calix[4]arenes.



Scheme 3.14: Synthesis of nonamer

Simple ^{1}H NMR analysis of the compound proved difficult to interpret because of the overlap of the generation 1 calixarenes resulting in series of multiplets. However COSY analysis gave valuable information regarding the coupling of the multiplets and allowed the assignment of peaks. Figure 3.16 shows the COSY spectra for the nonamer **272.**

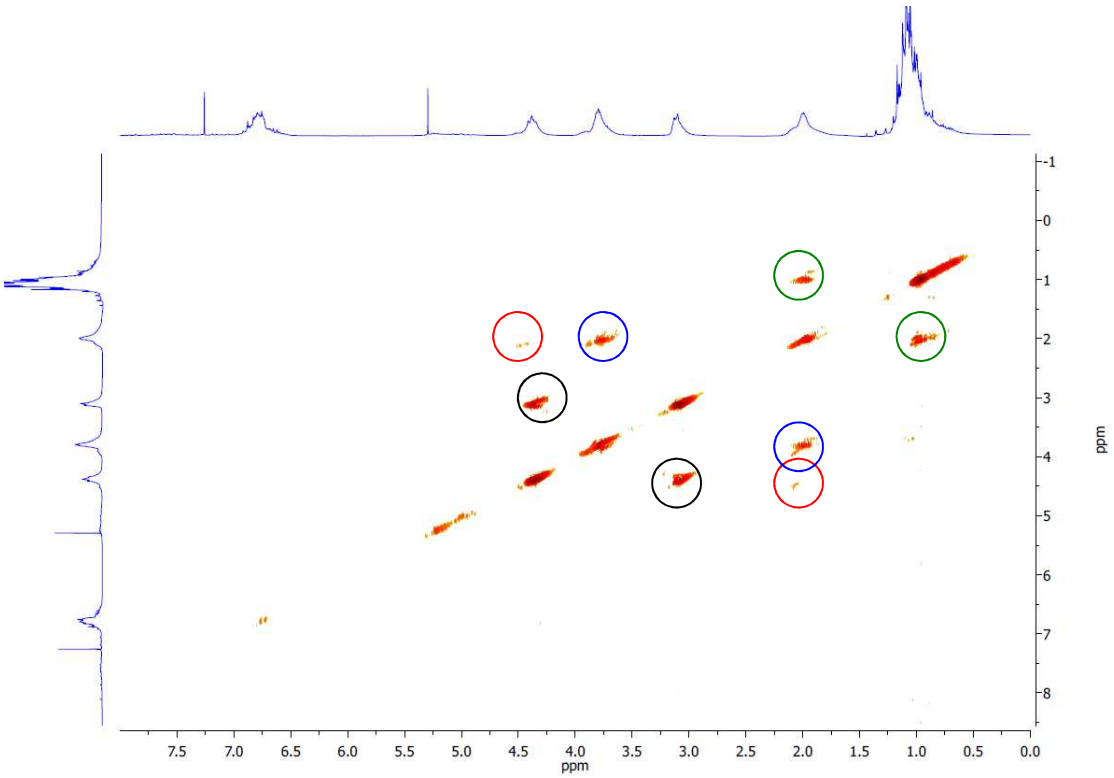


Figure 3.15: COSY acquisition for compound **272**

In the black circles, at 4.38 ppm and 3.07 ppm, it it is possible to observe the coupling between the methylene bridges. In blue, at 3.79 ppm and 2.07 ppm, is highlighted the coupling between the methylenes of the aliphatic chain, next to the oxygens and in the middle of the chain respectively. The multiplet at 2.07 ppm from the methylenes located in the middle of the aliphatic chains shows also coupling with the multiplet at 1.01 ppm which can be assigned to the terminal methyl groups of the propyl chain of the generation 1 calixarenes (circled in green). The last coupling highlighted in red at 4.47 ppm and 2.10 ppm, refers to the methylene protons next to the triazole ring and the protons in the aliphatic chain on the next carbon atom.

The signals for the click protons are normally observed as two singlets around 8 ppm and 5 ppm respectively. In our case, the eight triazole rings are in two different environments so we would expect to find two set of signals. Interestingly the ^{1}HNMR spectrum shows a number of signals in the “click” proton regions. A possible explanation of this unusual behaviour is suggested by the analysis of the mass spectrometry result obtained for this compound (figure 3.17). The spectrum shows two peaks. The first, m/z = 7505.1 corresponds to the mass of the molecular ion plus sodium. The second peak m/z = 7631.0 is greater than the other of 126 units. This correspond to two atoms of copper (Mw = 63). It is possible therefore that the copper atoms are trapped in the molecule structure and interact with the triazole rings complicating the signals given by the “click” protons.

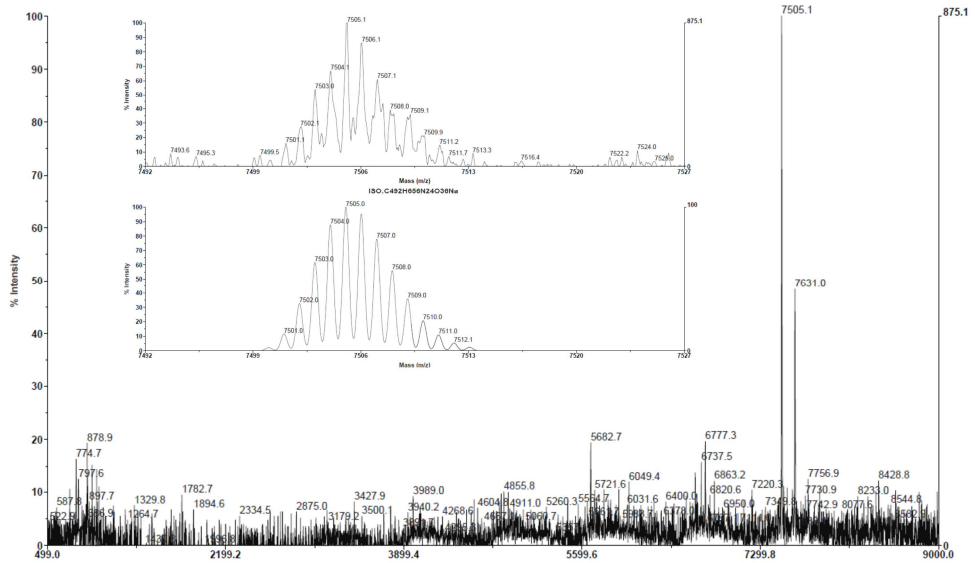
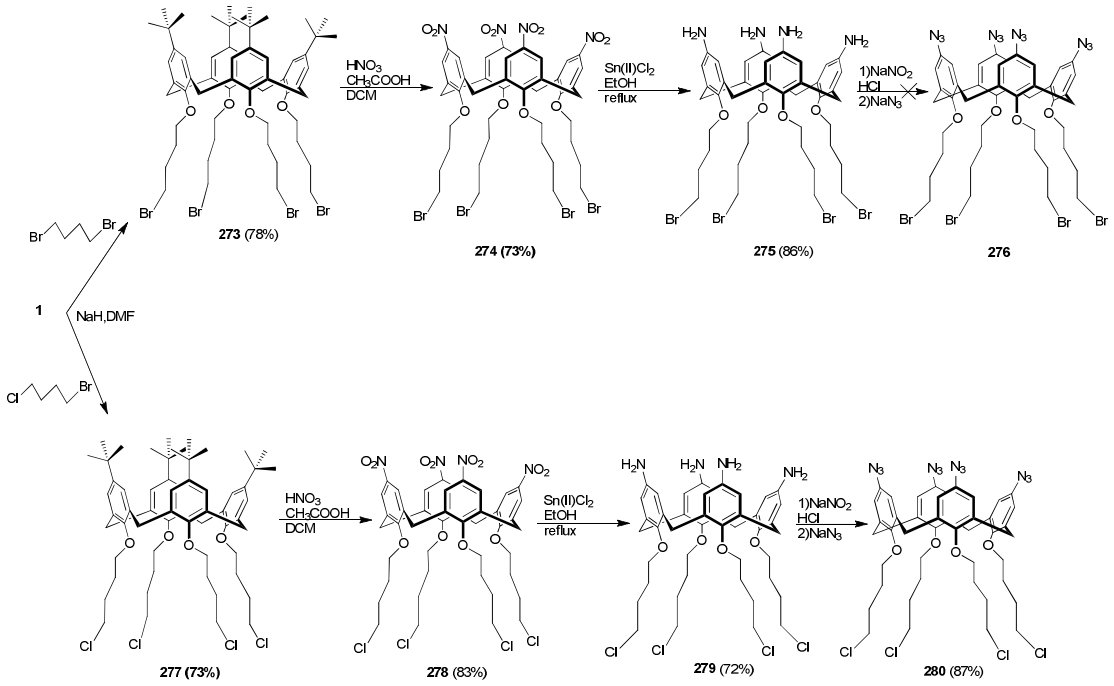


Figure 3.16: Mass spectrum of compound **272**

With this method we have been successful in introducing different calixarenes through triazole linkages in two distinct steps.

An alternative approach has been developed for the introduction of alkyne functionalised calixarenes both at the upper and at the lower rim of a central core in subsequent steps. The central core designed for this purpose featured four aromatic azido groups at the upper rim and four halo alkane chains at the lower rim, which could be readily converted into azido chains once the "click" reaction at the upper rim has been performed.

In a first attempt di-bromobutane was used to alkylate *p-tert*-butylcalix[4]arene (scheme 3.15).[21, 22] NaH in DMF was used as the base to lock the molecule in the cone conformation. *Ipso*-nitration of the compound[23] was carried out using as nitrating agent equal volumes of fuming nitric acid and glacial acetic acid. The product was recovered after recrystallisation from DCM/MeOH as a pale yellow solid (79 %). Subsequent reduction[24] of the nitro derivative **274** with tin chloride yielded the tetra amino compound **275** in quantitative yield.



Scheme 3.15: Synthesis of central core **280**

As described for the synthesis of **211** the four amino groups can be converted to azides *via* diazonium salt formation.[25] Despite the stoichiometric amount of sodium azide and the temperature used (0^oC), some of the bromines on the alkyl chain were also substituted by the azido anions. This problem was solved using 1-bromo-4-chlorobutane as the alkylating agent in the first step.[21, 22] The chlorine on the chain was not reactive enough to be substituted by the azido groups at 0^oC. Therefore the same set of reactions yielded the central core **280** featuring four aromatic azides at the upper rim and four halo alkyl chains at the lower rim. As was observed in previous examples for the synthesis of central the substitution of the aromatic amino

group with the azido group can be detected by the shift of signal for the aromatic protons from 6.07 for the amino derivative (green) to 6.31 for the tetra azido compound (purple) (figure 3.18).

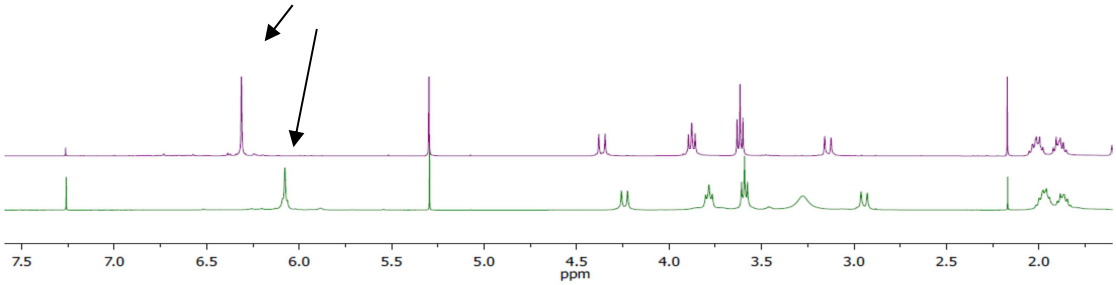
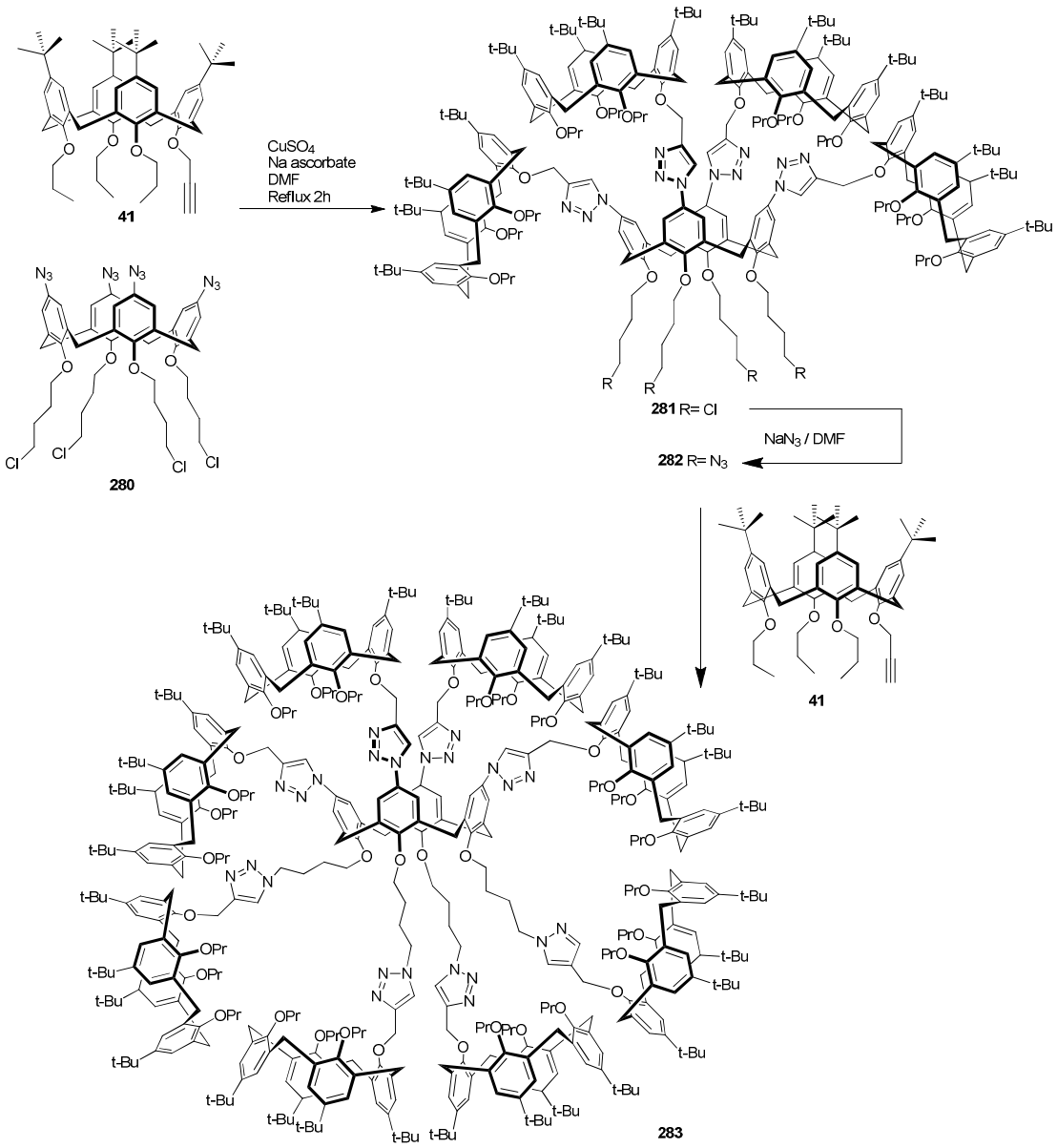


Figure 3.17: ^{1}H NMR spectra of compound **279** (green) and **280** (purple).

The molecule was successfully clicked using CuI [26] as a source of copper (I) ions to the propargyl functionalised generation 1 calixarene **41** (scheme 3.14). The product was obtained after purification by column chromatography as an off white solid (52%).

The pentamer **281** was heated at 70 ^{o}C in DMF in the presence of sodium azide. The chorines were successfully replaced and the tetraazido pentamer **282** was obtained by precipitation of the crude material from DCM/MeOH (48%). Reaction of this compound with four more equivalents of alkyne functionalised generation 1 calix[4]arene **41**.



Scheme 3.16: Synthesis of the nonamer **283**

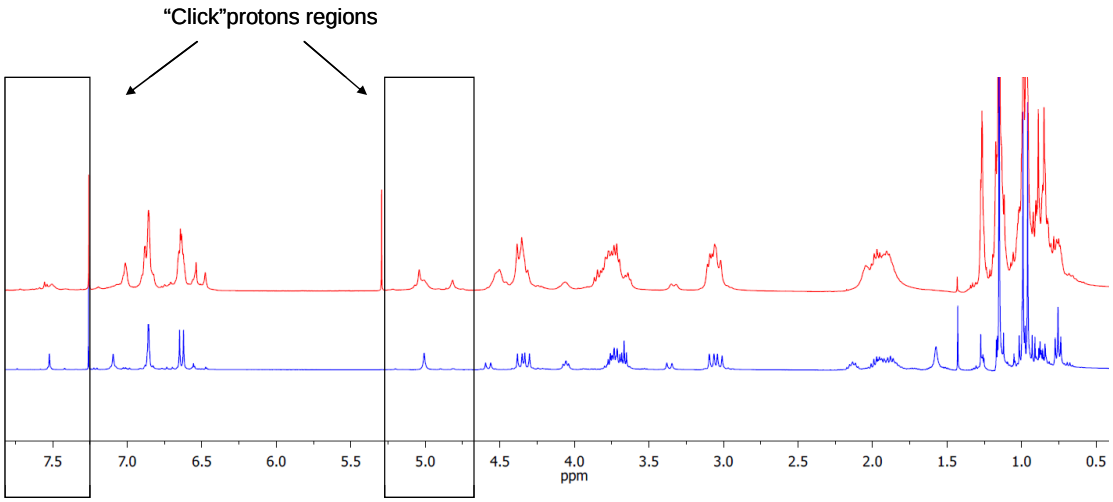


Figure 3.18: Comparison between the spectra of compound **281** (blue) and compound **283**.

Figure 3.19 shows the spectrum of the pentamer **281** (blue) against the spectrum of the nonamer **283** (red). Also in this case simple ^{1}H NMR of the nonamer is difficult to interpret due to the overlap of the generation 1 protons. The "click" protons give multiple broad signals. COSY analysis gave useful information about the coupling of the protons and is reported in figure 3.20.

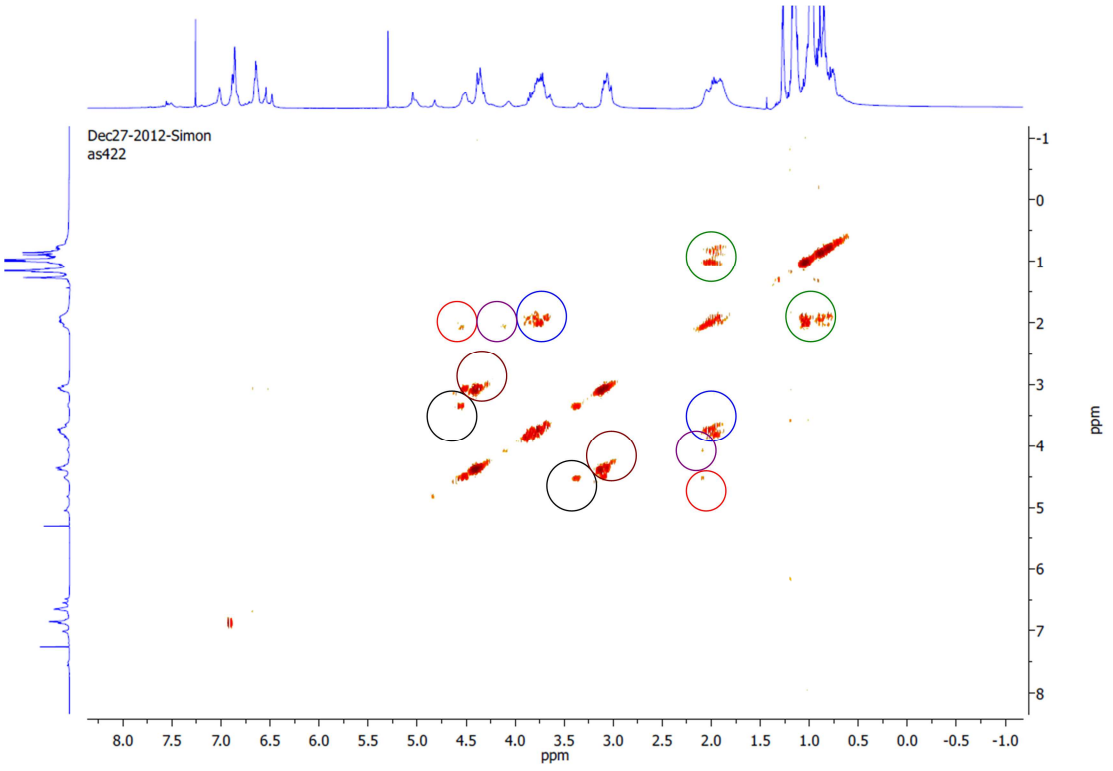


Figure 3.19: COSY acquisition for compound **283**

The COSY spectrum clearly shows the coupling of the methylene bridges of the generation 1 calixarenes (brown) at 4.38 and 3.11 ppm. In this molecule it is possible to distinguish also the coupling of the central core methylene bridges at 4.56 and 3.35 ppm (black). In green are highlighted the signals relative to the coupling of the terminal methyl protons of the propyl chain with the methylenes in the middle of the chain at 0.93 and 1.98 ppm respectively. These methylenes are coupled also to the ones next to the oxygens which give a signal at 3.76 ppm (blue). The protons of the methylenes located on the butyl chain next to the triazole ring (red) and the ones next to the oxygen on the other terminus of the aliphatic chain (purple) give two distinct signals at 4.52 and 4.01 ppm respectively. These protons are coupled to the ones in the middle of the chain which give a signal at 2.08 (red) and 2.06 (purple) ppm respectively.

The mass spectrometry analysis (figure 3.12) again shows the peak of the molecular ion plus sodium and another peak greater of 126 units which is probably due to the complex with two atoms of copper.

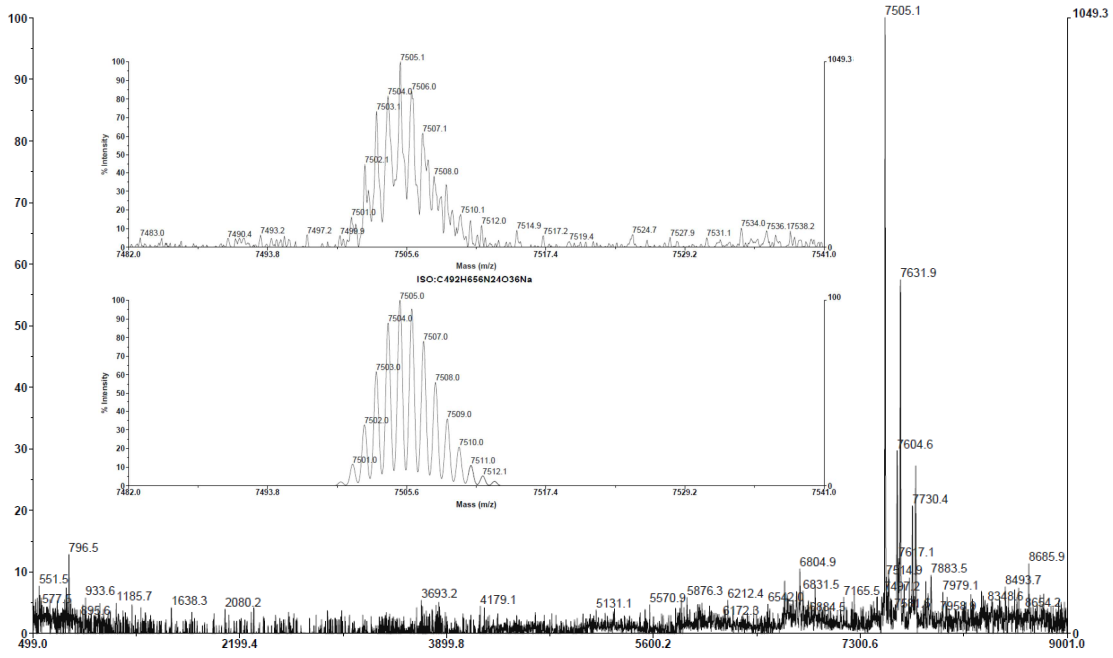


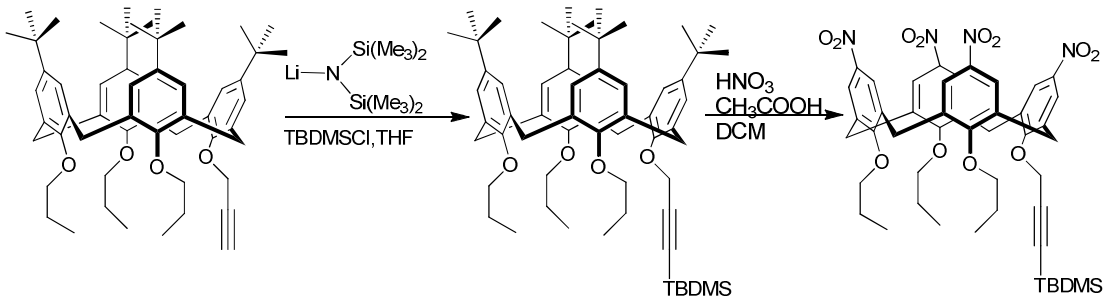
Figure 3.20: Mass spectrum of compound **283**

3.4 Synthesis of henicosamers

To synthesize a dentritic multicalixarene bearing twenty-one calixarene units, we opted for a convergent synthetic approach. The outer generation was composed of units of calixarene **41**.

The generation 1 calixarene was designed to bear four azido groups on the wide rim which would be able to react with the alkyne group of the generation 2 calixarenes, and a protected alkyne group on the lower rim, which after the formation of the pentamer could be deprotected and reacted with a tetrazido functionalised central core.

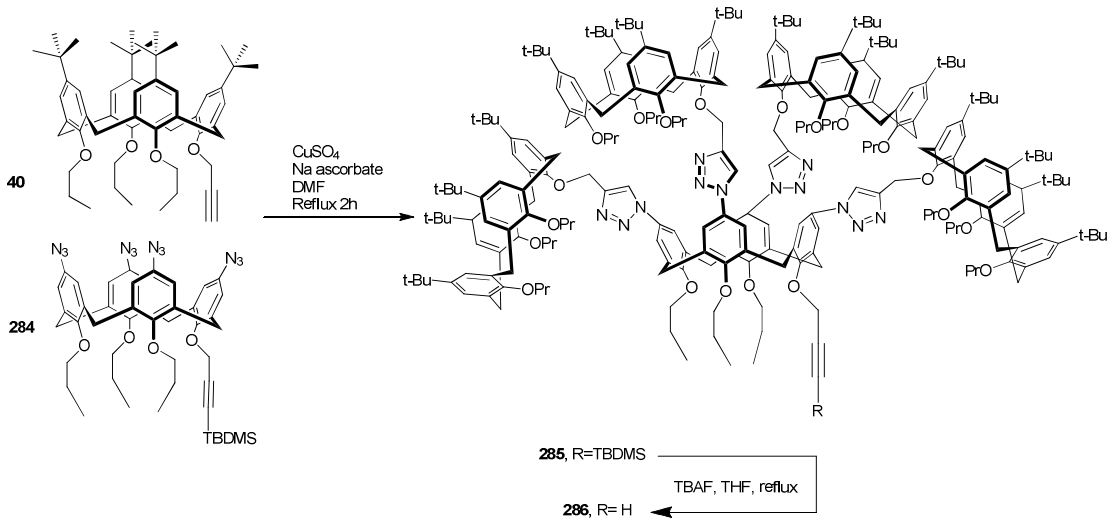
Starting from **41** the alkyne group was protected with TBDMS in the presence of LiHDA in THF. The product, recovered after precipitation from DCM/MeOH (98%), was nitrated using equal amounts of fuming nitric acid and glacial acetic acid (figure 3.16).



41 **42** **43**

Scheme 3.1730: Alkyne protection and *ipso*-nitration

The pale solid obtained after precipitation from DCM/ MeOH (85%) was reduced using the tin chloride method. The tetra amino-compound was dissolved in a mixture of 10% HCl and ethanol and allowed to react for twenty minutes with $NaNO_2$ to form a diazonium salt.[25] Subsequent addition of sodium azide yielded the tetra azido compound **284** which was recovered after column chromatography as an off white solid. The "click" reaction between generation 1 calixarene **284** and generation 2 calixarene **41** yielded the pentamer **285** (scheme 3.18).



Scheme 3.18: Synthesis of alkyne functionalised pentamer

The alkyne group could at this point be deprotected by treatment with TBAF in THF at reflux temperature and allowed to react with a tetra-azido functionalised central core. Two types of central core were tested. The first was locked in the cone conformation **16** and the second was locked it the 1,3-alternate conformation **34** (figure 3.22), their synthesis has been described in detail in chapter 1

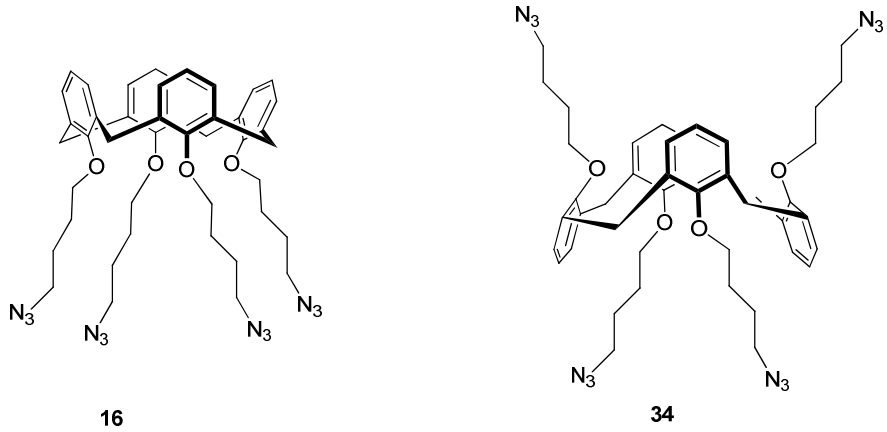
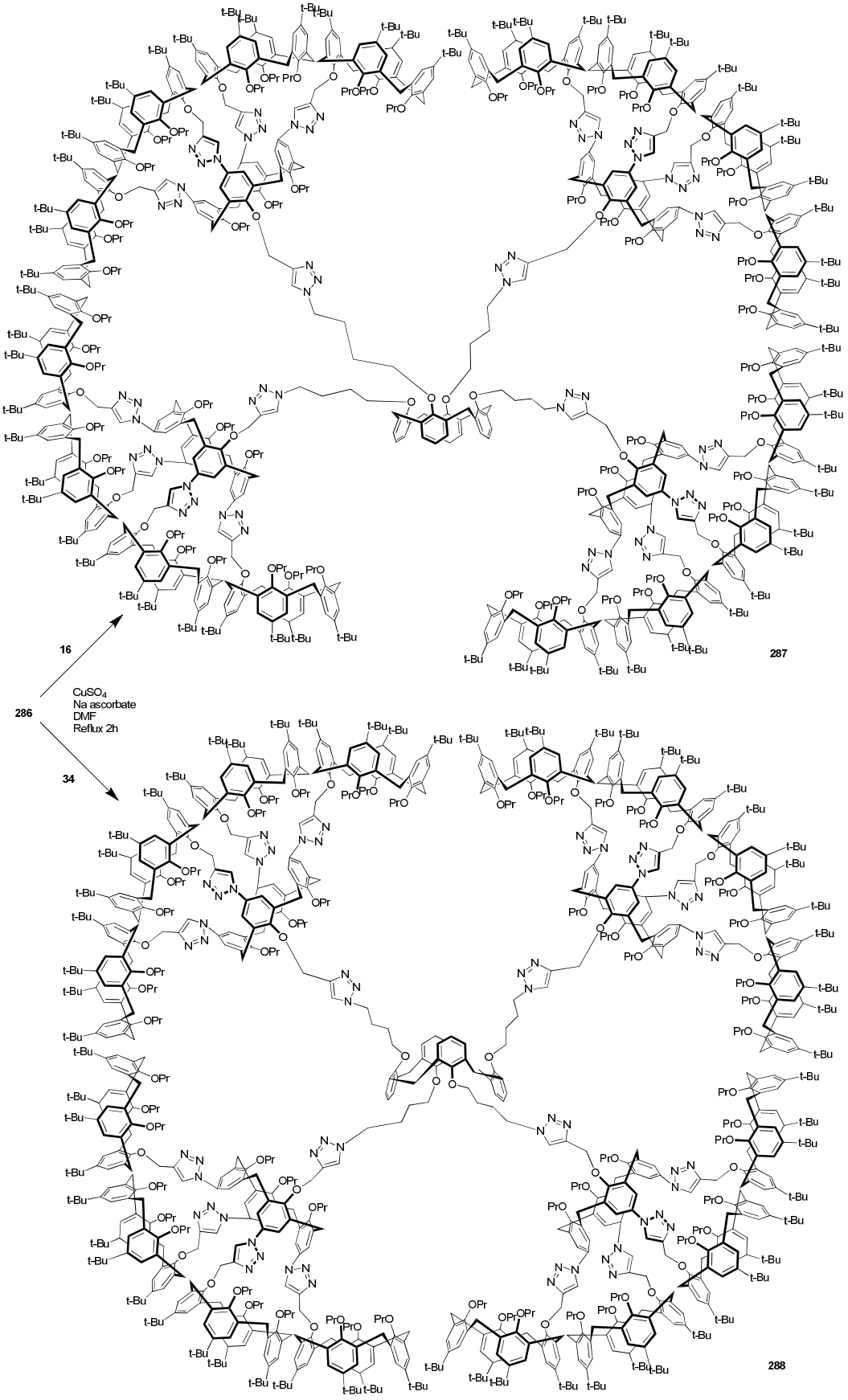


Figure 3.21: Azido functionalised central core in cone and 1,3-alternate conformation.

In the final step pentamer **286** was reacted with either central core **16** or **34** to yield the two structures containing twenty-one calixarene scaffolds, in the cone **287** (56%) and in the 1,3-alternate **288** (64%) conformation respectively (scheme 3.19). Copper sulphate and sodium ascorbate were used as a source of copper (I) ions.



Scheme 3.19: Synthesis of third generation multicalixarenes

Figure 3.23 displays a superimposition of the ^{1}HNMR spectra for the pentamer **285** (blue), its deprotected derivative **286** (red) and the heneicosamers **287** (green) and **288** (purple). The spectra can be divided in eight regions. The first, between 6 and 8 ppm (I), shows all the signals relative to aromatic protons. The peaks are really difficult to assign because of the lack of coupling with other protons. In the second region (II) can be observed the click protons and in the spectra of **285** and **286** the alkyne peaks. The section III and V fall all the signals for the methylene bridges. The fourth section (IV) displays all the methylenes of the alkyl chains next to the oxygens or to the triazole ring. In section VI, around 2.5, it is possible to notice the triplet given by the alkyne group present in compound **286** (red). Section VII includes all the protons in the middle of the aliphatic chains. The last section include the terminal methyls of the propyl chains, the *tert*-butyl protons and in the spectra for compound **285** the protons for the silyl protecting group.

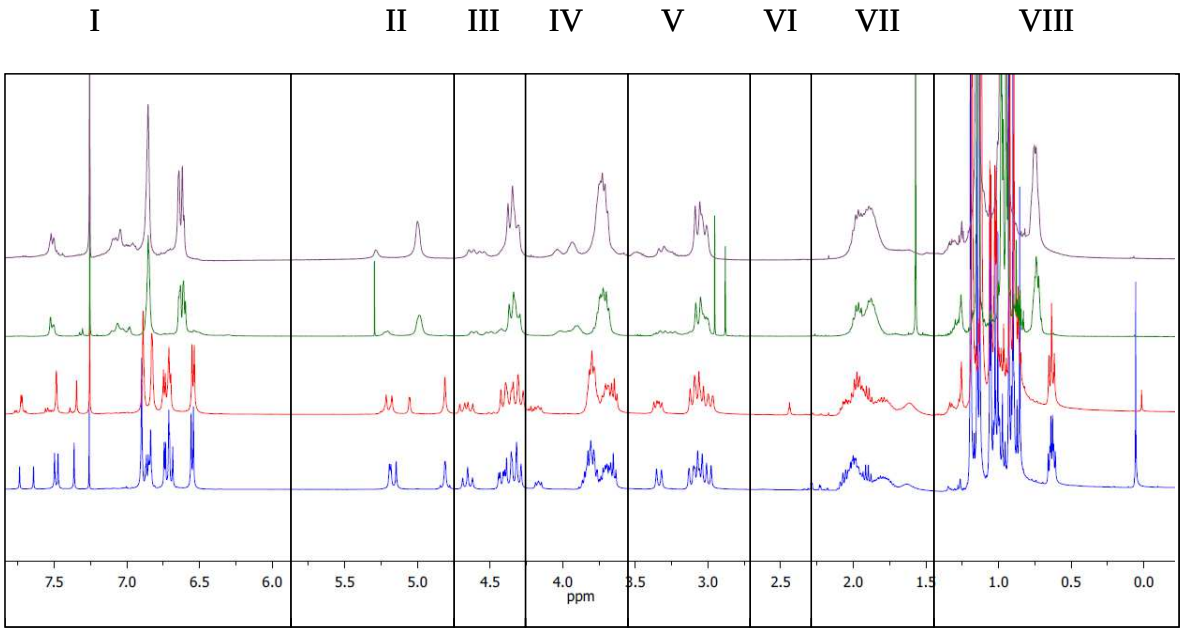


Figure 3.22: ^{1}H NMR spectra for compound 285 (blue), 286 (red), 287 (green) and 288 (purple)

Interestingly MALDI TOF analysis showed different masses for the two different conformations. The peak for the multicalixarene with the central core in the cone conformation corresponds to the molecular mass of the compound plus the molecular mass of a copper sulphate pentahydrate molecule plus two water molecules On the other hand the value observed for the multicalixarene with the central core in the 1,3-alternate conformation corresponded to the molecular weight of the compound plus the weight of two copper sulphate penthaydrate plus two water molecules.

3.5 Conclusions and future work

In this chapter we have described a new route towards the synthesis of calixarene based dendrimers bearing either nine or twenty-one calixarene units in their structure distributed over two and three generations respectively. The calixarene building blocks were linked to the central core through copper(I) catalysed azide-alkyne cycloaddition using either copper iodide or a mixture of copper sulphate and sodium ascorbate. This result was possible because of the design of central cores functionalised at the upper rim with four azido function which enables the lower rim to carry further functionalisation such as protected alkynes or alkyl chloride chains. The method allows a first “click” reaction to be performed on the upper rim and then following the activation of the functional groups on the lower rim for a second cycloaddition. With this approach in the near future it could be possible to synthesise third generation calixarene dendrimers fully functionalised on the outer rim. Another potentially valuable area to investigate would be the synthesis of hybrid central cores bearing azido functionalisations on the upper rim and both protected alkynes and alkyl chlorides on the lower rim. This would allow a series of “click” reactions and therefore to use the calixarene to anchor three different molecules.

3.6 References

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