INVESTIGATION OF ARTIFICIAL MALONYL GROUP CARRIERS AND MULTIFUNCTIONAL CYCLIC SCAFFOLDS TOWARDS THE MIMICRY OF PKS

Luis Angel Martinez Lozano

University of East Anglia

School of Chemistry

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The present thesis has been supervised by Dr George Richard Stephenson and Pr Philip Bulman Page and it is submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy.

DECLARATION

The research contained in this thesis is, to the best of my knowledge, original, except where due reference is made.

NAME: LUIS ANGEL MARTINEZ LOZANO

SIGNATURE:

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LIST OF ABBREVIATIONS

ACP	Acyl carrier protein
ACO	Acyl-CoA oxydase
AT	Acyl transferase
ATP	Adenosine triphosphate
Cbz	Benzyl carbamate
CHS	Chalcone synthase
^{13}C-NMR	Carbon nuclear magnetic resonance
CoA	Coenzyme-A
DCC	*N,N'*-dicyclohexylcarbodiimide
DH	Dehydratase
DIC	*N,N'*-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMA	*N,N* -dimethylacetamide
DMF	*N,N* -dimethylformamide
DMSO	Dimethylsulfoxide
EDG	Electron donating group
EPNP	Ethyl *p*-nitrophenyl phosphate
ER	Enoyl reductase
EWG	Electron withdrawing group
Fid	Free induction decay
^{1}H-NMR	Proton nuclear magnetic resonance
HPNP	2-Hydroxypropyl-4-nitrophenyl phosphate
HRMS	High resolution mass spectrometry
KR	Ketoreductase
KS	Condensing enzyme
K_a	Acid dissociation constant
K_{obs}	Observed rate constant
LRMS	Low resolution mass spectrometry

MAHO	Malonic acid half oxyester
MAHT	Malonic acid half thioester
MDa	Megadalton
*m*CPBA	*meta*-chloroperbenzoic acid
MHz	MegaHertz
Mp	Melting point
NBS	*N*-Bromosuccinimide
n-BuLi	*n*-Butyllithium
pK_a	Negative base-10 logarithm of the acid dissociation constant (pK_a = $-\log_{10} K_a$)
PKS	Polyketide synthases
PyBOP®	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
rt	room temperature
TBAB	Tetra-*n*-butylammonium bromide
TBAC	Tetra-*n*-butylammonium chloride
T_c	Coalescence temperature
Tfc	3-(trifluoromethylhydroxymethylene)-camphorate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TOF	Number of moles of converted starting material per moles of active sites and time for conversion.
TON	Number of synthesized molecules per number of catalyst molecules used.
TSTU	*N,N,N',N'*-Tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate

ABSTRACT

This thesis presents a new convenient method for the synthesis of malonic acid half esters and thioesters under solvent-free conditions. The relative acidity of a broad variety of MAHOs and MAHTs has been estimated by an innovative and simple method based on the determination of observed rate constants from hydrogen-deuterium exchange experiments of malonates. The results obtained from kinetic experiments allowed us to select the more suitable artificial malonyl carriers in decarboxylative aldol-type condensations.

In the second part of the thesis, the first example of a base and metal-free decarboxylative aldol condensation is described. Three different catalytic systems have been successfully employed to carry out mild condensations between malonates and aldehydes. One of these catalytic systems involves the first use of an antibiotic (valinomycin) in the catalysis of decarboxylative aldol condensations.

In the last part of the thesis, a new versatile and regioselective method to obtain monofunctionalised calix[4]arenes blocked in a *cone* conformation is described. A broad range of monoarmed calix[4]arenes have been synthesised and the properties of some of these calix[4]arenes studied. In addition, a simple method for the preparation of multifunctional calix[4]arenes blocked in the *cone* conformation has been developed, using a procedure based on the discovery of new reaction conditions for efficient Cannizzaro reactions of diformyl calix[4]arenes. Finally, the synthesis of a bio-inspired bifunctional mercapto-calix[4]arene has been carried out, in order to mimic part of the active site of a PKS. The new mercapto-calix[4]arene prepared, which carries two sulfhydryl groups in the upper rim, has been successfully loaded with two malonyl molecules and employed to mimic the condensing function of PKS.

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PKS and substituted phenols and thiophenols as artificial malonyl carriers.

1.1 Introduction to polyketide synthases (PKS) and malonyl group carriers.

1.1.1 PKS: A sophisticated type of molecular assembly line.[1]

Polyketide synthases (PKS) are a group of enzymes responsible for the synthesis of an important family of natural products known as polyketides. The exceptional activity of polyketides against cancer and other important diseases makes them one of the most relevant and promising type of natural substances with potential as lead compounds for pharmaceutical applications. For that reason, over the past decades scientists have devoted themselves to elucidate and understand the mechanistic pathways involved in the biosynthesis of polyketides by microorganisms and plants.

Polyketides are natural organic molecules presenting a wide variety in size and structural complexity. Nevertheless, all polyketides have a common origin as they are obtained from primary polyketone chains synthesised by polyketide synthases in microorganisms and plants. By the action of the PKS, the primary polyketone chain undergoes a number of specific transformations and cyclisations leading to the desired polyketide. The mechanism of biosynthesis of polyketides by PKS enzymes resembles a molecular assembly line where an initial polyketone chain is extended and modified by a sequence of finely programmed catalytic trasformations at specific positions in the chain (figure 1.1).

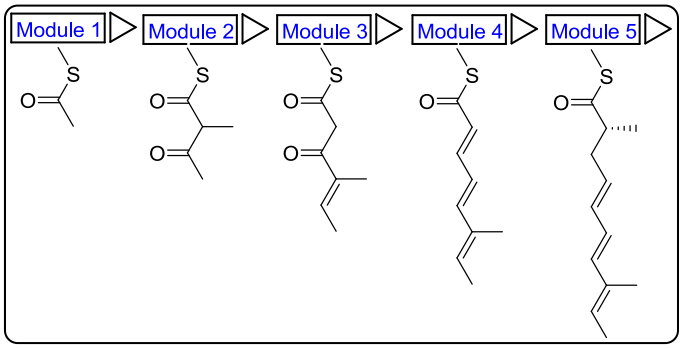


Figure 1.1 Schematic representation of polyketides biosynthesis by modular PKS.

Scientists have made important advances in the understanding of the mechanistic pathways involved in the biosynthesis of polyketides whilst organic chemists have dedicated great efforts to

develop novel syntheses of polyketides in a laboratory scale in order to study their pharmacological activity and potential applications in medicine.

The enormous interest in polyketides not only derives from their exceptional properties as immunosuppressants, antibiotics, antitumoral agents and antifungals but for their capacity to treat a great number of important diseases.

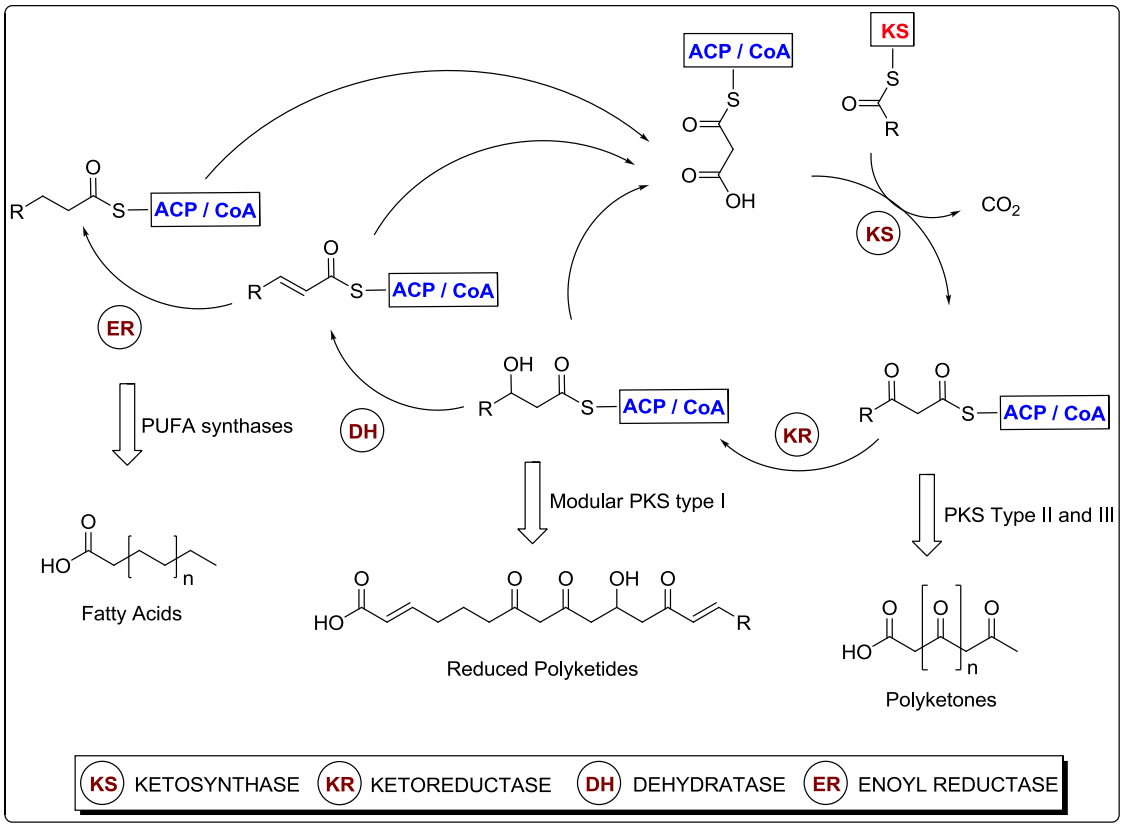


Figure 1.2 Synthesis of polyketones, polyketides and fatty acids catalyzed by different types of enzymes.[1]

To give a clear proof of the importance of polyketides in health care and thus in the pharmaceutical industry, the worldwide sales of drugs based on polyketides as active pharmaceutical ingredients reached more than fifteen billion dollars over the last decade which makes polyketides the best seller drugs around the world.[2]

The primary polyketone chain used in the synthesis of polyketides is generated from an acyl group and is extended through condensation reactions with malonyl groups. The condensation step

is repeated several times leading to the elongation and growth of the polyketide chain that will be further modified by other catalytic functions within the PKS (figure 1.2).

The modification of the primary polyketone chain is very specific and sophisticated. It may also lead to a broad range of metabolites as, for instance, the simple aromatic polyketide 6-methyl salicylic acid or the more complex macrocyclic structures like erythromycin-A (figure 1.3). The modifications carried out by the different catalytic functionalities within the PKS also involve extremely mild conditions at room temperature and in the absence of strong acids or bases.

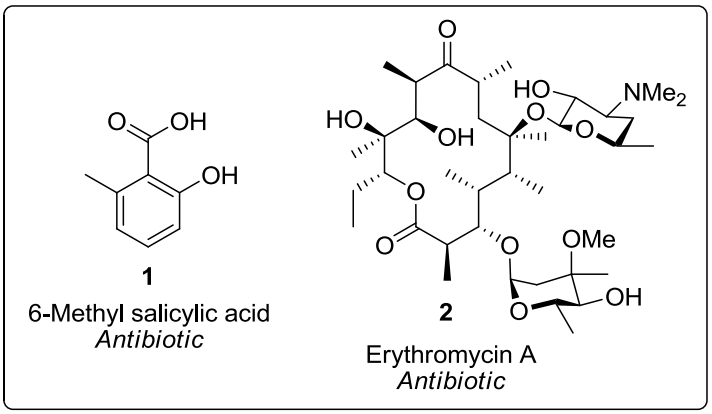


Figure 1.3 Structure of 6-methyl salicylic acid and erythromycin-A.[1]

The link between the polyketide chains and the enzyme is always accomplished by a sulfhydryl group that forms a thioester bond. Malonyl groups use the same type of linkage and are transported to the active site of the PKS by different carriers. The condensation between polyketide chains and malonyl thioesters is carried out in the active site of PKS following a decarboxylative Claisen condensation. As a result of a successive series of decarboxylative Claisen condensations, PKS is able to extend the length of the polyketide chains by two carbon atoms per condensation. The nascent polyketide chain is further modified by the PKS by other functions like ketoreductases (KR), dehydratases (DH) or aromatases allowing PKS to finely tune the functionality and structure of the new active metabolites obtained.

In the active site of PKS, hydrogen bond interactions between two amino acid residues (histidine and asparagine) and a thioesters linkage allows the activation of malonyl groups attached to Coenzyme-A and also facilitates the generation of a thioester enolate that is involved in the condensation reaction with a nearby polyketide chain attached itself to a cysteine residue within the

enzyme via a thioester bond. During the elongation step, the condensation between the malonyl groups and a polyketide chain is driven by the loss of a molecule of carbon dioxide during the decarboxylation of the malonyl group (figure 1.2). The mechanisms involved in further modifications of the nascent chain are catalyzed by several different functions present in the PKS and are generally very complex and shopisticated processes.

The mechanistic pathways for the biosynthesis of polyketides are very similar to those found in the biosynthesis of fatty acids by the fatty acid synthases (FAS). However, the enzymatic factors that dictate the generation of one specific type of natural product and instead of another remain unknown. There is not much information either about the type of chemical factors involved in the determination of the chain length, the degree of oxidation of the chain and also the type of cyclisation suffered by the linear polyketide chains.

Over the last years, advances in genetic engineering have allowed scientists to study in greater detail the complex mechanisms involved in PKS.[1] Functional modules in PKSs are formed by highly complex protein structures that catalyse not only single transformations but also cascade reactions. This particular feature makes PKS a natural and a highly versatile molecular machine capable of synthesizing both small and structurally simple biomolecules and larger and complex structures with molecular weights over a thousand Daltons.

The molecular structure of polyketides may also vary from very rigid multi-cyclic structures like the chemotherapy drug doxorubicin (aromatic polyketide), to more flexible molecules like pederin (isolated from hemolymph of female *Paederus* beetle) as another example of natural product with anti-tumoral properties (figure 1.4).

The functionalities that can be found in polyketides are very diverse and include, among others, functional groups like carboxylic acids, esters, ketones, olefins or aromatic rings. The stereochemistry and the functional groups present in the structure of the polyketides will determine their biological function in microorganisms and plants.

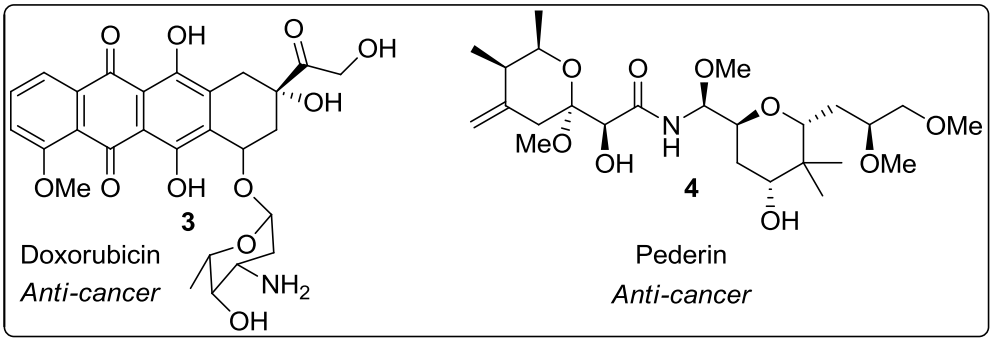
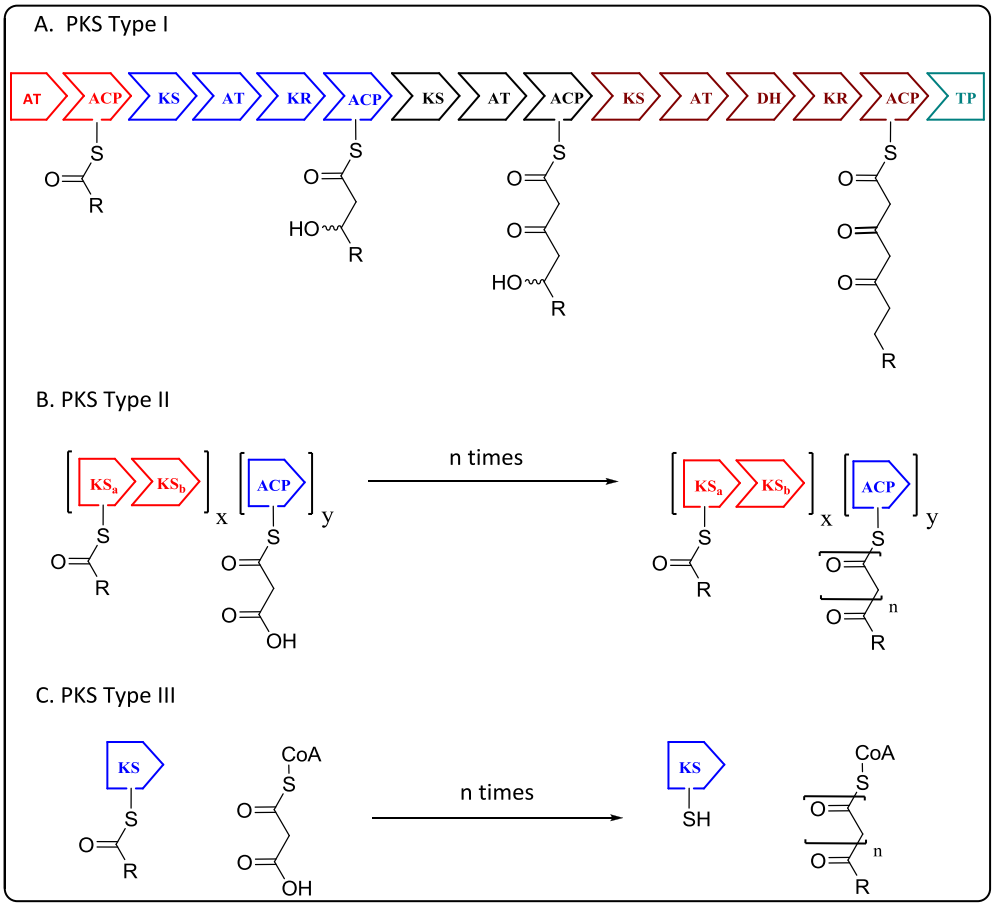


Figure 1.4 Molecular structures of polyketides doxorubicin and pederin.[1]

1.1.2 Classification of Polyketide Synthases.[1]

PKS can be classified in three different groups depending on the operating mechanism during the biosynthesis of polyketides (scheme 1.1). PKS type I presents several multifunctional subunits, each of them being able to perform a certain number of biosynthetic steps, normally following a non-iterative mechanism. PKS type II is formed by a group of discrete catalytic functions that can work in an iterative way in order to extend and modify the crescent polyketone chain. Finally, PKS type III is formed by a single active site capable of carrying out multiple biosynthetic steps in order to obtain the desired polyketide.

The properties of each type of PKS are summarized in the table 1.1. According to the mode of operation, only PKS type I follows a modular and an iterative mechanism respectively. The rest of PKS can only operate under iterative processes. The transport and activation of the malonyl groups used during the elongation step are different depending on the type of PKS involved. In PKS type I, the transport of malonyl groups to the active site of the enzyme is carried out by the acyl carrier protein (ACP), while in PKS type II and type III this task is conducted by acyl-CoA oxidase (ACO) and coenzyme-A (CoA) respectively.



Scheme 1.1 Synthetic mechanisms for each type of PKS.[1]

Finally, different types of PKS produce different types of polyketides. PKS type II and III carry out the biosynthesis of polyketides that contain aromatic groups in their structures whilst PKS type I can produce both aromatics and non aromatic polyketides.

Table 1.1 Main features for different types of PKS.[1]

Type of PKS	Mode of operation	Substrate activation	Products
I	Modular	ACP	Reduced
I	Iterative	ACP	Reduced and aromatic
II	Iterative	ACO	Aromatic
III	Iterative	CoA	Aromatic

1.1.2.1 PKS type I.

PKS type I consists in a group of enzymes characterised by presenting multiple functional groups located within the same polypeptide region which allows these types of enzymes to carry out different synthetic transformations in the same active site. PKS type I is involved in the synthesis of a structurally diverse class of natural products including polyketides like orsellinic acid or the more structurally complex squalestatin S1 also known as Zaragozic acid, a potent inhibitor of squalene synthase and therefore an inhibitor of sterol synthesis lowering plasma cholesterol (figure 1.5).

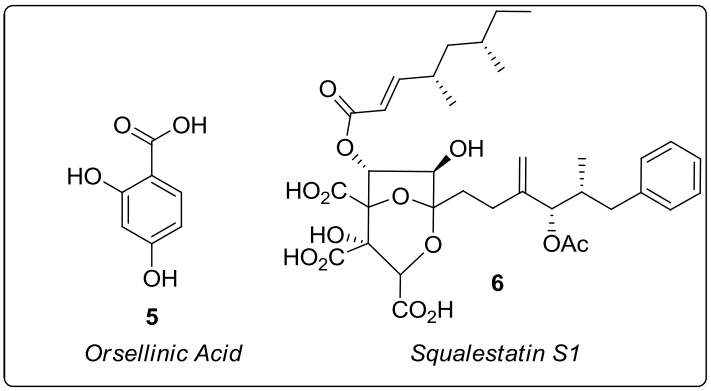


Figure 1.5 Example of polyketides produced by PKS type I.[1]

PKS type I can be, at the same time, classified in three different subcategories, according to the content of reductase functions in their catalytic sites:

A) Non-reducing PKS type I (NR-PKS-I).

B) Partially reducing PKS type I (PR-PKS-I).

C) Highly reducing PKS type I (HR-PKS-I).

The structure of non-reducing PKS type I and the functionalities present in its active site were published by Crawford et al. in 2008.[3] However, the structures and reaction mechanisms of PR-PKS-I and HR-PKS-I remain quite unknown. It has been found that PKS type I can produce long-chain polyunsaturated fatty acids (PUFA) by using a different synthetic pathway to the one

employed by PUFA synthases in eukaryote cells. This class of PKS type I can present up to nine different active functions cooperating in the same enzymatic region.[4] Since PKS type I can present several functions within the same protein, some of these enzymes are the largest proteins found in nature.[5] A good example of this is the enzyme MLSA-I from mycolactone polyketide synthase that presents eight different interconnected functions and a molecular mass of 1.8 MDa.[6]

1.1.2.2 PKS type II.

PKS type II take the form of a cluster of proteins where several enzymatic functions are located in the same region. Each of these groups of enzymatic functions or modules are able to carry out one specific transformation in an iterative process.[7] In this type of enzyme, there is a distinctive group of proteins known as “minimal PKS” responsible for the chain elongation step and also the condensation of malonyl and acyl thioesters. In general, “Minimal PKS” is made up of two ketosynthase condensing enzymes and an acyl carrier module where the nascent chain is attached through a pantothenyl arm.

Additional enzymatic subunits like ketoreductases, cyclisases, aromatases, transferases, oxygenases, glycosyl-transferases and N, O and C-methyltransferases assist to “minimal PKS” during the biosynthesis of complex polyphenolic polyketides.[8]

The specific function of some protein regions present in PKS type II enzymes remains unknown due to the difficulties of isolating highly unstable polyketide intermediates and to the great interdependence between different subunits within the enzymatic cluster respectively, making it extremelly challenging to reproduce the function of isolated enzymes.

1.1.2.3 PKS type III.

PKS type III are formed by a single and multifunctional active site capable of multitasking to perform the catalysis of Claisen condensations during the chain elongation step, the control of the number of cycles during the chain elongation process and the control of different intramolecular condensations and aromatizations in the polyketide during the folding step leading to active

aromatic metabolites. Due to the relatively low complexity of the synthetic pathways found in PKS type III, a good number of factors that determine the folding mechanism of the polyketide chain are well established in this type of enzymes.[9]

In contrast to PKS type II, where every type of synthetic transformation is carried out by a specific enzymatic subunit, in the case of PKS type III, all the biosynthetic transformations have place in the same active site.[10]

1.1.3 Natural malonyl group carriers: Coenzyme-A and ACP.

During the biosynthesis of polyketides and fatty acids, the transportation of malonyl units in the active site of PKS and FAS is required in order to extend polyketide chains. Coenzyme-A and ACP (acyl carrier protein) are able to covalently link malonyl groups through a thioester function allowing the transference of malonate units to the active site of the enzyme *via* a *trans*-thioesterification reaction. The thioester linkage plays a double role in this process. On one side, the lower stability of thioesters compared to esters facilitates the *trans*-esterification reaction and the transference of malonyl groups to the active site of PKS and FAS respectively. On the other side, the presence of a sulfur atom in the thioester linkage facilitates the formation of enolate thioesters that participate in the Claisen condensation during the chain elongation step.

1.1.3.1 Coenzyme-A.

Coenzyme-A constitutes, along with the acyl carrier protein, the most important acyl and malonyl carriers in the biosynthesis of polyketides and fatty acids, and has a key role in hundreds of synthetic and degradative processes in living organisms.[11] Coenzyme-A is an essential compound very much needed for the correct function of these enzymes, acting not only as a carrier but also as a cofactor. Coenzyme-A was first reported on 1945 by Lipmann *et al.*[12] and its name makes reference to its ability to co-catalyze acetylation reactions.

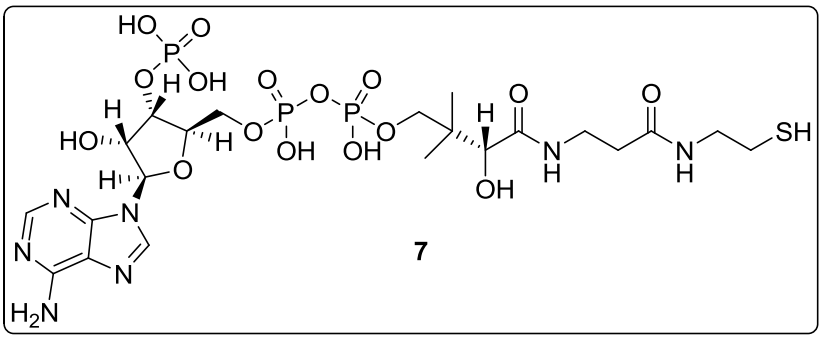
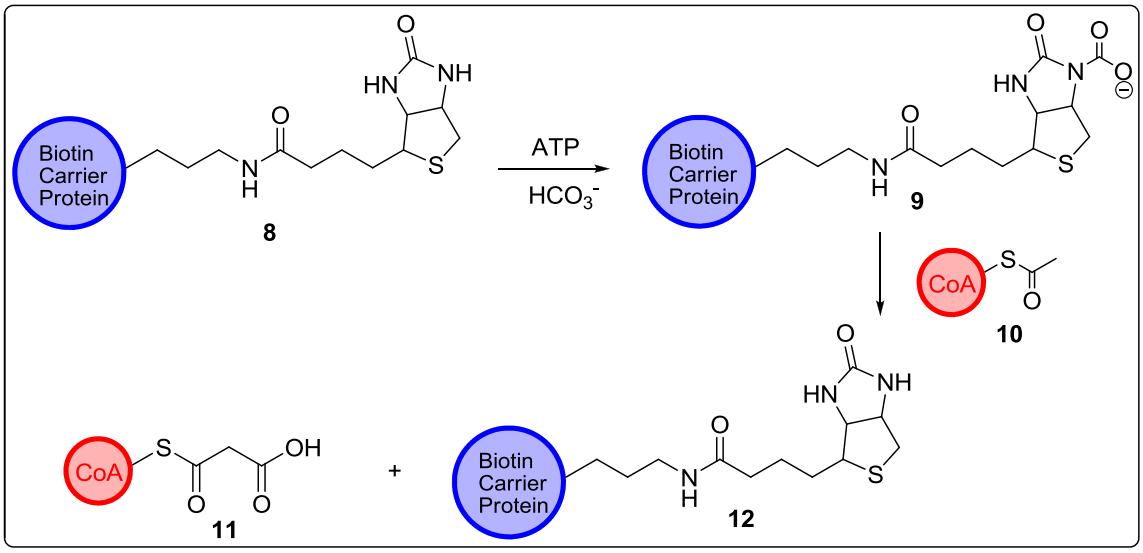


Figure 1.6 Chemical structure of coenzyme-A.[13]

The structure of coenzyme-A presents a 3'-phosphoadenosine group coupled to pantothenic acid by a diphosphate linkage in the 5' position of ribose (figure 1.6). The terminal carboxylic function is linked to β-mercaptoethylamine *via* a peptide bond.[13] Coenzyme-A participates in the synthesis of polyketides and fatty acids by carrying malonyl and acetyl groups forming malonyl-CoA and acetyl-CoA respectively. Malonyl-CoA is generated from acetyl-CoA, bicarbonate and a molecule of ATP (adenosine triphosphate) in an irreversible process catalyzed by acetyl-CoA carboxylase.[14]



Scheme 1.2 Formation of malonyl-CoA catalyzed by acetyl-CoA carboxylase.[14]

Acetyl-CoA carboxylase presents a biotin arm connected to a biotin carrier protein through the ε-amino group of a lysine residue. The formation of malonyl-CoA involves two synthetic steps. Initially, a carboxyl group is transferred from bicarbonate to the biotin fragment in the carrier protein

with the consumption of a molecule of ATP. In the second step, the biotinyl group transfers the carboxyl group to acetyl-CoA to generate malonyl-CoA (scheme 1.2).

1.1.3.2 Acyl carrier protein (ACP).

Nuclear magnetic resonance (NMR) was used to determine the structure of an acyl carrier protein domain in solution. The structure of an ACP domain from 6-deoxyerythronolide B synthase (DEBS), a modular PKS, was first time reported in 2007 by Alekseyev *et al.*[15] The reported ACP domain (ACP-2 of DEBS) contains three-helical bundles and an additional short helix in the second loop simirlarly to the structure found in the ACP of fatty acid synthases type I.

The function of ACP during the biosynthesis of polyketides is to accept the main polyketide chain from an acyl transferase (AT) and to collaborate with ketosynthase (KS) domains in the elongation of the polyketide chains and in the modification of carbonyl groups respectively. The acyltransferase domain (AT) is responsible for participating in the polyketide chain elongation and has the ability to specifically select the required building blocks needed for the condensation reactions that form the polyketide chain.[16]

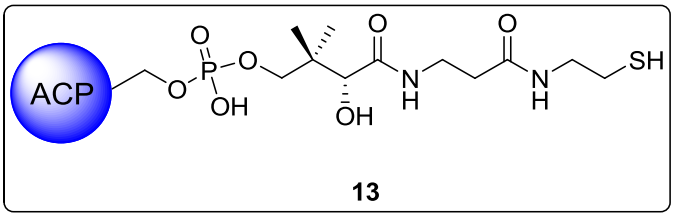


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Figure 1.7 Chemical structure of ACP linker.[17]

The ACP domain presents both a phosphopantetheine and a cysteamine terminal group where a sulfhydryl group is responsible for linking either malonyl groups, to transfer them to the active site of PKS, or the nascent polyketide chain during the elongation process (figure 1.7).[17] Other domains, like ketoreductase (KR), enoyl reductase (ER) or dehydratase (DH) participate in the modification of the new extended chain and determine the type of functional groups attached to the β-carbon of the polyketide chain.

The interaction between malonyl-ACP and the condensing enzyme (KS) in the active site of the PKS allows the decarboxylative Claisen condensation between malonic acid half thioesters and acyl thioesters leading to the generation of polyketone chains. The newly formed chains can be further modified by other functions like ketoreduction, dehydration or enoylreduction to generate modified polyketide chains that are once again, transferred to the KS domain by acyl transferase (AT) to begin a new cycle of chain elongation.

1.1.4 Artificial malonyl group carriers. Synthesis of MAHOs and MAHTs.

Malonyl coenzyme-A and malonyl ACP are natural half malonic acid thioesters involved in the transport and condensation of malonyl groups in the acive site of PKS. In the active site of chalcone synthase (CHS), one of the best known polyketide synthases, a cysteine residue is believed to react with an imidazolium-thiolate and histidine ion pair that enables its deprotonation.

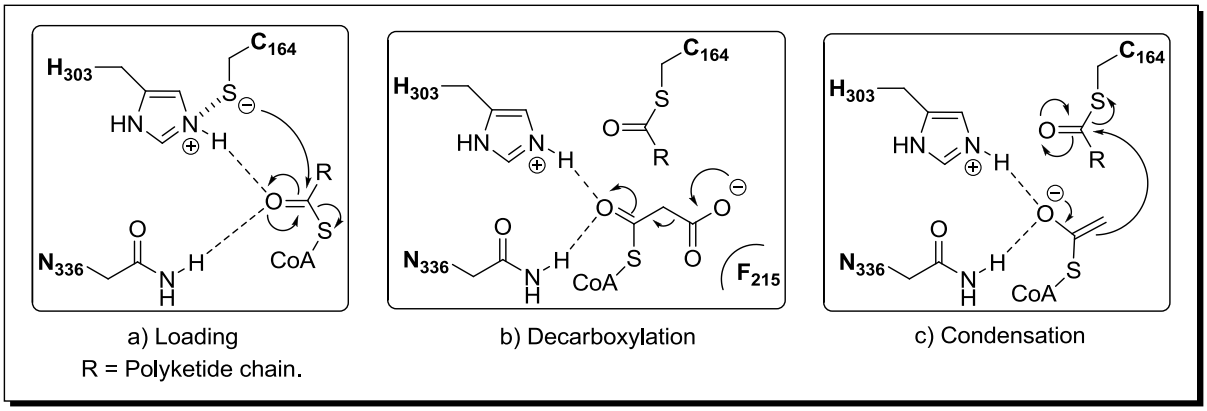


Figure 1.8 Proposed mechanism of substrate loading, malonyl decarboxylation and polyketide extension.[10]

Once cysteine is activated, it is able to accept the nascent polyketide chain involved in a decarboxylative condensation process (figure 1.8). The decarboxylation of malonyl-CoA is catalyzed by PKS by means of hydrogen bonding interactions between the malonyl group and the histidine and the asparagine residues that contribute to the formation and stabilization of the thioester enolate. The cysteine residue in KS transports the polyketide chain and also participates

in the condensation reaction with malonyl-CoA. During the condensation step, the polyketide chain is transferred and then the sulfhydryl group in the cysteine residue is free to accept a new poly-β-keto intermediate from coenzyme-A. After decarboxylative condensation and the subsequent chain elongation, the newly formed polyketide precursor is then transferred from Co-A to ACP. The sulfhydryl group in coenzyme-A is then available to accept a new malonyl group that can participate in a new decarboxylative Claisen condensation.

The key step in the activation of malonyl-CoA in the active site of PKS is therefore, the hydrogen bond interactions between amino acid residues and the malonyl group that enable the performance of mild decarboxylative Claisen condensations at room temperature and in the absence of either strong acids or bases.

A great effort has been deployed over the last years by organic chemists in the development of new bio-inspired mild decarboxylative aldol-type and Claisen condensations. Inspired by the biochemistry of polyketide (PKS) and fatty acid synthases (FAS), organic chemists have been able to develop mild decarboxylative processes for the formation of carbon-carbon bonds carried out at room temperature, in open vessels and in the presence of wet solvents.

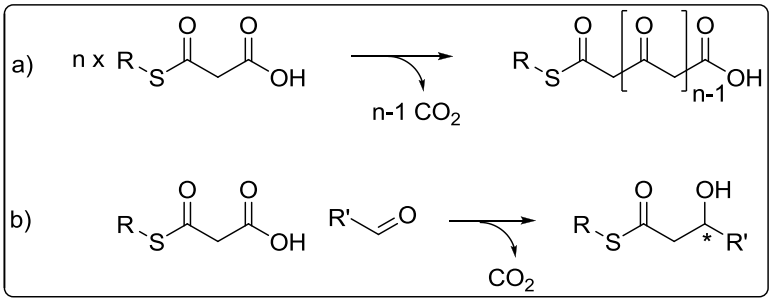
The mild catalysis of these important reactions has been generally achieved by the use of bases in combination with the use of coordinative metals that can effectively coordinate to carbonyl groups. A good example is the use of amine bases or imidazole derivatives in combination with divalent metals like magnesium or copper. Investigations about mild decarboxylative condensations have also led to the development of new stereoselective aldol-type condensations, successfully achieved due to the use of either chiral bases or ligands that coordinate to the formed enolate.[18]

However, the research carried out over the last two decades to develop bio-inspired decarboxylative condensations has been mainly focused on the nature of the catalyst, the use of different types of co-ordinative metals and the effects created by the solvent employed. Very little attention has been paid to the effect that malonyl carriers could induce on this type of reactions. In the vast majority of reported papers in the literature, only a very limited number of malonyl carriers are used in the formation of MAHOs and MAHTs. In just a few papers, however, several malonyl carriers are employed and also their role during decarboxylative condensations is compared. One of those papers found in the literature was reported by Matile et al. in 2001 where Claisen self-

condensations of MAHTs were carried out and seven different types of malonyl carriers were also screened.[19] In this study, important differences in the reaction yield were obtained when different malonyl carriers were used. Malonyl carriers bearing strong electron donating groups gave the best yields. However, malonyl carriers bearing strong electron withdrawing groups were not assessed, what reduces the scope of the study.

Substituents at the α-position of MAHOs and MAHTs respectively also represent an alternative way of tuning the behaviour of these malonates in mild decarboxylative condensations. In the same work, Matile and co-workers reported that the presence of a methyl group in the alpha position of malonates had a very negative effect in Claisen self-condensations, as no condensation products were obtained. Nevertheless, further investigations on the effect of different substituents at the α-position may lead to new and more efficient decarboxylative condensations.

The use of artificial malonyl carriers may eventually lead to extremelly mild and efficient aldol-type and Claisen condensations, affording new and useful strategies towards the formation of carbon-carbon bonds or the polymerization of malonates. The malonyl group carrier, linked through an ester or thioester function to a malonyl residue in MAHOs and MAHTs, may also facilitate the formation of stable enolates *via* intramolecular interactions. The use of chiral malonyl carriers may also induce stereoselectivity in the condensation reactions making them even more versatile processes (scheme 1.3).

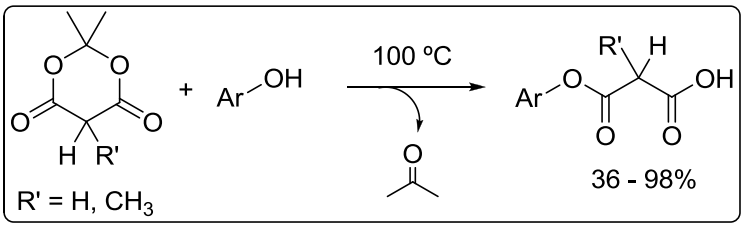


Scheme 1.3 (a) Polymerization of MAHTs and (b) decarboxylative aldol condensation of MAHTs with aldehydes.

A bio-inspired mild polymerization of malonates has never been achieved before and may potentially lead to the development of simple protocols for the preparation of important types of secondary metabolites such as polyketides. The effect of malonyl group carriers on the reactivity

and properties of MAHOs and MAHTs will be studied and described in detail in chapters 1 and 2 of this thesis.

Different methods were employed in the past for the synthesis of malonic acid half esters and thioesters. In 1976, Junek *et al.* reported the use of Meldrum's acid as a good malonyl equivalent in the preparation of MAHOs. In that work, the synthesis of a good number of esters was achieved by heating a mixture of phenol and Meldrum's acid at 100 ºC for 90 minutes (scheme 1.4). After purification, the desired malonic acid half esters were obtained in moderate to good yields (36 - 98%).[20]

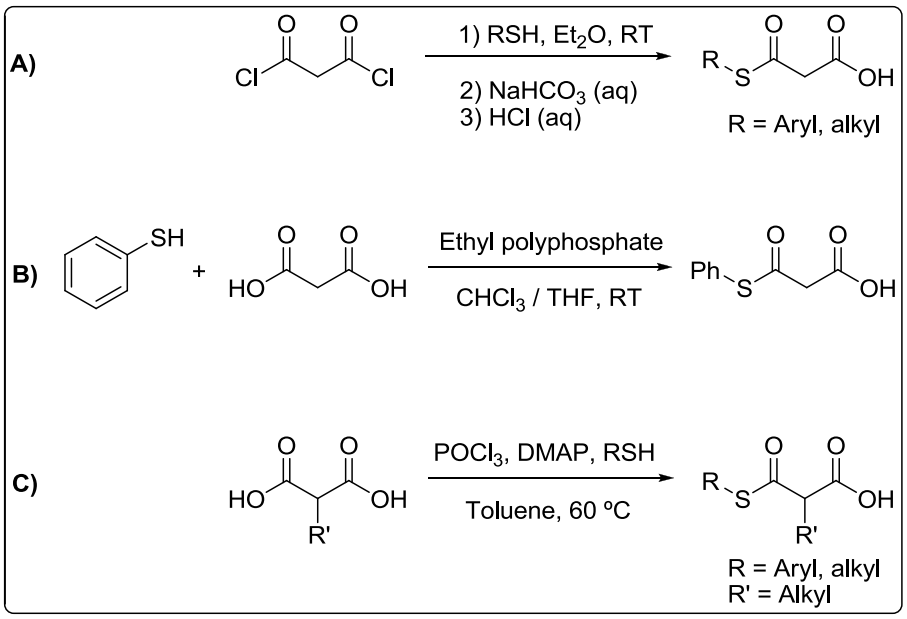


Scheme 1.4 Reaction between phenolic substrates and Meldrum's acid.

The majority of protocols towards the synthesis of malonic half esters described subsequently have involved the used of Meldrum's acid.[21]

Other methods employed malonic acid as malonyl group equivalent. In 2007, Levonis and co-workers reported the selective monoesterification of malonic acid catalyzed by boric acid.[22]

However, Meldrum´s acid was rarely used in the synthesis of MAHTs likely due to MAHTs instability with temperature. Other different malonyl equivalents were used instead in the preparation of half thioesters, such as malonyl chloride, or malonic acid. In the case of malonic acid, ethyl polyphosphate[23] or the catalytic system $POCl_3$/DMAP[24] were used in monoesterification reactions (scheme 1.5). Yields reported for the synthesis of MAHTs are generally lower than those for oxyester analogues.



Scheme 1.5 Alternative routes towards the synthesis of MAHTs under mild temperatures.

1.1.5 Aims.

The aim of the first chapter of this thesis is to develop a new and simple synthetic protocol that allows the preparation of malonic acid half oxyesters and thioesters, starting from readily available reagents. The new developed method will be employed in the preparation of multiple malonates, using different phenols and thiophenols as artificial malonyl carriers. The effect of the malonyl carriers in the relative acidity of the molecule will be study by ^{1}H-NMR spectroscopy.

1.2 Results and discussion.

1.2.1 Solvent-free synthesis of Malonic Acid Half Oxyesters and Thioesters.

As was explained in the introductory section of this chapter, both coenzyme-A and ACP use a thiol group in order to transport malonyl groups to the active site of PKS in the biosynthesis of polyketides. The thioester function involved in the linkage between Co-A or ACP and malonyl groups presents a double advantage. On one hand, it allows the mild transfer of malonyl groups to the active site of PKS by transesterification, which can be catalyzed by acyltransferases. On the

other hand, the thioester function facilitates the formation of enolate thioesters to participate in decarboxylative Claisen condensations during the chain elongation step.

From a chemical point of view, malonyl-CoA and malonyl-ACP can be considered as a special type of malonic acid half thioesters (MAHTs). In both cases, a sulfhydryl group in a structurally complex molecule is responsible for linking malonyl fragments. It is well known in the literature that MAHTs can undergo decarboxylative aldol-type and Claisen condensations under mild conditions in the presence of a base (tertiary amines, imidazole derivatives, etc.) and divalent metals. Decarboxylative condensations are also reported where malonyl groups are α-monosubstituted by aliphatic or aromatic groups. The presence of an extra substituent attached in the alpha position introduces a steric constraint that very often leads to high ratios of undesired decarboxylated product and therefore to poor yields in decarboxylative Claisen and aldol-type condensations.

In the same way, malonic acid half oxyesters (MAHOs) can also undergo aldol-type condensations under mild conditions. Decarboxylative Claisen condensations, however, are not so common in MAHOs under mild conditions and usually require the use of harsh conditions like very strong bases and coordinative metals.

In both, MAHTs and MAHOs, the type of malonyl group carrier attached to the molecule will determine their physical and chemical properties, and therefore will have an effect in the formation of enol or enolate (thio)esters in decarboxylative aldol-type and Claisen condensations.

The influence that malonyl carriers have on malonyl groups in decarboxylative condensations was never studied with detail in the past. This fact encouraged us to synthesise a broad variety of MAHOs and MAHTs, in order to study their properties and chemical behaviour in decarboxylative condensations. For our study, we concluded that the use of substituted phenols and thiophenols was very convenient. The majority of substituted phenols and thiophenols are commercially available, and a wide number of examples bearing very different substituents in different positions (*ortho, meta* and *para*) can be found. The wide availability of different phenols and thiophenols, allows the insertion of electron donating (EDG) and electron withdrawing groups (EWG) in the aromatic ring. The inductive or hyperconjugative effects caused by the substitution of the phenyl ring with EDGs and EWGs will be efficiently transmitted to the rest of the molecule.

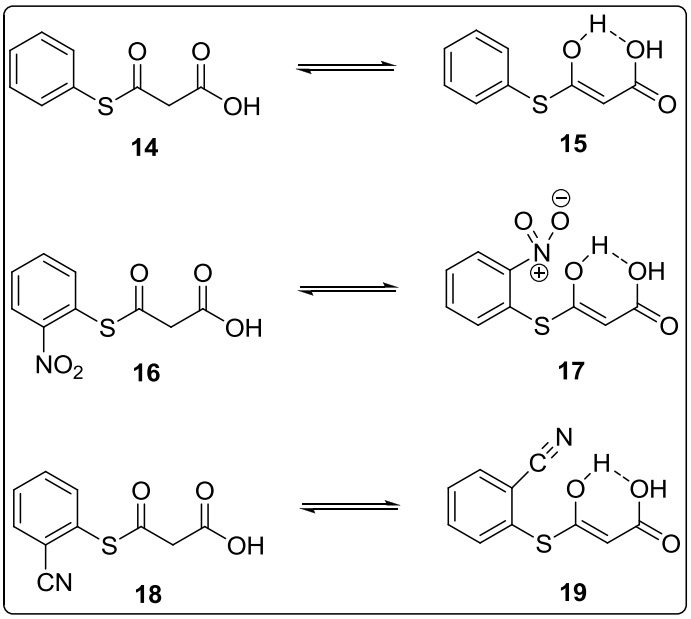
Another interesting aspect we considered when choosing phenols and thiophenols as malonyl group carriers was the fact that groups attached in the *ortho* position in the aromatic ring, may participate during the formation of enol or enolate species. The existence of such *ortho* effects might be confirmed by the comparison in reactivity between the *ortho*-substituted malonate and the *para*-substituted analogue, where the intramolecular interaction between the substituent and the formed enol or enolate is not possible.

We decided to tackle the study of malonates in two steps: in the first place, by studying the influence of malonyl group carriers on the physical properties of malonates, like acidity or pK_a values, and in the second place, by studying the reactivity of malonates bearing different malonyl carriers in decarboxylative condensations.

During the earliest steps of our investigation, we found surprisingly that pK_{a1} and pK_{a2} values for different types of MAHOs and MAHTs were either not determined or reported previously in the literature. In fact, only the apparent pK_1 values for a few examples of malonic acid half oxyesters were found published in the literature.[25] No pK_{a2} values for malonates were found in the literature using both SciFinder® and Reaxys® databases.

At the beginning of our research, some scientific publications found in the literature indicated that the use of different malonyl carriers led to the formation of malonates with different properties and reactivity. As mentioned in the introductory section, Matile *et al.* achieved the self-condensation of malonates when MAHTs were treated with benzimidazole derivatives and divalent magnesium salts. Different malonyl carriers based on thiophenols and other mercapto compounds were used for the preparation of malonic acid half thioesters. MAHTs, were used afterwards in decarboxylative Claisen self-condensation reactions. The experiments showed that different malonyl carriers afforded very different yields in this type of reaction, indicating that malonyl carriers were greatly influencing the reactivity of the malonates. In that work, malonyl carriers based on phenols and other alcohols were also employed in the preparation of MAHOs but, interestingly, MAHOs did not undergo Claisen self-condensation, showing once again the influence of malonyl carriers on the properties and reactivity of malonates in decarboxylative Claisen and aldol-type condensations.

Before embarking on the synthesis of MAHOs and MAHTs and their use in decarboxylative condensations, we studied theoretically how malonates could be influenced by substituted phenols and thiophenols. In first place, the presence of an oxygen or sulfur atom in the (thio)ester linkage, should exert a great influence on the pK_a and on the formation of enolates. The 2p-3p orbital overlapping between the heteroatom and the carbonyl carbon is much lower in the case of thioesters, which modifies the contribution of certain resonance structures, having a great impact on the pK_a value and on the stability of the thioester function. In second place, the electron density in the aromatic ring should also have a great influence on the p*K*a of MAHOs and MAHTs. The presence of electron donating or electron withdrawing groups in the aromatic ring produces inductive and hyperconjugative effects that can affect the pK_a value of malonates. As was mentioned before, groups attached alpha to the (thio)ester function have a great influence on the formation of enolates and on the pK_a values of malonates, and also introduce steric constraints in the molecule. Finally, we thought that substituents attached to the *ortho* position to the (thio)ester function, may contribute to the stabilization or destabilization of the enol form by intramolecular interaction with the carbonyl oxygen. Groups attached in the ortho position, unlike groups placed in the *para* position, are close enough to the (thio)ester function to create, for instance, an intramolecular hydrogen bonding, modifying the ability to form enol species (scheme 1.6). Because of that, we decided to prepare not only *ortho* substituted MAHOs and MAHTs, but also the *para* substituted analogues, in order to determine differences in pK_a values and reactivity.



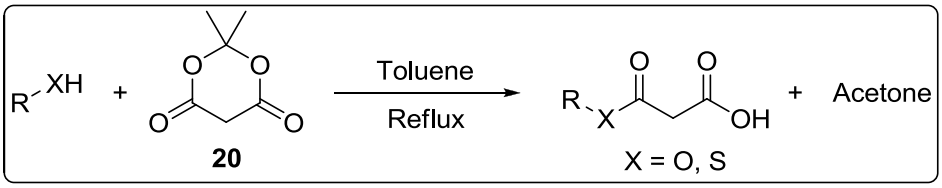
Scheme 1.6 Keto-enol equilibrium for some MAHTs.

Once all theoretical aspects were analysed, the study of malonates in mild decarboxylative condensations was divided in three well differentiated parts. First, the synthesis of a wide variety of MAHOs and MAHTs, derived from phenols and thiophenols bearing a wide range of substituents of a very different nature. Secondly, the determination of the relative acidity of the prepared malonates which should reflect their trend to produce enolate species. Finally, the use of the synthesised MAHOs and MAHTs in mild decarboxylative aldol condensations with aldehydes and ketones.

Under the best of circumstances, the selection of suitable malonyl group carriers may lead to the development of new mild decarboxylative aldol-type and Claisen condensations, likely to be used in the synthetic mild polymerization of malonates, never achieved to date, and also in the synthesis of polyketones, polyketides and fatty acids.

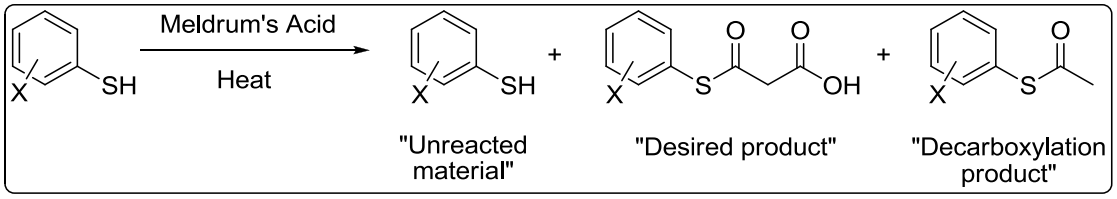
Prior to studying the effects of malonyl carriers on the properties and reactivity of MAHOs and MAHTs, the first task was the preparation of several examples of phenol and thiophenol based malonic acid half oxyesters and thioesters. From all the methods mentioned in the introductory section, the use of Meldrum's acid seems to be the simplest and most convenient protocol for the preparation of MAHOs and MAHTs. Meldrum's acid and a nucleophile (alcohols and thiols for our purpose) can be mixed, either under neat conditions or in an organic solvent, generally toluene, and the resulting mixture heated at 100 ºC (or above) for one to four hours, to obtain the desired

malonate after purification by column chromatography (scheme 1.7). In this type of reaction, the temperature plays an important role. Temperatures at 100 ºC or above are needed in order to activate the molecule of Meldrum's acid. At these temperatures, Meldrum's acid decomposes to produce acetone and the corresponding ketene, which can be trapped by different nucleophiles in the reaction mixture.



Scheme 1.7 Synthesis of malonic acid half oxyesters and thioesters from Meldrum's acid.

However, this methodology was not found to be efficient in the synthesis of MAHTs. In the synthesis of MAHTs using Meldrum's acid, yields are normally low and a mixture of products is obtained, being the main product the decarboxylation product, as a consequence of the decomposition of MAHTs at high temperature (scheme 1.8).



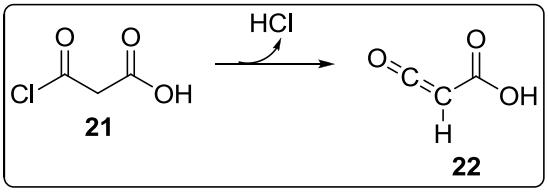
Scheme 1.8 Common reaction products in the synthesis of MAHTs from Meldrum's acid.

Attempts to reduce the ratio of decarboxylation product in the mixture by decreasing the reaction temperature were unsuccessful, since high temperatures are required to generate reactive ketenes from Meldrum's acid.

The lack of a suitable protocol for the preparation of MAHTs in the literature, made us consider the necessity to develop a new and more efficient method, suitable for both MAHOs and MAHTs. In order to prepare a wide variety of malonates, a quick and simple new protocol was needed. The new method also required the use of milder temperatures, around 50 or 60 ºC to avoid the decarboxylation of MAHTs during the reaction. Finally, a simple work up procedure and also a simple purification method were highly desirable.

Since malonates, and especially thioester malonates, decompose at elevated temperatures, we realised that the new method required the use of an alternative and more reactive source of malonyl moieties. Typical alternatives to the use of Meldrum's acid in the literature include malonic acid, malonyl chloride and alkyl malonates. Malonic acid needs to be activated by *N,N*'-dicyclohexylcarbodiimide (DCC) or *N,N*'-diisopropylcarbodiimide (DIC) in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP). However, the use of activating agents such as DCC and DIC in the reaction, leads to arduous purification processes as they are used in equimolar quantities. Malonyl dichloride is more reactive than malonic acid, though a catalyst is still needed and double esterification is also likely to occur, leading to mixtures of products and difficulties in purification. Last of all, the transesterification reaction of alkyl malonates requires the use of catalysts and can also lead to mixtures of products.

An exhaustive search in the literature of new types of malonyl derivatives, allowed us to find an unusual and very reactive malonic acid derivative. Half malonyl chloride **21** was first reported in 1908 to be used in the generation of ketenes.[26] In 1955 Gastambide *et al.* used half malonyl chloride to remove traces of alcohols from organic mixtures,[27] and after that, this compound has been barely used. Half malonyl chloride was described as a very reactive molecule, able to slowly decompose at room temperature to afford the corresponding ketene and hydrogen chloride (scheme 1.9).



Scheme 1.9 Spontaneous decomposition reaction of half malonyl chloride.

Half malonyl chloride can be prepared in a multi-gram scale by refluxing 1 equivalent of malonic acid and 1 equivalent of thionyl chloride in diethyl ether for two hours. After removing the solvent and volatiles, half malonyl chloride can be used without further purification. Due to its sensitivity to the atmospheric moisture, any attempts of purification by column chromatography or recrystallization are fruitless. Moreover, its analysis by solution ^{1}H-NMR spectroscopy and other wet analytical techniques becomes very difficult. A convenient method to estimate the purity of half

malonyl chloride is the determination of the melting point. The melting point of half malonyl chloride is 60-65 ºC, much lower than the melting point of malonic acid (135-136 ºC).[28] The other possible product in the reaction, malonyl dichloride, is a liquid at room temperature.

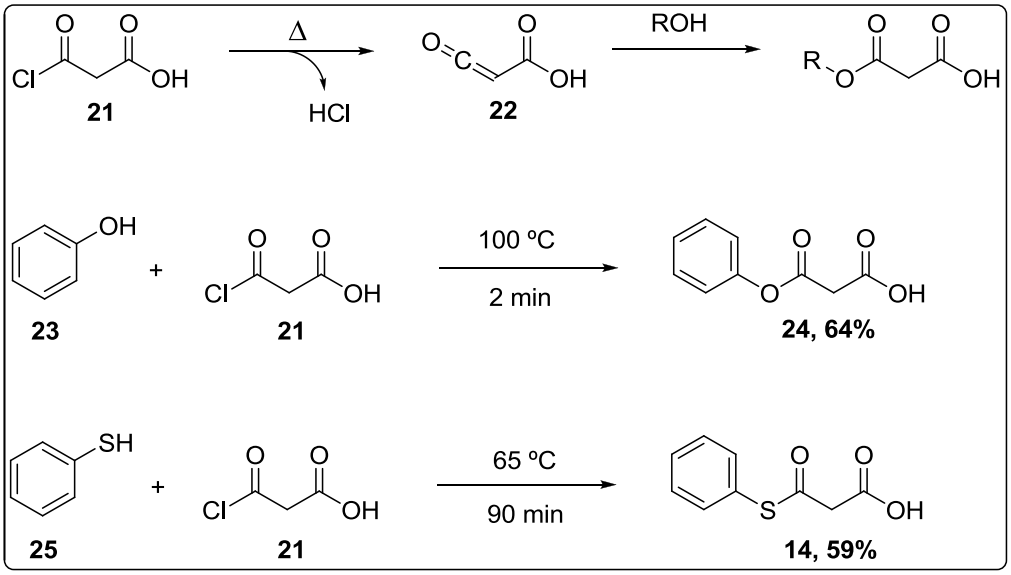
Half malonyl chloride decomposes very quickly when heated to afford hydrogen chloride and the corresponding ketene, which in the presence of water generates malonic acid. Half malonyl chloride, however, can be stored under nitrogen and at low temperature (−20 ºC) for several months without apparent decomposition.

Following the protocol described by Gastambide in 1955, half malonyl chloride was prepared in a multi-gram scale in our laboratory. The resulting pale yellow solid was reacted with phenol in anhydrous diethyl ether under a nitrogen atmosphere at room temperature. However, after stirring for 2 hours at room temperature, no reaction was observed by TLC. The addition of pyridine was required to promote the reaction. After an arduous purification to remove the excess of pyridine needed to promote the reaction, the desired 3-oxo-3-phenoxypropanoic acid was obtained as a white solid in a 27% yield.

Inspired by some previously reported methods that use Meldrum's acid as a source of malonyl groups the same reaction between half malonyl chloride and phenol was carried out under neat conditions. An equimolar mixture of reactants was gently heated, using for the purpose a heat gun, for a couple of minutes until the mixture melted and became homogeneous. The mixture was then allowed to reach room temperature and a crude sample was dissolved in dichloromethane. TLC analysis of the sample showed practically no starting material left and the generation of a new and more polar major product. The crude mixture was purified by column chromatography and, the isolated white solid analyzed by ^{1}H and ^{13}C-NMR spectroscopy. The obtained product was shown to be the desired malonic acid half oxyester (scheme 1.10).

This promising result was followed by an optimization of the process employed. Different parameters, such as the number of equivalents of half malonyl chloride, the reaction temperature and the reaction time were adjusted. As previously discussed, lower temperatures were used during the preparation of MAHTs whilst MAHOs were prepared in few minutes after heating the reaction mixture at 100 ºC. Phenols afforded the desired malonates in moderate to good yields with very small proportions of decarboxylation product. Thiophenols were reacted at 65 ºC for a longer

period of time compared to phenols to minimise decarboxylation byproduct and increase yields (scheme 1.10).



Scheme 1.10 Reactivity of half malonyl chloride under neat conditions.

Finally, a new convenient method, suitable for the synthesis of both MAHOs and MAHTs, was established. The new protocol allowed the preparation of a wide variety of aryl MAHOs and MAHTs in moderate to good yields on a multigram scale and employing simple work up and purification procedures (for MAHOs examples see figure 1.9; for MAHTs see figure 1.10).

Different substituted phenols and thiophenols were employed as nucleophiles during the solvent free esterification reactions. Several MAHOs and MAHTs, bearing both electron donating and electron withdrawing groups, were prepared in moderate to good yields. MAHOs and MAHTs bearing substituents attached in the *ortho* position were prepared along the *para* substituted analogues, in order to determine whether different reactivities between malonates were observed due to intramolecular interactions between substituents in the *ortho* position and the carbonyl oxygen.

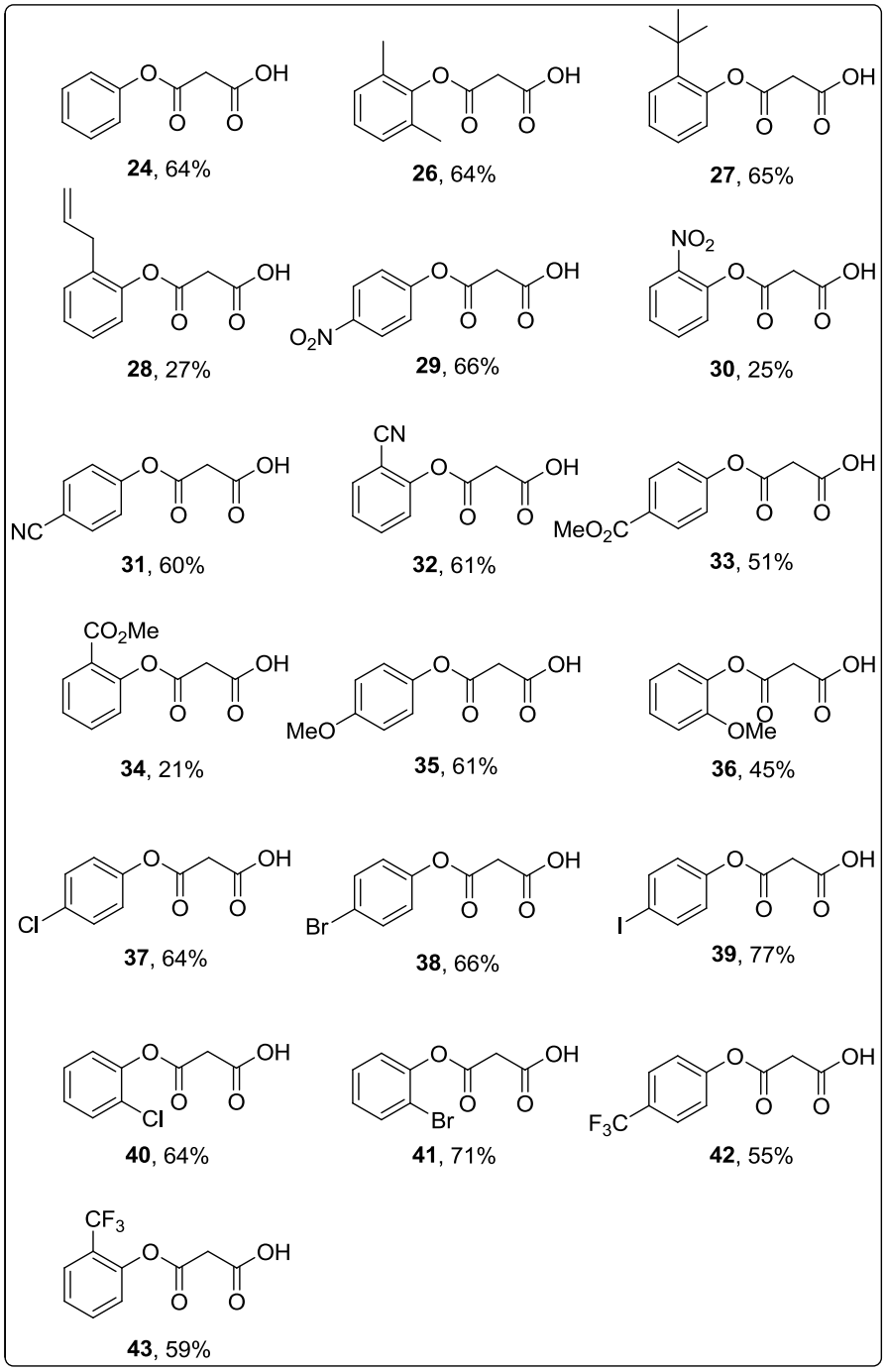
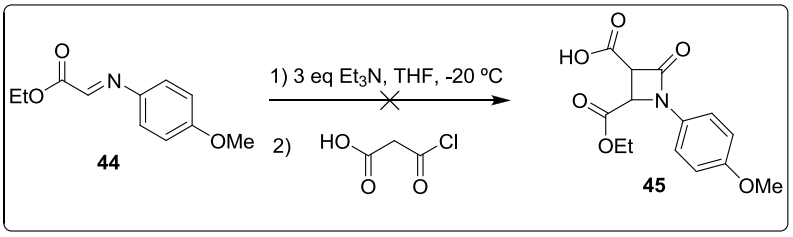


Figure 1.9 MAHOs prepared under the new methodology.

The reaction with half malonyl chloride did not afford the desired products when either phenol or thiophenol starting materials bore acid-sensitive groups in their structure. Hydrogen chloride, generated *in situ* after decomposition of half malonyl chloride, was responsible for the cleavage of those acid-sensitive groups leading in many cases, to the decomposition of the starting

materials and to very unsoluble reaction crudes, impossible to solubilise for further purification and characterization.

Due to the high reactivity and versatility of half malonyl chloride, the Staudinger reaction with the imine **44** was attempted (scheme 1.11).[29] However, no reaction was observed and only unreacted imine was recovered. The absence of reaction may suggest that the presumed formed carboxyl ketene may be involved in other processes like self-condensation or dimerization.



Scheme 1.11 Half malonyl chloride-imine attempted reaction at low temperature.[29]

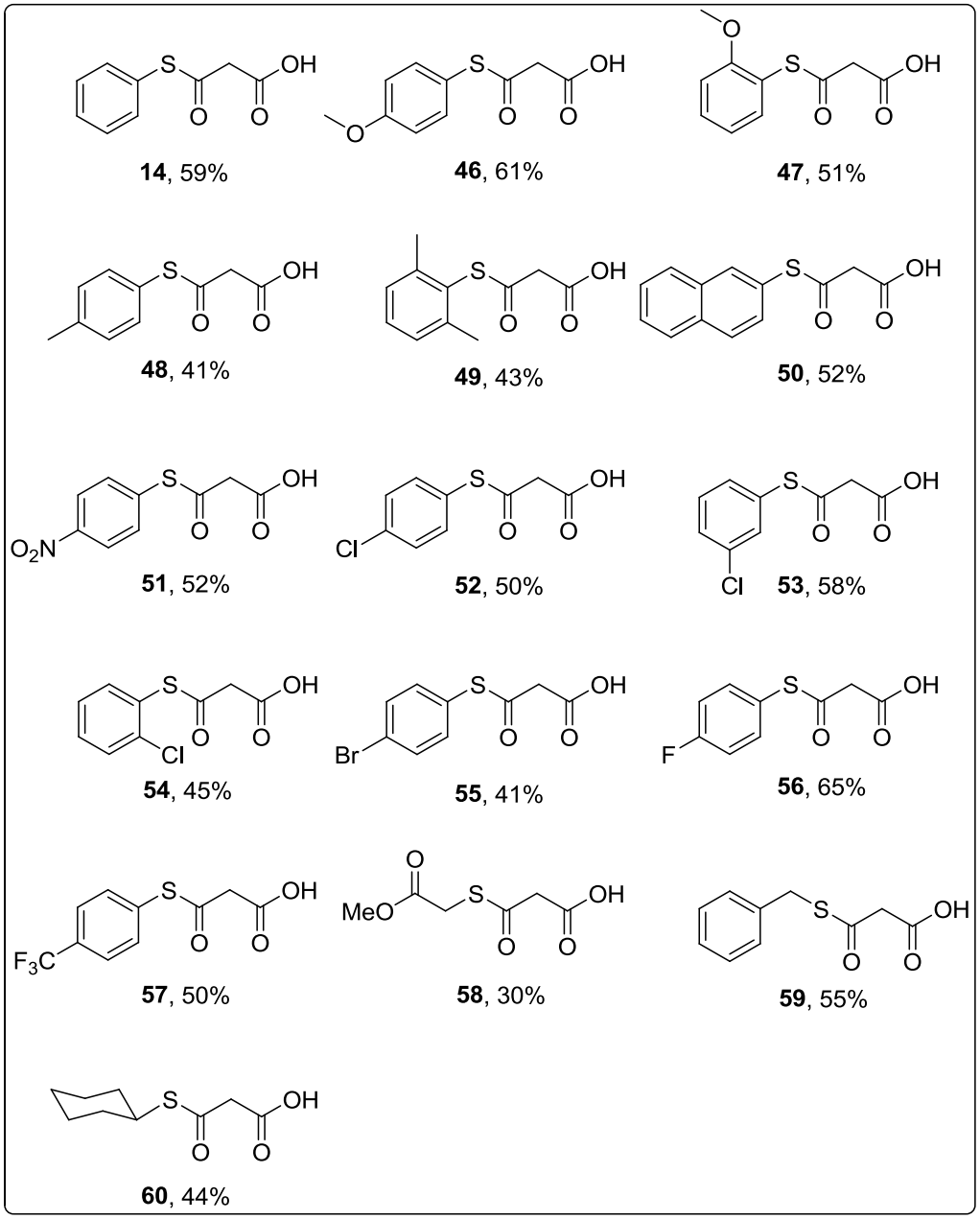


Figure 1.10 MAHTs prepared under the new methodology.

1.2.2 Determination of the relative acidity of MAHOs and MAHTs.

The synthetic method developed during the first stage of our research, allowed us to prepare pure samples of a variety of MAHOs and MAHTs for the study of the physical and chemical properties. During the synthesis of malonates, MAHTs showed less thermal stability than MAHOs, experiencing a quick loss of carbon dioxide at temperatures around 100 ºC. The melting points were also slightly different depending on the type of malonyl carrier present in the molecule, being in general higher for MAHTs. The solubility of malonates in both organic and aqueous solutions was different depending on the nature of the aryl group present in the molecule.

In order to study the behaviour of malonates during decarboxylative aldol-type condensations, we thought that determining the relative acidity of most of the synthesised MAHOs and MAHTs was important to find a correlation between the type of malonyl carrier used and the reactivity of malonates in decarboxylative aldol-type condensations. Since the first step of the decarboxylative aldol-type and Claisen condensations is the deprotonation of malonates, we were confident that the acidity of malonates should play an important role in the reactivity of malonates. In the same way that malonyl and acyl esters present a very different reactivity in aldol-type condensations, due to the enormous difference in pK_a values, smaller differences in the acidity of MAHOs and MAHTs should also lead to different reactivities in mild decarboxylative condensations.

The experimental determination of pK_a values in the laboratory is normally carried out in aqueous solution, where the rate of hydrogen exchange or acid-base reaction, carried out at different and constant pH values, leads to the calculation of pK_a values. The majority of malonates are only partially soluble in water. This fact prevented the use of pH regulated aqueous solutions in the determination of pK_a values for malonates and led us to the use of other alternative methods.

The relative acidity for organic molecules can be estimated by different methods using exclusively organic solvents for the purpose. As an example, the methods involving kinetic studies of hydrogen exchange reactions can lead to the determination of the relative acidity. Determining the hydrogen exchange rates can be carried out by different spectrometric techniques.

For our investigation, determining the absolute acidities or pK_a values was not essential. The values of the relative acidity for each molecule of malonate would allow us to establish a correlation between the acidity and the reactivity of malonates in aldol-type reactions. To illustrate this, the pseudo first order rate constants, which can be determined for MAHOs and MAHTs, could be linked to the relative acidity of these molecules and therefore provide some information with regard to, either the ease or the difficulty in the formation of an enolate from a specific malonate. Thus, we needed to establish which of the prepared MAHOs and MAHTs might afford the most suitable substrates for an eventual mild decarboxylative aldol condensation. Similarly, we needed to predict which phenols or thiophenols might contribute more positively to the formation of a reactive enol or enolate in decarboxylative aldol condensations. Finally, we were also interested in studying the effects of the different groups attached to the aryl units on the acidity and reactivity of the prepared molecules.

The full characterization of 3-oxo-3-phenoxypropanoic acid **24** to elucidate its structure was made by ^{1}H-NMR spectrometry. The NMR sample was prepared by dissolving 7 mg of MAHO **24** in 0.7 mL of methanol-d_4, and the NMR experiment was carried out at room temperature in an automated 400 MHz Varian spectrometer. However, the sharp peak corresponding to the hydrogens attached to the α-position between the ester and the acid moieties in the molecule was not present in the spectrum. This signal was observed when the product was dissolved in a non protic deuterated solvent like $CDCl_3$. At that moment, we realised the α-hydrogens of MAHOs and MAHTs were so acidic that they could be exchanged by deuterium in deuterated protic solvents within few minutes (figure 1.11).

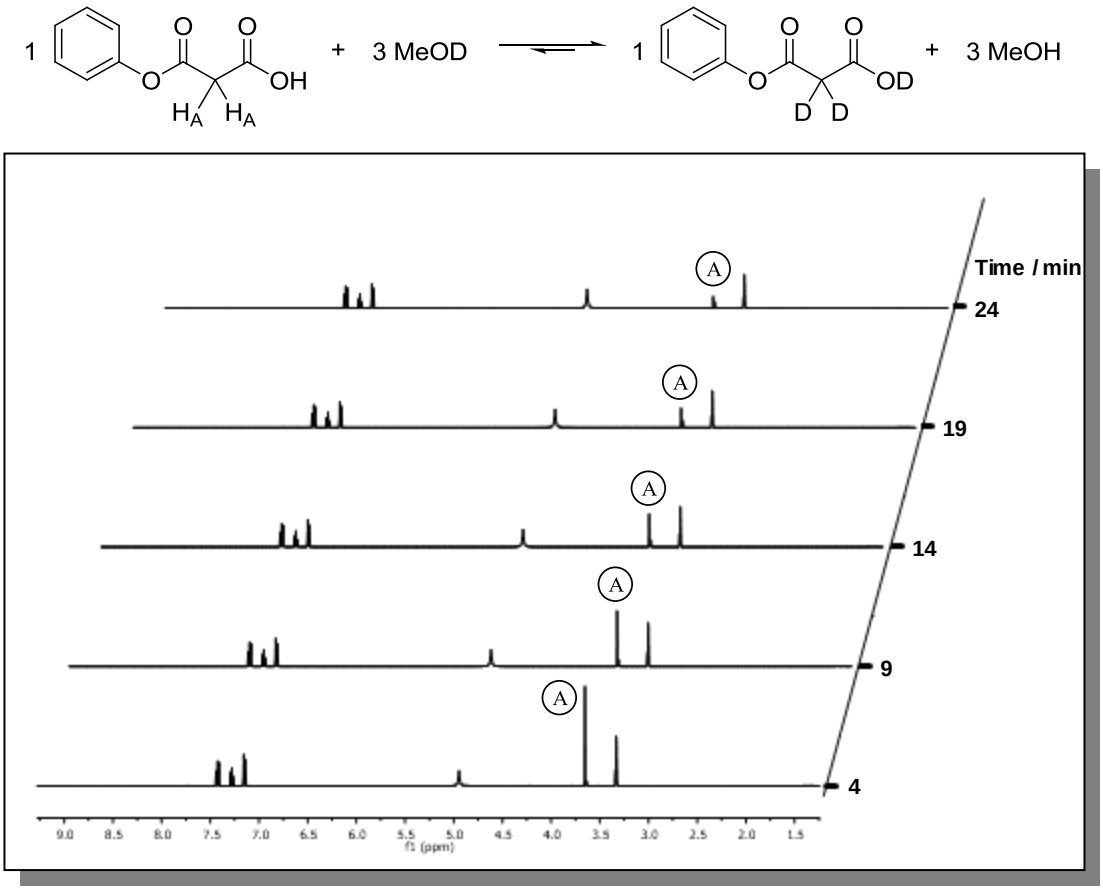


Figure 1.11 Progress of hydrogen-deuterium exchange for 3-oxo-3-phenoxypropanoic acid dissolved in methanol-d_4 followed by ^{1}H-NMR spectroscopy.

The fact that the exchange of the α-protons with deuterium occurred, provided us with a very useful and simple method to determine the relative acidity of MAHOs and MAHTs. The hydrogen-deuterium exchange has been previously and successfully employed for the determination of the rate constants and even pK_a values, when water was used as solvent.[30] Since the majority of MAHOs and MAHTs are only partially soluble in water, we thought it would be difficult to determine pK_a values from H-D exchange experiments. However, the fact that MAHOs and MAHTs were able to exchange their protons with deuterated methanol in only a few minutes, made possible to determine the observed rate constants by measuring the amount of deuterium incorporated to the molecule in a particular period of time. This type of exchange experiments were successfully applied in the past in order to determine the mentioned observed rate constants (K_{obs}) and pK_a values. In 1992, Richard and Amyes reported the measurement of the observed rate constants for ethyl thioacetate.[31] The deuterium exchange experiments, involving the α-protons of ethyl

thioacetate in 3-quinuclidinone buffers in deuterated water (D_2O), were followed by ^{1}H-NMR spectroscopy. In those experiments, the observed rate constants for the 3-quinuclidinone-catalyzed hydrogen-deuterium exchange were determined after exchange of 30-37% of the first α-proton in the methyl group of ethyl thioacetate.

In order to determine the effect that malonyl carriers may have in the acidity of malonates, the hydrogen-deuterium exchange experiments had to be performed under strictly controlled and consistent conditions. Preliminary exchange experiments for 3-oxo-3-phenoxypropanoic acid and 3-oxo-3-(*o*-methoxyphenoxy)propanoic acid were very promising, showing different hydrogen-deuterium exchange rates for α-hydrogens and, allowing the selection of optimal conditions for the exchange experiments. These preliminary experiments showed, as expected, that the rate of exchange was affected by the temperature inside the NMR spectrometer. Thus, the internal temperature in the NMR spectrometer was set up at 20 ºC and all experiments were carried out at this temperature. We thought that the presence of other protic species in the reaction media, such as residual water in the solvent, might affect the H-D exchange rates since, additional hydrogen-deuterium exchanges might occur. For this reason, all the exchange NMR experiments were carried out using the same bottle of deuterated methanol, always kept under an argon atmosphere.

Even though the concentration of deuterated solvent can be considered as constant during the NMR experiments, due to it is present in a huge excess, we decided to employ a constant concentration of malonates in the NMR samples to avoid any possible deviation by the different concentrations.

After optimizating the process, the best conditions found for the hydrogen-deuterium exchange NMR experiments were:

- Internal temperature in the spectrometer probe set up at 20 °C.
- Methanol-d_4 99.8 atom % D from Aldrich® used as solvent and as the deuterium source.
- 70 µM solution of MAHO or MAHT in methanol-d_4 (no buffer additive was employed).
- 5 mm Norell Standard Series NMR tubes.
- 500 MHz Bruker spectrometer.

In a typical H-D exchange experiment, a series of ^{1}H-NMR spectra (16 scans) were recorded every five minutes for a period of ninety minutes. After processing every spectrum obtained, the peaks were integrated and normalized. The value of the integral for the peak corresponding to the methylene group (α-position) was normalized and determined from the spectrum recorded every 5 minutes. The successive experiments showed to what extent, the value of the integral corresponding to the α-hydrogens was substantially decreasing every 5 minutes, indicating a fast deuterium exchange with methanol-d_4. In the case of 3-oxo-3-phenoxypropanoic acid **24**, the value of the integral dropped from 2 to 1.63 after four minutes of the beginning of the H-D exchange experiment (figure 1.12). Fourteen minutes later, the H-D exchange experiment afforded a value of the integral of 1.01, indicating that half of the hydrogens were already exchanged by deuterium. After 89 minutes of exchange experiment, practically all the α-hydrogens were substituted by deuterium and a value of the integral of 0.07 was obtained from the ^{1}H-NMR spectrum.

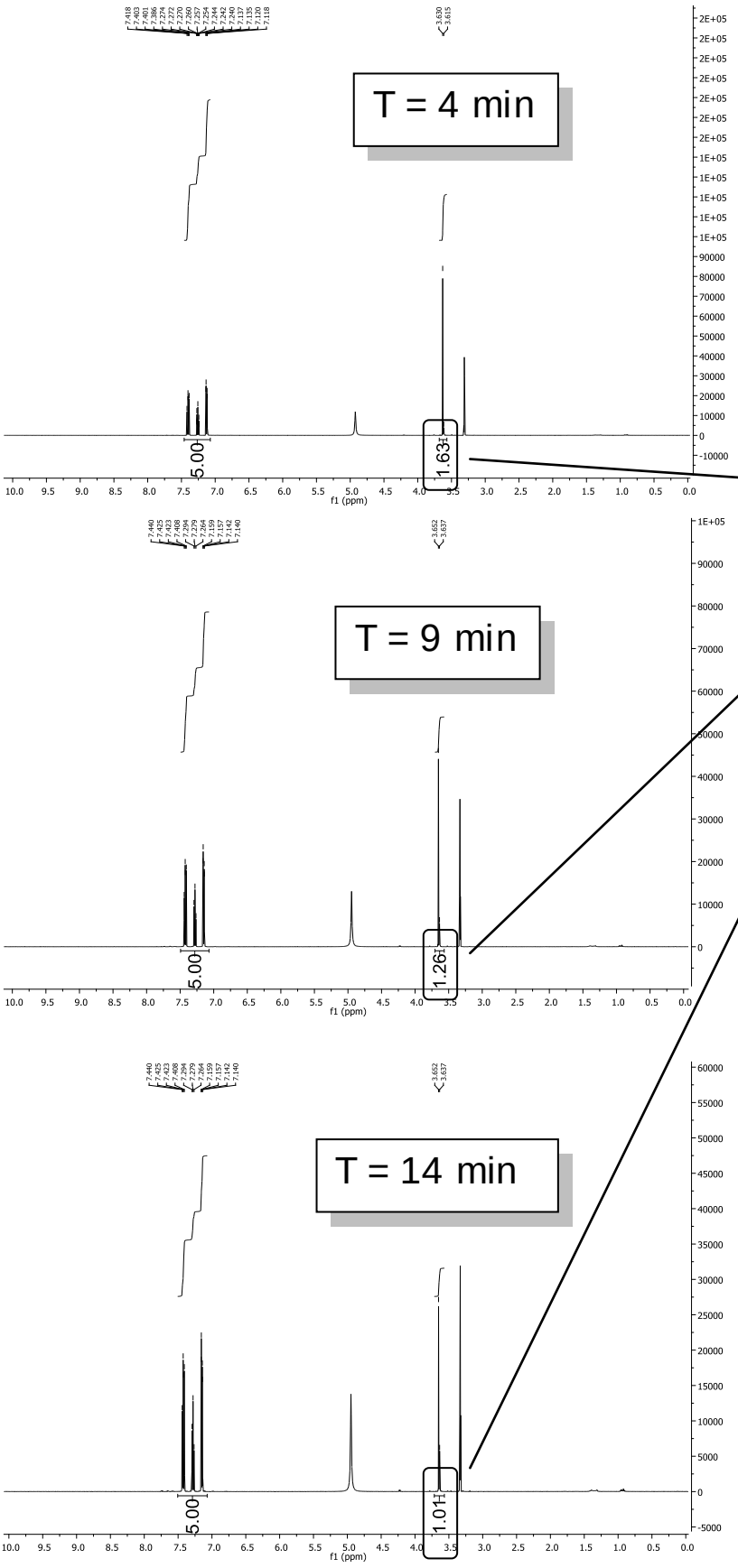


Figure 1.12 Determination of the % of methylenic protons in 3-oxo-3-phenoxypropanoic acid.

Time / min	Integral
0	2
4	1.63
9	1.26
14	1.01
19	0.81
24	0.65
29	0.53
34	0.43
39	0.35
44	0.29
49	0.24
54	0.20
59	0.17
64	0.15
69	0.12
74	0.11
79	0.09
84	0.08
89	0.07
94	0.07

The same experiment above was also performed for several MAHOs containing different types of malonyl carriers. The results obtained are summarized in the table 1.2. The majority of malonates containing electron withdrawing malonyl carriers present a higher rate of exchange, as a faster drop in the integral value with the time is observed, showing a clear trend. The exchange rates for MAHOs substituted in the *ortho* position and, the exchange rates for the *para* substituted analogues are always different. However, the differences in the exchange rates between *ortho* and *para* substituted analogues do not follow a clear trend.

Table 1.2 Integral values for methylenic hydrogens during H-D exchange experiments.

	MAHOs							
Time / sec	**2-CN**	**2-NO2**	**4-NO2**	**2-Cl**	**4-Cl**	**4-CN**	**4-MeO**	**2.6-Me**
0	2	2	2	2	2	2	2	2
240	1.661	0.995	1.227	1.212	1.726	0.9	1.703	1.485
540	1.233	0.445	0.633	0.622	1.374	0.429	1.456	1.082
840	0.948	0.235	0.362	0.356	1.132	0.245	1.264	0.816
1140	0.733	0.14	0.215	0.213	0.939	0.152	1.093	0.617
1440	0.572	0.09	0.138	0.138	0.782	0.105	0.957	0.473
1740	0.452	0.063	0.096	0.098	0.657	0.084	0.829	0.366
2040	0.359	0.053	0.072	0.075	0.555	0.072	0.723	0.287
2340	0.287	0.051	0.059	0.064	0.466	0.065	0.637	0.225
2640	0.232	0.046	0.052	0.055	0.392	0.063	0.56	0.18
2940	0.186	0.044	0.048	0.053	0.337	0.06	0.487	0.146
3240	0.156	0.044	0.046	0.05	0.29	0.06	0.422	0.119
3540	0.126	0.043	0.045	0.049	0.249	0.059	0.371	0.098
3840	0.11	0.042	0.045	0.05	0.217	0.06	0.332	0.079
4140	0.092	0.042	0.043	0.049	0.191	0.06	0.296	0.069
4440	0.079	0.043	0.044	0.049	0.171	0.06	0.262	0.062
4740	0.069	0.042	0.044	0.049	0.15	0.06	0.245	0.055
5040	0.031	0.042	0.044	0.049	0.137	0.06	0.218	0.05
5340	0.028	0.042	0.044	0.049	0.124	0.06	0.193	0.045
5640	0.05	0.042	0.044	0.049	0.118	0.06	0.183	0.043

	MAHOs							
Time / sec	**4-CO2Me**	**Phenol**	**4-Br**	**2-Br**	**2-COOMe**	**4-CF3**	**2-CF3**	**2-MeO**
0	2	2	2	2	2	2	2	2
240	0.987	1.649	1.89	1.918	1.762	1.664	1.435	1.655
540	0.556	1.248	1.72	1.704	1.517	1.39	1.12	1.245
840	0.346	1.001	1.582	1.529	1.362	1.189	0.867	0.98
1140	0.223	0.806	1.456	1.369	1.203	0.998	0.691	0.775
1440	0.152	0.648	1.347	1.242	1.07	0.861	0.548	0.617
1740	0.108	0.526	1.234	1.119	0.954	0.737	0.453	0.497
2040	0.081	0.429	1.133	1.009	0.834	0.639	0.365	0.4
2340	0.067	0.352	1.042	0.911	0.748	0.557	0.296	0.327
2640	0.058	0.291	0.955	0.825	0.662	0.484	0.25	0.272
2940	0.052	0.242	0.885	0.747	0.598	0.418	0.205	0.223
3240	0.05	0.202	0.805	0.686	0.523	0.366	0.171	0.185
3540	0.049	0.171	0.743	0.616	0.479	0.321	0.144	0.157
3840	0.05	0.146	0.683	0.566	0.424	0.283	0.122	0.137
4140	0.05	0.125	0.633	0.513	0.37	0.247	0.108	0.118
4440	0.05	0.109	0.585	0.468	0.343	0.215	0.097	0.102
4740	0.05	0.096	0.535	0.42	0.303	0.193	0.091	0.091
5040	0.05	0.086	0.498	0.382	0.288	0.18	0.08	0.082
5340	0.05	0.077	0.462	0.353	0.245	0.158	0.073	0.073
5640	0.05	0.072	0.426	0.324	0.231	0.144	0.066	0.067

When substituted thiophenols were used as malonyl carriers, the hydrogen-deuterium exchange rates were much higher than in the case of MAHOs. For many malonic acid half thioesters, almost 100 % of α-hydrogens were exchanged by deuterium after 30 minutes of the experiment. In the figure 1.13, the evolution of the peak for α-hydrogens in 3-oxo-3-phenylthiopropanoic acid **14** is shown. As it can be observed, the sharp peak for the α-hydrogens disappears after 9 minutes of exchange reaction. The integral values obtained for different MAHTs are summarized in the table 1.3.

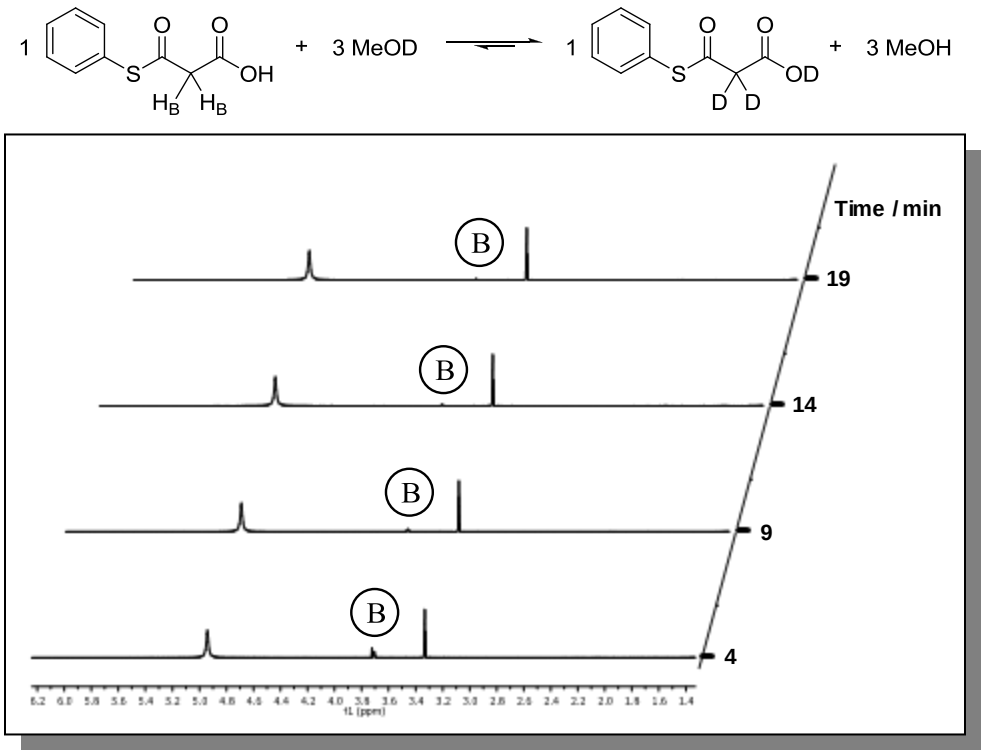


Figure 1.13 Evolution of methylene peak in exchange experiments for 3-oxo-3-phenylthiopropanoic acid in methanol-d_4.

Table 1.3 Integral values for methylene hydrogens of MAHTs during H-D exchange experiments.

	MAHTs						
Time / sec	**Thiophenol**	**2-Cl**	**4-Br**	**4-CF3**	**4-MeO**	**2-MeO**	**4-Cl**
0	2	2	2	2	2	2	2
240	0.557	1.17	0.306	0.596	0.974	1.524	0.946
540	0.201	0.585	0.07	0.233	0.523	1.075	0.373
840	0.095	0.32	0.047	0.122	0.357	0.787	0.162
1140	0.062	0.188	0.044	0.072	0.243	0.58	0.057
1440	0.053	0.117	0.044	0.052	0.17	0.435	0.046
1740	0.049	0.079	0.044	0.046	0.15	0.329	0.039
2040	0.046	0.06	0.044	0.045	0.138	0.25	0.037
2340	0.047	0.05	0.044	0.042	0.122	0.194	0.036
2640	0.046	0.044	0.044	0.044	0.111	0.153	0.036
2940	0.046	0.04	0.044	0.044	0.105	0.125	0.035
3240	0.046	0.038	0.044	0.044	0.108	0.104	0.034
3540	0.046	0.038	0.044	0.044	0.105	0.089	0.035
3840	0.046	0.036	0.044	0.044	0.105	0.079	0.035
4140	0.046	0.037	0.044	0.044	0.105	0.069	0.035
4440	0.046	0.037	0.044	0.044	0.105	0.064	0.035
4740	0.046	0.037	0.044	0.044	0.105	0.06	0.035
5040	0.046	0.037	0.044	0.044	0.105	0.058	0.035
5340	0.046	0.037	0.044	0.044	0.105	0.055	0.035
5640	0.046	0.037	0.044	0.044	0.105	0.056	0.035

The integral values obtained for both, MAHOs and MAHTs, were plotted against the time to represent the exchange curves as a better presentation of the experimental data gathered (figure 1.14).

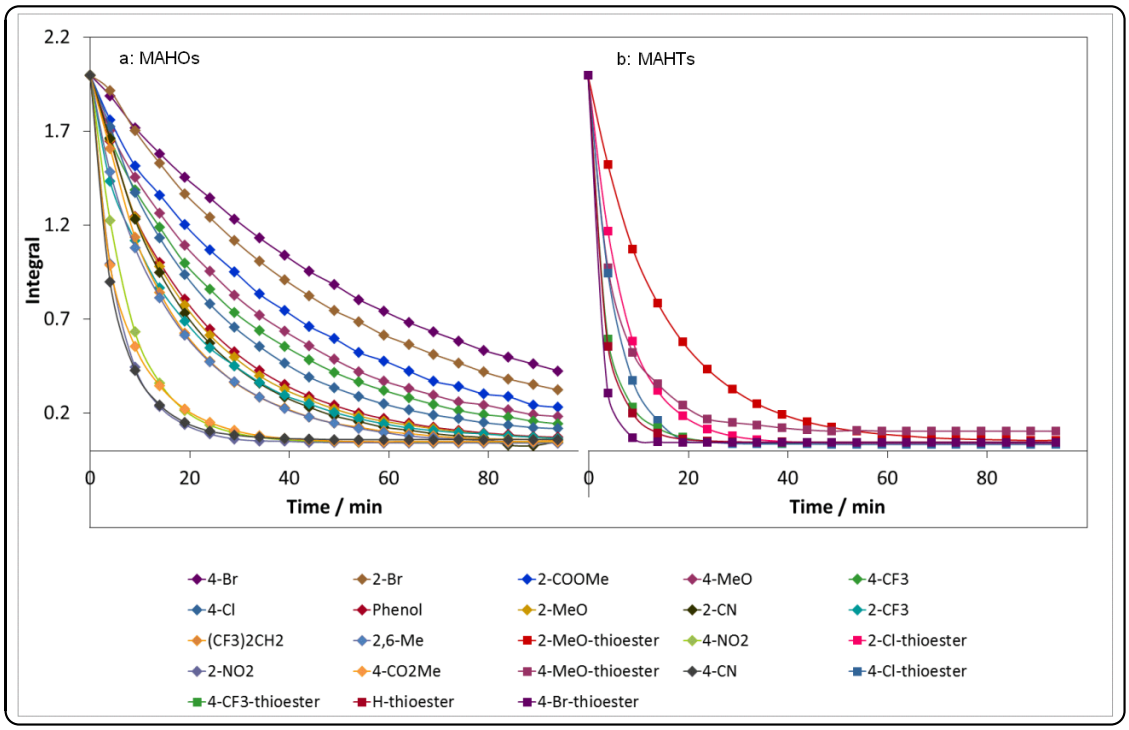


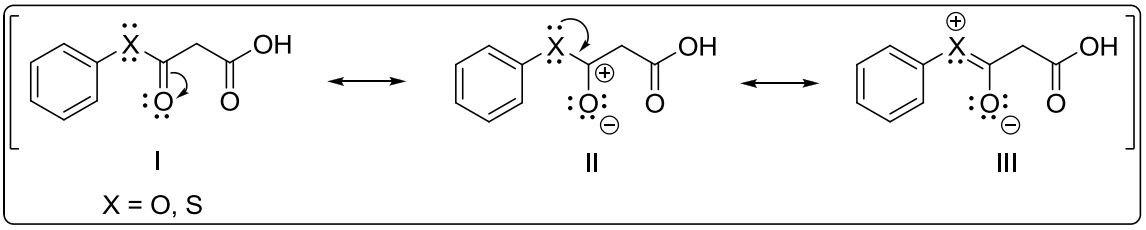
Figure 1.14 H-D exchange curves for MAHOs and MAHTs.

Some interesting findings and conclusions can be extracted from the exchange curves:

1) In broad terms, MAHTs present higher exchange rates than the corresponding oxyesters.
2) The compounds bearing strong electron withdrawing groups, experience faster deuterium-hydrogen exchange.
3) The position of the substituent in the aromatic ring (*ortho* or *para*) does not always produce the same effect, when malonates with substituents in different positions are compared.

To support the qualitative conclusions mentioned above, the molecular orbital theory applied to this type of compounds was looked at in detail. When half malonic acid oxyester and thioesters are compared, the only difference in their structures is the heteroatom linking the aryl group and the polyketone chain.

The different properties between esters and thioesters are well established in the literature. As an example, the different behaviour during acyl transfer reaction in oxyesters and thioesters was successfully explained in the past and supported by the molecular orbital theory. Considering all the possible resonance structures present in MAHOs and MAHTs, some differences in the contribution of every structure can be found (see scheme 1.12).



Scheme 1.12 Resonance structures for MAHOs and MAHTs.

The contribution of the interaction between orbitals 2p-3p in the sulfur atom is much weaker compared to the oxygen atom because of the smaller orbital overlap. As a consequence, the resonant structure III in thioesters provides a smaller contribution to the global electronic structure

of the molecule. Therefore, the carbonyl group in thioesters is less stabilized and more polarized, being more reactive functional groups.

The same principle can be applied to explain the acidity of the alpha protons. The higher contribution to the resonance hybrid from the structure II, generates a more electropositive carbon which could better stabilize the carbanion formed. Also, the higher positive density located in the carbon atom generates a weaker C-H bond in the α-position, making these protons more acidic.

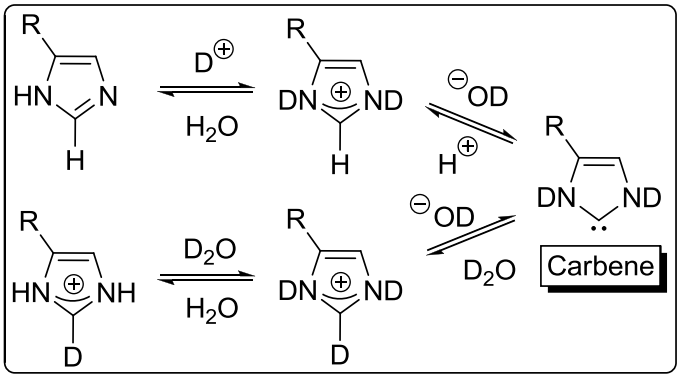
That may also explain why, in the case of thioesters, the keto-enol equilibrium could be shifted to the enol form (since the keto form is less stable). This theory also explains why electron withdrawing groups in the aromatic ring afford more acidic methylenic hydrogens. This, may be explained due to the lower availability of oxygen and sulfur lone pairs, as a consequence, the resonance structure III does not contribute much to the resonance hybrid.

1.2.3 Kinetic study of hydrogen-deuterium exchanges for MAHOs and MAHTs. Determination of the relative acidity from observed rate constants.

Hydrogen-deuterium exchange curves provide a good qualitative method to determine the relative acidity of MAHOs and MAHTs. However, the exchange curves for malonates presenting a similar relative acidity are difficult to compare and do not provide a quantitative and thus a measurable value.

As already mentioned, several methods based on spectrometry were used in the past to determine rate constants and pK_a values. In 2008 Miyagi and Nakazawa developed a method to determine the pK_a values of histidine residues in proteins by using mass spectrometry. The method was based on quantifying the amount of deuterium incorporated in the C2 of imidazole after incubation in deuterium oxide (scheme 1.13). In the mechanism of H-D exchange reaction, the rate-determinating step was the abstraction of the protonated imidazolium-C2 proton. The method, based on electrospray mass spectrometry allowed the determination of the rate constant which reflected the pK_a for the ionization of imidazole. Incubation of a peptide in heavy water resulted in partial H-D exchange in the C2 position of imidazole from the histidine residue and in a change in

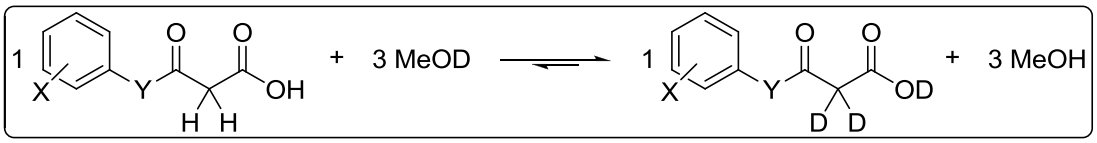
the relative abundance of isotopes obtained from the mass spectrum. Only peaks M and M+1 were used for the calculation of the observed rate constant (K_{obs}).



Scheme 1.13 Mechanism of H-D exchange reaction at the imidazole C2-position of histidine.[75]

In a similar way, we thought that the relative acidity of malonates could be determined by studing the changes caused by deuterium incorporation at the C2-position. We decided to calculate the observed rate constants during the first minutes of H-D exchange experiments. This decision was made based on the principle that equilibrium is not yet reached during the first minutes of H-D exchange, thus the reaction can be considered as a first order kinetic (n=1) and a linear correlation is obtained in order to determine the observed rate constant.

As it was mentioned before, the progress of the H-D exchange was monitored by ^{1}H-NMR spectroscopy. Considering the following reaction:



Scheme 1.14 H-D exchange reaction between malonates and deuterated methanol.

the rate equation for the H-D exchange reaction according to the concentration of the reactants would be:

$$-\frac{1}{3}\left(\frac{d[Malonate]}{dt}\right) = K \times \frac{1}{3} \times [Malonate]^{a} \times [MeOD]^{b} \qquad (1)$$

Since the hydrogen-deuterium exchange curves present an exponential shape, we will consider that the order of the kinetics of the reaction is 1. We will also consider that, the concentration of deuterated methanol, employed as solvent, is so elevated that it remains constant during the whole exchange experiment. Considering these assumptions in the equation 1:

$$\frac{d[Malonate]}{dt} = -K_{obs} \times [Malonate] \quad (2)$$

$$\frac{d[Malonate]}{[Malonate]} = -K_{obs} \times dt \quad (3)$$

$$\ln\left(\frac{[Malonate]}{[Malonate]_0}\right) = -K_{obs} \times t \quad (4)$$

In exchange NMR experiments, the relationship between the concentration of malonates at a particular time "t" and the initial concentration of malonates, can be obtained from the normalized values of the integral for the peak corresponding to the α-hydrogens:

$$f(z) = \frac{[Malonate]}{[Malonate]_0} \quad (5)$$

Considering that the initial concentration of hydrogenated MAHOs or MAHTs integrates for 2 protons at t=0 ($[Malonate]_0$=2), and also that the concentration of MAHOs and MAHTs at any time is given by the normalized integral value corresponding to the α-hydrogens ($I_{methylene,t}$), the equation 4 is rewritten as:

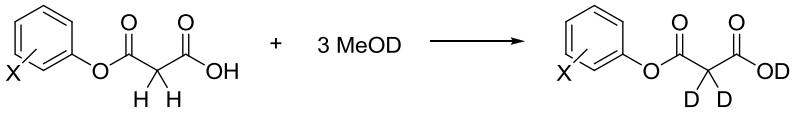
$$f(z) = \frac{[Malonate]}{[Malonate]_0} = \frac{I_{methylene,t}}{2} \quad (6)$$

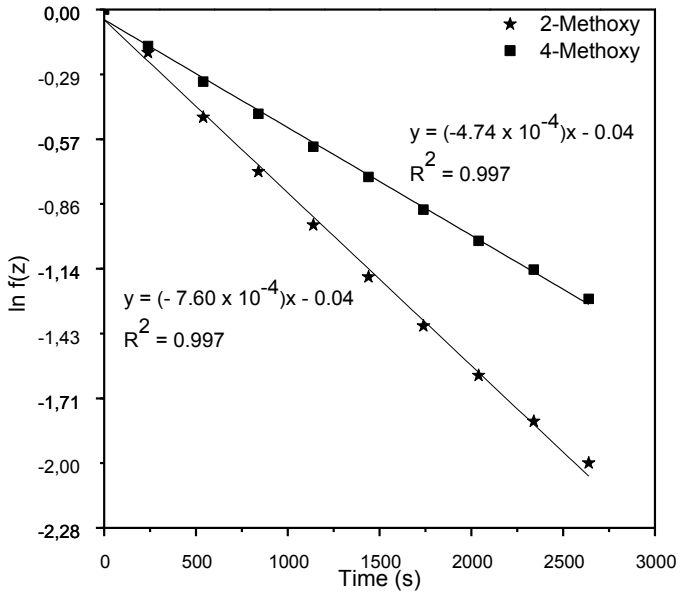
$$\ln\left(f(z)\right) = -K_{obs} \times t \qquad (7)$$

Finally, plotting $\ln\left(f(z)\right)$ against time (t) gives a linear correlation whose slope value determines the value of the observed rate constant for malonates.

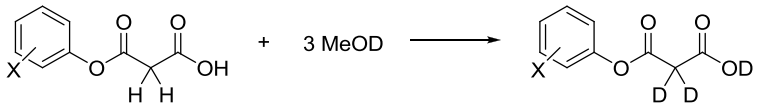
The calculated observed rate constants for different malonic acid half oxyesters are shown in the following plots.

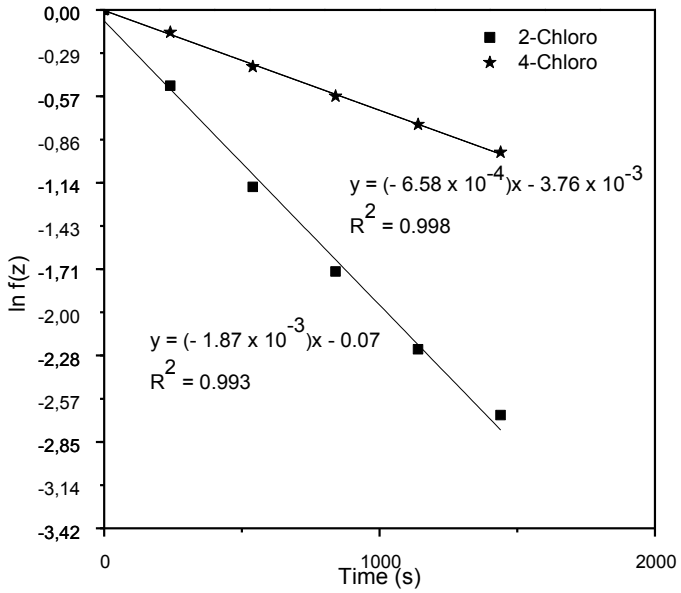
A) 3-Oxo-3-(2-methoxyphenoxy)propanoic acid (36) and 3-Oxo-3-(4-methoxyphenoxy)propanoic acid (35).



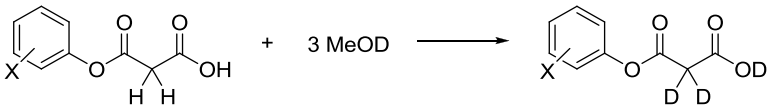


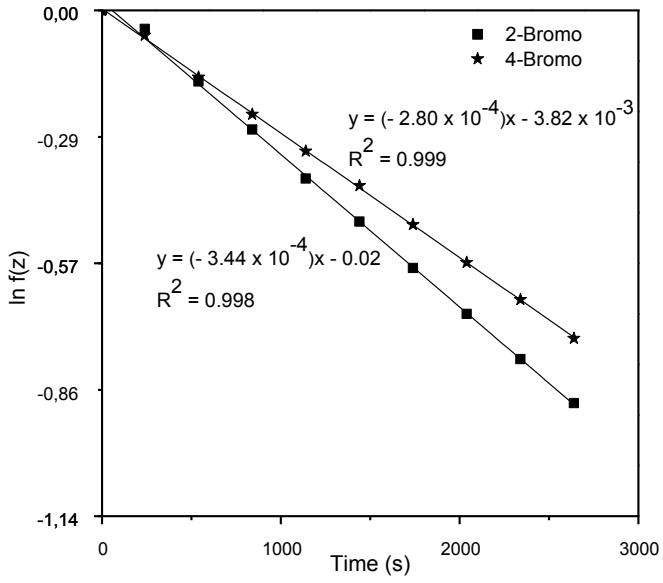
B) 3-Oxo-3-(2-chlorophenoxy)propanoic acid (40) and 3-Oxo-3-(4-chlorophenoxy)propanoic acid (37).



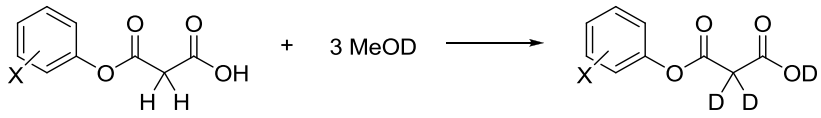


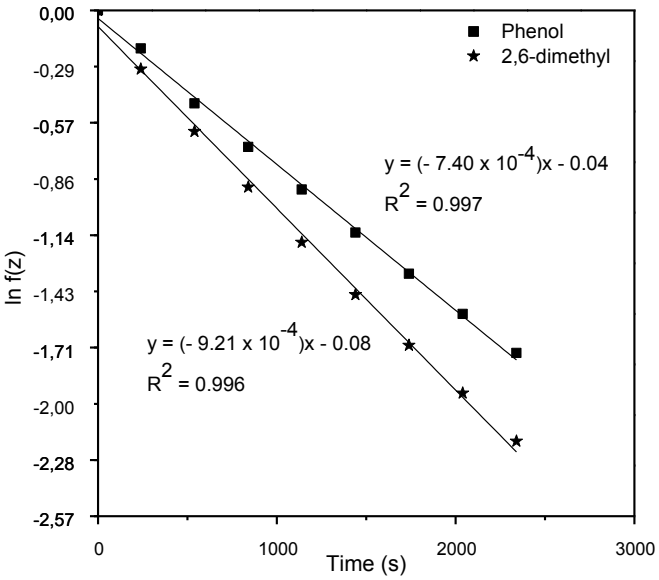
C) 3-Oxo-3-(2-bromophenoxy)propanoic acid (41) and 3-Oxo-3-(4-bromophenoxy)propanoic acid (38).



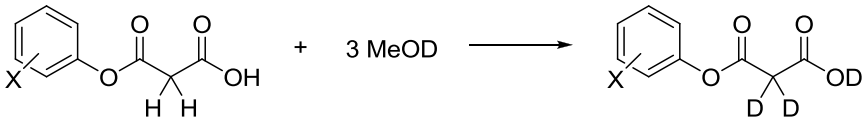


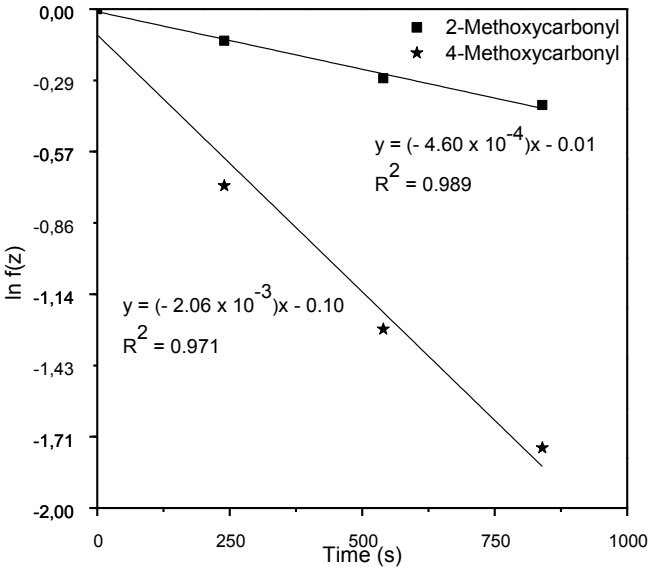
D) 3-Oxo-3-(phenoxy)propanoic acid (24) and 3-Oxo-3-(2,6-dimethylphenoxy)propanoic acid (26).



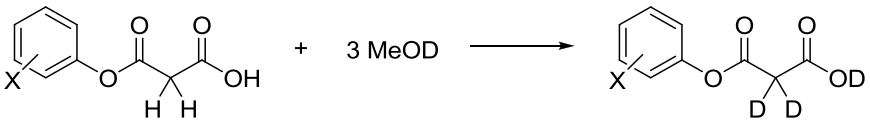


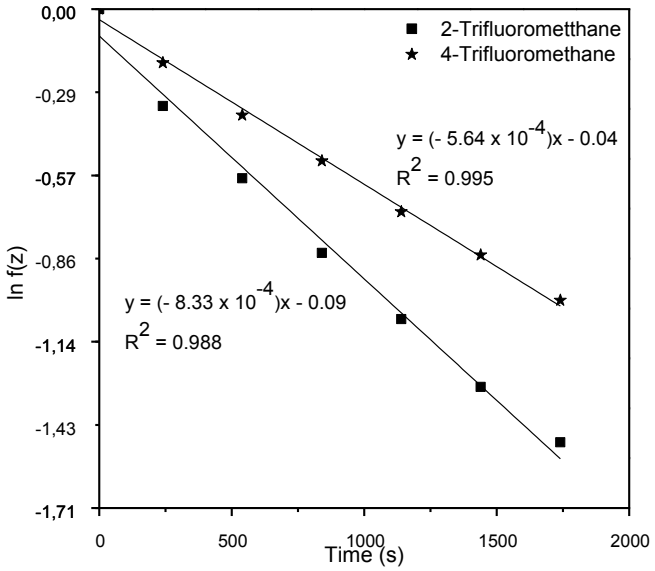
E) 3-Oxo-3-(2-(methoxycarbonyl)phenoxy)propanoic acid (34) and 3-Oxo-3-(4-(methoxycarbonyl)phenoxy)propanoic acid (33).



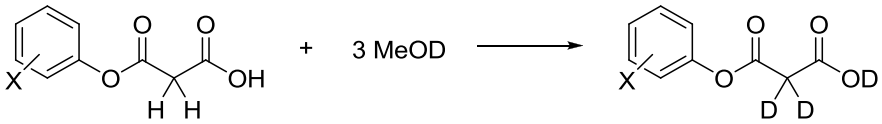


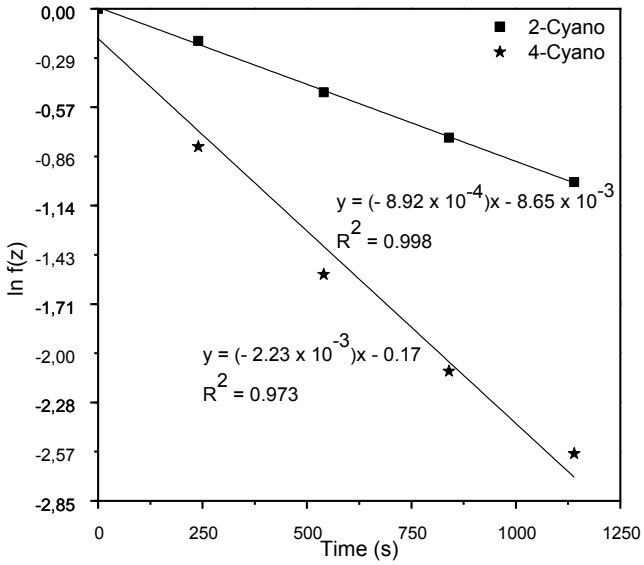
F) 3-Oxo-3-(2-(trifluoromethyl)phenoxy)propanoic acid (43) and 3-Oxo-3-(4-(trifluoromethyl)phenoxy)propanoic acid (42).



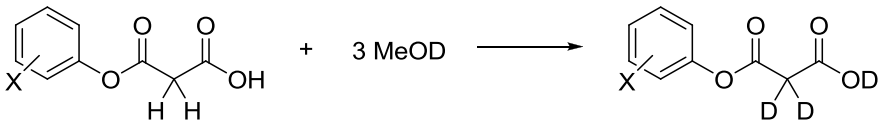


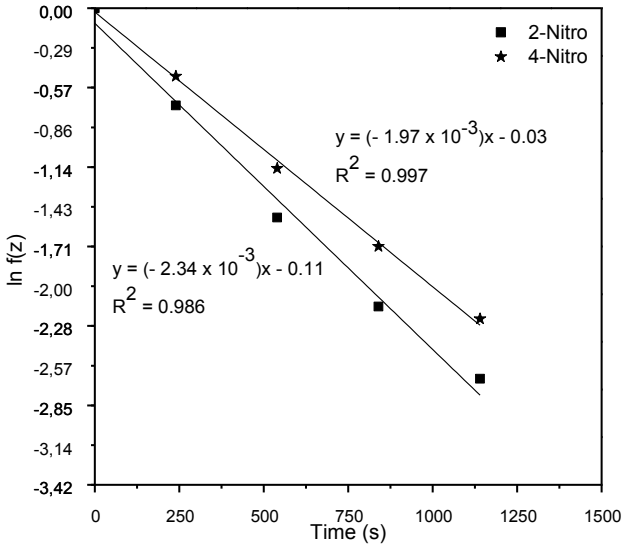
G) 3-Oxo-3-(2-cyanophenoxy)propanoic acid (32) and 3-Oxo-3-(4-cyanophenoxy)propanoic acid (31).





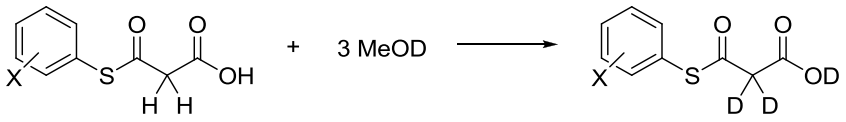
H) 3-Oxo-3-(2-nitrophenoxy)propanoic acid (30) and 3-Oxo-3-(4-nitrophenoxy)propanoic acid (29).

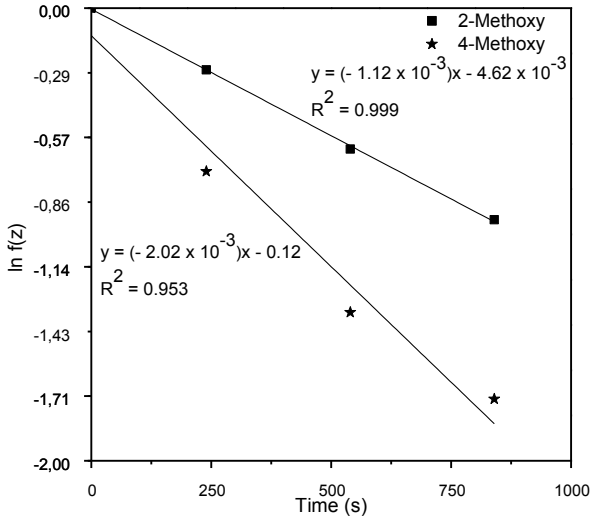




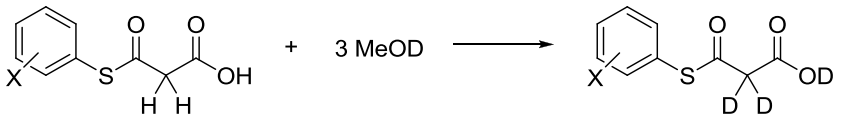
In the same way, the observed rate constants were calculated for malonic acid half thioesters from the representation of $\ln(f(z))$ against time:

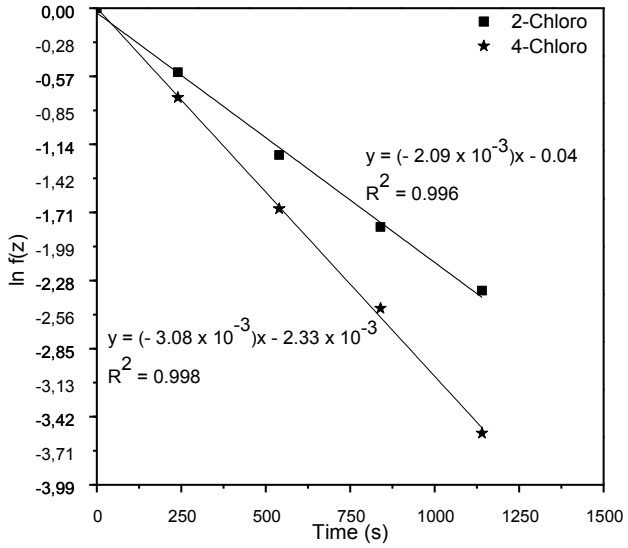
I) 3-Oxo-3-(2-methoxyphenylthio)propanoic acid (47) and 3-Oxo-3-(4-methoxyphenylthio)propanoic acid (46).



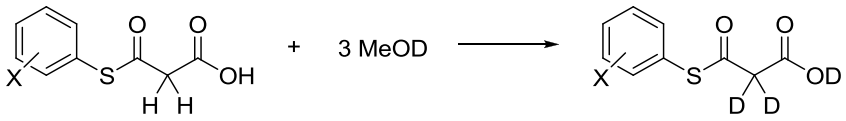


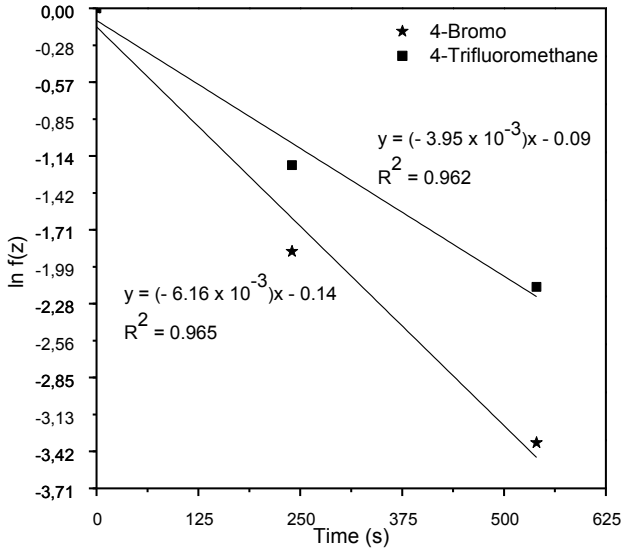
J) 3-Oxo-3-(2-chlorophenylthio)propanoic acid (54) and 3-Oxo-3-(4-chlorophenylthio)propanoic acid (52).



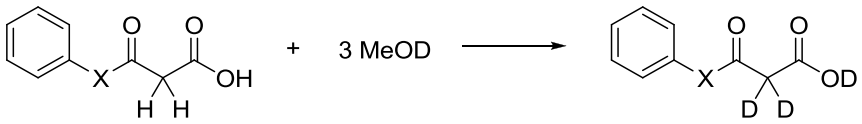


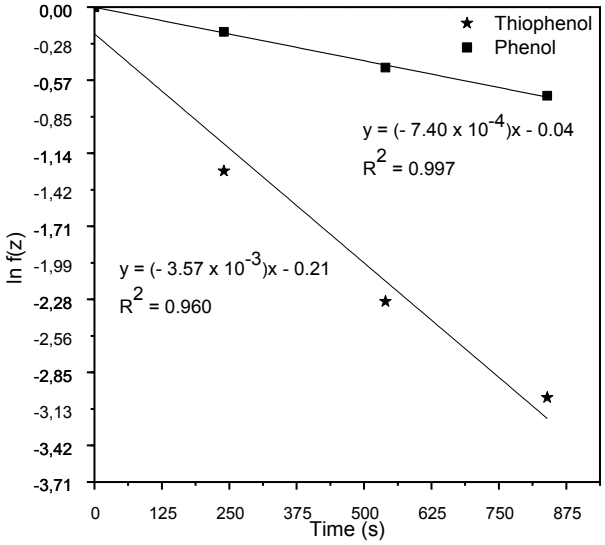
K) 3-Oxo-3-(4-bromophenylthio)propanoic acid (55) and 3-Oxo-3-(4-(trifluoromethyl)phenylthio)propanoic acid (57).





L) 3-Oxo-3-phenylthiopropanoic acid (14) and 3-Oxo-3-phenoxypropanoic acid (24).





The calculated observed rate constants by exchange experiments for each malonate studied are summarized in tables 1.4 and 1.5.

Table 1.4 Observed rate constant (K_{obs}) for MAHOs.

MAHO	K_{obs} / s^{-1}	Intercept	R^2
2-Methoxy	-7,60E-04	-4,53E-02	9,97E-01
4-Methoxy	-4,74E-04	-4,47E-02	9,97E-01
2,6-dimethyl	-9,22E-04	-8,23E-02	9,96E-01
Phenol	-7,40E-04	-4,09E-02	9,97E-01
2-Chloro	-1,87E-03	-7,30E-02	9,93E-01
4-Chloro	-6,58E-04	-3,76E-03	9,98E-01
2-Bromo	-3,44E-04	1,96E-02	9,99E-01
4-Bromo	-2,80E-04	3,82E-03	1,00E+00
2-Methoxycarbonyl	-4,60E-04	-1,04E-02	9,89E-01
4-Methoxycarbonyl	-2,06E-03	-1,03E-01	9,71E-01
2-Trifluoromethane	-8,33E-04	-9,20E-02	9,88E-01
4-Trifluoromethane	-5,64E-04	-3,62E-02	9,96E-01
2-Cyano	-8,92E-04	8,65E-03	9,99E-01
4-Cyano	-2,23E-03	-1,72E-01	9,73E-01
2-Nitro	-2,34E-03	-1,08E-01	9,86E-01
4-Nitro	-1,97E-03	-2,79E-02	9,97E-01

From the calculated data, it can be observed that, in the case of oxyesters, all the *ortho* substituted malonates present a higher rate constant than the corresponding *para* substituted derivatives, except for MAHOs presenting cyano and methoxycarbonyl groups as substituents. That means that, when the substituents are placed in the *ortho* position, MAHOs present higher relative acidities and a faster hydrogen-deuterium exchange reaction is observed in these cases.

Table 1.5 Observed rate constant (K_{obs}) for MAHTs.

MAHT	K_{obs} / s^{-1}	Intercept	R^2
2-Methoxy	-1,12E-03	-4,62E-03	9,99E-01
4-Methoxy	-2,04E-03	-1,21E-01	9,53E-01
2-Chloro	-2,09E-03	-3,91E-02	9,96E-01
4-Chloro	-3,08E-03	2,33E-03	9,99E-01
4-Bromo	-6,16E-03	-1,43E-01	9,65E-01
4-Trifluoromethane	-3,95E-03	-9,41E-02	9,63E-01
Thiophenol	-3,57E-03	-2,09E-01	9,60E-01

By comparing tables 1.4 and 1.5, it can be concluded that thioesters are, in the majority of the cases, more acidic than oxyesters as can be observed by the higher rate constants and therefore by faster hydrogen-deuterium exchanges. These conclusions can be better observed in figure 1.15 where the observed rate constant (K_{obs}) for each malonate is represented in a bar diagram.

Figure 1.15 Observed rate constants for malonic acid half oxyesters (MAHOs) and thioesters (MAHTs).

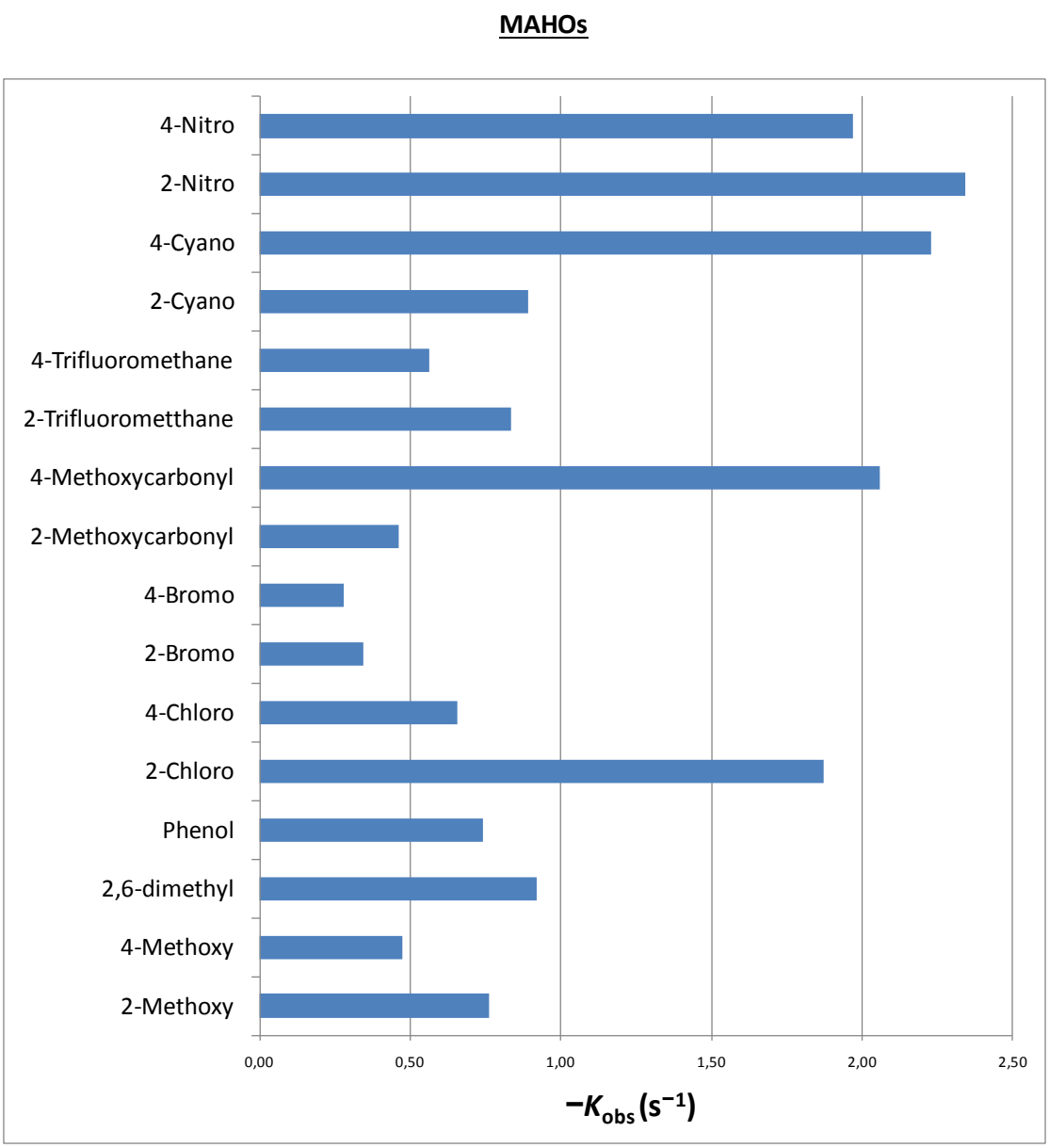
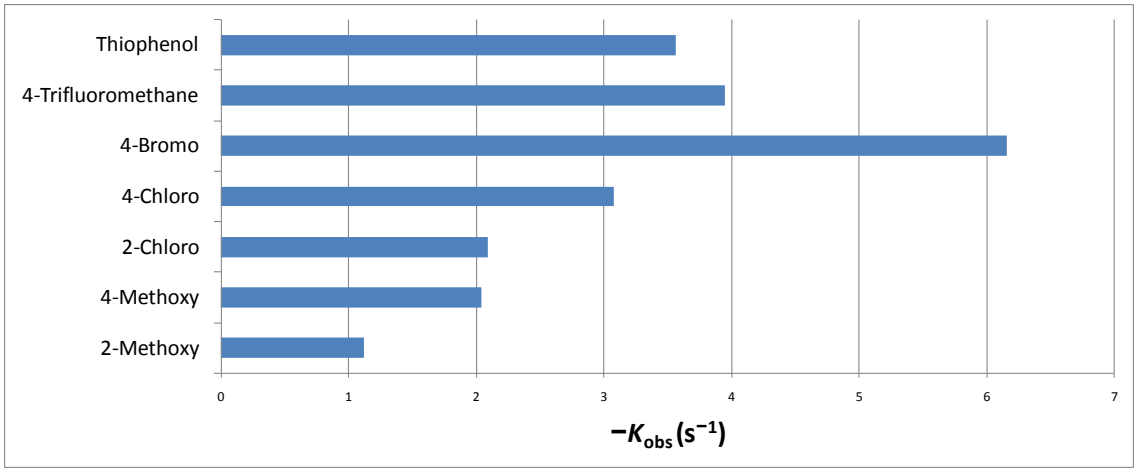


Figure 1.15 Continuation.

MAHTs



Important changes in exchange rates are found when strong electron withdrawing groups are attached to the aromatic ring, regardless of the position they hold within the aromatic ring. Malonates carrying electron donating groups are in general less acidic than malonates bearing electron withdrawing groups, although some exceptions are found.

However, the biggest difference in the relative acidity arises when MAHOs and MAHTs are compared with each other. Malonic acid half thioesters are in general much more acidic than the corresponding oxyesters and therefore, in principle, better candidates as enolate equivalents in decarboxylative aldol condensations.

1.3 Conclusions.

A convenient method for the synthesis of MAHOs and MAHTs has been successfully developed. The new method uses readily available reagents and avoids arduous work up and purification steps, however it is not compatible with compounds bearing acid-sensitive groups in their structure.

A broad range of MAHOs and MAHTs have been prepared in moderate or good yields. The relative acidity of these compounds has been estimated by H-D exchange experiments using ^{1}H-NMR spectroscopy and observed rate constants (K_{obs}) have been calculated.

In general, the observed rate constants obtained from H-D exchange experiments have proved MAHTs to be more acidic than MAHOs and malonates bearing EWG more acidic than those bearing EDG.

- Chapter 2 -

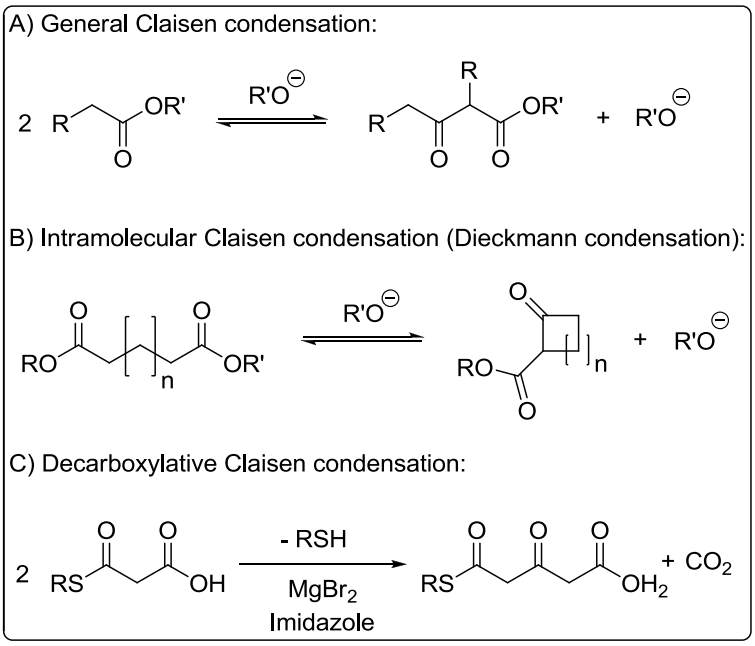
Base and metal-free decarboxylative aldol-type condensations.

3.1 Introduction to Claisen and aldol condensations.

3.1.1. The Claisen condensation.

By making use of Claisen condensations, PKS present in bacteria, fungi and plants are able to grow polyketide chains and synthesize a great number of active metabolites that form part of many relevant pharmaceutical drugs like tetracycline, daunorubicin, rapamycin, erythromycin or lovastatin.[32]

In the organic chemistry field, the Claisen condensation consists of the nucleophilic addition-elimination of an ester enolate to a carboxylic acid derivative such as an ester, a thioester or an acid chloride.[33] The Claisen condensation can be performed between the same two molecules (self-condensation) or between two different substrates.



Scheme 2.1 Different types of Claisen condensations.

In organic chemistry, Claisen condensations are normally achieved by treatment with strong bases and when the reaction proceeds intramolecularly it is known as the Dieckmann condensation (scheme 2.1). In decarboxylative Claisen condensations (scheme 2.1, c), a carboxylic group is attached to the enolizable α-carbon in the molecule, making the protons

attached to this position more acidic (lower pK_a). As a consequence, these types of molecules can be easily enolised by weak bases, allowing the formation of carbon-carbon bonds under mild conditions. Some examples of molecules that can undergo decarboxylative Claisen condensations are malonic acid half esters (MAHOs) and malonic acid half thioesters (MAHTs).

3.1.2. The aldol condensation.[33]

Another type of condensation that can be catalysed by bases is the aldol condensation. The aldol condensation is the addition of the α-carbon either of ketones or aldehydes to a carbonyl group. This reaction is typically catalyzed by strong bases such as alkoxides, amides or amines. Milder bases such as hydroxide salts can also be used but in this case the deprotonation at the α-carbon is not quantitative. However, enough amount of enolate is normally generated for the reaction to proceed.

The aldol condensation represents a very powerful and convenient method for the preparation of α,β-unsaturated aldehydes, ketones and esters and also for the preparation of β-hydroxy carbonyl compounds. Dehydratation of the β-hydroxyl carbonyl compound after the aldol addition is often spontaneous and prevents the isolation of this intermediate in some occasions.

The retro-aldol reaction is also possible from α,β-unsaturated or β-hydroxy carbonyl compounds by treatment with hydroxide salts. The aldol reaction can be also carried out under acidic catalysis. Thus, different protonic acids in conjuction with a chiral diamine have been effectively used in the past in the catalytic asymmetric direct aldol reaction of aldehydes in acetone.[34] In this type of catalysis, the dehydration process becomes much more common. The different types of aldol condensation are summarized in the table 2.1. Aldol condensations can be classified in four different groups depending on the nature of the starting materials involved in the reaction.

Table 2.1 Types of aldol reactions.

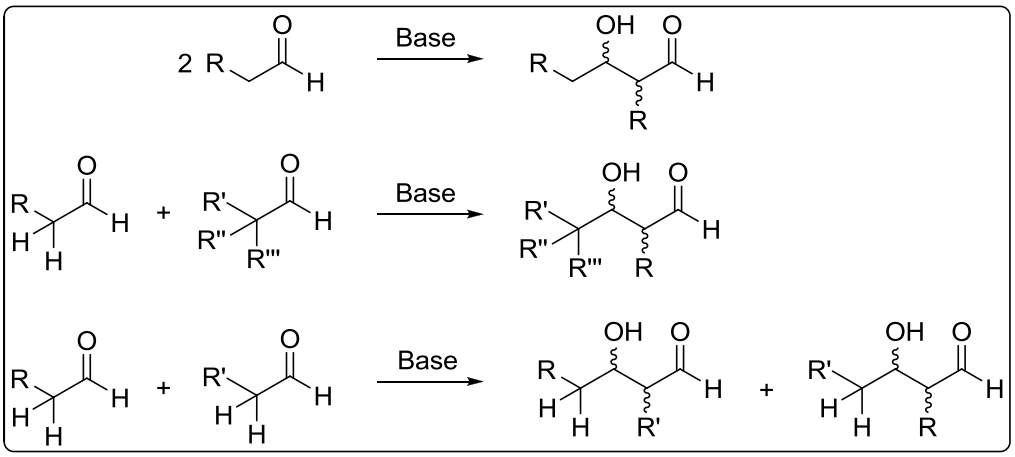
Reaction	Acive H component	Carbonyl compound	Subsequent reaction
Aldol reaction	Aldehydes or ketones	Aldehydes or ketones	Dehydration (may follow)
Aldol-type reaction	Ester or carboxylic acid derivatives	Aldehydes or ketones	Dehydration (may follow)
Knoevenagel reaction	Cyano or nitrocompounds	Aldehydes or ketones	Dehydration (normally follows)

3.1.6.1 Aldol condensation between two aldehydes.

The aldol reaction between two aldehydes can be divided in two subgroups:

- Condensation between the same type of aldehyde.
- Condensation that involves two different types of aldehydes (cross aldol condensation).

Aldehydes must present hydrogens in the α-position of the carbonyl unit and strong bases such us alkoxides and alkali metal amides are normally used to catalyse the reaction. In the case of cross aldol reactions, two different products are possible. In the simplest case, one of the aldehydes does not present hydrogens in the α-position and only one product is formed after the condensation (scheme 2.2). The β-hydroxy carbonyl compound formed during the aldol condensation is produced as at least two different isomers, since at least one stereogenic centre is formed during the reaction. In the second case, both types of starting aldehydes are enolizable. In this version, two different products can be formed and again, at least one stereogenic centre is created in each β-hydroxy carbonyl compound formed during the condensation reaction (scheme 2.2).

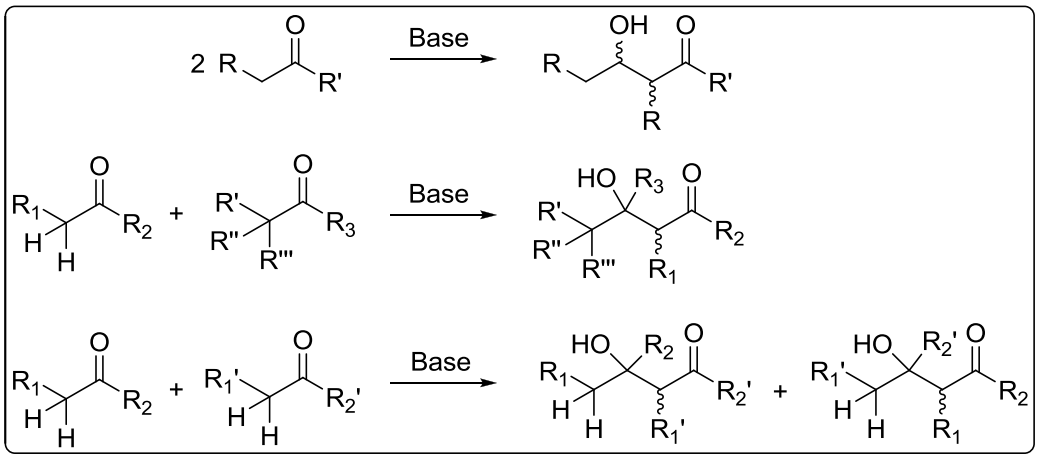


Scheme 2.2 Types of aldol condensations.

The most typical conditions to carry out cross aldol condensations involve the use of strong amide bases in aprotic solvents. One of the aldehydes is treated with the strong base at low temperature to stabilize the enolate anion and then, the second aldehyde is added to allow the condensation reaction. In the case of α-substituted carbonyl compounds, the aldol reaction leads to a mixture of diastereoisomers after the generation of two stereogenic centres during the reaction (scheme 2.2).

3.1.6.2 Aldol condensation between two ketones.

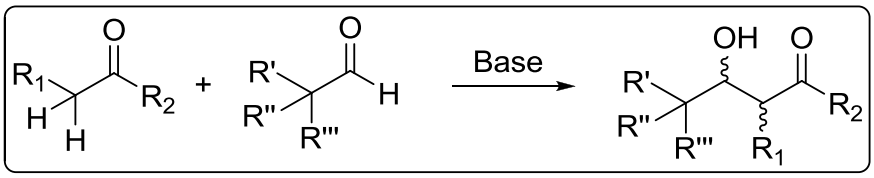
As in the previous case, the aldol reaction between two ketones can be performed using the same type of ketone or two different types (scheme 2.3). The reaction conditions are very similar to those employed during the aldol condensation of aldehydes. Hydroxide and alkoxide salts in protic solvents or amide bases in aprotic solvents and low temperature are the most common reaction conditions. In the case of asymmetric ketones, the nucleophilic attack normally proceeds via the less substituted α-carbon.



Scheme 2.3 Types of aldol condensations between ketones.

3.1.6.3 Aldol condensation between aldehydes and ketones (Claisen-Schmidt reaction).

The simplest case in this type of aldol condensation is the condensation between a ketone and an aldehyde without hydrogens in the α-position. This is known as the Claisen-Schmidt reaction. When enolizable aldehydes are used instead, complex mixtures of products are normally obtained, although aldehydes are more likely to act as electrophiles due to their higher reactivity (scheme 2.4).



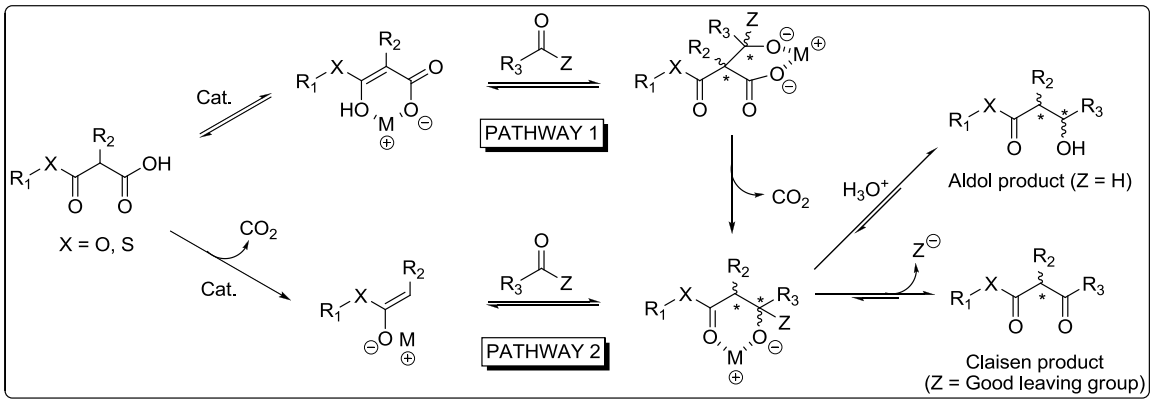
Scheme 2.4 Aldol condensation between ketones and non enolizable aldehydes.

2.1.3 Use of MAHOs and MAHTs in decarboxylative Claisen and aldol-type condensations.

As previously discussed, decarboxylative condensations are a mild and simple method for the generation of carbon-carbon bonds in organic chemistry. These types of bio-mimetic

decarboxylative processes allow the formation of new bonds in the absence of either strong bases or acids under mild temperatures. Malonic acid half esters and thioesters are the most common substrates used in this type of reaction. MAHOs and MAHTs can be activated by different types of catalysts, like for example mild bases. After activation, MAHOs and MAHTs can react with different types of electrophiles such as aldehydes, ketones and molecules containing carbon-carbon double bonds.

In nature, the Claisen condensation occurs by the formation of a thioester enolate, after initial decarboxylation of a malonate, which reacts in a second step with a nearby acyl thioester (scheme 2.5, pathway 2). However, mechanistically, there is a second route (pathway 1) to achieve Claisen or aldol-type condensation products, where the decarboxylation occurs after the addition step (scheme 2.5). Kinetic and NMR experiments of Me-MAHTs (α-methylated MAHTs) suggest that the addition of the enolate to the electrophile proceeds first, followed by the decarboxylation (pathway 1).[23]



Scheme 2.5 Possible mechanistic pathways in aldol-type and Claisen condensations.[23]

2.1.3.1 Use of MAHOs and MAHTs in decarboxylative aldol-type reactions.

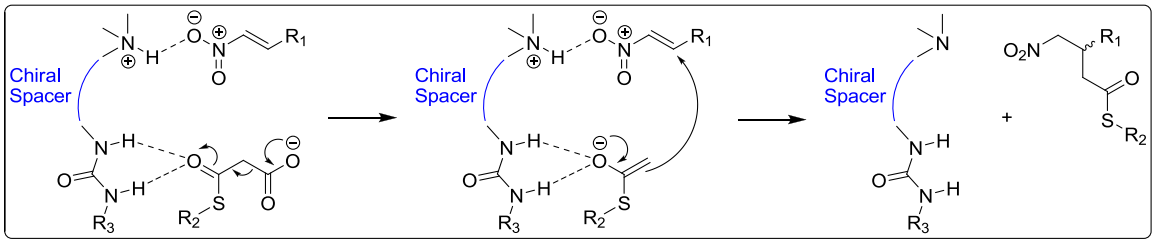
In 2003, Scott and co-workers reported the self-condensation of malonic acid half oxyesters catalyzed by an organic base. The use of Hünig's base (DIPEA or *N,N*-diisopropylethylamine) along TSTU (*N,N,N',N'*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate) as a promoter of the reaction, allowed the self-condensation of MAHOs in excellent yields at room temperature and in short periods of time (less than one hour).[35]

In 2005, List *et al.* reported the synthesis of (*E*)-α,β-unsaturated esters via a modified Doebner-Knoevenagel reaction between MAHOs and aldehydes (either aliphatic or aromatic), catalyzed by DMAP in *N,N*-dimethylformamide, obtaining excellent stereo and regioselectivity yields.[36]

In 2005, Shair *et al.* reported the catalytic aldol condensation between α-methylated MAHTs and aldehydes mediated by Cu(II) salts.[37] In 2006, Coltart and co-workers published a simple aldol addition of thioesters to aldehydes in the presence of Mg(II) salts and Hünig's base affording β-hydroxythioesters with excellent yields.[38]

In a similar way, Thomas *et al.* reported a decarboxylative Doebner-Knoevenagel condensation between MAHTs and aldehydes in 2007.[39] The reaction was carried out by dissolving an equimolar mixture of MAHT, aldehyde and 5-methoxybenzimidazole in THF and stirring the solution at room temperature after the addition of ytterbium triflate as catalyst (10 % mol). This methodology afforded good regio- and stereoselectivity and high yields for a variety of screened aldehydes, although further improvements are needed in the case of aliphatic aldehydes where yields obtained were poor.

The fact that PKS use two amino acid residues during the catalysis of the decarboxylative Claisen condensations in its active site, inspired organic chemists to use bi-functional catalysts in these types of reactions. In 2007, Wennemers *et al.* used cinchona alkaloid derivatives as bifunctional catalysts able to catalyse the decarboxylative Michael type addition of malonic acid half thioesters to nitro-styrene (scheme 2.6).[40]



Scheme 2.6 Bifunctional catalyst employed by Wennemers and co-workers in the Michael addition of MAHTs to nitro-olefins.[40]

In 2009, Fagnou and co-workers reported that both MAHOs and MAHTs can react with aldehydes *via* decarboxylative aldol-type reaction when either stoichiometric or catalytic amounts of triethylamine were present in the reaction mixture.[23]

The stereoselective synthesis of β-aminoesters from MAHTs, *via* decarboxylative Mannich reaction was also reported in 2007 by Ricci and co-workers.The Mannich reaction was catalyzed by chiral cinchona alkaloid derivatives in tetrahydrofuran at low temperatures, affording moderate enantiomeric excesses.[41]

In 2010, Rouden *et al.* published a ^{1}H-NMR mechanistic study of the triethylamine-promoted Mannich reaction between MAHOs and imines in DMF-d^{7}.[42]

Tan and co-workers reported the use of bicyclic guanidines as efficient catalysts in decarboxylative Manich reactions. *tert*-Butyl MAHTs and *N*-tosylimines were reacted in the presence of catalytic amounts of a guanidine derivative affording the desired Mannich products in good yields and with excellent enantiomeric excesses.[24]

2.1.3.2 Use of MAHOs and MAHTs in decarboxylative Claisen condensations.

The use of MAHOs and MAHTs in Claisen condensations started in 1975 when Scott and co-workers reported the use of catechol as a suitable template for promoting intramolecular Claisen condensations between malonyl and acyl groups.[43] Scott *et al.* used isopropylmagnesium salts as catalysts to afford catechol monoacetoacetate in a modest 30% yield. However, this reaction required the use of a strong base to proceed, and the reaction conditions were far harsher than the conditions used by PKS in plants, bacteria and fungi.

In 1978, Kobuke and Yoshida reported the first example of a mild Claisen condensation carried out under enzyme-free conditions. Imidazole and magnesium(II) acetate were used as catalytic system.[44] For the reaction, malonic acid half thioesters were employed instead of malonic acid half oxyesters. *N*-Butyl thiomalonate was reacted with one equivalent of phenyl thioacetate in the presence of imidazole and magnesium(II) acetate for 87 hours to afford the desired *n*-butyl acetothioacetate in a moderate 60% yield. When magnesium(II) acetate was not added to the mixture, no Claisen condensation was observed. As self-condensation of malonic acid half

thioesters was not observed during the experiments, it was inferred that the conditions employed were not suitable for MAHT polymerization and the mild synthesis of polyketones or polyketides in the laboratory. In an attempt to mimic the mechanism of the fatty acid and polyketides biosynthesis, the reaction between *n*-butyl thiolmalonate and methyl-*N,S*-diacetyl cysteinate was also attempted. However, very little product was formed under these conditions likely due to the poor performance of cysteinate as leaving group.

It was in 2001 when Matile *et al.* first found the conditions to perform a mild self-condensation of MAHTs in the presence of Mg(II) salts and imidazole.[19] In an attempt to find some suitable conditions for the controlled oligomerization of malonates under mild conditions and based on Kobuke *et al.* work, Matile and co-workers decided to optimize the initial conditions by modifying the leaving group, the base and the metal involved respectively. After some optimization work, the first example of malonic acid half thioesters self-condensation was achieved in a good 71% yield. However, oligomerization of MAHTs was not observed in the condensation experiments. This fact highlights indeed the suitability of those conditions to afford the mild synthesis of artificial polyketones and polyketides.

A few years later, in 2007, Scott *et al.* reported the first example of self-condensation of half malonic acid oxyesters under mild conditions. Based on the belief that primitive organisms used RNA instead of proteic enzymes for the catalysis of metabolic pathways, it was decided to use nucleoside malonates as starting material for the Claisen self-condensation. After some optimization work, a mild self-condensation of nucleoside malonates was achieved in nearly 90% yield after thirty hours of incubation at 37 ºC. The condensation experiments where carried out in aqueous acidic conditions and in some experiments, polynucleotides Poly-U or Poly-A were added in order to study the template effects that finally were not observed. Magnesium(II) salts were used as catalyst and this role was proven essential to make the reaction to proceed. As a control experiment, mono-phenyl malonate was incubated under the reaction conditions for the same time, and no Claisen self-condensation was observed.[45]

2.1.4 Aims.

The aim of this chapter is to develop a new mild decarboxylative aldol condensation under metal and base-free conditions. To achieve that, different ionophores will be employed as catalysts in a water-rich biphasic solvent. Using the new developed method, some of the MAHTs prepared in chapter 1 will be condensed with aldehydes and ketones.

2.2 Results and discussion.

2.2.1 Base and metal-free decarboxylative aldol condensation. Correlation between reactivity of MAHOs and MAHTs and observed rate constants.

Natural evolution allows living organisms to select the most convenient chemical processes to adapt to new and challenging environments. Thus, living organisms take advantage of a wide variety of thermodynamically favoured chemical reactions in order to synthesise biomolecules under mild conditions. A good example of that is the use of thermodynamically favoured decarboxylative Claisen condensations during the synthesis of polyketides and other metabolites in plants and microorganisms.

In the active site of PKS, the formation of thioester enolates from malonic acid half thioesters and the decarboxylative cross-Claisen condensation of malonates are catalyzed under mild conditions, allowing the generation of covalent carbon-carbon bonds. The use of malonic acid half thioesters by PKS as an ester enolate synthon inspires organic chemists to develop new and more environmentally friendly mild aldol-type and Claisen condensations based on decarboxylative processes taking place in polyketides biosynthesis.

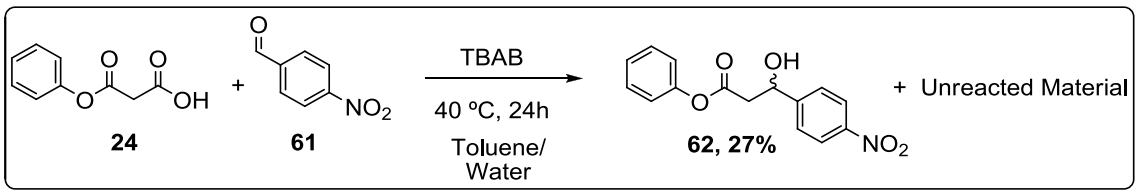
Although the use of MAHOs and MAHTs as enolate equivalents allowed organic chemists to develop bio-inspired aldol-type and Claisen condensations in the laboratory, the catalysis of these processes required very often the use of bases and coordinating divalent metals.

During the last decade, several works were published in relation to the development of mild deprotonations. Several molecules containing highly acidic hydrogens were activated under base-

free conditions. In 2009, Maruoka *et al.* reported the conjugate addition of 3-aryloxindoles to β-nitrostyrene catalyzed by quaternary ammonium salts in the absence of base. The reaction only required the use of catalytic amounts of a quaternary ammonium salt to promote the addition reaction. The formation of ion pairs between enolizable 3-aryloxindoles and quaternary ammonium salts was believed to be involved in the reaction mechanism.[46] In a similar manner, in January 2012 Maruoka *et al.* reported the reaction between α-substituted nitroacetates and formaldehyde catalyzed under base-free neutral conditions. Similarly to the previous work, Maruoka and co-workers were able to activate nitroacetate species by the use of catalytic amounts of a chiral quaternary ammonium salt acting as a phase-transfer catalyst.[47]

In our pursuit for an unprecedented base and metal-free decarboxylative aldol condensation, we thought that the presence of quaternary ammonium salts in a water-rich biphasic solvent could be a convenient method for the activation of MAHOs and MAHTs. Similarly to Maruoka's work, we thought the formation of ion pairs between malonates and quaternary ammonium salts, such as tetra-*n*-butylammonium bromide (TBAB), might favour the formation of malonyl enols and catalyse decarboxylative aldol condensations in the absence of base.

Preliminary studies of the decarboxylative aldol condensation between 3-oxo-3-phenoxypropanoic acid and *p*-nitrobenzaldehyde were carried out by reacting an equimolar mixture of the MAHO and the aldehyde in a biphasic solvent mixture water/toluene 50:50 v/v (scheme 2.7). One equivalent of tetra-*n*-butylammonium bromide was employed in an attempt to promote the reaction. The resulting reaction mixture was vigorously stirred at 40 ºC for 24 hours. After this time, the mixture was extracted with dichloromethane, and after drying over magnesium sulfate, the solvent was removed to afford a crude product. The ^{1}H-NMR spectrum of the crude product showed the presence of unreacted starting material and the desired product of aldol condensation **62**. The crude product was purified by column chromatography on silica gel (neat dichloromethane) and the desired product was obtained as a white solid in a modest 27% yield. ^{1}H-NMR, ^{13}C-NMR and mass spectrometry confirmed the white solid to be the desired product of aldol condensation.



Scheme 2.7 Decarboxylative aldol condensation between 3-oxo-3-phenoxypropanoic acid and *p*-nitrobenzaldehyde catalysed by TBAB.

At that point, we were delighted at being able to carry out an unprecedented decarboxylative aldol condensation under metal and base-free conditions. Although the reaction yield was only 27%, we were interested in the study of the reaction between *para*-nitrobenzaldehyde and other MAHOs and MAHTs previously synthesised.

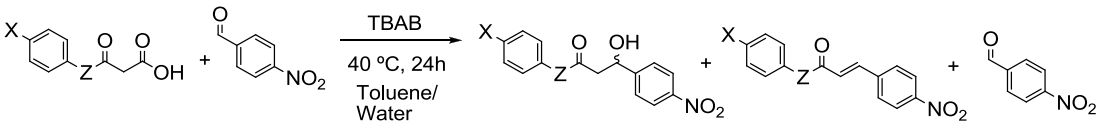
In a second set of experiments, we decided to carry out the reaction between *p*-chlorothiophenol half malonic acid and 4-nitrobenzaldehyde under the same reaction conditions. After 24 hours of reaction, TLC analysis showed that most of the starting material was consumed and that a new major compound was formed. After standard work up, ^{1}H-NMR of the crude product showed the desired product to be the majority component in the crude mixture (86% yield, calculated by peak integration). The same reaction did not work in the absence of TBAB or water.

Encouraged by this excellent result, we were interested in finding whether a correlation between the rate constants obtained from hydrogen-deuterium exchange experiments and the reactivity of malonates in this type of base-free decarboxylative aldol condensation could be established.

In a third set of experiments, we decided to carry out aldol condensations between 4-nitrobenzaldehyde and different MAHOs and MAHTs in order to study the connection between observed rate constants and reactivity of malonates. The reactions were performed using an equimolar mixture of malonate, aldehyde and a quaternary ammonium salt in a biphasic mixture water/toluene 50:50 v/v, at 40 ºC. The reaction mixtures were stirred for 24 hours at this temperature and after that time, the crude mixtures were extracted with dichloromethane and dried over magnesium sulphate. Finally, the solvent was removed under reduced pressure to afford the crude products that were analysed by ^{1}H-NMR spectroscopy. ^{1}H-NMR spectrum

allowed the identification of two new products in the crude product and also showed the presence of unreacted 4-nitrobenzaldehyde. The proportion of each product was estimated by integration of representative peaks, using the starting aldehyde as the limitating agent in the reaction. The results obtained for each MAHO and MAHT reacted are summarised in the table 2.2.

Table 2.2 Products ratio after base-free decarboxylative aldol condensation between malonates and 4-nitrobenzaldehyde.



Z =	X =	Hydroxyester	Elimination	Starting Aldehyde
O	OMe	27%	53%	20%
O	H	44%	29%	27%
O	Cl	42%	29%	29%
O	CF_3	35%	20%	45%
S	OMe	63%	7%	30%
S	H	74%	3%	23%
S	Cl	84%	2%	14%
S	CF_3	75%	2%	23%

In first place, we were delighted to observe that all the malonates tested were able to undergo decarboxylative aldol condensation. Secondly, we were gladly surprised about the relatively high reaction rate observed under these new mild reaction conditions. High conversions to the products were achieved after 24 hours of reaction. Thirdly, the presence of elimination product in the reaction mixtures was much lower in the case of thioesters. This fact could be explained by the influence that the thioester function has in the rest of the molecule. This influence was also observed during the H-D exchange experiments, where half malonic acid thioesters showed higher observed rate constants. The experiments showed that a change in the malonyl carrier can improve the selectivity of the reaction. Thus, the aldol reactions were in

general very selective to the β-hydroxythioester product when thiophenols were attached to the malonyl group (table 2.2).

After these experiments, we were quite confident of the achievement of the first example of decarboxylative aldol condensation under base and metal-free conditions. However, we were also concerned about the possibility that traces of amine in the commercially available quaternary ammonium salt could be actually acting as the catalyst in the aldol condensation. The quaternary ammonium salt employed during these bio-inspired experiments was tetra-*n*-butylammonium bromide 99.9% assay supplied by Sigma-Aldrich®. The material data sheet from this supplier stated that this quaternary ammonium salt may contain small amounts of tributylamine. Small amounts or even traces of amine in the reaction mixture could be acting as the true catalyst in our decarboxylative aldol experiments. We found it incredibly difficult to find a suitable method to remove the last traces of amine from the starting quaternary ammonium salt. We thought about the possibility of carrying out a recrystallisation of the quaternary ammonium salt in a mixture of organic solvents. However, even if we were able to obtain a very pure sample of quaternary ammonium salt free from amines by recrystallisation, the lack of a suitable analytical technique to detect traces of amine in the product seemed to make this methodology unfeasible.

Rather than questioning the presence of tributylamine in the ammonium salt as the likely catalyst in the reaction, we proposed a theoretical mechanism to explain the activation of malonates by TBAB. In that mechanism, the formation of malonyl enols would be favoured by the formation of ion pairs between MAHTs and TBAB. Malonyl enols would then react with aldehydes to afford β-hydroxythioesters and tetra-*n*-butylammonium hydroxide. TBAB would be regenerated in the aqueous phase after the reaction of tetra-*n*-butylammonium hydroxide with traces of previously formed hydrobromic acid (figure 2.1). As proving the absence of tertiary amines in commercial quaternary ammonium salts was not possible, we thought about the possibility of developing a similar catalytic system, based on the same principle, which avoided the use of quaternary ammonium salts.

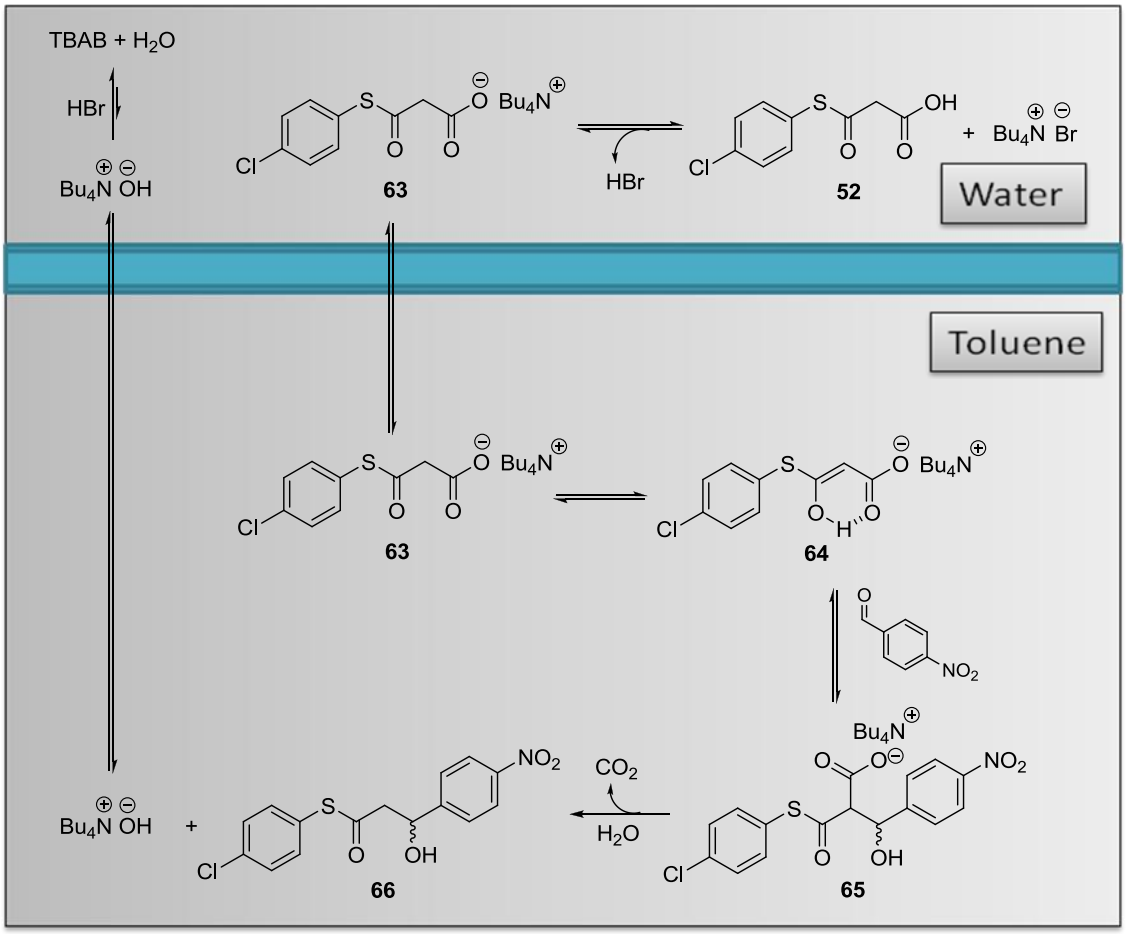


Figure 2.1 Proposed mechanism for the activation of MAHTs under base and metal-free conditions.

The new catalytic system needed to have the ability to form lipophilic ion pairs in an efficient manner. A suitable catalyst should be able to bind malonates and to transport them to the organic phase to assist during enolization and decarboxylative aldol condensation, in a similar way to the tetra-*n*-butylammonium cation. Therefore, the catalyst we were looking for was a substance able to behave as an ionophore.

An ionophore is a natural or artificial substance capable of complexing cations in a way that makes the whole complex that is formed soluble in hydrophobic media like organic solvents or the lipidic membranes in cells. The positive charge associated with these complexes is compensated by the counteranion which can promote different properties (figure 2.2).

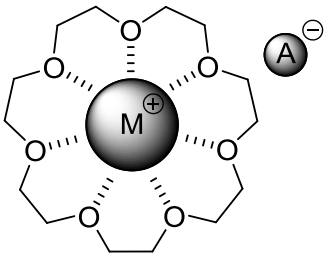


Figure 2.2 Schematic figure of ionophores based on crown ethers.

When an ionophore is solubilised in a biphasic mixture formed by an aqueous and an organic solution, these substances are able to transport specific cations from the aqueous phase to the hydrophobic phase. The complex cation-ionophore and the counteranion (that compensates the charge of the complex) can be solubilised in the organic phase. The hydrophobic structure of ionophores allows the complexation of cations and provides an effective hydrophobic cover for metals that can be solubilised in organic solvents and that can travel through lipidic membranes in cells.[48]

In a similar way to ionophores, quaternary ammonium salts also present a positively charged core, represented by a nitrogen atom, which is surrounded by hydrophobic aliphatic chains, increasing the solubility of the charged molecule and allowing their solubility in highly hydrophobic organic solvents. As ionophores, quaternary ammonium salts are also able to transport counteranions into hydrophobic phases.

Ionophores are, hence, a type of metal-binding compound that can be obtained either from natural sources or by synthetic methods. In biology, there are three classes of metal-binding compounds:

- Metal chelators.
- Metal shuttles.
- Metal ionophores.

Some examples of natural ionophores binding metals are dithiocarbamates, which can bind zinc and copper, and antibiotics like monensin (binding Pb), zincophorin (Zn) or valinomycin (K).[48] On the other hand, within the group of ionophores obtained by artificial means, the group formed

by macrocyclic polyethers, known as crown ethers, stands out. Calixarenes have also been employed as a new type of macrocyclic ionophore over the past decade.[49]

2.2.2 Alternative catalytic systems for base and metal-free decarboxylative aldol condensations.

The most common synthetic ionophores are crown ethers. Crown ethers are organic macrocycles able to transport ions from an aqueous phase to a hydrophobic phase. Many different crown ethers were synthesised in the past decades and their properties were widely reported in the literature (figure 2.3).[50]

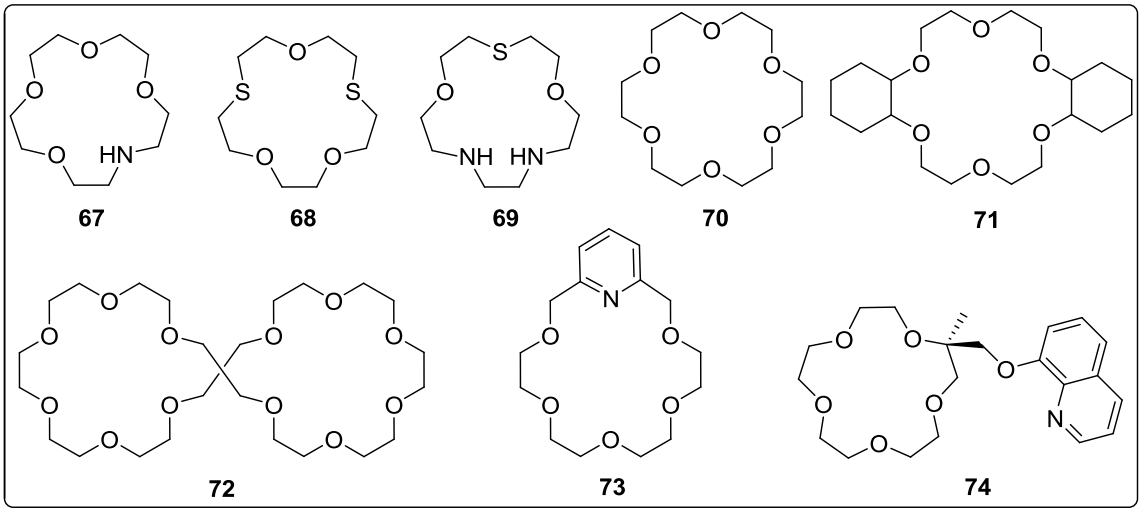


Figure 2.3 Examples of crown ethers reported in the literature.[50]

Since Pedersen *et al.* reported the first example of a crown ether in 1967,[51] it has been a great development in the synthesis of this type of macrocycle with the capacity to complex specific cations. Some of these macrocycles, like for instance Lariat ethers, were succesfully used as synthetic ionophores for many years. Although the vast majority of work involving crown ethers was related to the complexation of metals as the guest species, the complexation of ammonium cations and neutral molecules by crown ethers were reported in the literature. Many examples of crown ethers were successfully used as ion sensors due to their ability of changing the color of the solution when the macrocycle binds a specific metal. The applications of crown

ethers go from biological applications, acting as membrane amphiphiles or ion channels, to other applications like sensor chemistry or electrochemistry.[52]

With the definition of ionophore in mind, we thought about the possibility of using crown ethers or other ionophores as equivalent substitutes for quaternary ammonium salts during the catalysis of decarboxylative aldol condensations, acting as an ion transporter of cations and malonates into the organic phase.

At the same time we were looking for suitable ionophores to catalyse decarboxylative aldol condensations, Maruoka *et al.* reported the use of crown ether derivatives to carry out the conjugate additions of β-ketoesters to β-nitrostyrene.[53]

In Maruoka's work, a similar mechanism to the mechanism we postulated for the catalysis of base and metal-free decarboxylative aldol condensations by quaternary ammonium salts was proposed. In the proposed mechanism, the transference of an ion pair potassium-enolate to the organic phase was catalysed by the action of a crown ether and allowed the conjugate addition of β-ketoesters to β-nitrostyrene. Similarly to Maruoka's mechanism, we proposed a mechanism for the decarboxylative aldol condensation between MAHTs and aldehydes, catalyzed by the crown ether 18-crown-6. The proposed mechanim is shown in the figure 2.4.

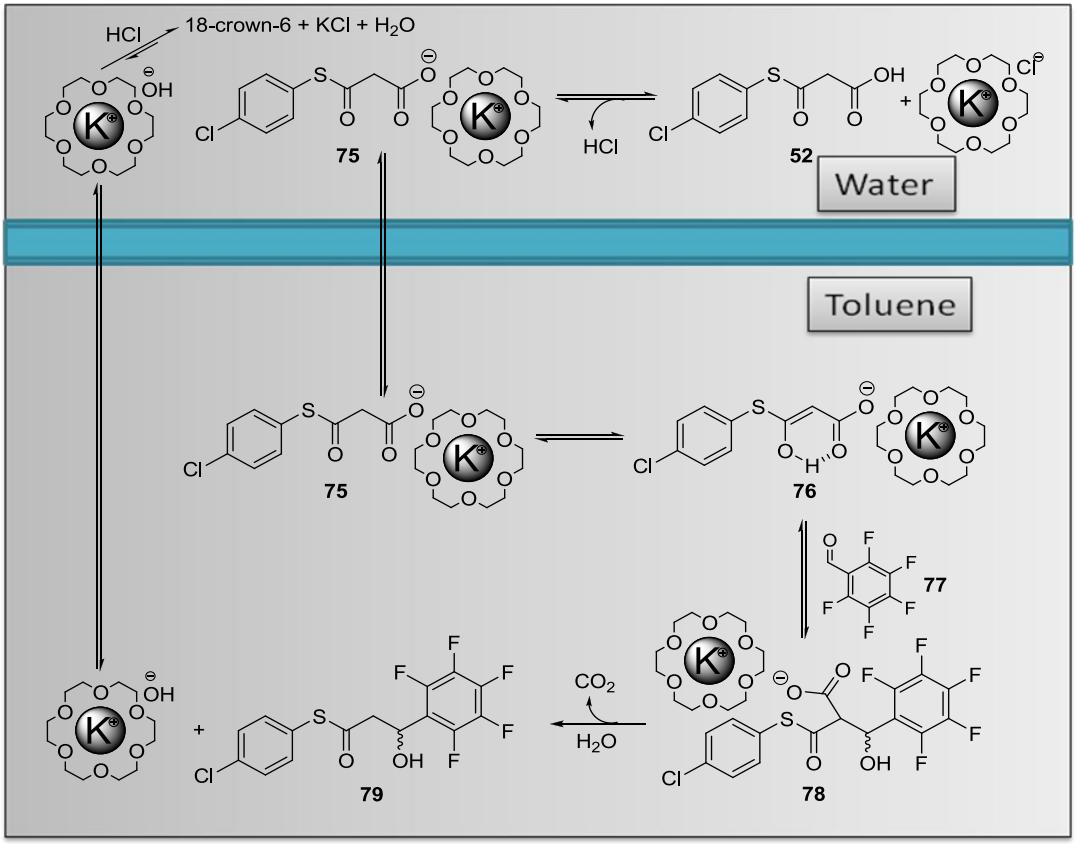
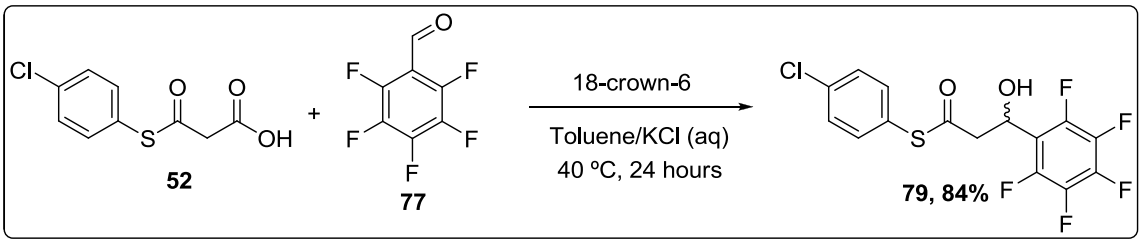


Figure 2.4 Proposed mechanism for the decarboxylative aldol condensation between MAHTs and pentafluorobenzaldehyde catalyzed by 18-crown-6.

Based on the theoretical mechanism, we decided to react *p*-chlorothiophenyl half malonic acid and pentafluorobenzaldehyde, but this time, using a catalytic system formed by potassium chloride and a crown ether. Equimolar amounts of MAHT and aldehyde were dissolved in a biphasic solvent mixture formed by toluene and a potassium chloride saturated aqueous solution. 18-Crown-6 (30% mol) was added to the mixure and the resulting solution was vigorously stirred for 24 hours at 40 ºC. After work up and purification by column chromatography, the desired aldol product **79** was obtained in a good 84% isolated yield (scheme 2.8). Similarly to the catalysis by quaternary ammonium salts, the reaction did not proceed when water, crown ether or potassium chloride were not added to the reaction mixture.



Scheme 2.8 Decarboxylative aldol condensation catalysed by a crown ether and KCl (aq).

With this experiment, we indirectly proved that a base-free catalysis using quaternary ammonium salts was in principle possible and, therefore, that the catalysis should also be able to be promoted by the use of TBAB in biphasic solvent mixtures, even in the absence of traces of tertiary amines.

We wanted to take these experiments further, so we thought about the possibility of carrying out a regioselective version of this new decarboxylative aldol reaction. We also thought of using a chiral ionophore to catalyze this type of reaction. We checked the properties of different chiral ionophores in the literature and finally, we decided that the chiral antibiotic valinomycin could be a good candidate for our purpose, mainly due to its great ability to coordinate alkali metals like potassium. Valinomycin (figure 2.5) is a biomolecule with a strong biological activity mimicing processes that are normally carried out by proteins, like the transport of cations across the cell membranes. An undesired transport of cations is normally poisonous for the cells. The main function of valinomycin is acting as an ion-carrier (antibiotic ion-carrier). The reason for the valinomycin activity is its ability to complex potassium cations and to create species with a hydrophobic external cover that allows the transport of potassium across lipidic membranes.[54]

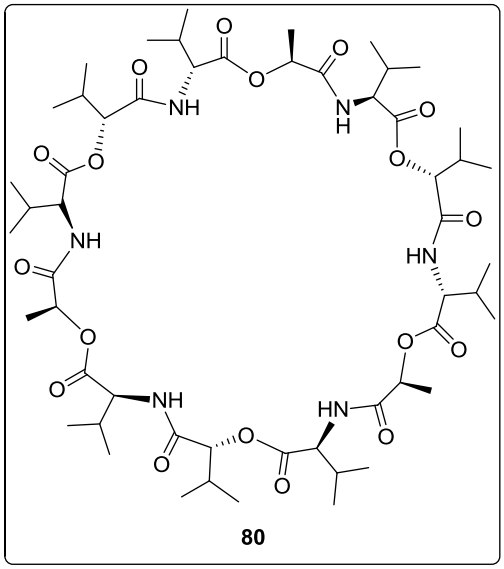
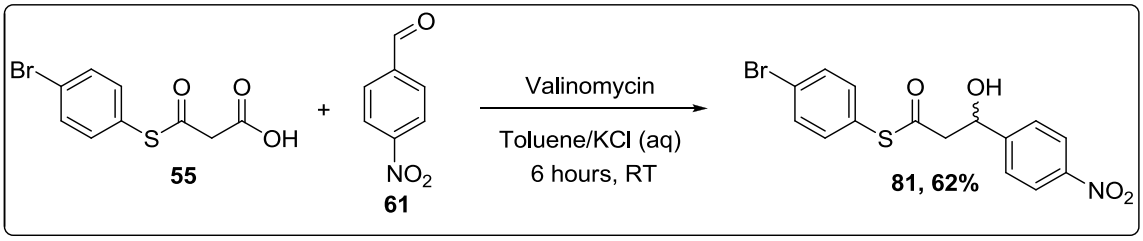


Figure 2.5 Structure of the antibiotic valinomycin.[54]

The reaction using valinomycin as ionophore proceeded with a very good yield (62% isolated product after six hours of reaction) and represented the first example in the literature of a decarboxylative aldol condensation catalyzed by an antibiotic (scheme 2.9).



Scheme 2.9 Decarboxylative aldol condensation catalysed by the antibiotic valinomycin.

The mechanism operating in this case must be very similar to the mechanism operating through the action of crown ethers. As a good potassium chelator, valinomycin should be able to transport potassium and the corresponding counteranion, malonate, to the organic phase. The formation of a malonyl enol and its reaction with *p*-nitrobenzaldehyde, would lead to the formation of β-hydroxy thioesters (figure 2.6). No enantiomeric excess could be observed in the product by measuring the specific rotation. Even though valinomycin presents a chiral structure, the resulting complex with potassium does not behave as a chiral counter cation for malonates. When valinomycin complexes potassium, the macrocycle folds in such a manner that an essentially non-chiral spherical complex is obtained, losing any capacity to induce stereoselectivity.[55]

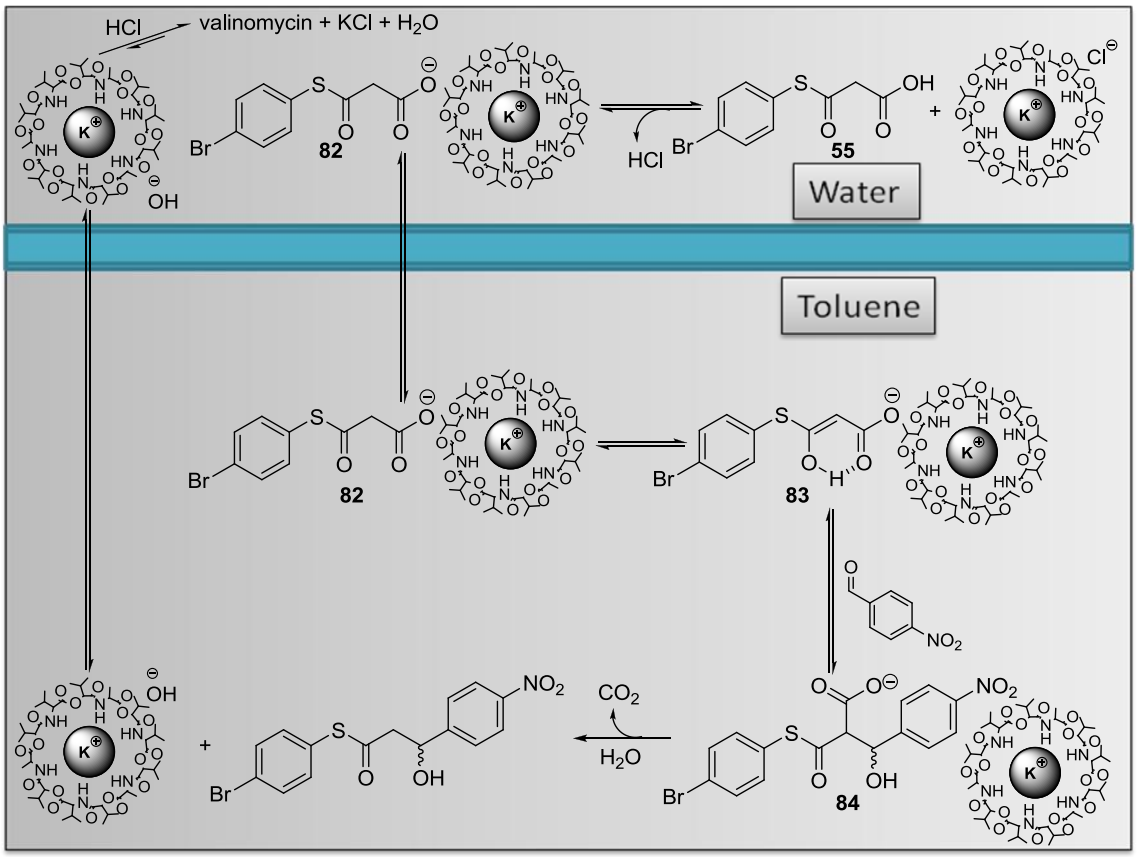


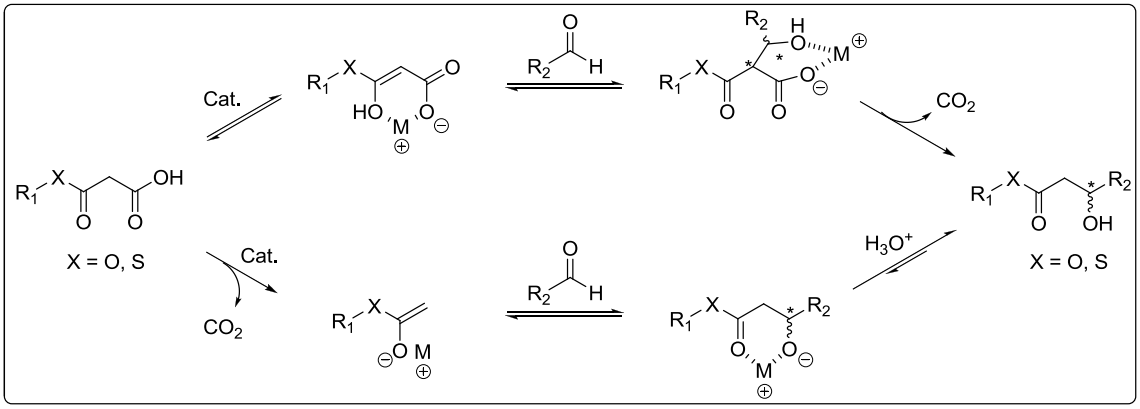
Figure 2.6 Proposed mechanism for the decarboxylative aldol condensation catalysed by the antibiotic valinomycin.

In summary, we were able to develop three different catalytic systems for carrying out the first example of metal and base-free decarboxylative aldol condensation between malonates and aldehydes. The three possible catalytic systems are:

- TBAB in water / toluene.
- Saturated KCl aqueous solution / toluene / crown ether.
- Saturated KCl aqueous solution / toluene / valinomycin.

2.2.3 Mechanism and scope of the reaction.

The reaction mechanism of base catalysed aldol-type condensations involves the deprotonation of α-hydrogens to generate enolate species that can react with different electrophiles. Strong bases and dry aprotic solvents are normally required in this type of reactions. However, the use of strong bases prevents the use of some important functional groups incompatible with the presence of strong bases. During the decarboxylative aldol reaction of malonates, the presence of a carboxylic group attached to the α-position favours the deprotonation of malonates and the formation of enolate esters. The great ability of MAHOs and MAHTs to undergo decarboxylative aldol condensation resides in two fundamental features. First, the presence of a carboxylic group attached in the alpha position weakens the bonds between the α-hydrogens and the carbon, increasing the acidity of those hydrogens and making more accessible the formation of an enolate ester. Moreover, after deprotonation of malonate, the formed carbanion is stabilized by both inductive and hyperconjugative effects in the molecule. Secondly, the presence of a carboxylic group in the molecule allows the reaction to be driven by the loss of carbon dioxide during the process. Although the mechanism for decarboxylative aldol condensations is well known, it is not clear whether decarboxylation takes place before or after the addition to the electrophile species, and both mechanisms have to be taken into account when new conditions for decarboxylative adol condensation are employed. Therefore, decarboxylative aldol condensation can follow two different mechanistic pathways (scheme 2.10).



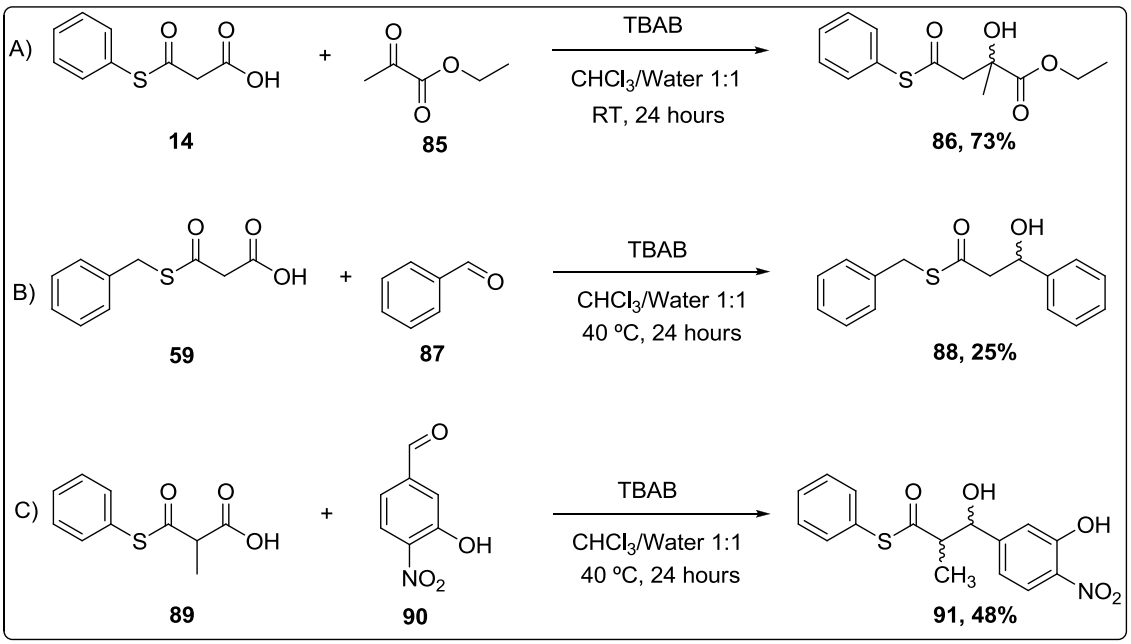
Scheme 2.10 Mechanistic pathways in decarboxylative aldol condensations.

In the case of our newly developed base-free decarboxylative aldol condensation conditions, the fact that decarboxylated malonate was not observed by ^{1}H-NMR spectroscopy during the reaction, suggests that the mechanism operating in this case must go through the formation of a tertiary carbon center previous to the decarboxylation (upper pathway).

The mechanism for the activation of malonates we propose is in agreement with the idea that acidic MAHOs and MAHTs can form lipophilic ion pairs with quaternary ammonium salts and potassium complexes. The formed lipophilic malonates in the organic phase can afford malonyl enols that can react with electrophiles present in the reaction media, such as ketones or aldehydes.

In order to study the scope of the reaction, different electrophiles were used in this type of decarboxylative aldol condensation and the results obtained, were compared with data gathered from the same reaction reported in the literature, carried out under basic catalysis (see scheme 2.11). When 3-oxo-3-phenylpropanoic acid was reacted with ethyl pyruvate in a biphasic mixture chloroform/water 50/50 v/v and in the presence of one equivalent of TBAB used as catalyst, the desired product was obtained in a 73% yield. The same reaction was reported in the literature under basic catalysis affording the desired product in 70% yield.[24] 3-(Benzylmercapto)-3-oxopropanoic acid was reacted with benzaldehyde in similar conditions to afford the desired beta-hydroxythioester product in a low 25% yield, showing the catalysis of this reaction proceed very slowly when poor electrophiles were used. The same reaction reported in the literature, carried out under basic catalysis and in the presence of copper (II), afforded the condensation product in a lower 22% yield.[56] Finally, we also evaluated our base and metal-free protocol when the aldehyde was bearing an unprotected hydroxyl group, which may potentially react with the formed thioester enol. 3-Hydroxy-4-nitrobenzaldehyde **90** was reacted with 3-(thiophenol)-3-oxo-2-methyl propanoic acid **89** to afford the desired mixture of diastereoisomers (scheme 2.11, c). Compound **91** had already been reported by Shair and co-workers in 2005,[37] and the absolute configuration of the secondary alcoholic stereogenic center was determined using the method developed by Kishi *et al.*[57] and based on ^{13}C-NMR experiments with $Pr(tfc)_3$. Thus, the *syn:anti* ratio was determined by ^{1}H-NMR analysis and found to be syn:anti 9/1. Very interestingly, when the ^{1}H-NMR spectrum for the mixture of diastereoisomers obtained through our TBAB-based method

was compared against ^{1}H-NMR data reported by Shair *et al.*, we found that the *syn:anti* ratio had inverted, leading to a moderate excess of *anti* diastereoisomers. The *syn:anti* ratio was calculated by ^{1}H-NMR analysis and found to be *syn:anti* 1/(3.6). Therefore, the mechanism operating under the influence of TBAB[58] afforded preferentially *anti* diastereoisomers, which is a relevant contribution to this type of aldol reaction and complements previous work reported in the literature.



Scheme 2.11 Aldol condensations catalysed by TBAB under base-free conditions and in the absence of metals.

2.3 Conclusions.

In conclusion, we have developed a simple and mild new method for the decarboxylative aldol-type condensation of malonates with aldehydes and ketones. A series of experiments show that the reaction can proceed without the presence of either base or metals, and can be promoted by substances such as tetra-*n*-butylammonium bromide, crown ethers or natural ionophores. This new methodology may be of a great interest in those areas of chemistry where substrates present functional groups that are incompatible with the presence of strong bases, as it is the case of biological and biochemical systems.

Interestingly, no self-condensation of MAHTs or MAOHs was observed during any of the aldol reactions performed so far. This might be due to the fact that thioesters or oxyesters are not electrophilic enough to react with the formed enols during the reaction. This explanation is in agreement with the fact that, non activated aldehydes, such as benzaldehyde, afforded very poor yields during metal and base-free decarboxylative aldol condensations.

In the last part of our research project, we were focused on the chemistry of calix[4]arenes as versatile cyclic scaffolds for the mimicry of enzymes and in the development of a bi-functional calix[4]arene capable to bond two subunits of malonic acid half thioester, to study synergistic effects in Claisen condensations and to mimic the condensing function of PKS.

- Chapter 3 -

Synthesis of multifunctional cyclic scaffolds and their applications in the mimicry of PKS.

3.1 Introduction to calixarenes as versatile scaffolds for the mimic of PKS.

3.1.1. The origin of calixarenes.[59]

In 1942, investigations in the field of phenol-formaldehyde chemistry carried out by Alois Zinke and Erich Ziegler led to the discovery of the first example of synthetic basket shaped molecules, known as calixarenes. The idea of protecting the para-position of phenols with alkyl groups during condensation reactions allowed Zinke and Ziegler the first preparation of a new class of synthetic macrocycles. The oligomerization of 4-*tert*-butylphenol produced a new class of basket-like molecules that further led on to a new area of chemistry based on calixarenes. Unlike cyclodextrins, that needed to be isolated from natural sources and whose production is expensive, or crown ethers which do not present a basket-like shape but a more discotic-like shape, the Zinke and Ziegler cyclic oligomers were the first example of artificial basket-like molecules prepared in the laboratory.

In 1970, Gutsche and co-workers, intrigued by the new advances in biochemistry and the mimicry of enzymes, decided to employ Zinke and Ziegler macrocycles as scaffolds in the mimicry of the active site of some enzymes. The unique shape of that new class of macrocycles made them very useful in the field of synthetic metalloenzymes and also in the field of molecular receptors. Moreover, calixarenes were presented as very versatile scaffolds for the synthesis of multifunctional macrocycles with potential applications in different disciplines of science.

The relevance of calixarenes as a new type of macrocycle was mainly based on the ease of their preparation and the great number of chemical modifications likely to occur in their structure depending on the nature of the substituents attached. One advantage of calixarenes is that most of them can be prepared in a multi-gram scale using straightforward one-pot protocols, following simple work up and purification methods. Moreover, these macrocycles can be prepared in different conformations that increase their versatility when used in many innovative applications such as host-guest supramolecular chemistry, smart catalysts or artificial sensors.

3.1.2. Definition and nomenclature.[59]

Calixarenes are organic macrocycles constituted by phenol subunits that are connected through the ortho positions by methylene bridges (figure 3.1). The name “calixarene” comes from the combination of the words “calix” (whose origin comes from “calix crater”, a type of Greek vase) and “arene”, making reference to the aromatic rings that form the macrocycle. The term “calixarene” was first established by Zinke and Ziegler in 1942 during their investigations in the phenol-formaldehyde chemistry.

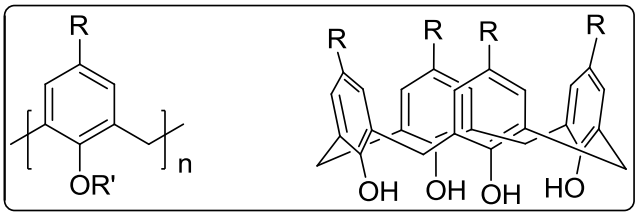


Figure 3.1 General structure of calixarenes (left) and structure of calix[4]arene.

In the systematic nomenclature, the size of the macrocycle is indicated by the number of phenol units in the structure included between the words *calix* and *arene* in brackets (calix[n]arene, where “n” is the number of phenol units in the macrocycle). The smallest calixarene presents three phenol units forming its structure but, calixarenes with over ten phenol units in their structure may also exist as an example of the larger calixarenes. There are different types of calixarenes depending on the size of the macrocycle (number of phenol units in the macrocycle), being 4, 6 and 8 the most thermodynamically stable and therefore, the most accessible calixarenes (table 3.1).

Table 3.1 Reaction yields of the one-pot syntheses of calix[n]arenes.

	Number of phenol rings in macrocycle				
Group in *para* position	**n = 4**	**5**	**6**	**7**	**8**
Me	-	-	-	22%	-
^{i}Pr	10%	-	26%	-	-
^{t}Bu	49%	10%	83%	6%	62%
Benzyl	-	33%	16%	-	12%

3.1.3. Calixarenes in the 21st century.

The number of publications based on calixarenes, regardless their applications, serves to illustrate the relevance of these cyclic oligomers within the past four decades.

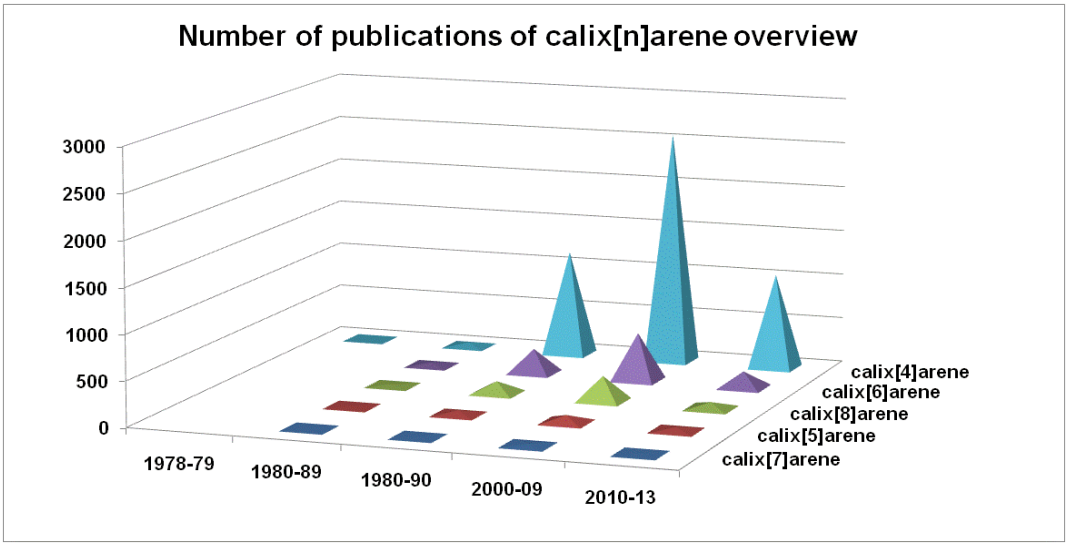


Figure 3.2 Number of publications related to calix[n]arene within the last five decades (*n*= number of aryl moieties participating in the cyclic oligomer).

By the end of 1990s, the number of publications related to calixarenes experienced a significant growth, reaching a considerable number of publications within the first decade of 21st century. Among the number of publications of calix[n]arenes, publications of calix[4]arenes are predominant compared to those calixarenes formed with more than four aryl moieties in their cyclic oligomeric structure (figure 3.2).

A combination of several factors explain why the number of calix[4]arene publications dominate over the number of publications of calix[6]arenes, calix[8]arenes and some other calix[n]arenes (figure 3.3). Calix[4]arenes have a great stability and are easily functionalized. Both advantages, combined with their relative rigidity and their conformational control, have made calix[4]arenes very versatile molecules.

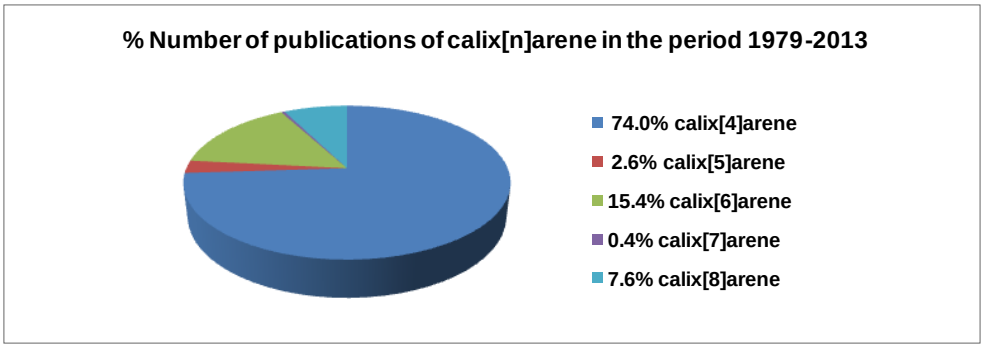


Figure 3.3 Number of publications of calixa[n]arene in percentage within the last four decades.

The vast majority of chemistry developed on calix[4]arenes corresponds to the *cone* conformation with tetra-functionalised and 1,3-difunctionalised upper rim respectively. The ease of synthesis and conformational control as well as their applications in coordination chemistry as complexing agents, have made calix[4]arenes the most explored macrocycles. However, the number of references relating to the preparation of 1,2-difunctionalised and monofunctionalised calix[4]arenes is much less abundant. Thus, a relative small number of synthetic protocols can be found in the vast literature of calix[4]arenes. In addition, most of the protocols found in the literature, either describe the synthesis of monosubstituted calix[4]arenes from the mono-halogenated calix[4]arene, treated with a slight excess of *n*-butyllithium,[60] or involve the use of hazardous substances like titanium(IV) chloride.[61] Due to the lack of control of the halogenation selectivity, mono-halogenation is considered as a low to moderate conversion process. Moreover, the isolation of mono-halogenated calix[4]arene is a very ardous process that affords low yields. Therefore, despite the major advances made in the calix[4]arenes chemistry within the past years, there are still few areas to develop such as finding more selective synthetic approaches as well as processes involving a greener chemistry. With regards to calix[4]arenes applications, there is further scope in areas such as host-guest interaction, chiral calixarenes and molecular machines.

3.1.4. Structure of calix[4]arenes.[59]

Three different regions can be differentiated in the structure of calixarenes:

- Lower rim, constituted by the phenolic hydroxyl groups.

- Methylene bridges, working as linkers between phenol moieties.
- The upper rim, which can be functionalized by the insertion of several functional groups (figure 3.4).

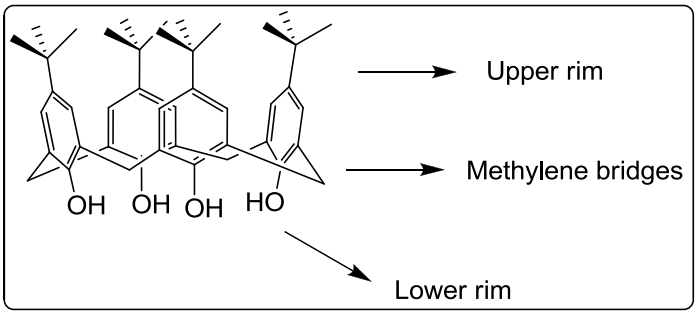


Figure 3.4 Functionalisable regions in calix[4]arenes.

Locking a conformation in place, as a single conformation, allows tuning of the reactivity and the properties of calix[4]arenes. Due to the free rotation between aromatic rings and methylene bridges in the macrocycle, calixarenes with free hydroxyl groups can adopt several different conformations, affecting their supramolecular and physical properties. Once the macrocyle is synthezised, different methods are employed to lock the structure in a single conformation such as, the insertion of bulky aliphatic or aromatic groups in the lower rim or, some more sophisticated methods introducing trans-annular bridges in the lower rim giving more rigid structures.

The number of conformations calixarenes can adopt depend on the size of macrocycle, type of groups attached in the lower rim and functionalities installed in the upper rim. Thus, the most common calixarene, calix[4]arene, can adopt up to four different conformations (figure 3.5).

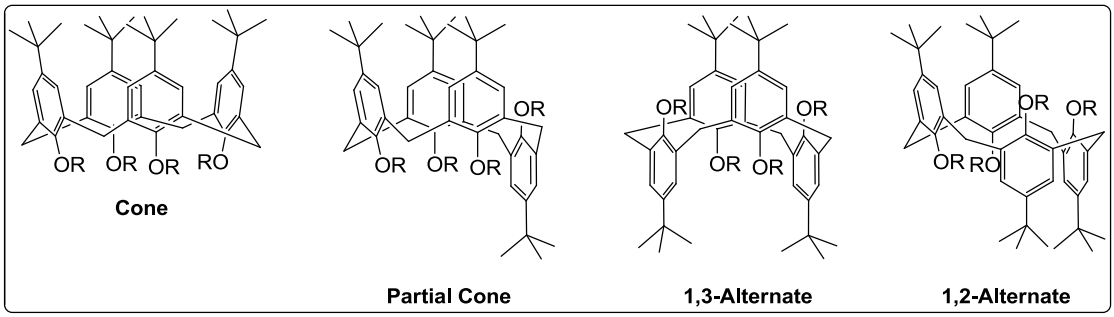


Figure 3.5 Different conformations adopted by calix[4]arene.

A simple method to determine the conformation adopted by calix[4]arenes has been well established in the literature.[62] The method is based on ^{13}C-NMR spectroscopy and allows the determination of the conformation in just a few minutes. De Mendoza and co-workers published the correlation found between the ^{13}C-NMR chemical shift of the methylene bridges and the orientation of the two phenol units attached to them for a series of different calix[4]arenes. The ^{13}C-NMR chemical shifts of the methylene bridges followed a clear trend with the neighbour phenols and their relative orientation. It was found that, when a methylene group was surrounded by two phenol moieties adopting a *syn* conformation, the ^{13}C-NMR chemical shift for that methylene bridge was always close to 31 ppm. However, when the neighbour phenol rings were pointing out in opposite directions, adopting an *anti* conformation, the ^{13}C-NMR chemical shift was always shifted downfield near to 37 ppm. The differences in the ^{13}C-NMR chemical shifts observed are believed to be due to steric factors. In the table 3.2, the ^{13}C-NMR chemical shifts of the methylene bridges for different calix[4]arenes are shown, as well as the conformations that can be inferred from the ^{13}C-NMR chemical shifts.

Table 3.2 Determination of the conformation of calix[4]arenes by ^{13}C-NMR chemical shift of the methylene bridges (R indicates the type of group attached in the lower rim and R' the group in the upper rim).

R	R'	Aromatic carbons	Ar_2CH_2 shift	Conformation
H	H	4	31.6	*cone*
H	Cl	4	30.7	*cone*
$COCH_3$	H	12	37.4, 30.7	*Partial cone*
$COCH_3$	^{t}Bu	4	38.2	*1,3-alternate*
COC_6H_5	^{t}Bu	4	30.2	*cone*

In the case of small calixarenes (four or five phenol moieties in the macrocycle), the conformation can be locked by the simple insertion of bulky groups or long aliphatic chains in the lower rim. The presence of bulky groups in the lower rim prevents the rotation of phenol rings through the macrocycle cavity and avoids the equilibrium between different conformations.

As discussed above, calixarenes presenting free hydroxyl groups in the lower rim can adopt different conformations in solution. In moderate and non polar solvents, calix[4]arene adopts a perfect *cone* conformation due to the hydrogen bond interaction between hydroxyl groups that stabilizes the structure. In this type of solvent, the ^{1}H-NMR spectrum of a calix[4]arene shows a

singlet peak with chemical shift between 8 and 10 ppm, for which the integral corresponds to four protons, confirming the equivalence of the hydroxyl groups in the lower rim as well as the symmetry of the molecule in the *cone* conformation. More polar solvents such as DMF or DMSO tend to interfere with the hydrogen bonding in the lower rim, disturbing the *cone* conformation. The *cone* conformation in calix[4]arenes can be locked by relatively simple methods. The treatment of calix[4]arene with sodium hydride followed by addition of 1-iodopropane allows the insertion of propyl chains in the lower rim blocking the conformation. The insertion of shorter aliphatic chains in the lower rim, like methyl or ethyl groups, does not provide enough bulkiness to avoid the free rotation of the phenol rings and therefore does not stop the equilibrium between conformations.

However, *O*-alkylated calix[4]arenes locked in the cone conformation, do not present a perfect basket shape. The impossibility of hydrogen bonding occurring in the lower rim, as a consequence of the *O*-substitution, obliges calix[4]arenes to adopt a more stable flattened *cone* conformation where two of the phenol moieties are facing parallel to each other with the other two pointing out of the cavity. This conformation is known as the *pinched cone* conformation and has an important role in the supramolecular properties of calix[4]arenes (figure 3.6).

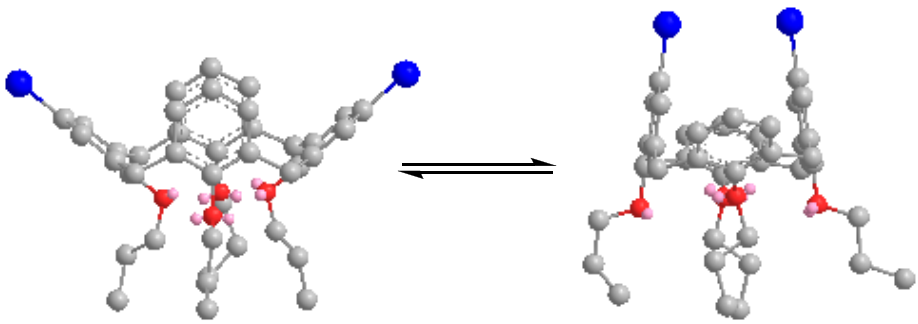
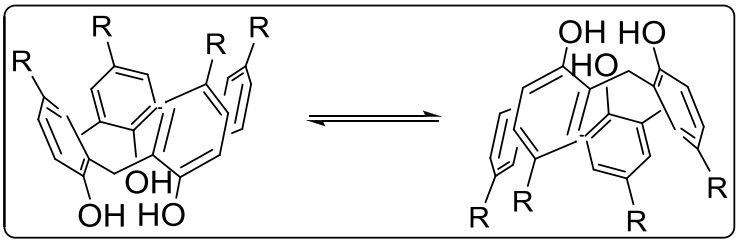


Figure 3.6 Equilibrium between two possible *pinched cone* conformations for tetrapropoxycalix[4]arene.

The preferred pinched cone conformation of *O*-substituted calix[4]arenes reduces the space available in the calixarene cavity and therefore, the capacity of calix[4]arenes to accept small molecules in their cavity. As a consequence, very few small molecules or group of atoms can be accommodated inside the cavity. Thus, the resulting host-guest complexes tend not to be very

stable, mainly due to the poor rigidity of the macrocyle with a constant conformational equilibrium between the two possible pinched *cone* conformations

In 25,26,27,28-tetrahydroxycalix[4]arene, geminal protons of the methylene bridges are not equivalent. Therefore, two doublets with integrals corresponding to four protons each are found in the ^{1}H-NMR spectrum. However, when the temperature is high enough, the interconversion between the two possible *cone* conformations makes the geminal protons equivalent and therefore a singlet peak, with a chemical shift between 3 and 4 ppm and integrating for eight protons, is obtained (scheme 3.1).



Scheme 3.1 Equilibrium between two equivalent *cone* conformations in 25,26,27,28-tetrahydroxycalix[4]arene.

At room temperature or lower temperatures, the typical signal for the hydrogens of the methylene bridges is a pair of doublets with ^{1}H-NMR chemical shifts laying between 3 and 5 ppm. The measured coupling constant for these doublets is 12-14 Hz, typical coupling values for geminal protons. Table 3.3 below shows the required rotation energy for the phenol moieties to rotate through the cavity of different calixarenes. The inversion energy has been calculated from NMR experiments carried out at different temperatures. From these experiments it can be concluded that the energy barrier is almost independent of the type of group attached to the *para* position of the aryl moieties, and also that the speed of interconversion greatly depends on the stablilty of the hydrogen bonds formed in the lower rim. When hydrogen bonds are disturbed in the presence of very polar solvents, the speed of interconversion increases and the energy barrier decreases.

Another interesting feature is that the protons of the hydroxyl groups in the lower rim are more acidic than the proton in monomeric phenol. This is also due to the hydrogen bonds occurring between the hydroxyl groups that weaken the hydrogen-oxygen bond, making those protons more labile.

Table 3.3 Energy barriers for the ring inversion of calix[n]arenes (R indicates the group attached in the upper rim and G_2-G_1 represents the Gibbs free energy of the transition state).

	$CDCl_3$		Py-d[5]	
n / R	**G_2-G_1/ Kcal/mol**	**T_c / K**	**G_2-G_1 / Kcal/mol**	**T_c / K**
4 / H	14.9	309	11.8	251
4 / *t*-Butyl	15.7	325	13.7	288
4 / C_6H_5	15.3	317	12.8	271
4 / *t*-Octyl	14.6	303	12.4	260
6 / *t*-Butyl	13.3	284	9.0	219
8 / *t*-Butyl	15.7	326	<9.0	<183

Melting points are normally high for calixarenes, especially for those where the hydroxyl groups in the lower rim are not substituted. In these cases, melting points are normally above 250 ºC. Some examples are *p-tert*-butylcalix[4]arene melting at 342-344 ºC, *p-tert*-butylcalix[6]arene melting at 372-374 ºC and *p-tert*-butylcalix[8]arene melting at 418-420 ºC.

Different protocols allow the isolation of calix[4]arene in different conformations. The formation of a specific conformer during the synthesis of calix[4]arene depends on different variables such as the solvent, the rate of the reaction, or the type of base used. In general, the cone conformation is favoured by a template effect when small alkali cations (lithium, sodium, etc.) are present in the reaction mixture. Alkali cations are able to strongly coordinate the four oxygens present in the lower rim of calix[4]arenes, favouring the formation of cone conformations. Bigger alkali cations, as cesium or potassium, do not allow tetra-complexation and can only stabilise the "1,3-alternate" conformation and the "partial cone" conformation.

Locking a conformation in large calixarenes requires arduous synthetic protocols. A standard protocol is the insertion of transannular ethyleneglycol bridges in the lower rim in combination with the attachment of bulky groups to the remaining hydroxyl groups to avoid conformational interconversion. In these cases, the groups attached in the lower rim can be further modified by reactions such as hydrolysis, transesterification, aminolysis, reduction or oxidation.

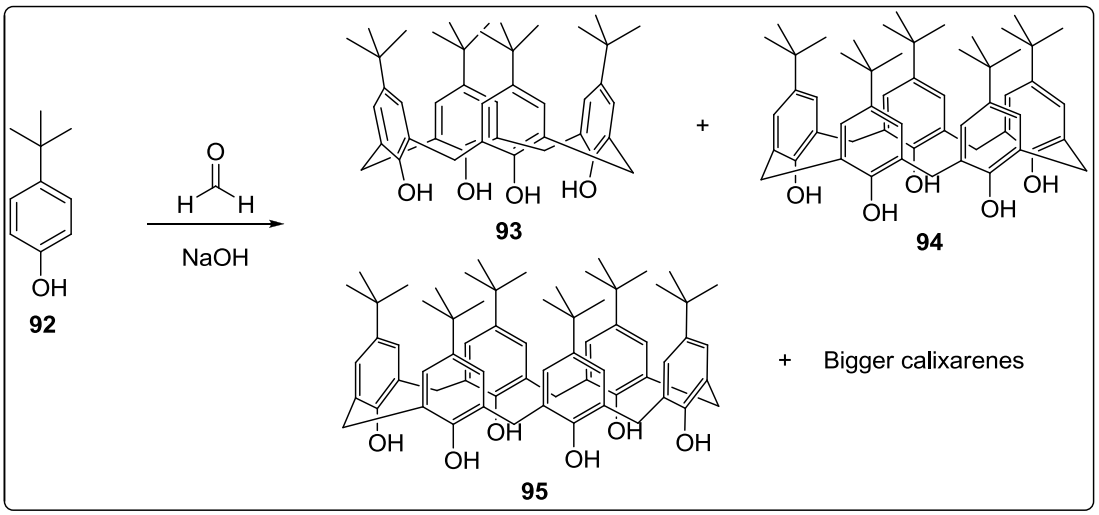
3.1.5. Synthesis of calixarenes.[59]

The synthesis of calixarenes can be performed by two different synthetic approaches. The most common method to synthesise calixarenes is the one-pot reaction of *para* substituted phenols and formaldehyde or paraformaldehyde. In this case, the oligomerization reaction of *para*

substituted phenols leads into a mixture of calixarenes with different sizes and the desired macrocycle is isolated from the reaction mixture generally purifying by recrystallization although column chromatography is also possible. The second approach involves a stepwise condensation of *para* substituted phenols. This method requires a higher number of synthetic and purification steps generally leading to lower overall yields. Although the second method is more arduous and time consuming, the step by step synthesis allows the preparation of calixarenes with different functionalities attached in the upper rim, being therefore a more versatile protocol. As starting materials, *ortho*-bromo *p*-alkylphenols are normally employed as monomers.

3.1.6.1 One-pot synthesis of calixarenes.

As mentioned above, the one-pot synthesis of calixarenes consists in the condensation of *p*-alkylated phenols and aqueous formaldehyde under basic catalysis where the cyclic oligomers are preferred over the lineal analogues. After removal of the water formed during the condensation step, the crude product obtained is formed by a mixture of different size calixarenes (scheme 3.2). The ratio of different calixarenes present in the reaction mixture will depend on the reaction conditions such as temperature, solvent, catalyst and *para*-alkylated phenol employed during the condensation. Calixarenes presenting an even number of phenol moieties forming the macrocycle can be obtained in high proportions and can be easily prepared in a multigram scale with moderate to good yields over 60%. The condensation reaction can be tuned in order to generate size-specific calixarenes and even odd calixarenes like calix[5]arene in a gram scale in the laboratory.



Scheme 3.2 One-pot synthesis of calixarenes from *para*-*tert*-butylphenol and formaldehyde.

Calixarenes containing four, six or eight phenol units forming the macrocycle have been normally found to be the major products in this kind of oligomerization. Long reaction times favour the formation of calix[4]arene as it is the more thermodynamically stable product. Thus, calix[4]arene can be obtained from a mixture of calix[6]arene and calix[8]arene by heating at high temperatures (above 200 ºC) in the presence of catalytic amounts of base. Calix[8]arene is the kinetic product. Large cyclic oligomers can be obtained when *para*-substituted phenols bearing large bulky groups are employed during the condensation reaction.

One inconvenience in the one-pot synthesis is the impossibility to obtain calixarenes bearing different groups or functionalities in the upper rim. This can only be achieved by following a multi-step or stepwise protocol.

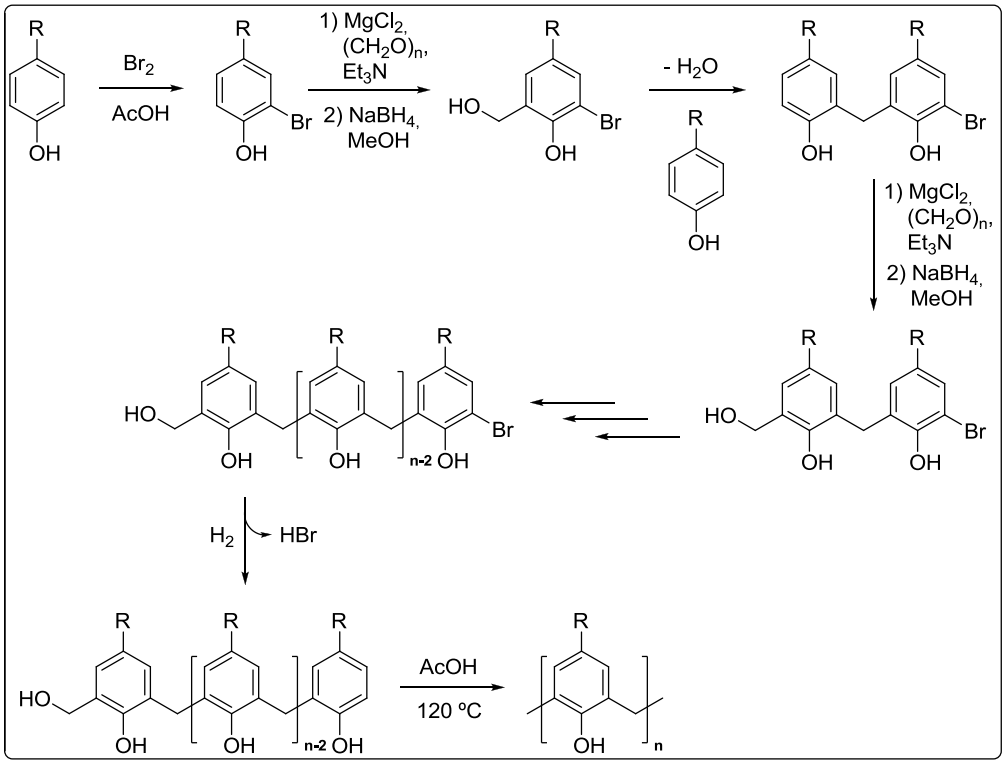
Interestingly, the one-pot condensation reaction between *para*-*n*-alkyl and *para*-phenyl substituted phenols and formaldehyde, under basic catalysis, has never been achieved. This fact has been explained by the low solubility of the formed intermediates, preventing the formation of cyclic oligomers.

3.1.6.2 Stepwise synthesis of calixarenes.[59]

The first stepwise synthetic approach for the preparation of calixarenes was developed by Hayes and Hunter in the 1950s and allowed the preparation of calixarenes with different groups

attached in the *para* position of the phenol units in the upper rim. *Ortho*-bromo *p*-alkylphenols were normally employed as starting material in this kind of condensation reactions.

The stepwise synthesis of calixarenes involved the preparation of linear oligomers, using *o*-bromo *p*-alkylphenol as monomer and the newly formed lineal oligomer could be cyclised in the final step (scheme 3.3).



Scheme 3.3 Stepwise synthesis of calixarenes.

The most frequent protocol employed in the formation of phenol-based oligomers used the repetition of several hydroxymethylation-condensation steps. The cyclization step was carried out after dehalogenation of the linear oligomer and, in the presence of very low concentrations of oligomer to avoid intermolecular reactions.

Due to the high number of steps involved in the process, overall yields tend to be low, even when each synthetic step proceeded with moderate or good yield. As was mentioned before, this synthetic approach allowed the preparation of calixarenes with different substituents attached in the upper rim. However, due to the reaction conditions employed during the condensation of phenol units, groups attached in *para* position ought to be carefully selected to avoid undesired reactions.

3.1.6. Reactivity of calix[4]arenes.[59]

Three different regions, lower rim, upper rim and methylene bridges, define the reactivity of calix[4]arenes. Calix[4]arenes obtained from the condensation of *p*-substituted phenols in the one-pot reaction, can be mainly modified in two different regions. The simplest and most common approach to obtain calix[4]arene derivatives, is the alkylation of hydroxyl groups in the lower rim. This can be easily achieved by esterification, sulfonylation or nuceophilic substitution, after the base mediated deprotonation of the hydroxyl groups.

Another alternative, is the functionalization of the upper rim after de-alkylation of calix[4]arenes. This functionalization is normally carried out by electrophilic aromatic substitution, allowing the insertion of several types of electrophiles in the upper rim like halogens, nitro groups or aminomethyl groups.[63] However, the lower rim is historically the most functionalized region in calix[4]arenes due to the presence of nucleophilic hydroxyl groups.

The methylene bridges represent the most difficult area to functionalize in calix[4]arenes and only a few examples of this type of derivatives are so far reported in the literature.[64] The method employed in calix[4]arenes generally consisted of the selective bromination of these positions with NBS to achieve the monobromination of each methylene bridge, followed by an interconversion of functional group such as nucleophilic substitution or Friedel-Crafts reaction.

In the chemistry of calix[4]arenes, selective reactions towards the synthesis of mono-, bis- or tri-functionalized macrocycles are challenging and also play an important role in the development of versatile multifunctional calix[4]arenes.

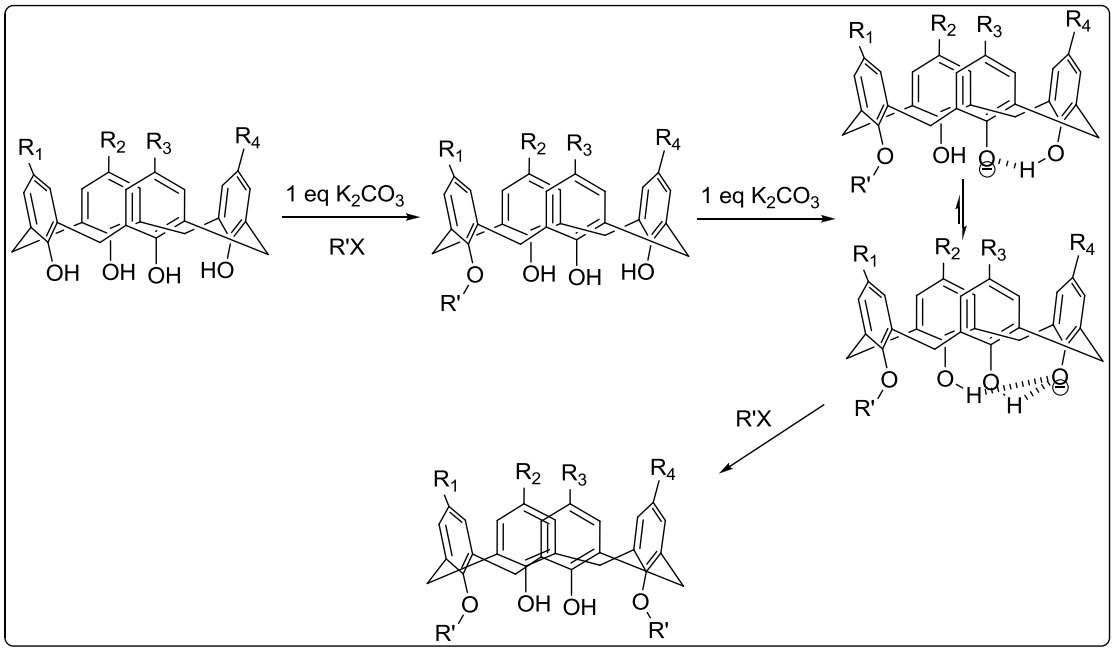
3.1.6.1 Reactions involving hydroxyl groups.

Different synthetic strategies involving the hydroxyl groups in the lower rim of calix[4]arenes allow the selective functionalization of both, the lower and the upper rim. Initial modifications carried out in the lower rim have a great influence in the reactivity of the *para* positions in the upper rim. Derivatization of calix[4]arenes normally begins with modifications in the lower rim. [65]

Very selective and efficient methods were developed for the synthesis of *O*-substituted calix[4]arenes. These methods allowed the mono-, bis-, tri- and tetra-substitution of calix[4]arenes, depending on the choosen strategy.[66]

Regioselective reactions in the lower rim of calix[4]arenes play an important role in the preparation of useful macromolecules employing calix[4]arene as building blocks. The selective mono-functionalization of the lower rim is achieved through several methods. The most popular approach is the direct monoalkylation of the lower rim, using a base and equimolar quantities of an alkylating electrophile. Examples of this approach are, the use of potassium carbonate in acetonitrile, cesium fluoride in *N,N*-dimethylformamide (DMF),[67] sodium hydride in toluene or barium hydroxide in DMF.[68] Tetra-*O*-substituted calix[4]arenes and 1,3-distal di-substituted calix[4]arenes are also employed as starting substrates in the synthesis of mono-*O*-substituted derivatives. A good example of that, is the controlled cleavage of ethers in the lower rim by the use of the right number of trimethylsilyl iodide equivalents.[69] Mono-ester calix[4]arenes can also be obtained from 1,3-diesters by treatment with a mild base like imidazole.[70]

The most regioselectively efficient reactions in the lower rim of calix[4]arenes are either the protection of all the hydroxyl groups in one synthetic step or the 1,3 functionalization. 1,3-Distal alkylation or acylation of hydroxyl groups are successfully achieved by the use of two equivalents of a weak base like potassium carbonate. The high selectivity is explained by the stability of certain intermediate species during the *O*-functionalization (scheme 3.4).



Scheme 3.4 Reaction mechanism for 1,3-*O*-functionalization of calix[4]arenes.

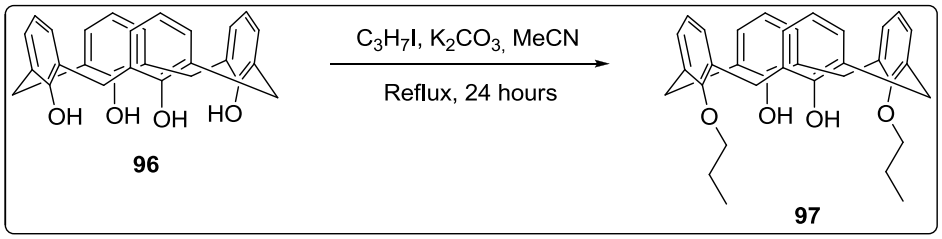
The second deprotonation in the lower rim of calix[4]arenes generates two possible intermediates with different stability. Deprotonation in the position 3, leads to an oxyanion that can be greater stabilised by the action of two hydrogen bonding interactions. This difference in stability is enough to promote the regioselective formation of 1,3-distal calix[4]arenes when two equivalents of a mild base are used during the reaction.

Formation of 1,2-proximal *O*-substituted calix[4]arenes was also achieved in the past but yields were normally much lower than in the case of 1,3-distal derivatives, due to the absence of good selective synthetic protocols. Selective tri-functionalization of the lower rim from unsubstituted calix[4]arene is carried out by tri-esterification, using mild bases like imidazole derivatives.

Complete *O*-substitution in the lower rim, as the simplest process, can be carried out under basic or acidic conditions. The treatment with a strong base, to activate the hydroxyl groups, followed by the addition of an electrophiile is the most common method to obtain complete substitution in the lower rim. Hydroxyl groups can also react in the presence of good electrophiles and certain catalysts.

3.1.6.2 Blocking the conformation of calix[4]arenes.

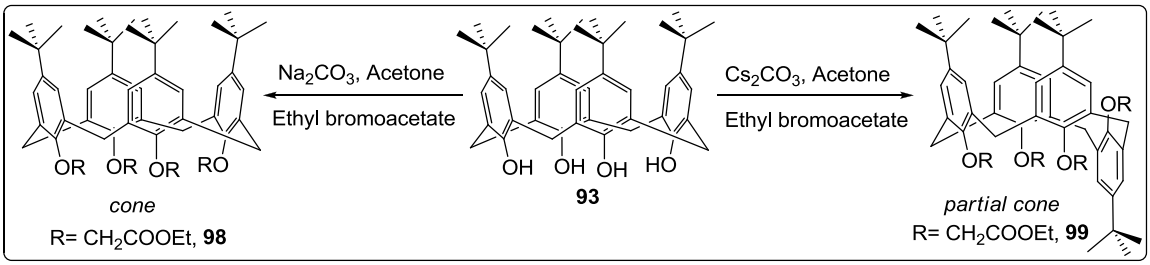
Blocking a specific conformation for calix[4]arenes was possible, both by the use of selective protocols, and protecting groups (scheme 3.5). In general, partially *O*-alkylated calixarenes can adopt different conformations (cone, partial cone, 1,3-alternate, etc.). Since free hydroxyl groups allow phenol rings to rotate around the methylene bridges through the central cavity, *syn* and *anti* conformers are obtained.



Scheme 3.5 Selective 1,3-distal-*O*-alkylation of calix[4]arenes.

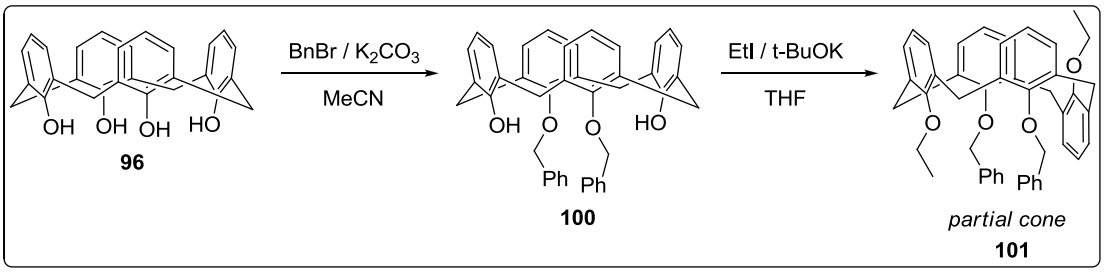
When 1,3-diether calix[4]arenes are *syn* conformers, a *cone* conformation is obtained and a pair of doublets (AX system) is observed by ^{1}H-NMR spectroscopy for the methylene bridges, where the doublets are separated by 0.6 ppm. *Anti*-1,3-diethers, also show one pair of doublets (AB system) by ^{1}H-NMR however, in this case, the gap between doublets is smaller with a value of 0.2 ppm.

The regioselectivity of the reactions performed on the hydroxyl groups in the lower rim is modulated by the bulkiness of the substituents introduced and template effects, mainly due to the formation of metal complexes in the course of the reaction,[71] the nature of the solvent used and the nature and bulkiness of the functional groups attached in the upper rim (scheme 3.6).

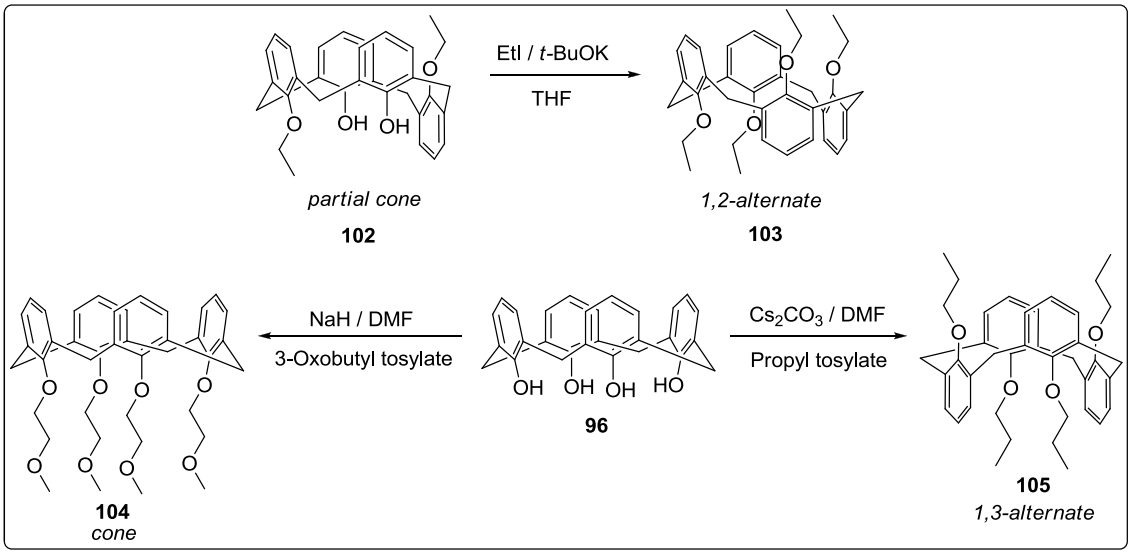


Scheme 3.6 The treatment of 5,11,17,23-tetra-*para*-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene with sodium carbonate and ethyl bromoacetate in acetone yields quantitatively, the corresponding calix[4]arene derivative in the *cone* conformation, whilst the treatment with cesium carbonate affords quantitatively the corresponding calix[4]arene derivative in the partial *cone* conformation.

The partial *cone* conformation can also be obtained in good yields by a two step synthetic approach starting from 25,26,27,28-tetrahydroxycalix[4]arene (scheme 3.7).



Scheme 3.7 The treatment of 25,26,27,28-tetrahydroxycalix[4]arene and benzyl bromide with potassium carbonate in acetonitrile affords 25,27-dibenzyloxy-26,28-dihydroxycalix[4]arene. Further treatment with ethyl iodide and potassium *tert*-butoxide in tetrahydrofuran, affords the corresponding calix[4]arene derivative in the partial *cone* conformation.

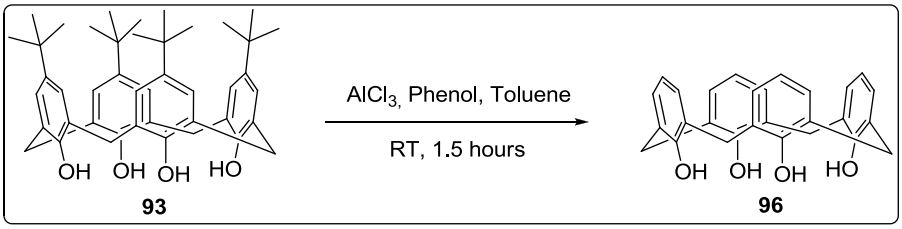


Scheme 3.8 Synthesis of 1,2-alternate calix[4]arene from a partial *cone* calix[4]arene intermediate and 1,3-alternate calix[4]arene from 25,26,27,28-tetrahydroxycalix[4]arene respectively. The template effect of the metal ion, as well as the bulkiness of the functionalities attached to the lower rim of the calixarene, are also represented to demonstrate their importance in modulating the conformational selectivity.

The 1,2-alternate and 1,3-alternate conformations are obtained with moderate yields. The 1,2-alternate conformation is obtained by a two-step approach whilst, the 1,3-alternate conformation is easily obtained in one step from 25,26,27,28-tetrahydroxycalix[4]arene (scheme 3.8).[72,73]

3.1.6.3 Chemistry in the upper rim of calix[4]arenes.

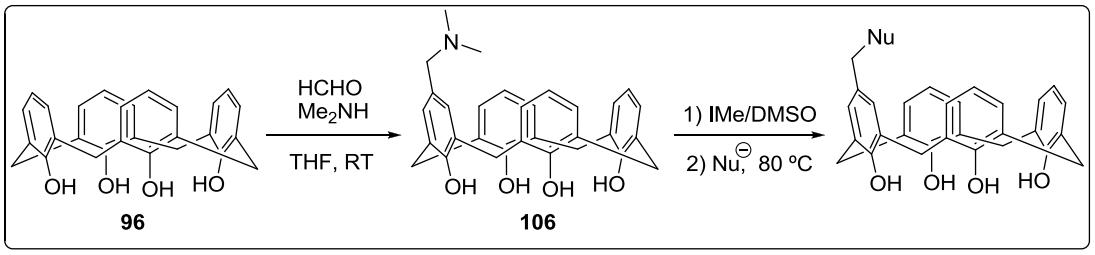
Tert-butyl groups attached to the upper rim of calix[4]arenes can be easily removed by different protocols such as, the treatment with aluminium(III) chloride and phenol as transalkylating acceptor (scheme 3.9) or, the use of trifluoroacetic acid (TFA) in combination with sodium dithionite.[74]



Scheme 3.9 De-*tert*-butylation reaction of calix[4]arene catalyzed by aluminium(III) chloride.

The cleavage of functionalities from the upper rim is very important in calixarenes, since the free *para* position can be easily functionalized (i.e. electrophilic aromatic substitution). The same reactivity principles observed in phenol derivatives can be successfully applied on calixarenes. Reactions such as nitration, halogenation, sulfonation, chloromethylation, aminomethylation, acylation, metal-halogen exchange or C-C couplings are some representative examples. Fries and Claisen rearrangements are also possible.

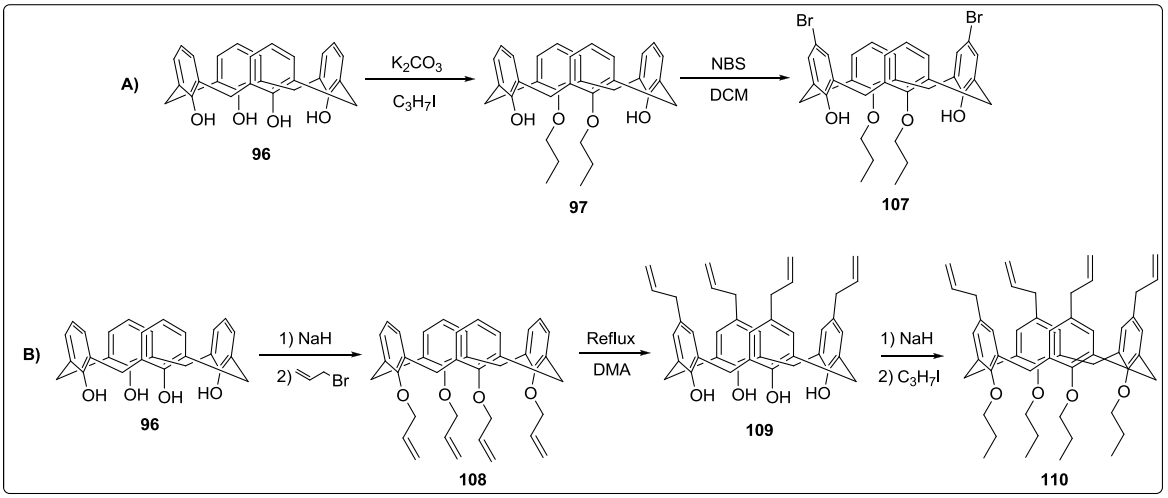
The *p*-quinone methide route is an important protocol, developed by Gutche in the 1990s, that allowed an easy functionalization of the upper rim. The protocol consisted in the aminomethylation of the *para* position of plain calix[4]arene (scheme 3.10). Further conversion of the amino group into a quaternary ammonium salt, allowed its substitution by other nucleophiles like cyanides, hydroxides, methoxides, malonates, phenoxides among others.



Scheme 3.10 *p*-Quinone methide route. Selective synthesis of monoarmed calix[4]arenes.

Selective functionalization of the upper rim in calixarenes is very important. Nevertheless, most of the selective functionalizations in the upper rim described up to the present are achieved by carrying out firstly a regioselective functionalization of the hydroxyl groups in the lower rim. The initial and partially selective functionalization of hydroxyl groups in the lower rim induces a different reactivity in the *para* positions of the phenol moieties of the macrocycle. *O*-alkylated phenol units, show lower reactivity towards electrophilic aromatic substitution (S_EAr) in the *para* positions than non *O*-alkylated analogues due to the different electronic effects caused in the ring by the substitution (scheme 3.11).

To date, the most successful examples of selective functionalization of hydroxyl groups in the lower rim are, the de-alkylation of partially *O*-alkylated calix[n]arenes (n=4, 6) under carefully controlled conditions, and the selective *O*-alkylation such as 1,3-*O*-dialkylation and the selective Claisen rearrangement in calix[4]arenes (scheme 3.11).



Scheme 3.11 Upper rim functionalization of calix[4]arenes. A) Selective 1,3-*O*-dipropylation of calixarene 10. B) Functionalization of calix[4]arenes by Claisen rearrangement.

3.1.6.4 Chemistry in the methylene bridges of calix[4]arenes.

There are two further available positions to functionalyse calixarenes though they are much less common. One of them is the outer face region of calixarenes, formed by the external area around the benzene rings that can be functionalised by coordination chemistry, i.e. formation of metal complexes with low-valence transition metals.[75] The second are the methylene bridges joining the phenolic units together in the cyclic structure. The monosubstitution at these positions opens the scope of functionalising calixarenes, as the methylene protons are not equivalents, being the equatorial position the preferred one.[76]

Further modification of calix[4]arenes allows the preparation of a great number of macrocycles with potential applications in supramolecular chemistry,[77] metal sequestering[78] and catalysis.[79]

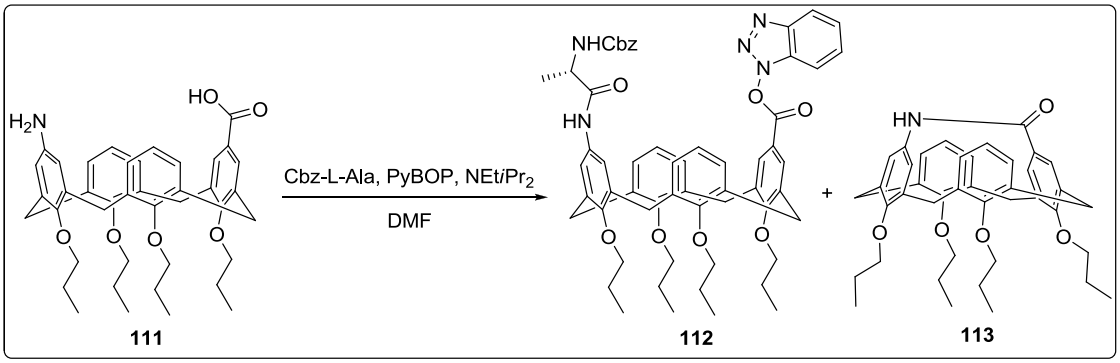
3.1.6.5 Transannulation reactions in calix[4]arenes.

Transannulation reactions, the generation of bicyclic molecules from a medium or large ring, are also involved in the synthesis of natural products like terpenes, polyketides, steroids and alkaloids. Transannulation reactions can be achieved by the use of different processes like aldol condensation, Diels-Alder cycloaddition, ionic cyclisation, atom-transfer reaction or radical cyclisation.[80]

The application of transannulation reactions in calixarenes was very limited in the past and only a few examples were reported in the literature.

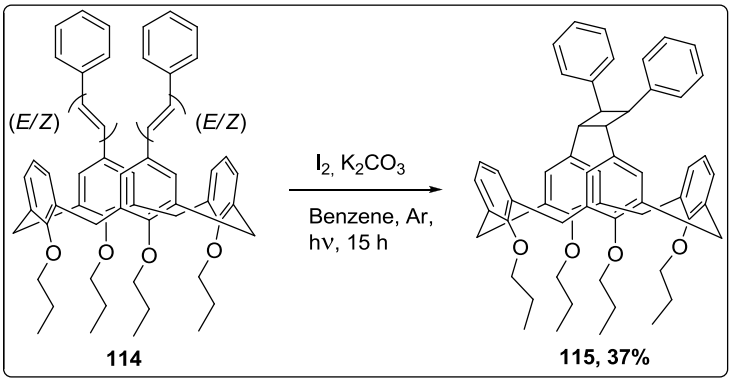
In 2003, Neri and co-workers reported the synthesis of transannular spirodienona calixarenes under the action of strongly basic oxidizing systems.[81] Later, in 2008, Ungaro *et al.* reported the 1,3-transannulation of calix[4]arenes in the upper rim. When the 1,3-disubstituted calix[4]arene **111** was treated with Cbz-alanine in the presence of PyBOP and a base, an intramolecular reaction between the amino group and the activated carboxylic acid had taken place unexpectedly (scheme 3.12). The ^{1}H-NMR spectrum of the crude product showed the aromatic

protons in *ortho* to the amino (NH) and carbonyl (CO) groups to be highly shielded, affording unusual low chemical shifts of 5.5 and 5.6 ppm respectively.[82]



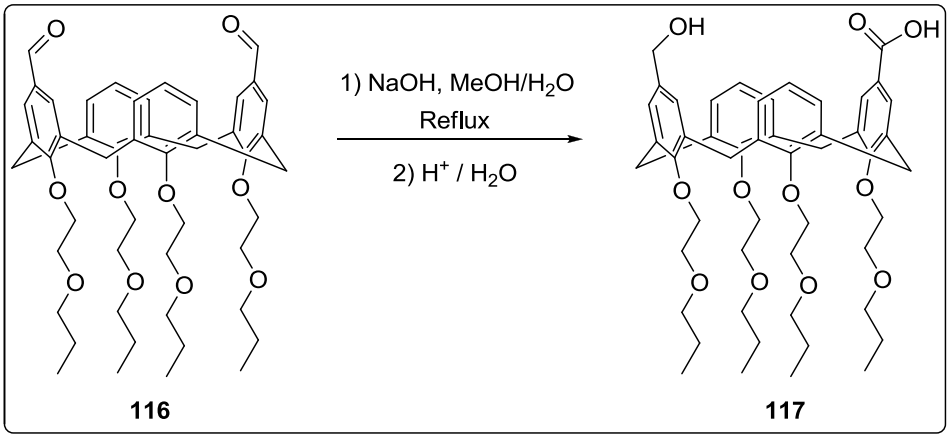
Scheme 3.12 Intramolecular condensation between amino and carboxylic groups during the synthesis of peptidocalix[4]arenes.

A photochemical transannulation reaction of calix[4]arene **114** was reported in 2010 by Diker and co-workers when, a solution of this compound in benzene was irradiated with a mercury lamp in the presence of iodine and potassium carbonate to afford a 37% yield of the desired product via the transannular [2 + 2] cycloaddition reaction of the stilbene units (scheme 3. 13).[83] Again, the upfield shift for the meta-phenolic protons at 5.5 and 5.9 ppm, confirmed the transannular reaction.



Scheme 3.13 Transannular [2 + 2] cycloaddition reaction of the stilbene units in calix[4]arene 114.

In 2012, Casnati *et al.* reported the Cannizzaro transannular disproportionation of 1,3-distal diformyl calix[4]arene locked in the cone conformation.[84] Under strongly basic conditions, the 1,3-distal dialdehyde could undergo disproportionation while the 1,2-proxymal isomer was unable to disproportionate. The reaction was reported to proceed *via* a transannular transition state with a 3-atom bridge and the product formation rate was consistent with an intramolecular process, showing a first-order dependence with the substrate concentration (scheme 3.14). The insertion of bulky groups in the lower rim of calix[4]arenes locks the conformation and allows a better control of the structure. However, the insertion of bulky groups still allows certain degree of flexibility, being possible the rotation through the bridged methylene groups connecting the phenol rings. This makes possible the interconversion between a pure *cone* conformation with C_{4V} symmetry and, a flattened *cone* conformation (the already mentioned pinched *cone* conformation) with symmetry C_{2V}. The pinched *cone* conformation allows calix[4]arenes to locate the functional groups installed on 1,3-distal positions very close to each other, favouring the performance of transannular reactions.



116 **117**

Scheme 3.14 Intramolecular Cannizzaro reaction of calix[4]arene 116 in aqueous methanol.

3.1.7. Biomimetic applications of calix[4]arenes.[85]

In 1970, Gutsche, intrigued by the new advances in the biochemistry and enzyme mimic fields, proposed calixarenes as a convenient scaffold for the mimicry of enzymes. Calix[4]arenes represent a very suitable scaffold for the mimicry of enzymes due to their basket shape structure

and their moderate flexibility that allow the functional groups, in both the upper and lower rim, to adapt themselves to guest molecules by small changes in the conformation, i.e. changing from pure cone to pinched cone conformations through low energy barriers. Moreover, the fact that calix[4]arenes present eight different, easily functionalizable positions, allow an easy access to multifunctional calix[4]arenes where synergic effects between different functional groups in the molecule are possible.

Supramolecular chemistry has become a high-profile topic over the last decades and plays an important role in the mimicry of enzymes. Supramolecular chemistry is an area of the chemistry studying the non covalent interactions between molecules and their organization in space. In an attempt to reproduce the chemistry of natural processes, chemists are working to control weak interactions such as dispersion and electrostatic forces and hydrogen bonding, which could contribute into a supramolecular coordination, leading to regio- or enantioselective processes. The design of macromolecular receptors is a representative example of the importance of non-covalent interactions. Artificial receptors contain suitable and complementary functionalities that can interact non-covalently with specific molecules. The design of artificial receptors, metalloenzymes or smart materials, normally requires the use of molecular scaffolds. Calixarenes, particularly calix[4]arene, are one of the macromolecules attracting more attention as molecular scaffolds and play an active role in the enzyme-mimic field. Their macrocyclic structures are able to reproduce the active site of enzymes by the insertion into their structures of active functional groups. These types of biomimetic structures are important because of their potential applications, not only to produce artificial catalysts or receptors but also to be used in the study of the mechanisms carried out by natural enzymes.

The most important features as a scaffold of an enzyme based, artificial calixarene are:

- **Molecular recognition:**

  The scaffold must present different functionalities able to selectively interact with the target substrates. Very often, such functionalities are also able to orientate those substrates before the reaction occurs, inducing a predetermined regio- or enantioselectivity.

- **Turnover rate:**

  The artificial enzyme-based, bio-inspired scaffolds must be able to perform the reaction a great number of times before becoming partially or fully inactive.

- **Reaction rate:**

  Reactions must be considerably accelerated in the presence of these scaffolds as catalysts.

- **Mild conditions:**

  The catalysis performed by these scaffolds must be suitable to occur under mild conditions of room temperature, atmospheric pressure, absence of strong bases or acids, etc.

- **Low complexity:**

  Biomimetic structures must present just the necessary active functions to carry out the catalytic activity as only the structure in the enzymatic active pocket is imitated.

- **Broad range solubility:**

  Good solubility properties in different solvent systems to widen the scope to different processes.

In order to design a biomimetic structure, there are two main approaches to the mimicry of enzymes. The first approach is focused on the enzymatic function regardless of the complex structural features or supramolecular interactions. The second is based on the type of interactions occurring between the substrates and the enzyme.

3.1.7.1 Mimicry of the enzymatic function.[85]

To mimic the enzymatic function of an enzyme, robust and long life catalytic systems having weak interactions with the substrates are required. Those characteristics lead to high turnover frequencies (TOF) and turnover numbers (TON) at room temperature and atmospheric pressure. The interactions between the substrates and the catalytic system must be strong enough to

minimize the transition state energy barrier. The formation of adducts should be avoided though. The structure of the catalytic system must also provide a minimum flexibility for the substrates to accommodate properly and react after adopting a specific orientation.

Macromolecules possessing the features described above are used in the catalysis of a variety of bio-inspired reactions. Some of these molecules are cyclodextrins, macrocyclic polyethers and calixarenes.

One of the most common and studied biochemical reactions, is the cleavage of phosphate esters catalyzed by metalloenzymes like phosphorous nuclease, DNA-polymerase, phospholipase-C, and alkaline-phosphatase.

Several metallic cations such as Zn(II), Fe(III), Mn(II) or Mg(II) are found in the active site of these enzymes. The metallic cation activates the phosphate group allowing the cleavage of the molecule through a five member transition state. *p*-Nitrophenyl esters (HPNP) are used as convenient substrate models for the cleavage of phosphate esters due to their simple quantification by UV-VIS spectroscopy (λ_{max} = 400 nm).

3.1.7.1.1 Hydrolysis catalysed by Zn(II)-complexes.[86]

Calix[4]arenes fuctionalised with a single or several pyridine chelating agents in the upper rim are described in the literature and their catalytic properties have been reported. The calixarenes containing pyridine type ligands in their structure are able to strongly complex divalent metals like Zinc(II). The compounds shown below (figure 3.7) are effectively used in the hydrolysis of several substrates involved in natural processes like phenyl phosphates.

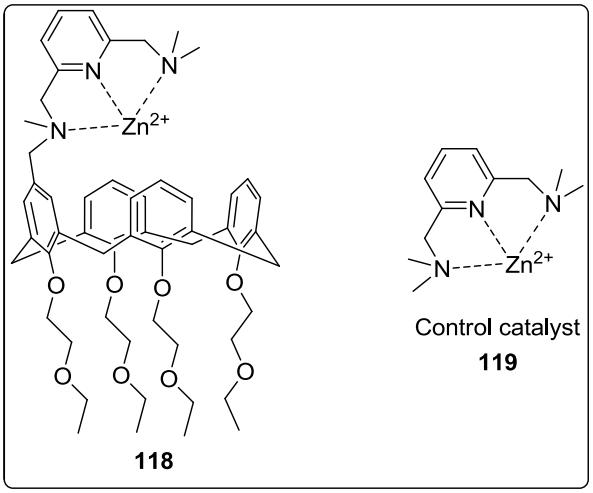


Figure 3.7 Calix[4]arene-based model for metallo-phosphodiesterases.

Monoarmed calix[4]arene **118** loaded with Zn(II) is reported to have six times more catalytic activity than the control compound **119**. This enhancement in the catalytic activity is explained by the contribution of the hydrophobic effects in the calix[4]arene cavity what facilitates the stabilization of a nearby metal-water bond which participates in the catalysis to a certain extent.

Analogous di-substituted complexes were shown to be fifty times more active that the monoarmed complexes (figure 3.8). This is explained by a synergic effect between the two metallic centres. Thus, the bis-armed macromolecule was able to coordinate the RNA model substrate 2-hydroxypropyl-4-nitrophenyl phosphate (HPNP) at two different points where one metallic centre was coordinated to the phosphate group and the other was coordinated to the hydroxyl group. The reaction was finally achieved by an intramolecular cyclization (figure 3.8). An increase in the rigidity of the calix[4]arene scaffold resulted in a negative effect in the catalytic efficiency, being the activity reduced eight times (figure 3.9).

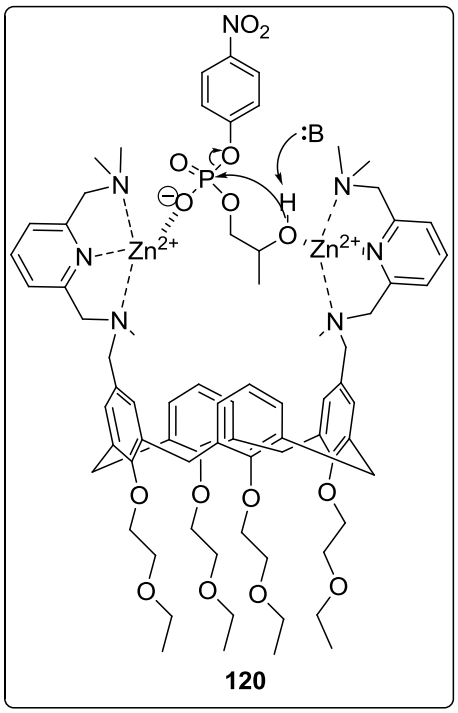


Figure 3.8 Proposed mechanisms for HPNP cleavage by calix[4]arene complex 120.

The insertion of a third metallic centre in the upper rim enhanced the reaction rate by 40%, what was explained by a cooperative effect. It was also found that tri-metallic complexes were able to form more stable adducts, decreasing the substrate affinity.

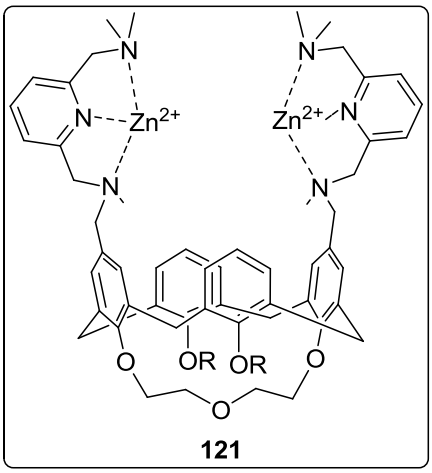


Figure 3.9 Rigid bis-armed calix[4]arene complex for the catalysis of hydrolysis reactions.

3.1.7.1.2 Hydrolysis catalysed by Cu(II)-complexes.[85]

The hydrolysis of phosphate esters is carried out by phosphoesterase metalloenzymes and several other amino acid residues. During the hydrolysis of phosphate monoesters, residues of histidine, serine and arginine, along with two metallic atoms of zinc and magnesium respectively, are found to be key elements in the catalysis of this type of reactions.

During the mimicry of these enzymes, the amino acid residues are replaced by simpler organic bases able to perform the same function (i.e. in the mimic of RNase A, imidazole and other amino derivatives).

Di-substituted calix[4]arenes complexing Cu(II) are reported in the literature and their activities in the cleavage of HPNP and ethyl *p*-nitrophenyl phosphate (EPNP) are found to be much higher than in the case of mono-substituted analogues where a cooperative effect is not possible. In this kind of system, the pK_a for the solvation water bound to the copper atom is found to be lower than expected, by showing again the hydrophobic effect generated by the aromatic cavity of calix[4]arenes allowing a catalysis under neutral conditions.

More complex bifunctional calixarenes containing copper (II) chelators and hydroxymethyl groups have also been tested in transesterification reactions of HPNP. Other bi-functional calix[4]arene complexes bearing extra amino groups showed a high activity in the catalysis of intramolecular transesterification of HPNP.

3.1.7.1.3 Mimicry of acyltransferases.

Imidazole derivatives are frequently used as good acceptor-donor species of acyl groups. Calix[4]arenes bearing imidazole groups in the upper rim are reported in the literature and their catalytic properties well studied (figure 3.10).[87] The presence of a nucleophilic group in the calixarene scaffold resulted in an enhancement in the hydrolysis rate by a 52%. When the calixarene cavity was not present in the catalytic system and the control substrate was used instead, the catalytic activity decreased by a 13%. The di-substituted calixarene generated an increase of the initial reaction rate by 50% compared with the mono-substituted catalyst by showing

a cooperative effect. At pH values close to neutral, half of the imidazole moieties are protonated, which makes bi-functional imidazole calixarenes good acid-base catalysts for the hydrolysis of esters in aqueous solution. This catalysis is efficiently applied to the hydrolysis of *p*-nitrophenyl benzoate.

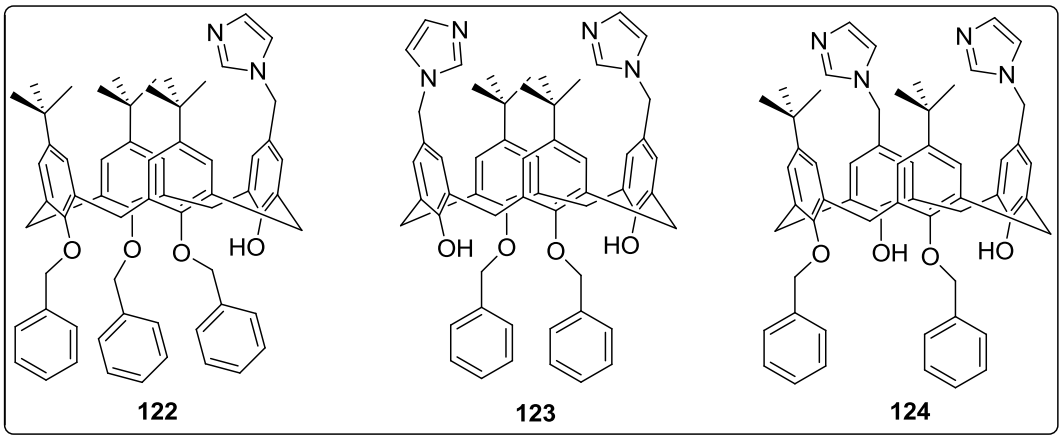


Figure 3.10 Imidazole substituted calix[4]arenes.[87]

3.1.7.1.4 Mimicry of ribonucleases.

Lanthanide complexes have proved to be good catalysts in the bio-mimetic hydrolytic cleavage of HPNP. The first examples of bio-mimetic macromolecules based on regioselective ribonucleases were reported by Shinkai *et al.* in 1991, where water soluble sulfonate calix[4]arenes were used for the cleavage of cyclic phosphates of cytidine, adenosine, uridine and guanosine (figure 3.11).[88] The regioselective cleavage of cyclic ribonucleosides phosphate was achieved in acidic media, where cyclodextrins resulted to be only active in neutral or alkaline conditions.

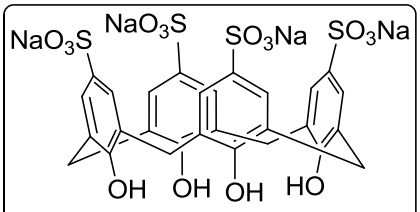


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Figure 3.11 Synthetic water-soluble calix[4]arene employed in the regioselective cleavage of 2',3'-cyclic phosphates.[88]

Very low catalytic activity was shown by bigger macromolecules like calix[6]arene and calix[8]arene. This could be explained because of the high flexibility and number of conformations these macrocycles can adopt. It was also found that the calixarene cavity was essential for the regioselectivity. Thus, the regioselectivity was not improved when a monomeric analog (4-hydroxy-benzenesulfonic acid) was used in the reaction. The electrostatic interactions between the macromolecule and the substrate played an important role in the catalytic process.

3.1.7.2 Mimicry based on the structure of enzyme active sites.

The complexity and high molecular weight of enzymes make impossible the exact synthetically-achieved reproduction of the proteic structure in the laboratory. The study of the active site and surrounding areas in enzymes showed the catalysis of the enzyme sites presented many similarities to the catalysis synthetically performed in the laboratory.

One of the big challenges of the enzyme mimicry is the preparation of artificial ligands, able to reproduce the coordination sphere of the metallic atoms occuring in the active sites of enzymes.

3.1.7.2.1 Structural models based on calixarene-Cu(I) complexes.

Calix[6]arene derivatives are used to form Cu(I) complexes (figure 3.12).[89] In this type of complexes, the metallic centre is protected by the calixarene scaffold, avoiding undesired metal-metal interactions. The complex can also accept small molecules carrying coordinative atoms like nitrogen, with nucleophilic lone pairs. A NMR study of the coordination of several nitriles with the

metallic center has provided interesting conclusions about the binding of different nitriles to these mono-nuclear copper complexes.

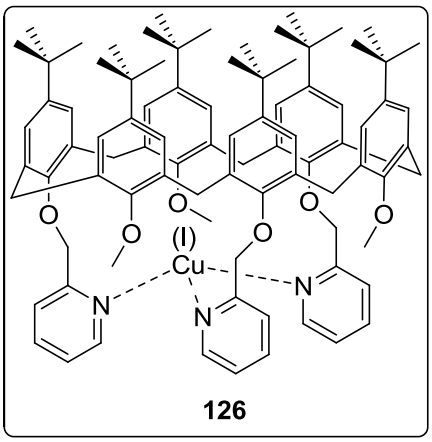

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Figure 3.12 Tridentate N-ligand based on calix[6]arene.[89]

These types of complexes present an available coordinative site that can selectively interact with small molecules like acetonitrile or allylnitrile, but not with bulkier nitriles. It was also observed that the calixarene cavity protected the metal centre from undesired reactions such as dimerization, and could act as a molecular funnel.

3.1.7.2.2 Structural models based on calixarene-Zn(II) complexes.

A biomimetic calix[6]arene-Zn(II) complex was reported by Sénèque *et al.* where the metallic atom is tri-coordinated by three histidine residues (figure 3.13).[90] In this particular complex, the highly "Lewis acidic" Zn(II) cation was placed in a tetrahedral environment presenting a labile site which was facing the calixarene pocket. This position, which was initially occupied by a water molecule, could be exchanged by small molecules like amines, amides, alcohols, sulfoxides, nitriles, as it was determined by H-NMR spectroscopy. Interestingly, secondary, tertiary and aromatic amines did not show substantial complexation. These studies concluded that this kind of calixarene was able to selectively complex small molecules depending on structural features of the guest molecule such as types of functionalities present in the structure or size of the guest molecule. In addition, these macrocyclic systems proved to be very versatile, allowing the tuning of

steric and electronic properties.[91] In the same work, the X-ray structures of the corresponding ethanol and formamide aducts were presented. These structures showed that the hydrogen bonding and CH/π interactions are essential in order to stabilise the complex formed by host and substrate.

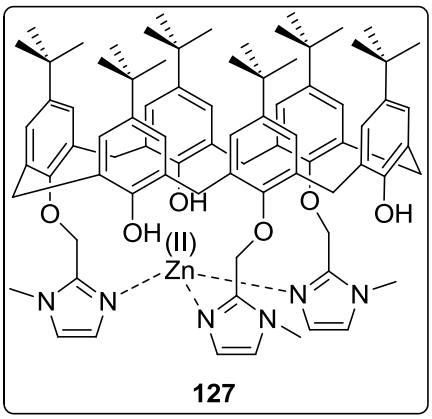

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Figure 3.13 Biomimetic calix[6]arene-based Zinc complexe.[90]

A different study focused on the nature of the coordinative nitrogen source, finding that imidazole, benzimidazole and pyrazole were also good nitrogen donors in the formation of stable tetrahedral zinc complexes. The calixarenes adopted a *cone* conformation presenting a cavity which could accept small molecules like acetonitrile. Interestingly, pyridine did not prove to be a good ligand in this kind of Zn(II) complex.[92]

3.1.8 Aims.

The aim of the last part of our research is to develop new synthetic protocols towards the efficient preparation of upper rim mono- and multi-substituted calix[4]arenes locked in the *cone* conformation. These synthetic methods will be employed in the preparation of a new bio-inspired calix[4]arene, in an attempt to mimic the condensing function of polyketide synthases.

3.2 Results and discussion.

3.2.1. Regioselective synthesis of monosubstituted calix[4]arenes locked in the *cone* conformation.

A common representation for the operating mechanism of PKS involves the use of different functional modules that participate in the catalysis of specific transformations during the biosynthesis of polyketides in microorganisms and plants. Each functional module is sequenced in such as specific manner that the polyketide chain can be finely tune to produce a very specific type of biomolecule (figure 3.14).

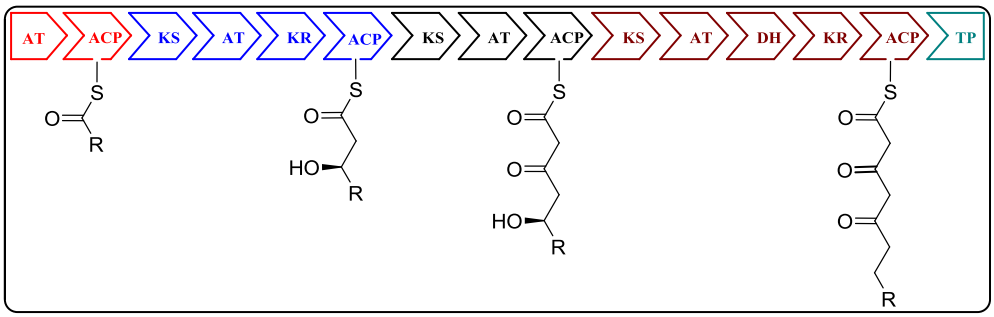


Figure 3.14 Schematic representation for the modular mechanism of PKS.

Within this context, polyketide synthases present the ability to bring together a variety of active functions that are specialised in the catalysis of important biochemical transformations. This idea of bringing together a group of catalysts, so that cascade reactions can be performed in the same reaction media, motivated organic chemists in the use of cyclic scaffolds for the mimicry of enzymes. Of all the macrocycles known in the literature, calixarenes, and more specifically calix[4]arenes, appear to be a very convenient type of macromolecule for the mimicry of enzymes. This is due to its great versatility, the high control on its structural conformation and the substantial number of functionalizable positions within the macrocyclic structure. Thus, calixarenes have been used for a long time as scaffolds and also successfully employed in the mimicry of enzymes like for example aldolases, acyltransferases or ribonucleases. However, most of the studies involving mimicry of enzymes have been based on the use of metallic centres anchored in calixarenes and not so much attention has been devoted to the use of calixarenes in organocatalysis. Thus, the

preparation of multifunctional calixarenes bearing different functional groups, similarly to modular PKS, which are able to act as independent organocatalytic centres, correspond to a novel field of investigation which present many challenges.

In addition to our investigations of the properties of artificial malonyl carriers and mild decarboxylative condensations we were equally interested in the use of cyclic scaffolds for the mimicry of enzymes that were able to catalyse decarboxylative Claisen condensations. Bearing this idea in mind, we decided to develop new synthetic methods that allowed the preparation of mono, di and multi-functionalised calix[4]arenes with applications in biomimetic catalysis. Our initial idea was to design a macrocyle capable of mimicking the active site of a PKS where two thiol groups are placed nearby (figure 3.15).

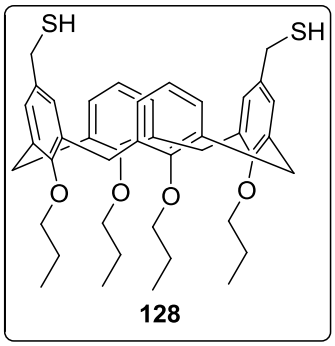


Figure 3.15 Bio-inspired mercapto-calix[4]arene to mimic part of the active site of a PKS.

Furthermore, the preparation of multifunctional calix[4]arenes bearing different organocatalysts at the upper rim might lead to the mimic of some PKS functions such as chain elongation (KS), reduction of carbonyl groups (KR) or dehydration reactions (ER) (figure 3.16).

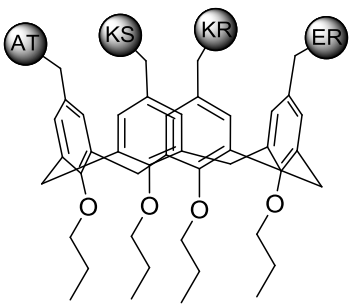


Figure 3.16 Multifunctional calix[4]arene bearing different biomimetic functions.

With the idea of multifunctionalising calixarenes in mind, we started the synthesis of new calix[4]arenes with potential applications in catalysis and enzyme mimicry. Different approaches can be followed during the preparation of multifunctional structures based on calix[4]arene. One option may be the insertion of different and compatible functional groups in the macrocycle acting as selective and independent catalysts. These functional groups might be attached to either the upper or to the lower rim (figure 3.17).

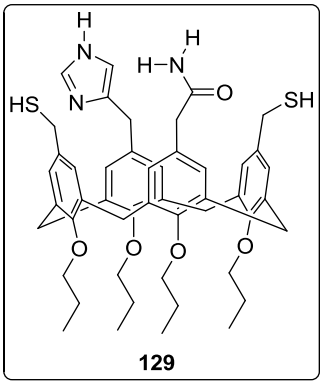


Figure 3.17 Multifunctional calix[4]arene inspired in the active site of PKS.

Another approach may be the preparation of several monoarmed calix[4]arenes bearing different functionalities, followed by the oligomerisation of several units of selected monofunctionalised calix[4]arenes, acting as a chemical toolbox (figure 3.18).

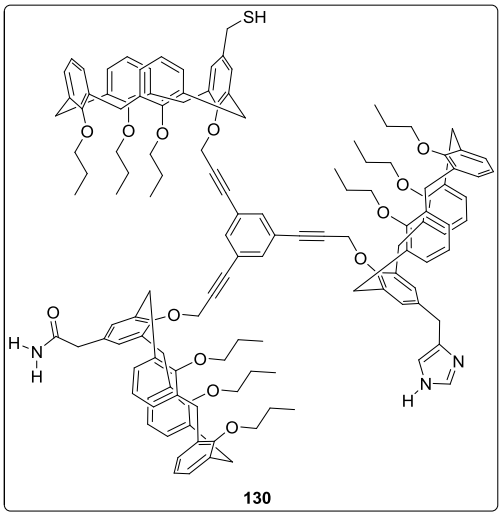
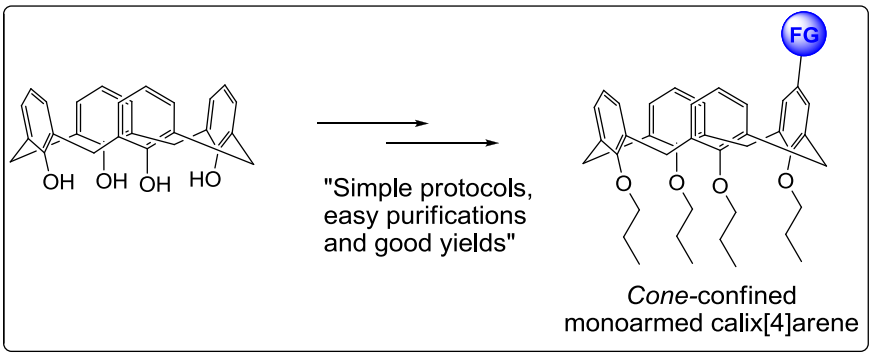


Figure 3.18 Multifunctional oligomer formed by the linkage of three different monoarmed calix[4]arenes.

During the first stage of our investigations in the field of calixarenes, we were interested in the regioselective functionalisation of the upper rim of calix[4]arenes, particularly in the selective monofunctionalisation.

As was explained in the introductory section, only a few protocols were reported in the literature with regards to the monofunctionalisation of calix[4]arenes. However, none of them afforded calix[4]arenes locked in the *cone* conformation. The development of a selective and a simple method for the preparation of monoarmed (monofunctionalised) calix[4]arenes locked in the *cone* conformation may be very useful in the chemistry of this type of macrocycle. The study of the properties of simple monoarmed calix[4]arenes, may be greatly helpful in the preparation and understanding of more complex multisubstituted calix[4]arenes. Moreover, monosubstituted calix[4]arenes might be employed as a suitable starting material in the synthesis of multisubstituted calix[4]arenes.

Another motivation that encouraged us to prepare monoarmed calix[4]arenes was the fact that very few synthetic protocols were available in the literature. In our opinion, a simple and highly regioselective protocol for the preparation of monoarmed calix[4]arenes was needed (scheme 3.15).

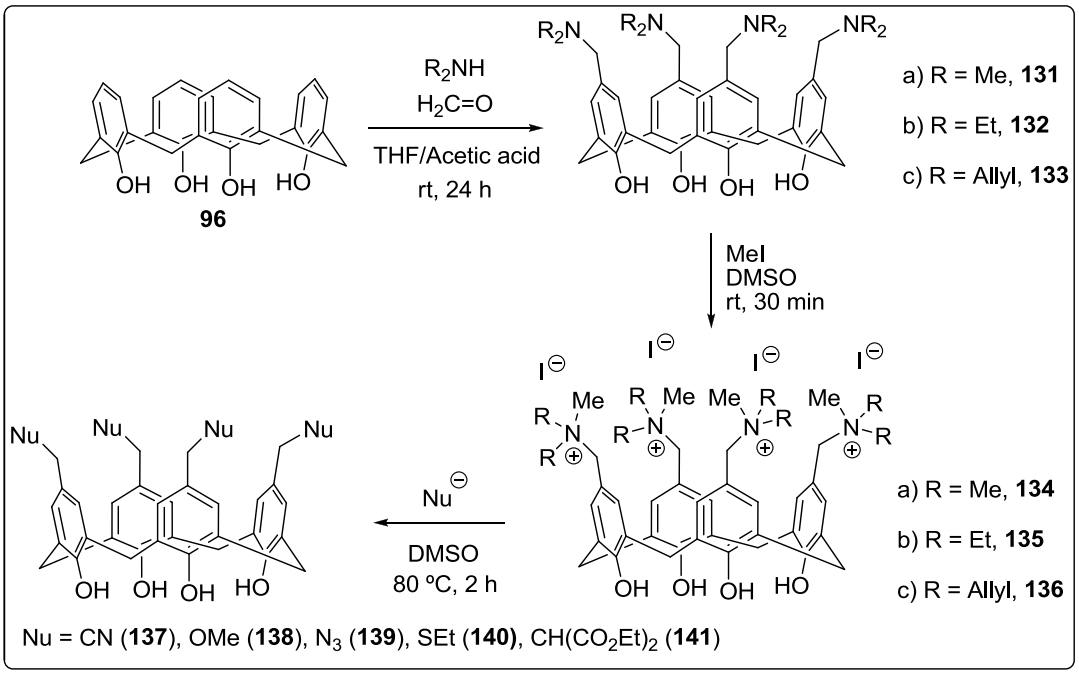


Scheme 3.15 Schematic monofunctionalisation of calix[4]arenes locked in the *cone* conformation.

In 1988, David Gutche and Kye Chun Nam published the reaction between calix[4]arene **96**, formaldehyde and a secondary amine to generate tetraaminocalixarenes in high yields.[93] Although the first attempts to carry out this reaction led to a mixture of polymers, the use of lower reaction temperatures and the use of a mixture of polar solvents (tetrahydrofuran and acetic acid) allowed

the preparation of Mannich bases in high yields (70-90%), after stirring the reaction mixture for 24 hours at room temperature. The tertiary amino groups installed in the upper rim of the calix[4]arene, were easily converted into the corresponding quaternary ammonium salts by treatment with an excess of iodomethane (scheme 3.16).

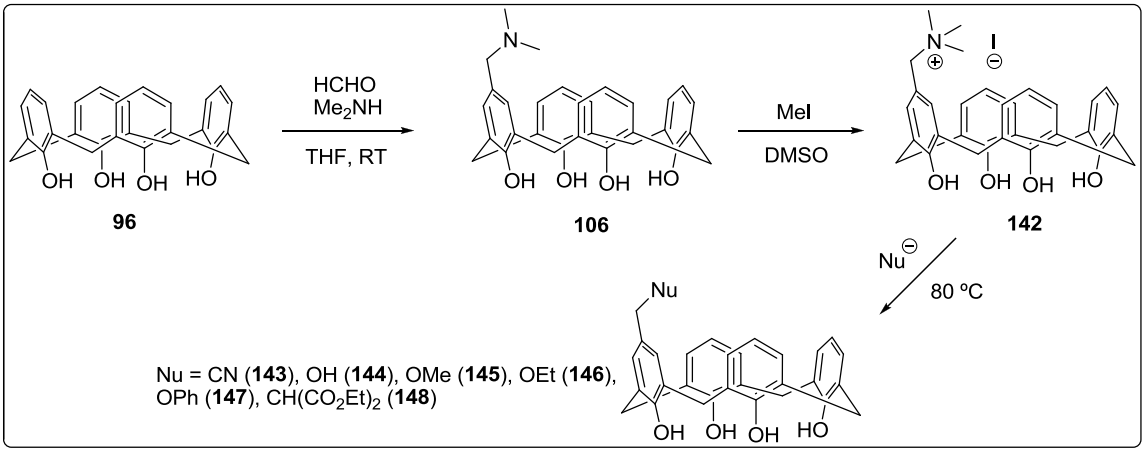
It is known that trialkyl benzyl quaternary ammonium salts are unstable when heated around 80 ºC or above,[94] or when irradiated with intense UV light.[95] Trialkyl benzyl quaternary salts decompose to form tertiary amines and highly reactive quinone methides. Thus, when a solution of calix[4]arene **134** in dimethylsulfoxide was heated at 60 ºC in the presence of a nucleophile, the amino group was efficiently substituted. The use of different nucleophiles allowed the synthesis of several tetrasubstituted calix[4]arenes (scheme 3.16).



Scheme 3.16 Upper-rim functionalisation of calix[4]arene *via* the *para* quinone methide route.[93]

The proposed formation of *para* quinone methide in calix[4]arene was supported by the observation that, when the hydroxyl groups in the lower rim were alkylated, the formation of *para* quinone methide was prevented and, as a consequence, the substitution reaction in the upper rim did not proceed.

As already mentioned, in 1994, Gutsche *et al.* developed a selective method for the preparation of monosubstituted calix[4]arenes. The use of tetrahydrofuran as solvent in the synthesis of Mannich bases allowed the selective formation of monosubstituted amino calix[4]arenes.[96] Treatment with iodomethane, to form the corresponding quaternary ammonium salt, followed by heating at around 80 ºC in the presence of a nucleophile, led to the preparation of a variety of monoarmed calix[4]arenes. Quaternized amino groups could be substituted by nucleophiles such as alkoxides, aryl oxides, Grignard reagents, secondary amines or alkyl sodium malonates (scheme 3.17).



Scheme 3.17 Synthesis of OH-free monosubstituted calix[4]arenes by Gutsche et al.[96]

Although in the paper published by Gutsche and co-workers the selective formation of monoamino calix[4]arene was described, no explanation was given for such selectivity. In our opinion, the exclusive isolation of monoamino calix[4]arene was mainly due to solubility issues. We believe calix[4]arene **106** must exist in a zwitterionic form that would explain its low solubility in the majority of organic solvents like THF, methanol, chloroform, diethyl ether, acetonitrile or toluene. Calix[4]arene **106** was only found to be soluble in very polar solvents like *N,N*-dimethylformamide, dimethylsulfoxide, *N,N*-dimethylacetamide or acetic acid. This would also explain, why tetrasubstituted calix[4]arenes were obtained as major product when acetic acid was used as part

of the solvent system, since the use of a highly polar solvent allowed solubilization and further reaction of intermediates.

In our opinion, the *para* quinone methide route represents one of the few methods reported in the literature that truly allows the access to monoarmed calix[4]arenes through a selective, simple and efficient process. The route allows the preparation of monoarmed calix[4]arenes in high yields, is easily scalable and requires short reaction times and simple purifications.

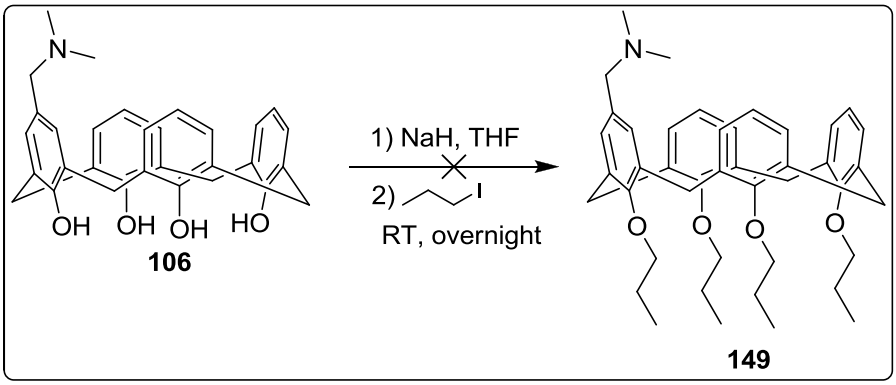
However, there is an important aspect that the *para* quinone route does not cover. The conformation of monoarmed calix[4]arenes obtained by this method is not locked in the *cone* conformation. To achieve conformational stability is crucial in order to control the properties and reactivity of calix[4]arenes. Besides, the *para* quinone route affords calix[4]arenes where free hydroxyl groups located in the lower rim can be involved in a number of side reactions. In the previously mentioned paper published by Gutsche *et al.*, only tetraamino calix[4]arenes were blocked in the *cone* conformation by treatment with sodium hydride, followed by *p*-bromobenzenesulfonyl chloride in anhydrous tetrahydrofurane. However, no examples of monoamino calix[4]arenes locked in a *cone* conformation were reported in that publication.

We thought that the lack of a convenient protocol for the *O*-alkylation of monoamino calix[4]arenes was a good opportunity to continue Gutsche's work and to create a method to generate *O*-alkylated monoarmed calix[4]arenes blocked in the *cone* conformation, and in that process, employ normal, stable and widely used substituents on the lower rim OH groups rather than the unusual and reactive 4-bromobenzenesulfonyl group chosen by Gutsche for his tetrasubstituted examples.

In order to obtain *O*-alkylated monoamino calix[4]arenes, *O*-propylation using 1-iodopropane might be the most convenient option. Once inserted, propoxy groups are reasonably inert to side reactions and their bulkiness prevents phenol rings from rotating through the calixarene cavity. *O*-propylation of calix[4]arenes is a very well known process in the literature and the reaction conditions are well established. Standard conditions involve initial deprotonation of hydroxyl groups by a strong base, like sodium hydride, in an anhydrous solvent, followed by the addition of the

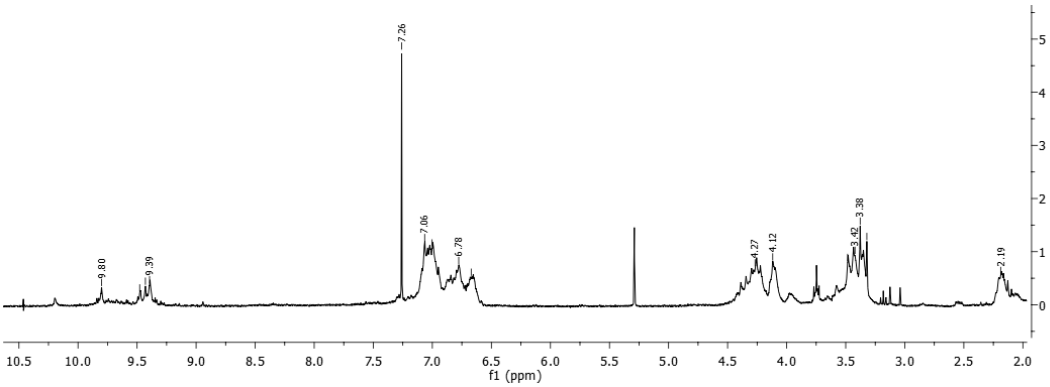
alkylating agent (in most cases 1-iodopropane). Stirring the reaction mixture overnight at room temperature normally affords the desired *O*-alkylated calix[4]arene in high yields.

However, attempts to *O*-propylate calix[4]arene **106** following the standard conditions described above were unsuccessful (scheme 3.18).



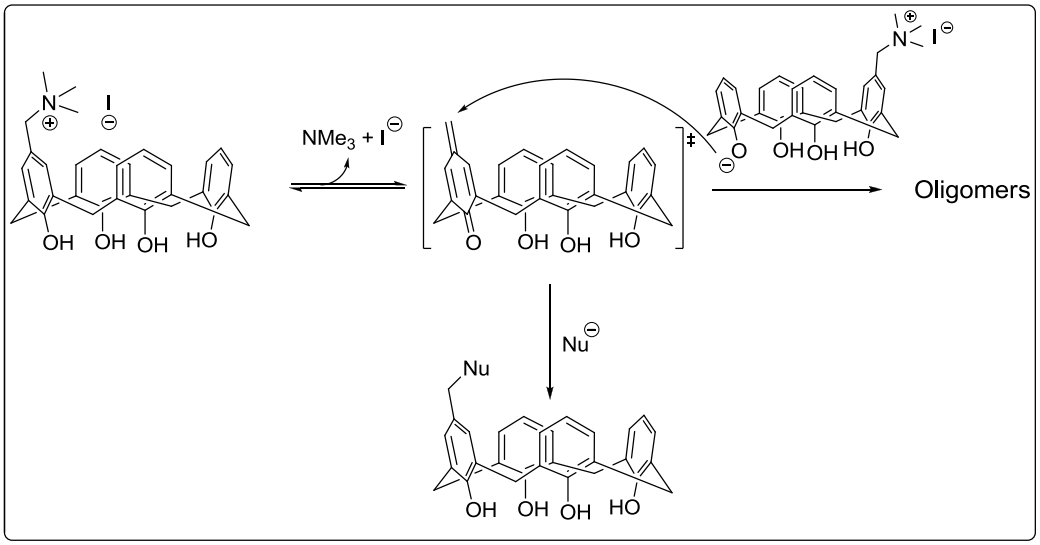
Scheme 3.18 Attempted O-propylation of calix[4]arene 106 following standard conditions.

When calix[4]arene **106** was deprotonated with six equivalents of sodium hydride in THF and treated with an excess of 1-iodopropane, a pale yellow solid was obtained as the only product in the reaction mixture. The resulting pale yellow solid was analysed by ^{1}H-NMR and the resulting spectrum showed very broad peaks that were not consistent with the structure of the desired calixarene **149** (spectrum 3.1).



Spectrum 3.1 ^{1}H-NMR spectrum of isolated product after attempting *O*-alkylation in calix[4]arene 106.

After considering the possibility of side reactions that might be taking place, we thought that the obtained product may consist in a mixture of oligomers resulting from the self-condensation of calixarenes. We also realised that the amino group in the upper rim may react with 1-iodopropane to form the quaternary ammonium salt that can decompose easily to afford the *p*-quinone methide. In that case, deprotonated hydroxyl groups in the lower rim of calixarenes may act as nucleophiles and react with *p*-quinone methides to produce oligomers (scheme 3.19).



Scheme 3.19 Proposed mechanism for the formation of calix[4]arene oligomers.

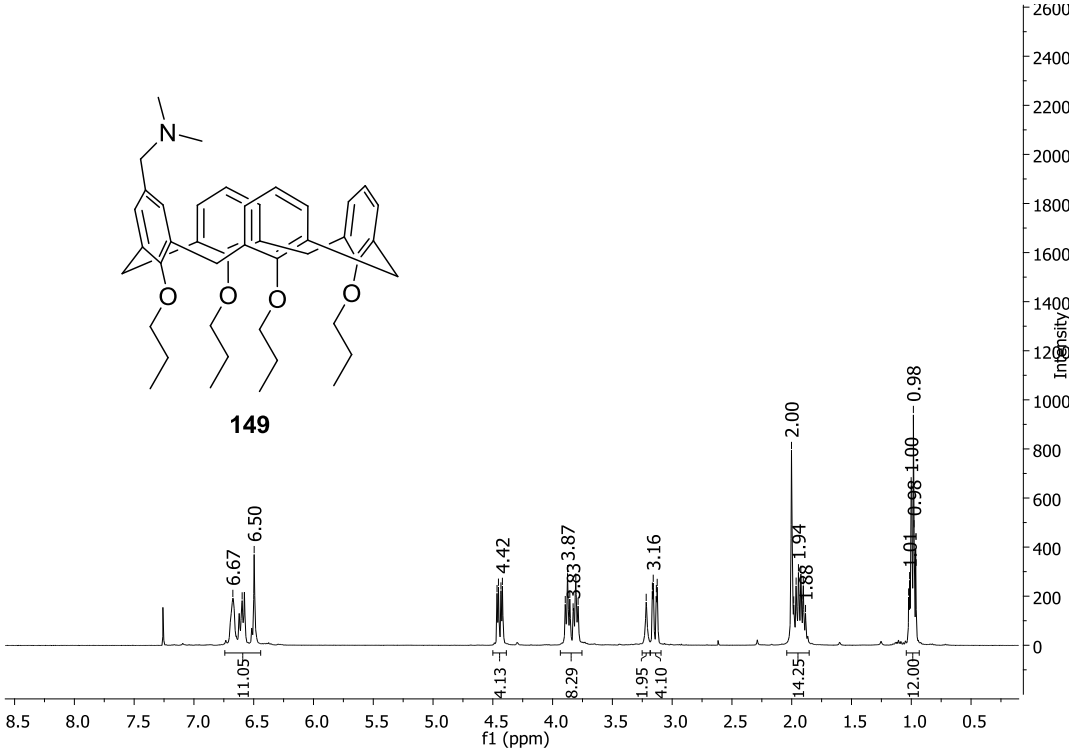
Thus, we thought we were experiencing a problem of selectivity between *O*- and *N*-alkylation. The *O*- versus *N*-alkylation competitions are well known in the literature and some studies have been carried out in order to determine how the *O*/*N* alkylation ratio can be modulated under different conditions. Smith and Robertson studied the competition between *O*- and N-alkylation in the reaction between oxime salts and methyl and benzyl bromide.[97] That study showed that, the affinity of electrophiles for either one nucleophilic centre or another depends on factors like type of solvent, electronic nature and structure of alkylating agent and concentration of reactants. For instance, it was found that for benzophenone oxime salts the use of bulky electrophiles and polar solvents in the alkylation reaction increased the proportion of *O*-alkylated product.

Similarly to Smith and Robertson, we thought we may also be able to find reaction conditions to favour *O*-alkylation. Therefore, we decided to study the effect of different solvents and alkylating agents in the *O*-propylation reaction of monoamino calixarene **106** with the hope of improving the selectivity and obtaining the desired monoarmed *cone* calix[4]arenes in moderate or good yields.

The use of an excess of sodium hydride, followed by 1-iodopropane in tetrahydrofuran at room temperature (table 3.4, entry 1) afforded a complex mixture of oligomers. The use of slightly bulkier electrophiles, such as propyl methanesulfonate in tetrahydrofuran, stopped the oligomerization but in this case, starting material was recovered as the major product.

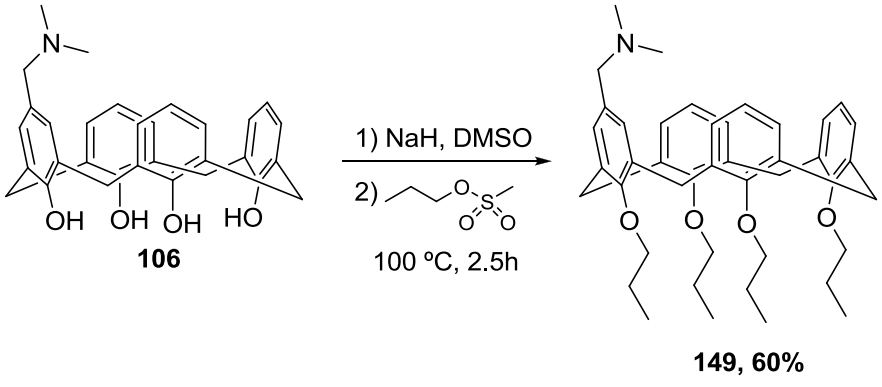
It was reported in the literature that, the addition of quaternary ammonium salts to the reaction media could increase the solubility of organic salts and make the reactions more efficient.[98] The addition of 3 equivalents of tetra-*n*-butylammonium chloride to the reaction mixture (entry 2) when THF was employed as solvent, did not produce any positive effect in the reaction and a mixture of oligomers was obtained once again as the main product.

The use of a more polar solvent like dimethylsulfoxide, instead of tetrahydrofuran, led to much promising results. When the propylation of calixarene **106** was attempted in DMSO, at 100 ºC, using propyl methanesulfonate as alkylating agent, the desired product was firstly obtained in a poor 15% yield (entry 7). This time, the isolated product showed a ^{1}H-NMR spectrum with sharp peaks in which all the chemical shifts and peak integrals were consistent with the desired mono-armed propylated calix[4]arene **149** (spectrum 3.2). The new monoamino calix[4]arene **149** was fully characterized by HRMS, ^{1}H and ^{13}C-NMR spectroscopies and FT-IR (ATR) spectroscopy. Surprisingly, when dimethylsulfoxide was used as solvent, the addition of tetra-*n*-butylammonium chloride (TBAC) to the reaction mixture, just before the addition of the alkylating agent, significantly increased the reaction yield from a poor 15% to a moderate 60% (entry 8). This reaction was performed several times, confirming its reproducibility and also the great contribution of TBAC to the selectivity and to the yield in the reaction, when DMSO was used as solvent (scheme 3.20). In contrast, the use of 1-iodopropane in DMSO always led to complex mixture of calixarenes.



Spectrum 3.2 ^{1}H-NMR spectrum of new monoarmed calix[4]arene 149.

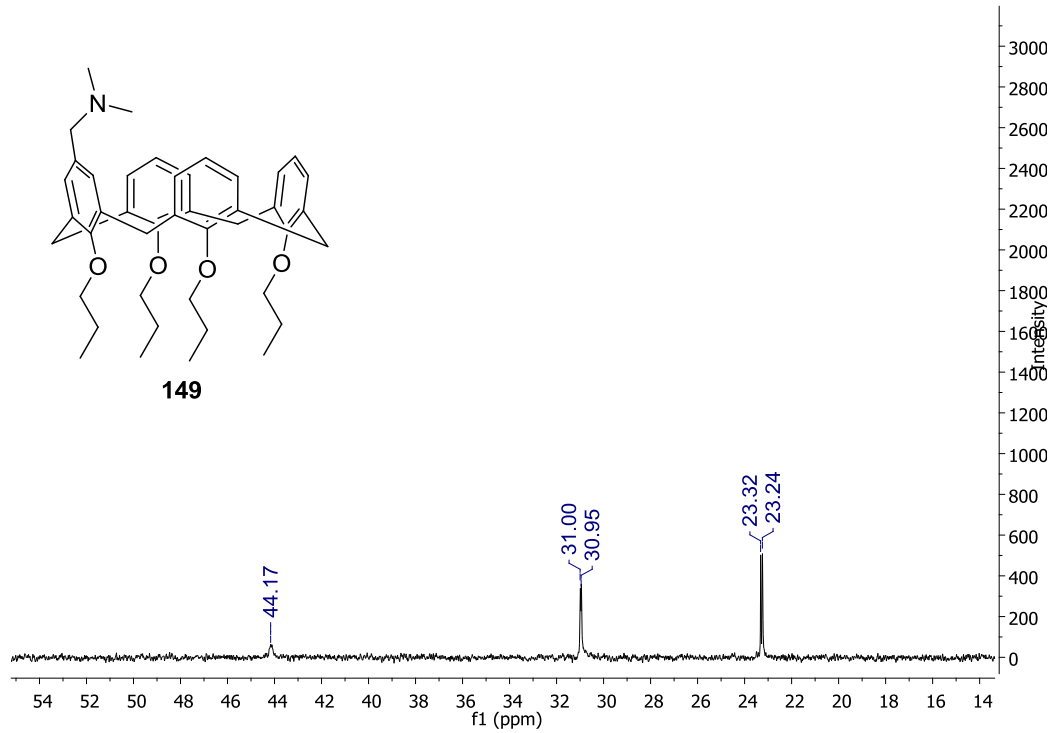
In addition to this study, we also investigated the use of microwave irradiation in the *O*-alkylation reaction. Microwave irradiation is known by reducing reaction times and increasing selectivities, affording in general, cleaner raction crudes and higher yields.



Scheme 3.20 Optimised reaction conditions for O-alkylation of compound 106.

The alkylation reaction of monoamino calixarene **106** under microwave irradiation when 1-iodopropane was used as alkylating agent and DMSO as solvent, afforded the desired product in a modest 24% (table 3.5, entry 1). The use of other solvents like *N,N*-dimethylacetamide, led to a complex mixture of calixarenes and the use of formamide afforded only starting material. The use of *N,N*-dimethylformamide gave the best results (entries 6, 7 and 8) although the combination with quaternary ammonium salts led to complex mixtures of calixarenes (entries 3,4 and 5). The use of 1-iodopropane as alkylating agent and DMF as solvent afforded the desired product in a moderate 55% yield after microwave irradiation at 90 ºC for 80 minutes (table 3.5, entry 7)

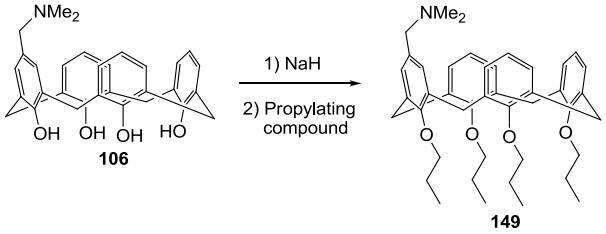
After the optimization work carried out and summarized in tables 3.4 and 3.5, which involved the screening of several solvents, electrophiles and reaction conditions, we were able to develop two suitable protocols (table 3.4, entry 8 and table 3.5, entry 7) for the preparation of *O*-propylated monoarmed calix[4]arenes in a moderate yield. The desired product obtained by the means of both methods, thermal heating and microwave irradiation, was found to be locked in the *cone* conformation. This was proved by ^{13}C-NMR spectrometry, following the method reported in the literature by De Mendoza and co-workers that was previously explained in the introductory section. The ^{13}C-NMR spectrum of the isolated product showed two peaks for the methylene bridges at 31.00 and 30.95 ppm. These values proved that all the phenol rings were orientated in the same direction and therefore, the new monoamino calix[4]arene was adopting a *cone* conformation, locked by the bulkiness of propyl chains in the lower rim (spectrum 3.3). The fact that two different types of methylene bridges were found in the ^{13}C-NMR spectrum was not surprising. This can be explained by the formation of a pinched *cone* conformation, where two opposite phenol rings are pointing outward.



Spectrum 3.3 ^{13}C-NMR spectrum of *cone* 5-(*N,N*-dimethylaminomethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene.

The developed method allowed the first and simple preparation of a monoarmed calix[4]arene confined in the *cone* conformation. The method was also highly selective to upper rim monofunctionalisation and allowed the preparation of *cone* monoarmed calix[4]arenes on a multi-gram scale with very simple work ups and purifications.

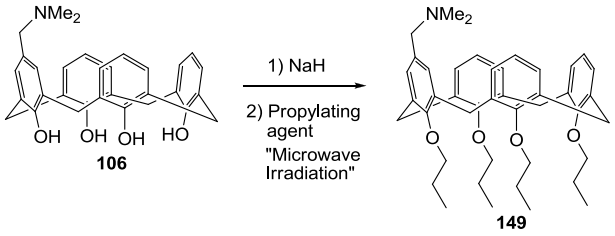
Table 3.4 Optimization reactions towards the *O*-alkylation of monoarmed calix[4]arene 106.



Entry	Solvent	Electrophile	Temperature / ºC	Ammonium Salt	Time / h	Product	Yield
1	THF	1-Iodopropane	21	-	24	Oligomers	-
2	THF	1-Iodopropane	21	TBAB	24	Oligomers	-
3	THF	Propyl methanesulfonate	21	-	24	Starting material	-
4	THF	Propyl methanesulfonate	21	TBAB	24	Oligomers	-
5	DMSO	1-Iodopropane	100	-	2.5	Mixture of calixarenes	-
6	DMSO	1-Iodopropane	100	TBAC	2.5	Mixture of calixarenes	-
7	DMSO	Propyl methanesulfonate	100	-	2.5	Desired product	15%
8	DMSO	Propyl methanesulfonate	100	TBAC	2.5	Desired product	60%
9	DMF	Propyl methanesulfonate	100	TBAC	2.5	Mixture of calixarenes	-

We thought that monoamino calix[4]arene **149** may have a great potential as a precursor of many other monoarmed calix[4]arenes blocked in the *cone* conformation. Calix[4]arene **149** may act as a "gate compound", where the amino group installed in the upper rim may be converted into different useful functionalities. Therefore, we started to look for possible different transformations that allowed the conversion of bis-alkyl benzylamines into other functional groups.

Table 3.5 Optimization reactions towards the *O*-alkylation of calix[4]arene 106 under microwave irradiation. Reactions were carried out at 90 °C for a period of 80 minutes.



Entry	Solvent	Electrophile	Ammonium Salt	Product	Yield
1	DMSO	1-Iodopropane	-	Desired product	24%
2	DMA	1-Iodopropane	-	Mixture	-
3	DMF	Propyl methanesulfonate	TMBC	Mixture	-
4	DMF	Propyl methanesulfonate	TEAC	Mixture	-
5	DMF	Propyl methanesulfonate	TBAC	Mixture	-
6	DMF	Propyl methanesulfonate	-	Desired product	52 %
7	DMF	1-Iodopropane	-	Desired product	55 %
8	DMF	Propyl *p*-toluenesulfonate	-	Desired product	34 %
9	DMF	Propyl trifluoromethanesulfonate	-	Decomposition	-
10	Formamide	1-Iodopropane	-	Starting Material	-

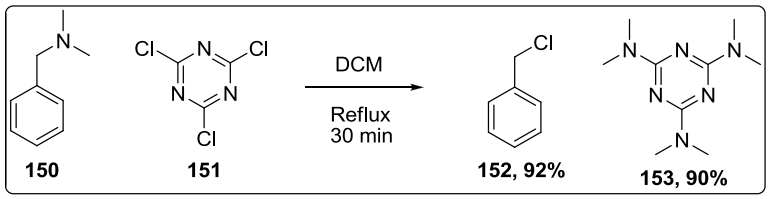
Quaternization of the tertiary amine, followed by treatment with a nucleophile and heating was not a likely transformation, since Gutsche *et al.* already proved the high stability of the quaternary amines attached to the calixarenes. Our initial strategy was focused on the transformation of monoarmed amino calix[4]arene **149** into a few other monoarmed calix[4]arenes, bearing versatile functionalities, which were able to act as starting materials for a broad family of monosubstituted calix[4]arenes.

Two key functionalities were identified as greatly versatile groups to install in the upper rim of monoarmed calix[4]arenes. These groups were:

- Halogens: Benzyl chlorides or bromides can afford a great number of new functionalities by simple nucleophilic substitution.

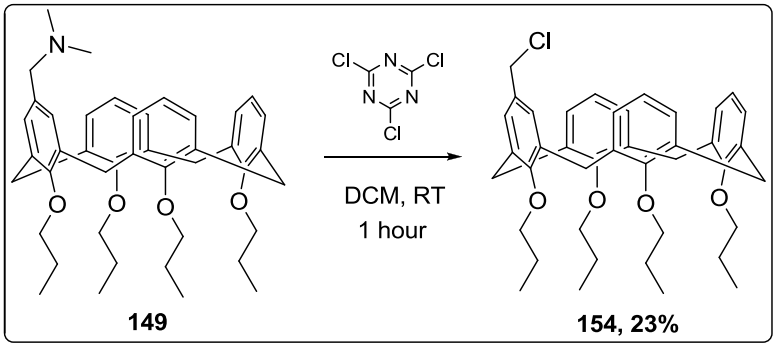
- Aldehydes: The formyl group can be transformed into many interesting compounds like imines, carbon-carbon double bonds (Knoevenagel reaction), alcohols or carboxylic acid derivatives.

After a thorough search in the literature, the transformation of tertiary benzylamines into the corresponding benzyl chloride derivatives was found in a paper published on 2009 by Kolesinska *et al.*[99] The reaction of interest was part of a wider work related to the preparation of melamine derivatives. In that work, cyanuric chloride was efficiently reacted with *N,N*-dimethylbenzylamine **150** to afford *N,N*-dimethylaminotriazine **153** in 90% yield, along benzyl chloride that was obtained as byproduct in 92% yield (scheme 3.21).



Scheme 3.21 Synthesis of benzyl chloride from *N,N*-dimethylbenzylamine and cyanuric chloride.

Treatment of calixarene **149** with cyanuric chloride in dichloromethane at room temperature for one hour, afforded the desired chloromethyl calix[4]arene **154** in a low 23% yield (scheme 3.22).

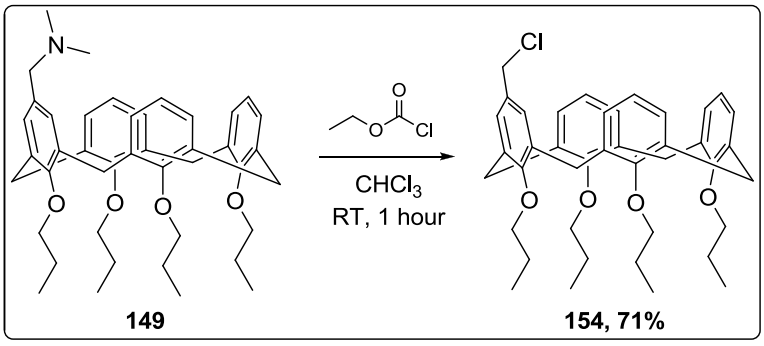


Scheme 3.22 Synthesis of chloromethyl calix[4]arene 154 using cyanuric chloride.

Attempts to increase the reaction yield by extending the reaction time or increasing the reaction temperature were unsuccesful. In the paper published by Kolesinska's and co-workers, it was explained that, for the reaction to proceed, the three chlorine atoms of cyanuric chloride should be substituted by amines, in order to generate an unstable compound that decomposed to form benzyl chloride and melamine derivatives. When only one or two of the chlorine atoms were substituted by amines, no formation of products was observed. That suggests that in our case, three monoarmed calixarenes must react with one molecule of cyanuric chloride before decomposition and formation of products occur. Due to the bulkiness of calixarenes, the formation of trisubstituted species might be difficult which might explain the low yields obtained.

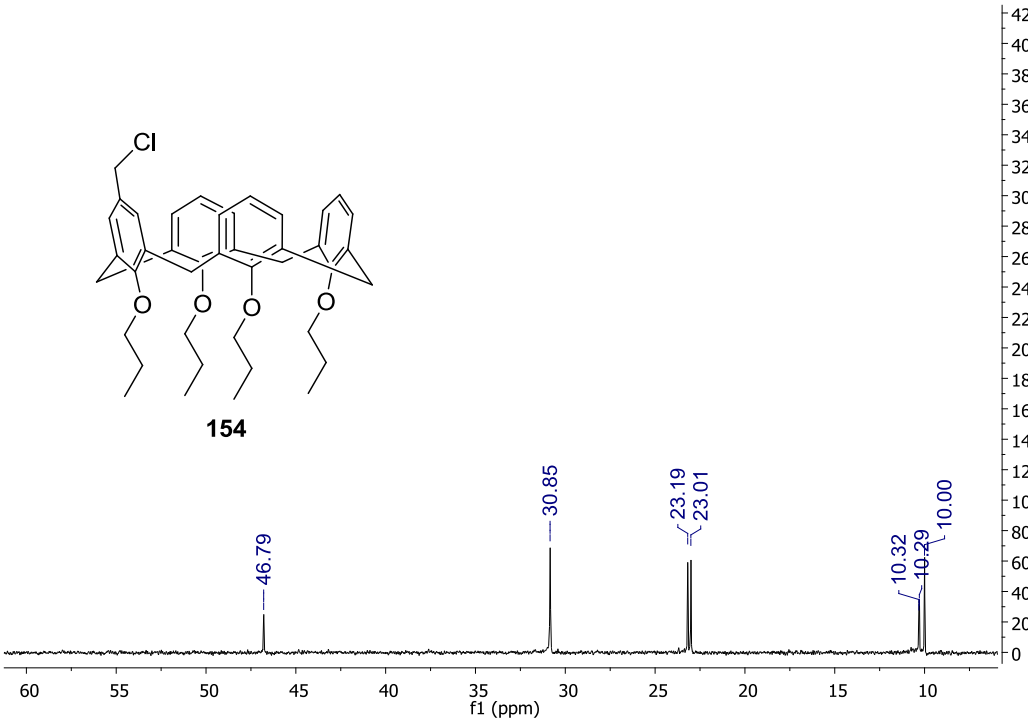
We decided then, to look for a more suitable route with higher yields and lower reaction times. Knabe *et al.* reported the conversion of *N,N*-dimethyl-4-methoxybenzylamine into the corresponding benzyl chloride by treatment with ethyl chloroformate.[100] An electron donating group in the *para* position of benzylamines was essential for the reaction to proceed.

In order to increase the yield of this interesting reaction, ethyl chloroformate was used instead of cyanuric chloride and the mixture with calix[4]arene **149** heated in chloroform at 55 ºC for 30 minutes. After this time, the solvent and volatiles were removed under reduced pressure and the crude product was filtered through silica to afford the desired calix[4]arene **154** in a moderate 65% isolated yield. Finally, the yield was increased up to 71 % yield when the mixture was stirred at room temperature in chloroform for one hour (scheme 3.23).



Scheme 3.23 Synthesis of chloromethyl calix[4]arene 154 using ethyl chloroformate.

The *cone* conformation was kept during the functional group interconversion as could be confirmed by ^{13}C-NMR spectroscopy (spectrum 3.4).

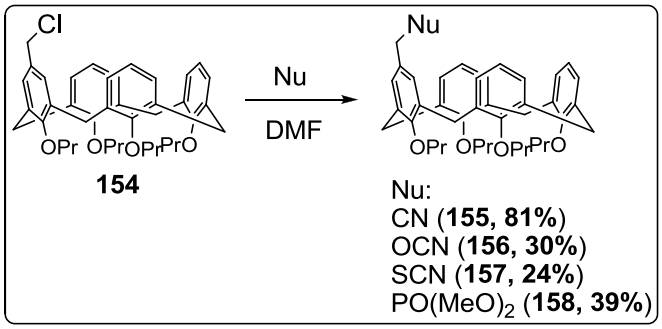


Spectrum 3.4 ^{13}C-NMR spectrum of *cone* 5-(chloromethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene 154.

A peak at 30.85 ppm was found in the ^{13}C-NMR spectrum for the bridge methylenes of calix[4]arene **154,** confirming the *cone* conformation.

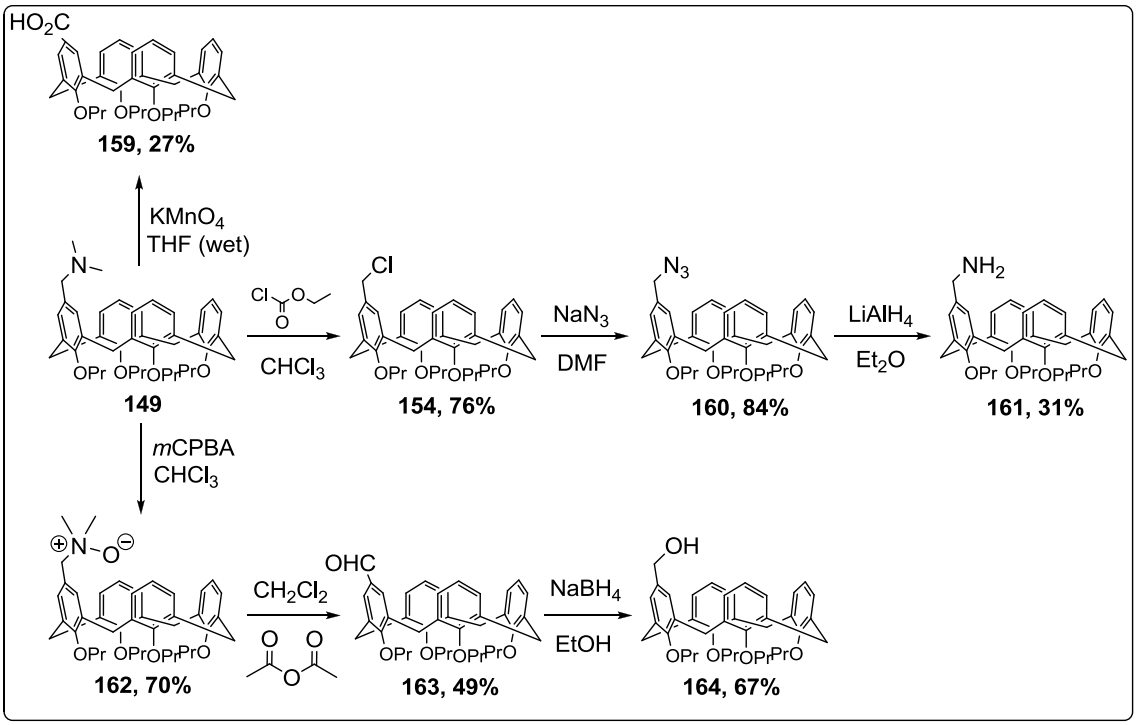
In this way, we have created a simple and efficient protocol to afford the versatile chloromethyl calix[4]arene in good yields after an easy purification. In a route that we named as the "Chloroformate Route", chloromethyl calix[4]arene **154** was employed in a range of nucleophilic substitutions acting as the electrophile and affording several new monosubstituted calix[4]arenes blocked in the *cone* conformation (conformations were confirmed in each case by ^{13}C-NMR spectroscopy). Reaction of chloromethyl calix[4]arene **154** with nucleophiles such as cyanide, cyanate, thiocyanate or phosphite was successfully carried out (scheme 3.24). Treatment of **154** with trimethylphosphite at 95 ºC for 48 hours afforded the Arbuzov product in 39 % yield. This compound is a precursor for Horner-Wadsworth-Emmons reactions. Treatment of **154** with

potassium cyanide in DMF afforded the corresponding cyanomethylcalixarene in a good 81% yield, which can also be used in 'click' chemistry.



Scheme 3.24 Synthesis of monoarmed calix[4]arenes by nucleophilic substitution.

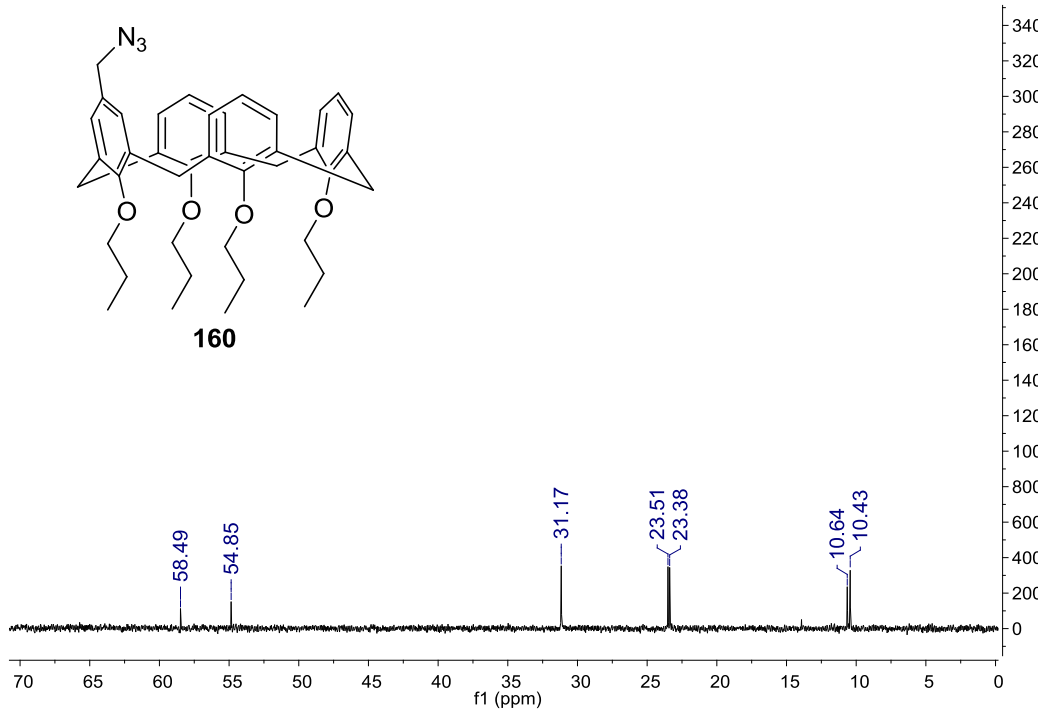
Monosubstituted amino calix[4]arene **149** was also successfully employed as "gate compound" during the synthesis of new monoarmed calix[4]arenes (scheme 3.25). Calixarene **149** was converted into the corresponding mono-formyl calix[4]arene by oxidation of the amino group with *meta*-chloroperbenzoic acid (*m*CPBA), followed by reflux in toluene in the presence of acetic anhydride (Polonovski rearrangement). In the Polonovski rearrangement, the nucleophilic oxygen in the *N*-oxide is able to attack an electrophile, normally acetic anhydride or acyl chloride, to give an imminium salt which, after a series of rearrangements, affords the desired aldehyde (scheme 3.25). We named this route as the "*N*-oxide route". The "*N*-oxide route" afforded for first time a selective methodology for the preparation of mono-formyl calix[4]arene in good to moderate yield without the use of neither pyrophoric *n*-BuLi nor difficult purifications. The access to the mono-formyl calix[4]arene can allow the use of many synthetic transformations like reduction to the alcohol, formation of imines, reductive amination, Horner–Wadsworth–Emmons reaction, Wittig reaction, aldol condensation, oxidation to the acid, etc. The formyl group in calix[4]arene **163** was reduced to the alcohol by treatment with sodium borohydride, affording calix[4]arene **164** in 67% yield (scheme 3.25).



Scheme 3.25 Functional group interconversion of calix[4]arene 149.

A second transformation was the direct oxidation of dimethylamino calix[4]arene to the carboxylic acid by treatment with potassium permanganate in wet tetrahydrofuran. This reaction afforded the desired compound **159** in a moderate yield (27%) and produced useful mono-formyl calixarene as a side product though can be easily removed by column chromatography.

Treatment of calix[4]arene **154** with sodium azide in DMF afforded the azide compound **160** in the *cone* conformation and in 84 % yield (spectrum 3.5). This compound is of particular interest in 'click' chemistry. Calix[4]arene **160** was successfully reduced to the corresponding aminomethyl calix[4]arene **161** by treatment with lithium aluminium hydride (scheme 3.25).



Spectrum 3.5 ^{13}C-NMR spectrum of *cone* 5-(azidomethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene 160.

In conclusion, we were able to obtain a series of new transformations that allowed the preparation of many types of monosubstituted calix[4]arenes blocked in the *cone* conformation.

3.2.2. Applications of monosubstituted calix[4]arenes.

Our new methodology allowed for first time a simple access to monoarmed calix[4]arenes. Our desire was to use one of these new monoarmed calix[4]arenes in both supramolecular and sensor chemistry. Our first idea consisted in the use of these monoarmed macrocycles as sensors for small molecules or metals. We thought about the possibility of installing a ferrocene group on the upper rim of calix[4]arene (figure 3.19). The cavity could be then targeted by different metals and the interaction measured by fluorescence spectroscopy. In this way, hydroxymethyl calix[4]arene **164** was reacted with the ferrocene derivative **165** to afford, as far as we are aware, the first example of monoferrocenyl calix[4]arene **166**. A 50 µM solution of ferrocene calix[4]arene **166** in acetonitrile (0.5 ml) was mixed with a 250 µM solution of a heavy metal ($CdCl_2$, $SnCl_2$ and

$CuCl_2$) and the fluorescence emission measured after irradiation at 240 nm. We found that the fluorescence intensity was strongly quenched when Cu(II) was present in the mixture, showing a high sensitivity to this metal. Softer bivalent metals like cadmium or tin did not have much effect in the fluorescence intensity. This experiment illustrates how this new type of calix[4]arene can be used as sensor (figure 3.19). A likely explanation for the fluorescence quenching may be the strong electron transfer from the carbonyl group in the upper rim of calix[4]arene **166** to the metallic centre.

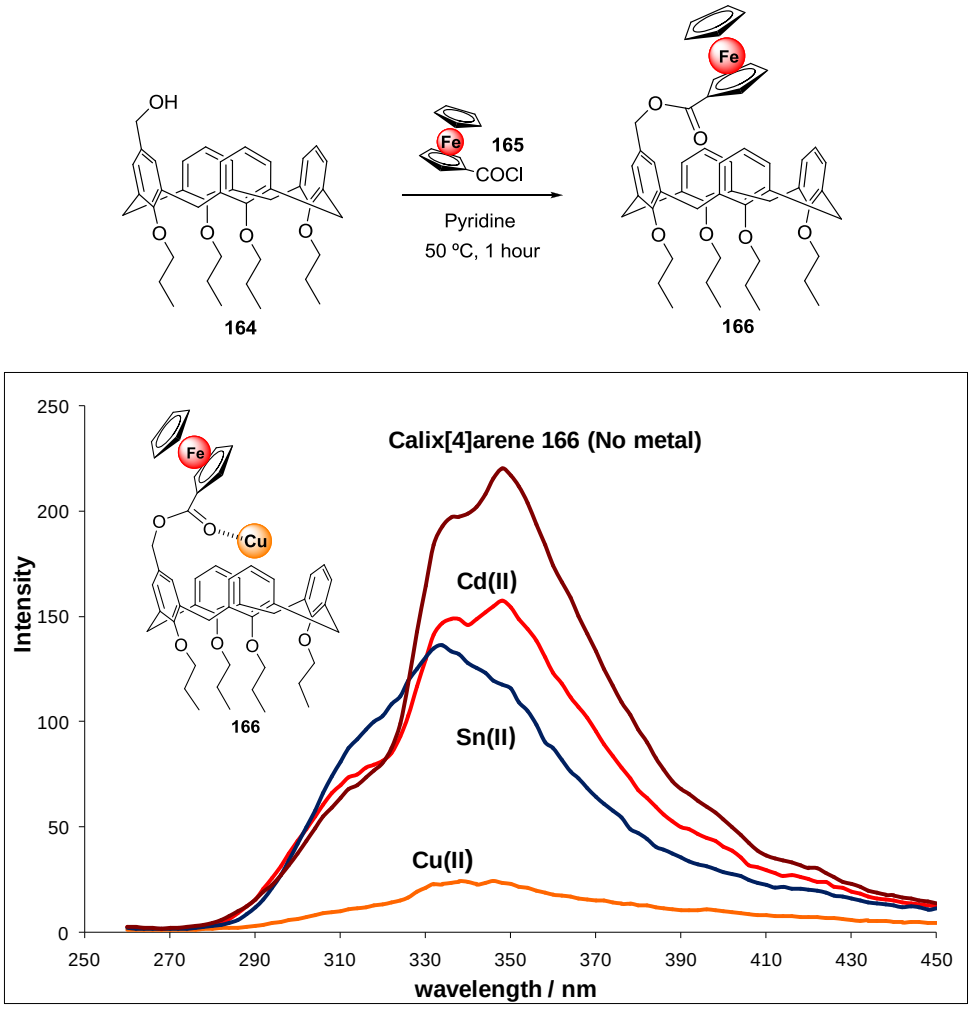
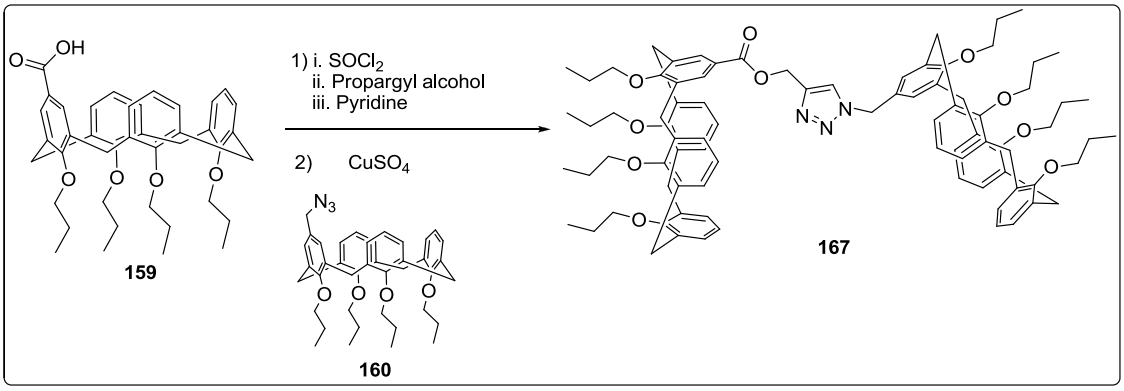


Figure 3.19 Synthesis of monoarmed ferrocenyl calix[4]arene and its use as a sensor for metals.

The second application was linked to supramolecular chemistry and it was related to the possibility of forming host-guest complexes. We thought monoarmed calix[4]arenes were very good

scaffolds for the preparation of nanocapsules. The formation of nanocapsules could be easily achieved by the condensation of suitable pairs of complementary monoarmed calixarenes by simple reactions such as esterification or “click chemistry”. To illustrate that, we reacted azidomethylcalix[4]arene **160** and the propargyl ester of carboxy calix[4]arene **159** to afford an example of a head to head asymmetric dimer based on calix[4]arenes and prepared by a simple method (scheme 3.26).



Scheme 3.26 Synthesis of head to head dimer based on monoarmed calix[4]arenes.

Compounds such as the dimer **167** may be used as a host molecule for smaller species such as small organic molecules or quaternary ammonium salts. The fact that the formed dimer is asymmetric may lead to host-guest complexes where the guest molecule may adopt a preferred orientation, allowing molecular recognition.

3.2.3. Highly efficient Cannizzaro reaction. Easy access to multifunctional calix[4]arenes.

The development of a simple methodology that allows the generation of chiral functionalised calix[4]arenes still remains a challenge. Only a few methods for the synthesis of chiral calixarenes are reported in the literature, and all of them require of a large number of synthetic steps.[101] The abilty to prepare inherently chiral multisubstituted calix[4]arenes is of great importance as this would allow their use in relevant fields of science. Chiral calix[4]arenes may potentially be used as

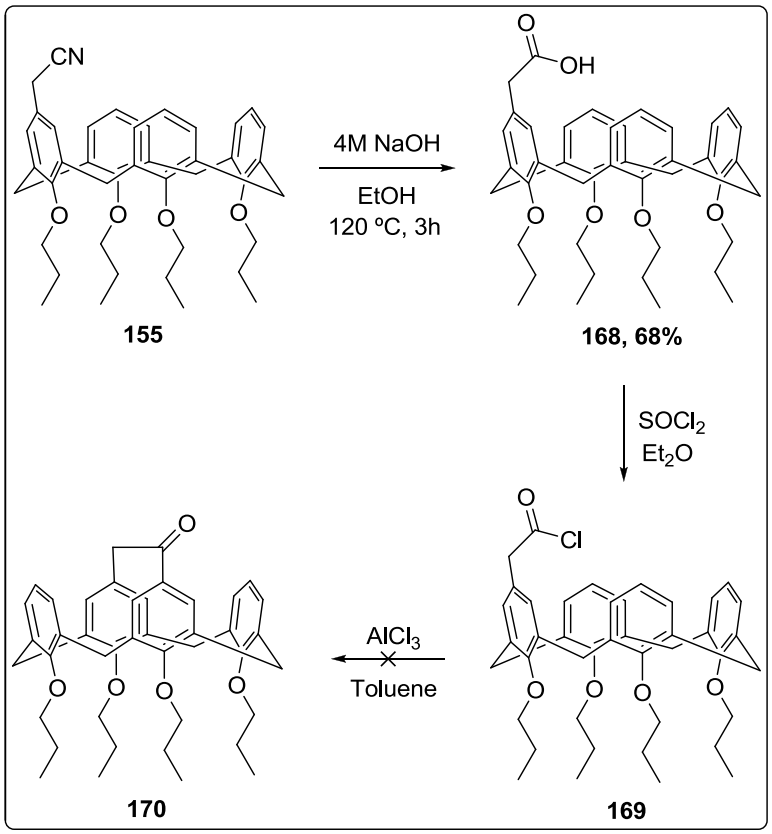
a new family of chiral ligands, as new stationary phases employed in the fabrication of chiral chromatographic columns or as a new family of chiral organocatalysts in organic chemistry.

Building on our research on monosubstituted calix[4]arenes, we wondered whether some of the monoarmed calix[4]arenes described before could be used as starting materials for the preparation of multisubstituted calixarenes and eventually, for the preparation of more sophisticated chiral calixarenes. We also wondered whether a functional group installed on the upper rim of monoarmed calix[4]arenes could modulate the insertion of a second different functional group in the available *para* positions on the upper rim. In that case, the presence of two different functional groups in the upper rim could then modulate the insertion of a third group, leading directly to chiral calix[4]arenes in only a few synthetic steps.

In order to control the insertion of a second functional group in the upper rim, we considered two different possibilities. First, the insertion of a chiral functional group, able to orientate the insertion of a second functional group in the upper rim, and secondly, the insertion of a functional group capable of reacting intramolecularly and selectively with one of the available *para* positions on the upper rim of calix[4]arenes. Both methods would allow the selective installation of two different functional groups on the upper rim of calix[4]arenes. The selective addition of a third different functional group, would directly lead to chiral calix[4]arenes. We decided to start our investigations towards the synthesis of multifunctional calix[4]arenes with the second approach which involved the use of an intramolecular reaction. This decision was taken after studing the transannular reactions for calix[4]arenes described in the introductory section.

The fact that alkylated calix[4]arenes normally adopt a pinched *cone* conformation, makes it possible that two opposite aromatic rings can be placed very close each other. We thought this conformation could allow the reaction between the first introduced functional group and the opposite *para* position in the macrocycle. By way of illustration, the insertion of an acid chloride functionality on the upper rim could allow intramolecular Friedel-Crafts acylations. The formation of a pinched *cone* conformation, may ideally lead to selective acylation of the opposite phenol ring (scheme 3.27). In that case, an upper rim 1,3-bridged calix[4]arene would be selectively formed between two opposite aromatic rings. The resulting ketone from the Friedel-Crafts acylation could

be then derivatized to afford a chiral calix[4]arene. The regioselective insertion of a third group on the upper rim of these type of chiral calix[4]arenes could be achieved by asymmetric induction, leading to chiral tri- and tetrasubstitued calix[4]arenes.

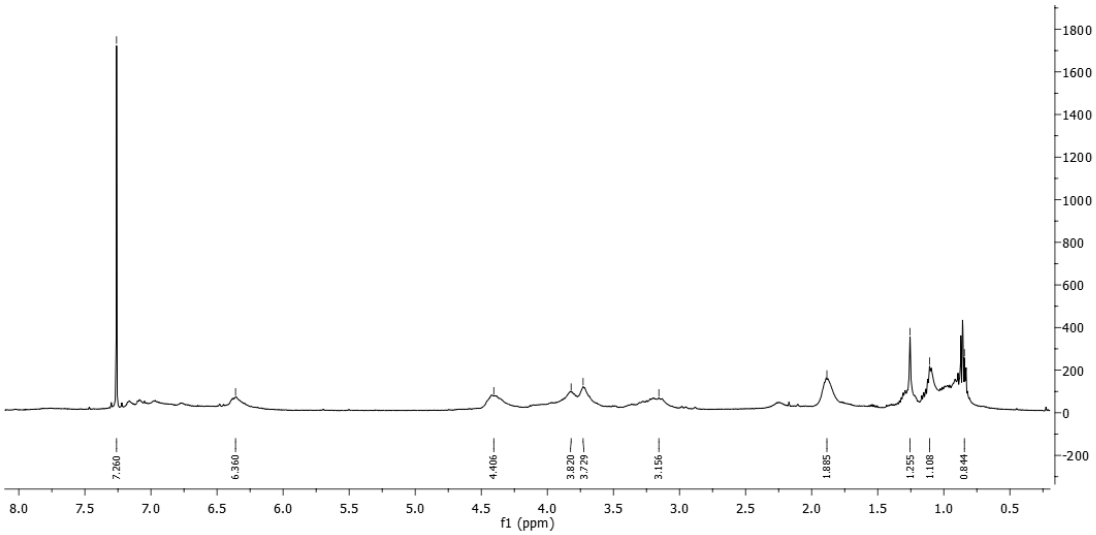


Scheme 3.27 Attempted transannular reaction in calix[4]arene 169.

Compound **168** was successfully prepared by alkaline hydrolysis of cyanomethyl calix[4]arene **155**. Compound **155** was dissolved in ethanol and a 4M NaOH solution added. The mixture was microwave irradiated for 3 hours at 120 ºC to afford acid **168** in 68% yield. The carboxylic goup was transformed into an acid chloride in a second step, by treatment with thionyl chloride and the resulting calixarene was submitted without further purification to the next step to avoid any decomposition. Intramolecular Friedel-Crafts acylation of calix[4]arene **169** was attempted using a diluted solution of this compound in dry toluene and aluminium trichloride was employed as Lewis acid catalyst. The solution was stirred at room temperature overnight. After the

work up, ^{1}H-NMR spectrum of the crude product showed very broad peaks (spectrum 3.6) that may suggest the formation of polymeric material. This result could be explained by the intermolecular polymerization of calix[4]arene **169**.

Intramolecular transannular reaction also did not proceed when compound **169** was treated with boron trifluoride as Lewis acid. Again, very broad peaks were observed by ^{1}H-NMR spectroscopy.



Spectrum 3.6 ^{1}H-NMR spectrum of isolated product obtained after the treatment of monoarmed calix[4]arene 169 with $AlCl_3$ in dry toluene.

At that point, we realised that the intramolecular reaction of **169** may be badly affected by entropic factors. This reaction may require high structural order, placing two opposite aromatic rings close to each other in a pinched *cone* conformation at the same time that, the carboxylic group was conveniently orientated so that aromatic electrophilic substitution in the opposite ring could proceed. During the intermolecular Friedel-Crafts acylation, however, the formation of a pinched *cone* conformation is not required for the reaction to proceed.

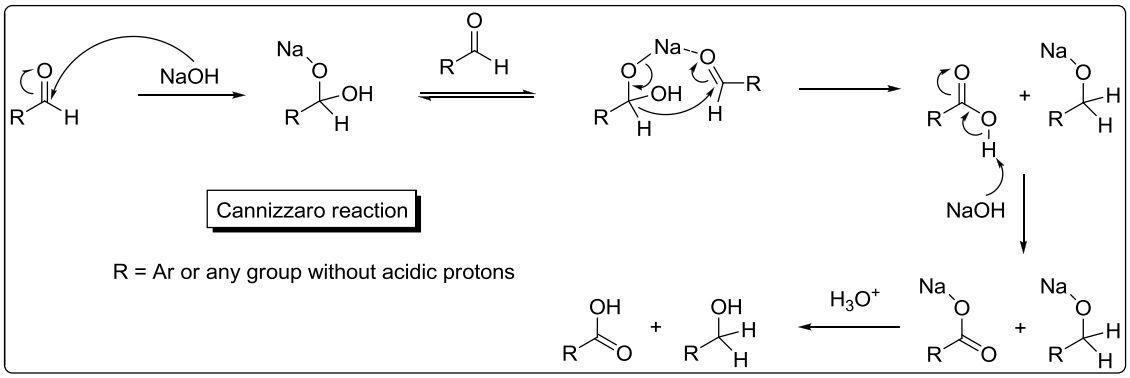
The distance between opposite phenol rings however should not be a problem for an intramolecular reaction to proceed. As was explained in the introduction, several examples of

intramolecular reactions have been reported where the formation of a two-atom transannular bridge has been successfully achieved.

In our opinion, the reaction should be more entropically favoured when the two functional groups involved in the reaction were placed in the *para* position of different and opposite aryl moieties. In this way, the formation of a suitable pinched *cone* conformation should be enough to allow the intramolecular reaction between opposite functional groups.

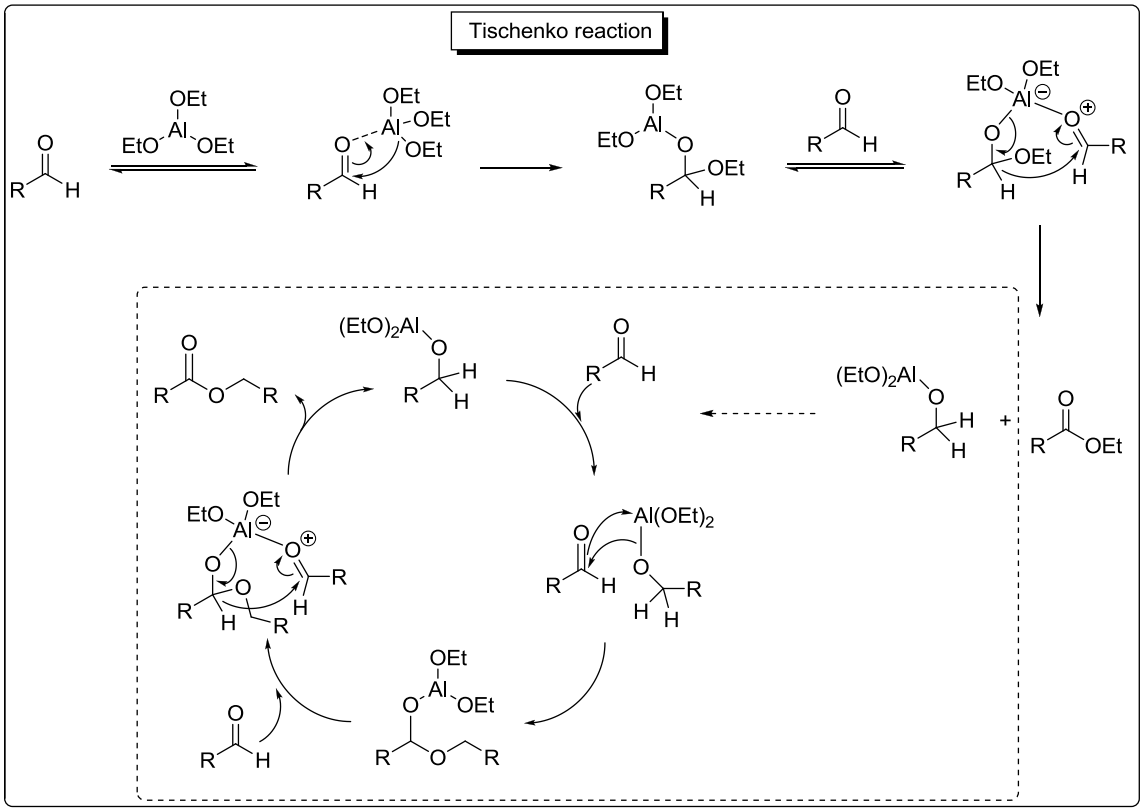
Following this hypothesis, we decided to prepare a 1,3-disubstituted calix[4]arene and attempt an intramolecular and transannular reaction in the macrocycle.

We thought 1,3-diformyl calix[4]arene **171** may be a good candidate for our intramolecular reactions since aldehydes can undergo self-reaction, leading to disproportionation and affording two different products. Depending on the catalyst used, the disproportionation of aldehydes can lead to different type of products. When hydroxide salts are used as catalyst, the resulting products from the condensation of two aldehydes are an acid and an alcohol (Cannizzaro reaction, scheme 3.28, *vide infra*).



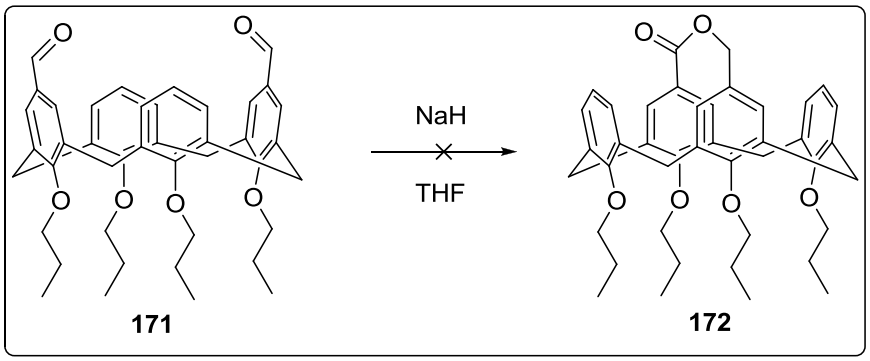
Scheme 3.28 Mechanism of Cannizzaro reaction.

However, when aluminium alkoxides are employed as catalysts, two aldehydes are also able to disproportionate and condense to afford an ester function (Tischenko reaction, scheme 3.29).



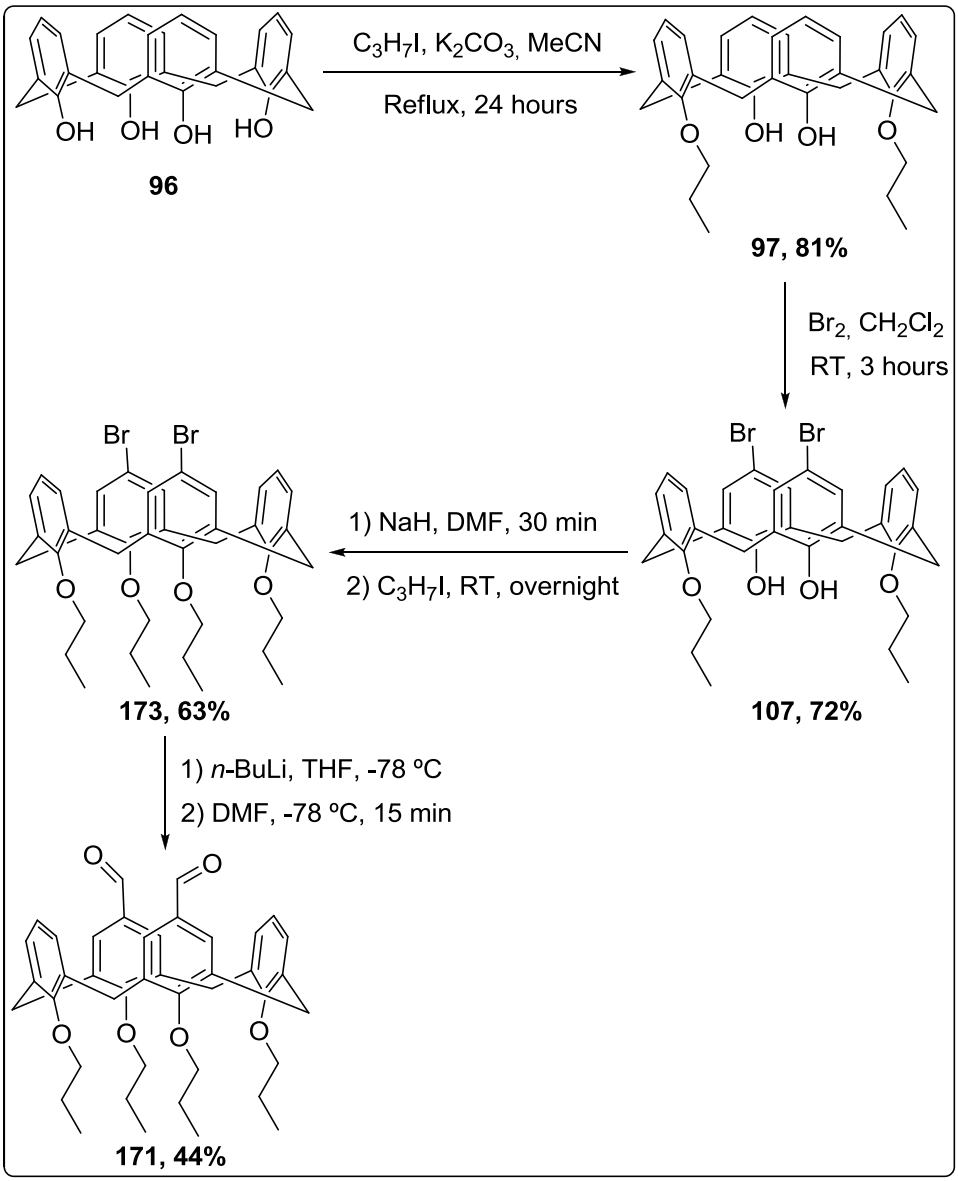
Scheme 3.29 Mechanism of Tischenko reaction.

We decided to continue our investigations on transannular reactions attempting the intramolecular Tischenko reaction of 1,3-diformyl calix[4]arene **171**, whose product should be a more rigid disubstituted calix[4]arene (scheme 3.30).



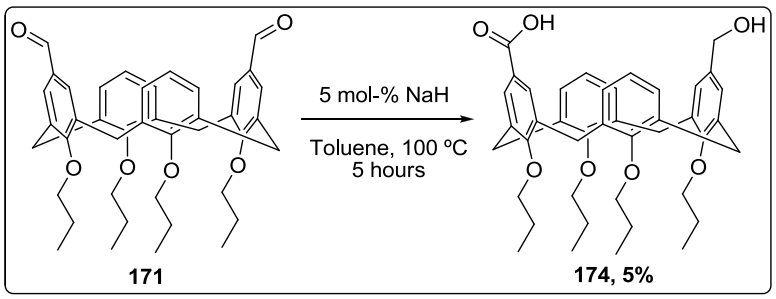
Scheme 3.30 Attempted intramolecular Tischenko reaction in calixarene 171.

The starting material **171** was synthesised from calix[4]arene **96** in a 16% overall yield following a protocol described in the literature (scheme 3.31).[102] In the first step, calixarene **96** was selectively *O*-alkylated in the positions 1 and 3 using potassium carbonate and 1-iodopropane. In the second step, the upper rim was selectively brominated followed by further *O*-alkylation in the lower rim. In the last step, dibromo calix[4]arene **173** was treated with *n*-butyllithium at –78 ºC, followed by the addition of anhydrous *N,N*-dimethylformamide to afford diformyl calix[4]arene **171** in 44% yield.



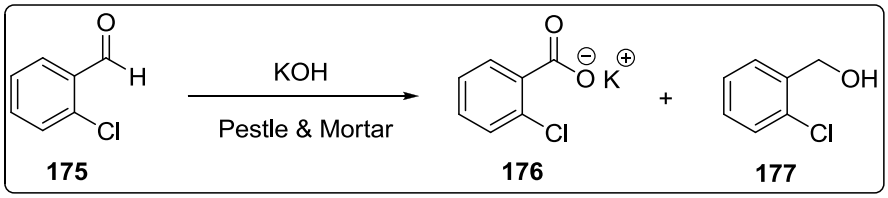
Scheme 3.31 Stepwise synthesis of diformyl calix[4]arene 171.

Several catalysts were used in the intramolecular Tischenko reaction. In first place, a thioalkoxide salt was employed, but no reaction was observed. In a second attempt, sodium hydride was used as a catalyst,[103] and a new product was formed in the reaction. However, this product was isolated in very low yield (5%). Furthermore, the obtained product was not the desired Tischenko product but one which resulted from a Cannizzaro reaction (scheme 3.32). To explain the unexpected reactivity we assumed that small amounts of water in the solvent employed during the reaction (toluene) could be generating small amounts of sodium hydroxide after reacting with sodium hydride and, the sodium hydroxide generated *in situ* may be catalysing the intramolecular Cannizzaro reaction.



Scheme 3.32 Cannizzaro product obtained after attempting a Tischenko reaction in calix[4]arene 171.

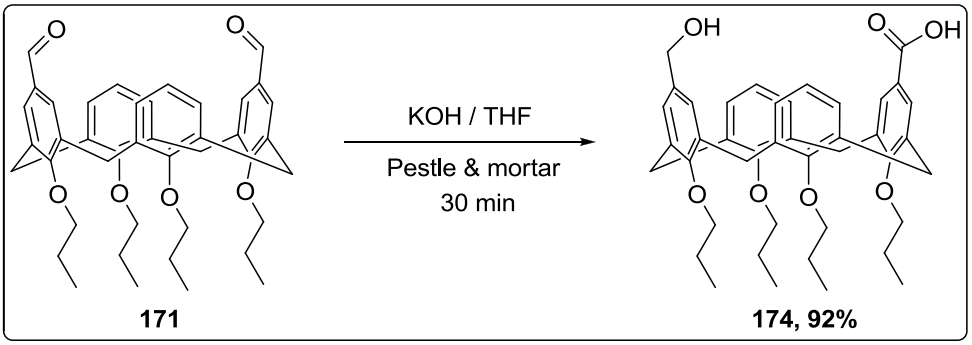
At that point, we realised the Cannizzaro reaction could be equally useful in the preparation of bi-functional calix[4]arenes starting from symmetrical calixarenes. Therefore, we decided to turn our efforts into finding a more suitable catalyst for this type of reactions in calix[4]arenes. This reaction is not new in calix[4]arenes and as discussed in the introductory section, an intramolecular Cannizzaro reaction had already been achieved by Casnati *et al.* However, this reaction proceeded only in moderate yield (60%), after very long reaction times, and we decided to optimize reaction conditions in order to obtain a more efficient reaction. A literature reference was found in which the intermolecular Cannizzaro reaction of 2-chlorobenzaldehyde was successfully achieved in only a few minutes, after grinding a neat mixture of potassium hydroxide and the aldehyde (scheme 3.33).[104]



175 176 177

Scheme 3.33 Solvent-free Cannizzaro reaction of 2-chlorobenzaldehyde.[104]

In order to reduce the reaction times reported by Casnati *et al.*, we thought this solvent-free approach could be useful. Thus, calixarene **171** was placed in a glass mortar to which was added an excess of potassium hydroxide along a few drops of THF and the resulting mixture, was ground for five minutes. TLC analysis of the resulting paste revealed that most of the calixarene **171** had disappeared and a new and more polar product had formed. The grinding operation was continued for five more minutes after placing the glass mortar in a heater at 90-100 ºC and until complete consumption of the starting material was observed by TLC. After cooling down, the resulting white solid was dissolved in 2M hydrochloric acid aqueous solution and then extracted with diethyl ether. After drying over anhydrous magnesium sulfate and removing the solvent under reduced pressure, the desired product was obtained in yields over 90% (scheme 3.34).

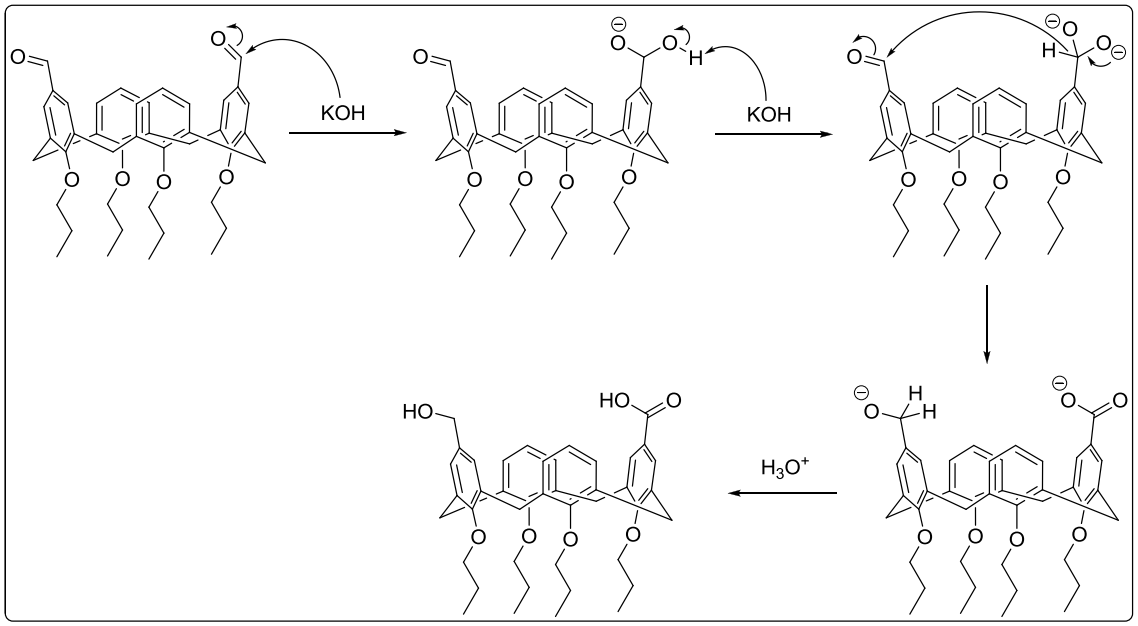


171 174, 92%

Scheme 3.34 Highly efficient Cannizzaro reaction of calix[4]arene 171.

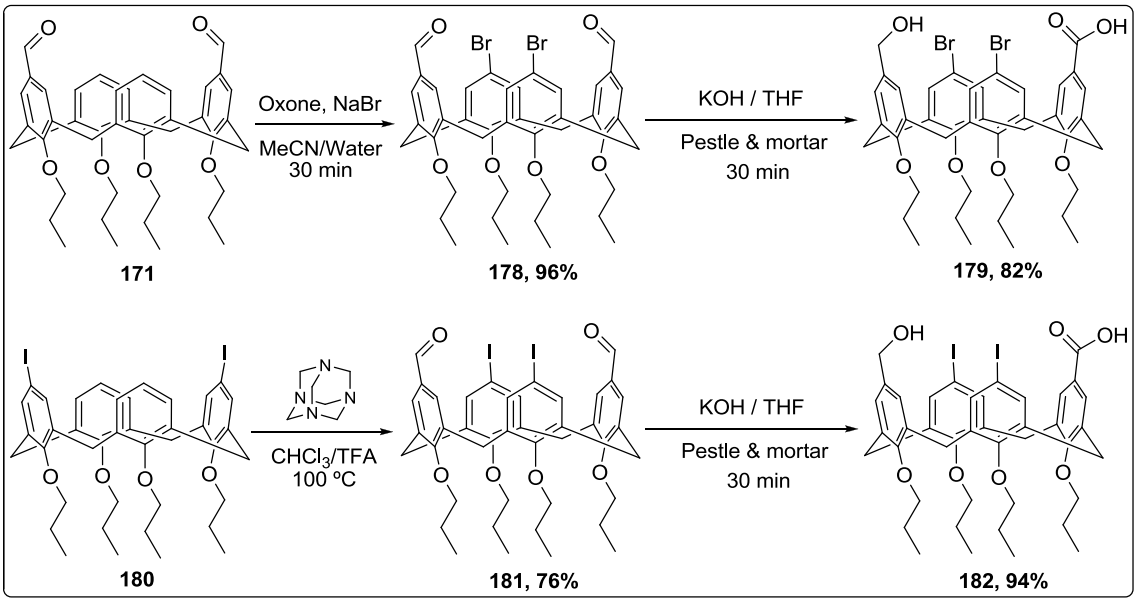
The fact that close to quantitative yields were obtained in the reaction, strongly indicated that the Cannizzaro reaction was taking place intramolecularly. Otherwise, a mixture of products, bis-

alcohol and bis-carboxylic acid together with the desired product, may have been obtained instead, due to random intermolecular reactions. The generally accepted mechanism for the Cannizzaro reaction involves the attack of hydroxide anion to one formyl group to form a hydrate monoanion, which may not be reactive enough to transfer a hydride anion. The initial monoanion, may then be deprotonated by a second hydroxide anion in a rate-determinating step, to afford a highly reactive species capable of transferring a hydride anion to the opposite aldehyde. This aldehyde would then afford a more stable negatively charged calixarene derivative that, after neutralization with aqueous HCl solution, would afford the desired product (scheme 3.35).



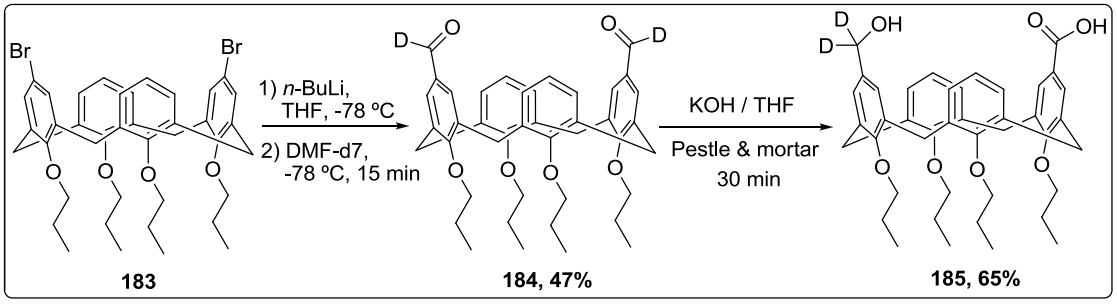
Scheme 3.35 Proposed mechanism for intramolecular Cannizzaro reaction of calix[4]arene 171.

We were delighted with the results obtained from such a simple reaction and we wondered if the reaction would still work efficiently if other bulkier groups were attached to the available *para* positions, making the resulting product more versatile. To test this, the bromo- and iodo-analogues of calixarene **171** were prepared (scheme 3.36). The Cannizzaro reaction of **178** and **181** afforded the desired products in high yields (82% and 94%, respectively). These new products are more versatile than calix[4]arene **174** and could be further functionalised through different C-C coupling reactions, leading to chiral tetrasubstituted calix[4]arenes in only a few synthetic steps.



Scheme 3.36 Synthesis of halogenated diformyl calix[4]arenes and intramolecular Cannizzaro reaction.

Finally, we also prepared a deuterated version of calix[4]arene **171** (scheme 3.37). Cannizzaro reaction of calixarene **184** afforded the deuterium labelled compound **185** in moderate 65% yield.



Scheme 3.37 Synthesis and intramolecular Cannizzaro reaction of deuterated bis-formyl calix[4]arene 184.

In a cross-over experiment, a mixture of deuterated and non deuterated calix[4]arene **171** was grinded with KOH for 30 minutes to afford a mixture of Cannizzaro products (figure 3.20). As predicted, ^{2}H-NMR spectra of starting materials and products showed how the single peak corresponding to the deuterium labelled formyl groups was replaced by a new single peak at around 4.0 ppm, which is consistent with the chemical shift of deuterium attached to a benzylic

carbon and it is in agreement with the ^{2}H-NMR spectra of benzyl alcohol-d_7 previously reported in the literature.[105]

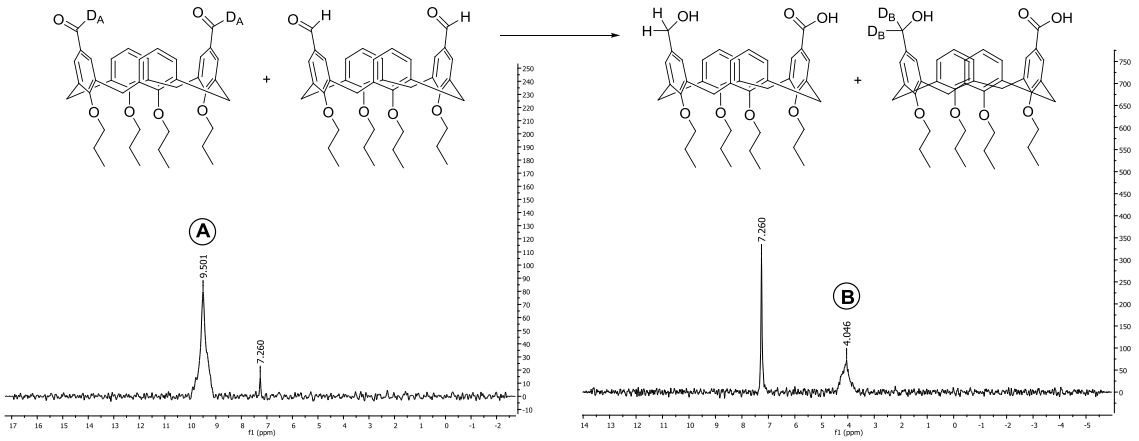


Figure 3.20 D-NMR spectrum before and after Cannizzaro reaction of an equimolar mixture of deuterated and non deuterated bis-formyl calix[4]arene 171.

Although further experiments will need to be carried out in the future in order to confirm the intramolecular nature of this type of reaction, the fact that very high yields have been obtained during our investigations strongly suggest that the reaction must proceed intramolecularly. Otherwise, a mixture of different calix[4]arenes should have been observed by ^{1}H-NMR spectroscopy (figure 3.21).

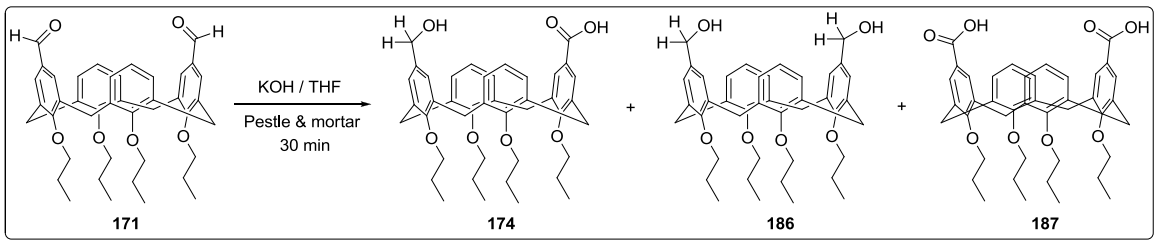


Figure 3.21 Expected mixture of products after a hypothetical non intramolecular Cannizzaro reaction of calix[4]arene 171.

After developing a simple and more efficient protocol for the Cannizzaro reaction of diformyl calix[4]arenes, we decided to address the more challenging issue of a PKS mimic by preparing a new bifunctional mercapto-calix[4]arene which could act as malonyl group carrier.

3.2.4. Synthesis of bio-inspired mercapto-calix[4]arenes. Mimicry of PKS.

The last part of our research was greatly influenced by the investigations described in the previous chapters of this thesis. This last part links the synthesis of calix[4]arenes with the synthesis of MAHTs and also, with the development of new mild decarboxylative condensations. We were convinced that the experience and knowledge acquired during every different stage of our research would be very valuable in the development and preparation of the more advanced bio-inspired macromolecules with potencial applications in catalysis and many other fields.

We decided to attempt the mimicry of one of the key functions that are carried out in the active site of PKS. We were interested in the preparation of a cyclic scaffold able to carry out intramolecular Claisen condensations, mimicking the chain elongation step of PKS.

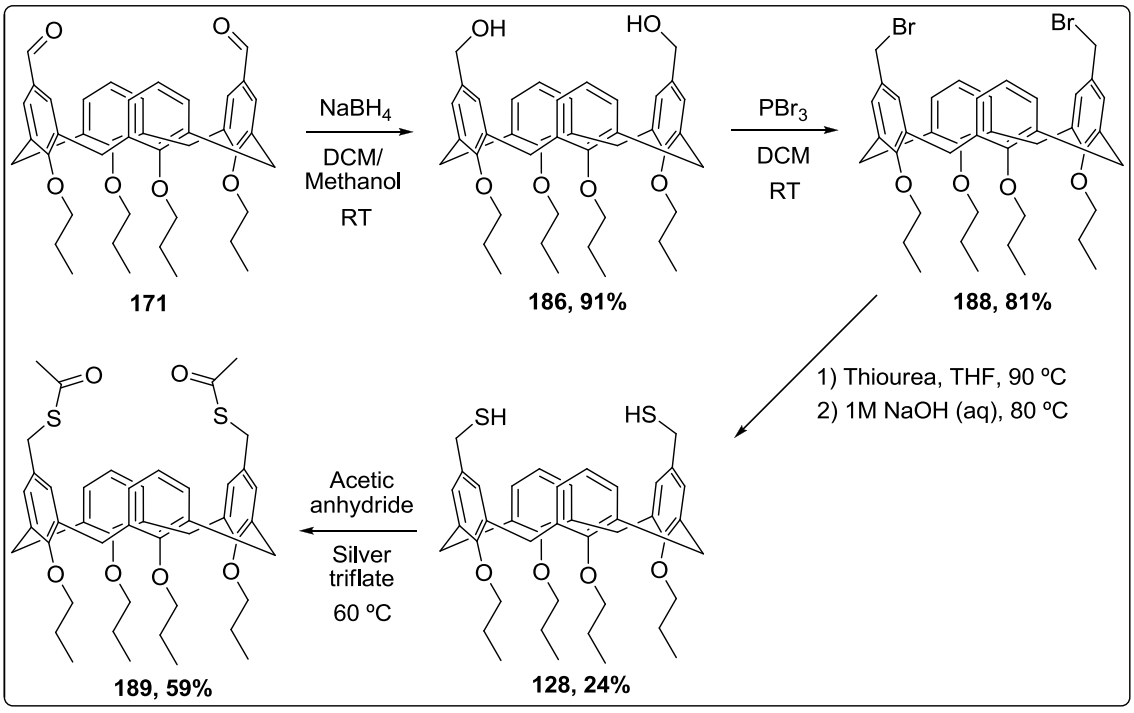
In the active site of a PKS, two sulfydryl groups are placed close to each other so a Claisen condensation can be performed during the chain extension step of polyketide biosynthesis. In a similar way, we thought bifunctional mercapto-calix[4]arenes could mimic the principle of this process. In plants and microorganisms, the Claisen condensations between malonyl-CoA and the polyketide chain are catalyzed by several amino acid residues in the active site of PKS. In our case, we thought it was a good idea to employ the experience gained during the catalysis of mild decarboxylative aldol condensations, to develop suitable experimental conditions for the catalysis of an intramolecular, metal and base-free, decarboxylative Claisen condensation on the upper rim of calix[4]arenes. Thus, we thought that, for our bio-mimetic approach, quaternary ammonium salts could be trialled during the catalysis of intramolecular Claisen condensations.

Our synthetic plan was divided in three different stages:

- Synthesis of a bifunctional mercapto-calix[4]arene.
- Attachment of malonyl groups to the upper rim of mercapto-calix[4]arene.
- Intramolecular decarboxylative Claisen condensations in calix[4]arenes.

A) Synthesis of a bifunctional mercapto-calix[4]arene.

We started the synthesis of mercapto-calix[4]arenes from the same starting material employed for the intramolecular Cannizzaro reactions. In the first step, bis-formyl calix[4]arene **171** was successfully reduced to the diol calixarene **186** by treatment with an excess of sodium borohydride in a mixture of DCM/methanol. The desired product was obtained in 91% yield (scheme 3.38). The hydroxyl groups in calixarene **186** were efficiently substituted by bromine atoms after treatment with phosphorous tribromide in dry dichloromethane. In the next synthetic step, the bromine atoms were replaced by sulfhydryl groups in a two step reaction initiated by the nucleophilic substitution at the bromomethyl groups in calixarene **188** by thiourea, followed by basic hydrolysis of the resulting salt, affording the desired bifunctional thiol calix[4]arene **128** in a low 24% yield.



Scheme 3.38 Synthesis of bifunctional thiol calix[4]arene 128, inspired in the active site of PKS.

In order to confirm the formation of calix[4]arene **128**, thiol groups were acetylated. Compound **128** was treated with an excess of acetic anhydride and a catalytic amount of silver triflate to obtain the desired acetylated product **189** after 10 minutes of reaction (scheme 3.38). Calix[4]arene **189** was fully characterised and the data obtained was in agreement with the proposed structure.

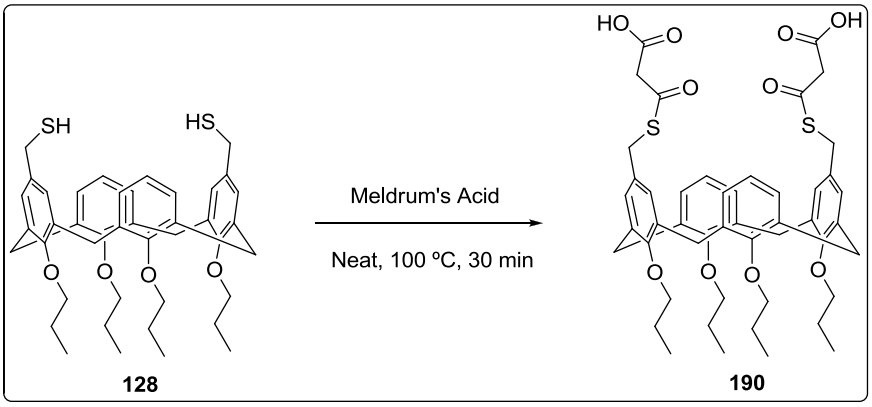
B) Attachment of malonyl groups to the upper rim of mercapto-calix[4]arene **128**.

The next stage was the loading of malonyl groups onto the prepared mercapto-calix[4]arene. To do so, we used the same methodology that was employed during the preparation of MAHOs and MAHTs in chapter 1. The reaction between freshly prepared half malonyl chloride and mercapto-calix[4]arene **128** was attempted under neat conditions. The mixture was heated at 65 ºC for 90 minutes. After cooling, and adding dichloromethane, TLC analysis showed the formation of a new product and the consumption of most of the starting thiol calix[4]arene. However, after standard work up and purification by column chromatography on silica gel, the isolated product proved not to be the desired product. The isolated product seemed in fact to be a complex mixture of oligomers that exhibited ^{1}H-NMR spectrum with very broad peaks.

At that moment, we realised that the strong acidic conditions used during the reaction with half malonyl chloride could be protonating the sulfhydryl groups in calix[4]arene **128** and promoting the cleavage of sulfydryl groups, followed by the polymerization of calix[4]arenes through thioetherification reaction.

To avoid the cleavage of sulfhydryl groups, and to confirm that strong acidic conditions were incompatible with this type of substrate, we decided to attempt the reaction again but changing from the half malonyl chloride to Meldrum's acid as the activated malonic acid. We thought the reaction with Meldrum's acid should proceed under neutral conditions, avoiding the formation of hydrogen chloride during the esterification reaction. A neat mixture of thiol calix[4]arene **128** and Meldrum's acid was heated at 100 ºC for 30 minutes. After this time, the mixture was cooled to room temperature and dichloromethane was added. TLC analysis of the crude product showed the

consumption of most of the starting calix[4]arene. A new product was formed which was purified by column chromatography on silica gel (eluting with dichloromethane followed by a mixture of dichloromethane / diethyl ether = 4:1, V/V) and a white solid was obtained. ^{1}H-NMR and MALDI-TOF mass spectrometry proved the solid to be the desired product obtained in a modest 38% yield (scheme 3.39).



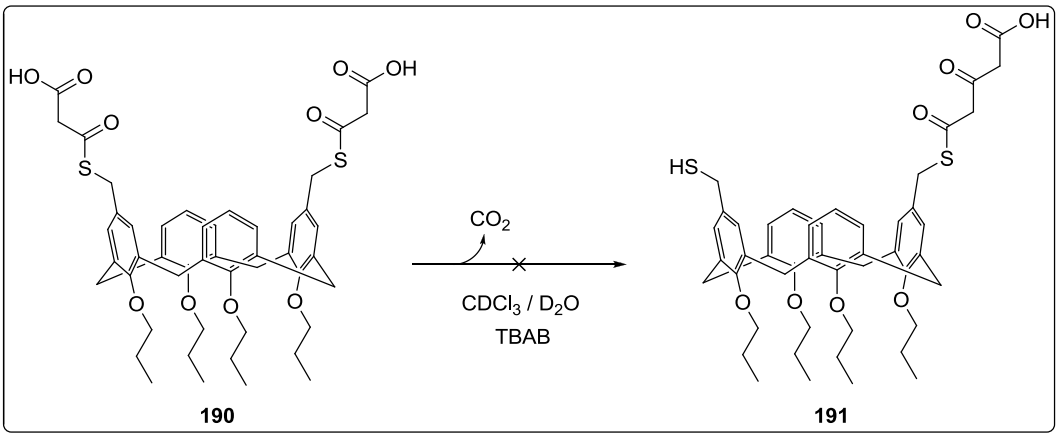
Scheme 3.39 Synthesis of new malonyl mercapto-calix[4]arene 190.

C) Intramolecular decarboxylative Claisen condensations in calix[4]arenes.

The last stage of our research plan was the study of intramolecular and decarboxylative Claisen condensation in malonyl mercapto-calix[4]arene **190**. We decided to attempt the intramolecular Claisen condensation of malonates under different mild conditions. For the purpose, we used both reported conditions from the literature and the conditions previously employed during the performance of base and metal-free decarboxylative aldol condensations described in chapter 2.

Several samples of calix[4]arene **190** were dissolved in deuterated chloroform and the intramolecular Claisen condensation was attempted using different catalytic systems. Each test reaction was followed by ^{1}H-NMR spectroscopy to monitor changes in the starting material and also to check the formation of new products in the reaction.

In the first attempt of intramolecular Claisen condensation, a mixture of compound **190** (8 mg) and tetra-*n*-butylammonium bromide (1 equivalent) were dissolved in a biphasic mixture formed by chloroform-d and deuterium oxide 1:1 (total volume = 7 mL) (scheme 3.40).



Scheme 3.40 Attempted intramolecular Claisen condensation of bis-malonyl calix[4]arene 190.

The reaction mixture was stirred for 72 hours at room temperature. ^{1}H-NMR spectrum of the mixture were recorded every 24 hours in order to follow the evolution of reactants. During the first 24 hours of reaction, all α-hydrogens in malonates were exchanged by deuterium coming from deuterium oxide. However, no changes in the starting material were observed as all the signals for malonyl calix[4]arene remained unchanged. After 72 hours of reaction, no further changes in the ^{1}H-NMR spectrum were detected confirming that quaternary ammonium salts were not a suitable catalyst for the transannular decarboxylative Claisen condensation and showing that the use of a bifunctional scaffold, to place two molecules of malonate nearby, was not in itself enough to promote the intramolecular reaction.

In the second attempt of intramolecular Claisen condensation, 8 mg of malonyl calix[4]arene **190** were dissolved in 7 mL of chloroform-d along one equivalent of imidazole and 0.5 equivalents of magnesium(II) bromide. The mixture was stirred for 72 hours at room temperature and monitored by ^{1}H-NMR spectroscopy. After this time no changes in the ^{1}H-NMR spectrum were observed, indicating that the system imidazole/magnesium(II) was also not suitable for the catalysis of this type of intramolecular decarboxylative condensation.

In the last attempt, 8 mg of calixarene **190** were mixed with 1 equivalent of diisopropylethylamine and 0.5 equivalents of magnesium(II) bromide in 0.7 mL of chloroform-d. The solution was stirred for 72 hours at room temperature and the reaction monitored by ^{1}H-NMR spectroscopy as in the two previous experiments. After that time, ^{1}H-NMR spectrum of the reaction mixture revealed no changes. In these two last cases, however, no H-D exchange was observed, since only aprotic $CDCl_3$ was used as solvent.

The obtained results confirmed that, as was found by Matile *et al.*, mild decarboxylative Claisen condensations are very sensitive processes where fine tuning of reaction conditions is required in order to promote the self-condensation of malonates.

Further studies need to be carried out in order to determine the effect that the calix[4]arene scaffold may induce in this type of condensation. In the catalysis of intramolecular Claisen condensations by TBAB, the use of a scaffold to place two malonates in close proximity had no clear benefits. The use of higher amounts of base or the use of stronger bases, in combination with the use of other metals different to the employed magnesium(II), may lead to the first example of intramolecular Claisen condensation in calix[4]arenes.

3.3 Conclusions.

A selective and versatile method to prepare monoarmed calix[4]arenes, that involves simple work up and purification, has been developed. Using this methodology, several examples of monosubstituted calix[4]arenes have been synthesised, some of them showing properties with potential applications in sensors and nanocapsules.

A highly efficient protocol for the Cannizzaro reaction of diformyl calix[4]arenes has been discovered. The new reaction conditions reduce reaction times, avoid the use of solvents and allow the preparation of upper rim trisubstituted calix[4]arenes in only five synthetic steps.

Finally, a bio-inspired mercapto-calix[4]arene bearing malonic acid fragments in the upper rim has been successfully synthesised. Intramolecular Claisen condensations have been attempted under different conditions but no reaction has been observed so far. In future works, additional solvents, bases, metals and quaternary ammonium salts should be screened in order to obtain the

suitable conditions that can promote a Claisen condensation in this type of calix[4]arene. The successful catalysis of Claisen condensations in calix[4]arenes may allow the synthesis of polyketones in a controlled manner.

- Chapter 4-

Experimental part

4.1 General experimental methods.

Solvents were purchased from ROMIL® (Cambridge, UK) or Sigma-Aldrich® and used as supplied. All commercially available chemicals and reagents were used as supplied. All reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an atmosphere of nitrogen. All new products were characterized by ^{1}H-NMR, ^{13}C-NMR, IR and MS. ^{1}H and ^{13}C-NMR spectrum were recorded on 300 MHz and 400 MHz Varian spectrometers and 400 MHz and 500 MHz Bruker spectrometers and unless otherwise specified, deuterated chloroform was used as the solvent. NMR solvents were purchased from Apollo Scientific Limited® or Sigma-Aldrich® and dried over type 4Å molecular sieves prior to use. $CDCl_3$ was further filtered through basic alumina. Chemical shifts (δ) are reported in ppm and referenced to the residual solvent signal ($CDCl_3$: δ = 7.26 ppm for ^{1}H-NMR and δ = 77.16 ppm for ^{13}C-NMR spectrum). Peak multiplicities are designated as: singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), multiplet (m), broad (br). Coupling constants are reported in Hertz (Hz). Ion mass/charge (*m/z*) ratios are reported as values in atomic mass units. Low-resolution mass spectrometry (LRMS) was performed in a Shimadzu Maldi-TOF mass spectrometer and high-resolution mass spectrometry (HRMS) was performed in a QTOF-MS-ES+, a FTMS+pNSI, or a FTMS-pAPCI mass spectrometer, depending on the molecule. Microwave syntheses were performed on a Personal Chemistry Emrys Creator. Melting points were recorded using open capillary tubes on melting point apparatus. Thin layer chromatography was performed on Merck aluminum plates coated with 0.2 mm silica gel-60 F254. Flash column chromatography was performed on silica gel (Kieselgel 60).

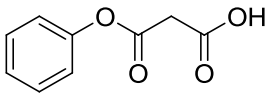
4.2 Protocols and experimental data for MAHOs and MAHTs.

4.2.1 General procedure for the synthesis of MAHOs.

Malonyl monochloride (0.3 g, 2.45 mmol, 3 equivalents) and the corresponding phenol (0.82 mmol, 1 equivalent) were stirred neat at 100 °C for two minutes during which time the reaction mixture became liquid and homogeneous. After cooling to room temperature, to the resulting mixture (solid or oil) was added dichloromethane to produce a precipitate which was collected by filtration and the residual organic solvent was removed under reduced pressure affording an impure product. Subsequent purification by flash column chromatography using dichloromethane first, followed by dichloromethane / diethyl ether (9.5:0.5, V/V) afforded the desired MAHO.

4.2.2 Experimental data for MAHOs.

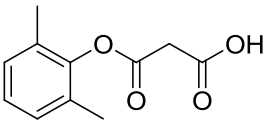
i) 3-Oxo-3-phenoxypropanoic acid (24).[20]



24

White solid (0.114 g, 77% yield). Mp 66 - 68 °C, chloroform (lit. 71 ºC, benzene). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.99 (br s, 1H, COO*H*), 7.42-7.37 (m, 2H, Ar-*H*), 7.29-7.24 (m, 1H, Ar-*H*), 7.15-7.13 (m, 2H, Ar-*H*), 3.69 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.79, 165.07, 150.39, 129.68, 126.49, 121.42, 41.26 ppm. FTIR [ATR] 1751, 1695, 1592, 1522, 1483, 1459, 1417, 1321, 1279, 1243, 1223, 1191, 1143, 1070, 1019, 1007, 968, 939, 909, 826, 749, 692 cm^{-1}. HRMS Calcd for $C_9H_8O_4$ $[M + Na]^+$ 203.0320, found 203.0325.

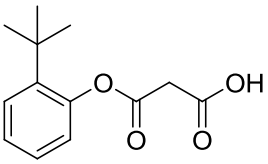
ii) 3-Oxo-3-(2,6-dimethylphenoxy)propanoic acid (26).[20]



26

White solid (0.113 g, 66% yield). Mp 102 - 104 °C, chloroform (lit. 122 °C, benzene). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.08 (s, 3H, Ar-*H*), 3.73 (s, 2H, CH_2), 2.18 (s, 6H, CH_3). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.83, 164.19, 147.85, 130.20, 128.86, 126.49, 40.89, 16.37 ppm. FTIR [ATR] 1751, 1694, 1616, 1591, 1477, 1442, 1409, 1380, 1321, 1279, 1244, 1223, 1165, 1147, 1092, 1038, 1017, 991, 964, 938, 911, 836, 801, 776, 732, 695 cm^{-1}. HRMS Calcd for $C_{11}H_{12}O4$ [M + Na]$^{+}$ 231.0633, found 231.0627 (FTMS-pNSI spectrometer used in this case).

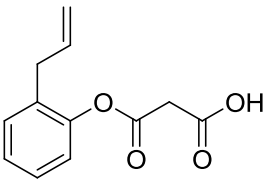
iii) 3-Oxo-3-(2-tert-butylphenoxy)propanoic acid (27).



27

Pale yellow oil (0.126 g, 65% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40 (dd, 1H, *J* = 7.5, 1.5 Hz, Ar-*H*), 7.25-7.18 (m, 2H, Ar-*H*), 7.05 (dd, 1H, *J* = 7.5, 1.5 Hz, Ar-*H*), 3.72 (s, 2H, CH_2), 1.34 (s, 9H, CH_3). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 165.24, 149.00, 141.06, 127.53, 127.21, 126.45, 123.73, 41.73, 41.68, 34.58, 30.31 ppm. FT-IR (ATR) 1761.5, 1716.5, 1605.5, 1576, 1488, 1469.5, 1442, 1407.5, 1395.5, 1364.5, 1324.5, 1284.5, 1256.5, 1200.5, 1180, 1138.5, 1125.5 cm^{-1}. MS (MALDI-TOF) calculated for $C_{13}H_{16}O_4$ [M]$^{+}$ 236.3, found 236.0. HRMS Calcd for $C_{13}H_{15}O_4$ [M − H]$^{-}$ 235.0976, found 235.0976 (FTMS-pNSI spectrometer used in this case).

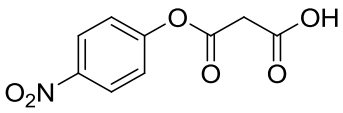
iv) 3-Oxo-3-(2-allylphenoxy)propanoic acid (28).



28

Pale yellow oil (0.049 g, 27% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.19-7.12 (m, 3H, Ar-*H*), 7.01-6.99 (m, 1*H*, Ar-*H*), 5.85-5.77 (m, 1H, C*H*=CH_2), 4.99-4.96 (m, 2H, CH=C*H*$_2$), 3.61 (s, 2H, C*H*$_2$), 3.25 (d, 2H, J = 6.5 Hz, C*H*$_2$-Ar). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.90, 164.86, 148.59, 135.87, 131.94, 130.73, 127.66, 126.83, 122.18, 116.41, 41.17, 34.52. FT-IR (ATR) 1764, 1745, 1698, 1580, 1480, 1436, 1398, 1331, 1295, 1273, 1207, 1176, 1165, 1153, 1093, 1056, 1009, 952, 910, 845, 791, 702, 677 cm^{-1}. HRMS calculated for $C_{12}H_{11}O_4$ $[M - H]^-$ 219.0663, found 219.0665 (FTMS-pNSI spectrometer used in this case).

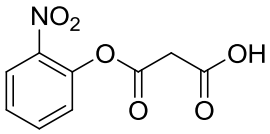
v) 3-Oxo-3-(4-nitrophenoxy)propanoic acid (29).[25]



29

White solid (0.122 g, 66% yield). Mp 73 - 75 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.29 (d, 2H, *J* = 8 Hz, *part A system AX*), 7.33 (d, 2H, *J* = 8 Hz, *part X system AX*), 3.74 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.18, 163.86, 154.88, 145.87, 125.49, 122.46, 41.19 ppm. FTIR [ATR] 1770, 1717, 1683, 1615, 1589, 1523, 1487, 1418, 1391, 1335, 1267, 1204, 1184, 1152, 1127, 1011, 966, 944, 858, 780, 738, 694, 671 cm^{-1}. HRMS Calcd for $C_9H_7NO_6$ $[M + Na]^+$ 248.0171, found 248.0163.

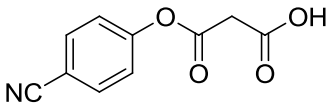
vi) 3-Oxo-3-(2-nitrophenoxy)propanoic acid (30).



30

White solid (0.046 g, 25% yield). Mp 92 - 93 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.13 (dd, 1H, *J* = 8, 1.6 Hz, Ar-*H*), 7.69 (td, 1*H*, *J* = 8, 1.6 Hz, Ar-*H*), 7.45 (t, 1H, *J* = 8 Hz, Ar-*H*), 7.32 (dd, 1*H*, *J* = 8 Hz, 1.6 Hz, Ar-*H*), 3.79 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.84, 164.15, 143.63, 141.51, 135.18, 127.41, 126.12, 125.29, 40.84 ppm. FTIR [ATR] 1778, 1704, 1524, 1343, 1265, 1209, 1121, 1082, 965, 936, 920, 872, 857, 816, 795, 731, 695, 672, 656 cm^{-1}. HRMS Calcd for $C_9H_7NO_6$ $[M + Na]^+$ 248.0171, found 248.0164.

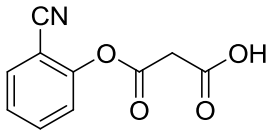
vii) 3-Oxo-3-(4-cyanophenoxy)propanoic acid (31).[24]



31

White solid (0.101 g, 60% yield). Mp 94 - 95 °C, chloroform (lit. 76 - 80 ºC) ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, 2H, *J* = 8 Hz, *part A system AX*), 7.28 (d, 2H, *J* = 8 Hz, *part X system AX*), 3.71 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.04, 164.00, 153.52, 133.97, 122.69, 118.11, 110.50, 41.20 ppm. FTIR [ATR] 1771, 1704, 1601, 1500, 1427, 1399, 1339, 1318, 1300, 1274, 1230, 1201, 1168, 1137, 1018, 970, 919, 864, 824 cm^{-1}. HRMS Calcd for $C_{10}H_7NO_4$ $[M + Na]^+$ 228.0273, found 228.0269.

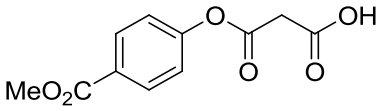
viii) 3-Oxo-3-(2-cyanophenoxy)propanoic acid (32).



32

Colorless solid (0.103 g, 61 % yield). Mp 81 - 84 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.39 (br s, 1H, COO*H*), 7.70-7.62 (m, 2H, Ar-*H*), 7.39-7.35 (m, 2H, Ar-*H*), 3.78 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.79, 163.76, 151.77, 134.41, 133.51, 126.93, 123.22, 114.80, 106.84, 41.05 ppm. FTIR [ATR] 1770, 1712, 1603, 1487, 1450, 1402, 1323, 1302, 1276, 1253, 1217, 1179, 1140, 1101 cm^{-1}. HRMS Calcd for $C_{10}H_7NO_4$ $[M + Na]^+$ 228.0273, found 228.0284.

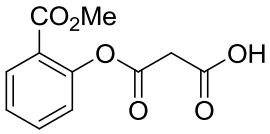
ix) 3-Oxo-3-(4-methoxycarbonyl)phenoxy)propanoic acid (33).



33

White solid (0.119 g, 61% yield). Mp 92 - 94 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.08 (d, 2H, *J* = 8 Hz, *part A system AX*), 7.21 (d, 2H, *J* = 8 Hz, *part X system AX*), 3.92 (s, 3H, COOCH_3), 3.70 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.04, 166.41, 164.43, 153.91, 131.44, 128.37, 121.54, 52.49, 52.45, 41.22 ppm. FTIR [ATR] 1768, 1721, 1696, 1602, 1556, 1506, 1434, 1411, 1336, 1279, 1216, 1152, 1110, 1094, 1017, 959, 940, 925, 864, 844, 802, 756, 696 cm^{-1}. HRMS Calcd for $C_{11}H_{10}O_6$ $[M + Na]^+$ 261.0364, found 261.0364.

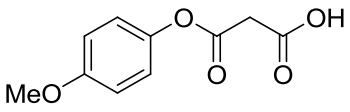
x) 3-Oxo-3-(2-(methoxycarbonyl)phenoxy)propanoic acid (34).



34

White solid (0.041 g, 21% yield). Mp 64 - 66 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.04 (dd, 1H, *J* = 8, 1.6 Hz, Ar-*H*), 7.59 (td, 1H, *J* = 8, 1.6 Hz, Ar-*H*), 7.35 (t, 1H, *J* = 8 Hz, Ar-*H*), 7.16 (d, 1H, *J* = 8 Hz, Ar-*H*), 3.87 (s, 3H, COOC*H*$_3$), 3.77 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.48, 165.70, 164.89, 150.18, 134.29, 132.04, 126.76, 123.72, 122.83, 52.55, 52.53, 40.87 ppm. FTIR [ATR] 1755, 1708, 1607, 1578, 1485, 1435, 1400, 1337, 1300, 1266, 1204, 1147, 1131, 1081, 1042, 963, 933, 886, 841, 810, 746, 700, 675, 656 cm^{-1}. HRMS Calcd for $C_{11}H_{10}O_6$ $[M + Na]^+$ 261.0375, found 261.0367.

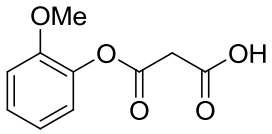
xi) 3-Oxo-3-(4-methoxyphenoxy)propanoic acid (35).[24]



35

White solid (0.105 g, 61% yield). Mp 92 - 93 °C, chloroform (lit. 88 - 90 ºC) ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.05 (d, 2H, *J* = 8 Hz, *part A system AB*), 6.89 (d, 2H, *J* = 8 Hz, *part B system AB*), 3.80 (s, 3H, OC*H*$_3$), 3.67 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.34, 165.59, 157.73, 143.88, 122.21, 114.68, 55.75, 41.08 ppm. FTIR [ATR] 1749, 1713, 1601, 1509, 1434, 1335, 1202, 1185, 1148, 1099, 1032, 844, 829, 754, 656 cm^{-1}. HRMS Calcd for $C_{10}H_{10}O_5$ $[M + Na]^+$ 233.0426, found 233.0419.

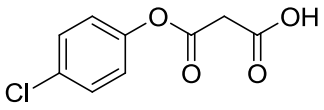
xii) 3-Oxo-3-(2-methoxyphenoxy)propanoic acid (36).



36

Colorless oil (0.078 g, 45% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.56 (br s, 1H, COO*H*) 7.24-7.20 (m, 1H, Ar-*H*), 7.08 (d, 1H, J = 4 Hz, Ar-*H*), 6.98-6.92 (m, 2H, Ar-*H*), 3.82 (s, 3H, OCH_3), 3.70 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.21, 164.85, 151.01, 139.41, 127.56, 122.69, 120.95, 112.71, 56.04, 40.68 ppm. FTIR [ATR] 1764, 1732, 1606, 1499, 1458, 1440, 1409, 1308, 1281, 1255, 1194, 1171, 1130, 1109, 1041, 1022, 940, 920, 857, 829, 796, 748, 666 cm^{-1}. HRMS Calcd for $C_{10}H_{10}O_5$ $[M + Na]^+$ 233.0426, found 233.0433.

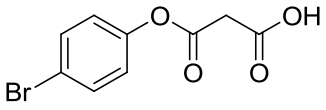
xiii) 3-Oxo-3-(4-chlorophenoxy)propanoic acid (37).[20]



37

White solid (0.113 g, 64 % yield). Mp 93 - 95 °C, chlroform (lit. 97 °C, cyclohexane). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.36 (d, 2H, *J* = 8 Hz *part A system AX*), 7.06 (d, 2H, *J* = 8 Hz, *part X system AX*), 3.68 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.57, 164.72, 148.81, 131.99, 129.79, 122.83, 41.15 ppm. FTIR [ATR] 1755, 1694, 1589, 1510, 1486, 1447, 1402, 1333, 1294, 1243, 1200, 1162, 1146, 1083, 1013, 951, 933, 843, 815, 789, 741, 711, 687 cm^{-1}. HRMS Calcd for $C_9H_7O_4{}^{35}Cl$ $[M + Na]^+$ 236.9931, found 236.9937.

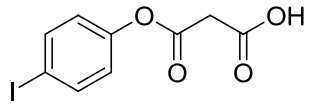
xiv) 3-Oxo-3-(4-bromophenoxy)propanoic acid (38).[101]



38

White solid (0.147 g, 69% yield). Mp 121 - 122 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.51 (d, 2H, *J* = 8 Hz, *part A system AX*), 7.03 (d, 2H, *J* = 8 Hz, *part X system AX*), 3.68 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.53, 164.60, 149.37, 132.76, 123.24, 119.69, 41.19 ppm. FTIR [ATR] 1759, 1734, 1695, 1482, 1446, 1398, 1334, 1295, 1199, 1164, 1145, 1064, 1012, 933, 843, 704, 681 cm^{-1}. HRMS Calcd for $C_9H_6O_4{}^{79}Br$ $[M - H]^-$ 256.9455, found 256.9468 (FTMS-pNSI spectrometer used in this case).

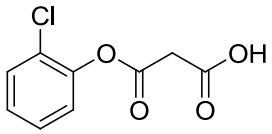
xv) 3-Oxo-3-(4-iodophenoxy)propanoic acid (39).



39

Colourless oil (0.193 g, 77% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.70 (d, 2H, *J* = 9 Hz, *part A system AX*), 6.90 (d, 2H, *J* = 9 Hz, *part X system AX*), 3.68 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.57, 164.54, 150.18, 138.77, 123.60, 90.71, 41.17 ppm. FT-IR (ATR) 1761, 1717, 1639, 1611.5, 1583.5, 1559, 1540, 1530, 1488.5, 1452.5, 1408, 1366, 1321, 1285, 1211.5, 1184, 1167.5, 1137, 1087.5 1050, 1037 cm^{-1}. HRMS Calcd for $C_9H_6O_4I$ $[M - H]^-$ 304.9316, found 304.9314 (FTMS-pNSI spectrometer used in this case).

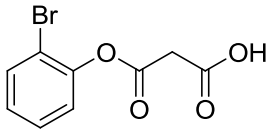
xvi)3-Oxo-3-(2-chlorophenoxy)propanoic acid (40).



40

Colourless oil (0.120 g, 68% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.45 (dd, 1H, *J* = 8, 1.6 Hz, Ar-*H*), 7.32-7.28 (m, 1H, Ar-*H*), 7.24-7.18 (m, 2H, Ar-*H*), 3.75 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.30, 164.02, 146.60, 130.58, 128.01, 127.71, 126.84, 123.62, 40.82 ppm. FT-IR [ATR] 1769, 1716, 1585, 1475, 1449, 1408, 1326, 1261, 1208, 1134, 1121, 1060, 1029, 946, 928, 865, 824, 796, 747, 710, 686, 658 cm^{-1}. HRMS Calcd for $C_9H_7O_4{}^{35}Cl$ $[M + Na]^+$ 236.9931, found 236.9931.

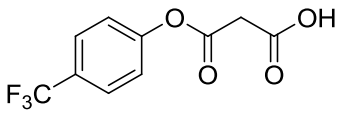
xvii) 3-Oxo-3-(2-bromophenoxy)propanoic acid (41).



41

Pale yellow solid (0.151 g, 71% yield). Mp 59 - 60 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.62 (dd, 1H, *J* = 8, 1.6 Hz, Ar-*H*), 7.35 (td, 1H, *J* = 8, 1.6 Hz, Ar-*H*), 7.21-7.14 (m, 2H, Ar-*H*), 3.76 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.23, 164.03, 147.86, 133.63, 128.75, 128.02, 123.66, 115.99, 77.36, 40.91 ppm. FT-IR [ATR] 1770, 1721, 1623, 1582, 1556, 1542, 1471, 1445, 1408, 1327, 1259, 1207, 1133, 1046, 1026, 947, 929, 864, 823, 799, 749, 706, 672, 651 cm^{-1}. HRMS Calcd for $C_9H_7O_4{}^{79}Br$ $[M + Na]^+$ 280.9425, found 280.9438**.**

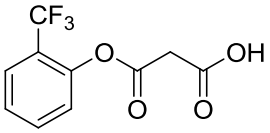
xviii) 3-Oxo-3-(4-(trifluoromethyl)phenoxy)propanoic acid (42).



42

White solid (0.112 g, 55% yield). Mp 89 - 89 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.62 (s, 1H, br), 7.67 (d, 2H, *J* = 8 Hz), 7.27 (d, 2H, *J* = 8 Hz), 3.72 (s, 2H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.7, 164.3, 152.8 (q, $J_{C\text{-}F}$ = 1.4 Hz), 128.9 (q, $J_{C\text{-}F}$ = 33.1 Hz), 127.1 (q, $J_{C\text{-}F}$ = 3.7 Hz), 123.9 (q, $J_{C\text{-}F}$ = 273.1 Hz) 122.0, 41.2 ppm. FT-IR [ATR] 1761, 1723, 1613, 1514, 1434, 1416, 1327, 1294, 1217, 1167, 1119, 1100, 1065, 1018, 970, 936, 856, 813, 795, 727, 682 cm^{-1}. HRMS Calcd for $C_{10}H_7O_4F_3$ $[M + Na]^+$ 271.0194, found 271.0189.

xix)3-Oxo-3-(2-(trifluoromethyl)phenoxy)propanoic acid (43).



43

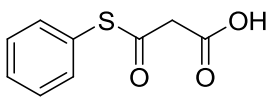
White solid (0.120 g, 59% yield). Mp 81 - 83 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 10.06 (s, 1H, br), 7.68 (d, 1H, *J* = 8 Hz), 7.60 (t, 1H, *J* = 8 Hz), 7.38 (t, 1H, *J* = 8 Hz), 7.31 (d, 1H, *J* = 8 Hz), 3.73 (s, 2H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.7, 164.2, 147.7 (q, $J_{C\text{-}F}$ = 1.9 Hz), 133.4, 122.9 (q, $J_{C\text{-}F}$ = 4.8 Hz), 126.6, 124.2, 122.9 (q, $J_{C\text{-}F}$ = 31.9 Hz), 122.9 (q, $J_{C\text{-}F}$ = 273.7 Hz), 41.00 ppm. FT-IR [ATR] 1785, 1713, 1615, 1588, 1494, 1458, 1426, 1349, 1322, 1269, 1203, 1173, 1126, 1051, 997, 967, 937, 919, 870, 827, 805, 764, 703, 680 cm^{-1}. HRMS Calcd for $C_{10}H_7O_4F_3$ $[M + Na]^+$ 271.0194, found 271.0195.

4.2.3 General protocol for the synthesis of MAHTs.

A neat mixture of malonyl monochloride (0.2 g, 1.63 mmol, 3 equivalents) and the corresponding thiophenol (0.54 mmol, 1 equivalent) was stirred at 65 °C for two hours. After cooling to room temperature, to the resulting mixture (solid or oil) was added dichloromethane. The resulting precipitate was collected by filtration and the residual organic solvent was removed under reduced pressure affording an impure product. Purification by flash column chromatography using dichloromethane and then dichloromethane / diethyl ether (9.5: 0.5 V/V) afforded the desired MAHT.

4.2.4 Experimental data for MAHTs.

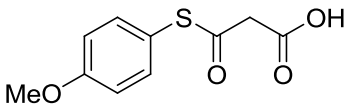
i) 3-Oxo-3-(phenylthio)propanoic acid (14).[24]



14

Colourless oil (0.063 g, 59% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.45 (s, 5H, Ar-*H*), 3.72 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 190.04, 170.98, 134.62, 130.18, 129.58, 126.56, 48.42 ppm. FT-IR [ATR] 1695 (br), 1584, 1478, 1441, 1399, 1300, 1237, 1157, 1094, 1039, 1021, 997, 936, 915, 814, 744, 706, 688 cm^{-1}. HRMS Calcd for $C_9H_8O_3S$ $[M + Na]^+$ 219.0092, found 219.0101.

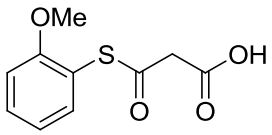
ii) 3-Oxo-3-(4-methoxyphenylthio)propanoic acid (46).[40]



46

Pale yellow oil (0.075 g, 61% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.35 (d, 2H, *J = 8 Hz*, *part A system AX*), 6.96 (d, 2H, *J = 8 Hz*, *part X system AX*) 3.83 (s, 3H, CH_3), 3.70 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 191.67, 170.17, 161.28, 136.29, 117.16, 115.26, 55.57, 47.98 ppm. FT-IR [ATR] 1723, 1704, 1593, 1574, 1496, 1463, 1441, 1408, 1292, 1251, 1175, 1107, 1027, 936, 828, 799, 745, 720, 701 cm^{-1}. HRMS Calcd for $C_{10}H_{10}O_4S$ [M + Na]$^+$ 249.0198, found 249.0204.

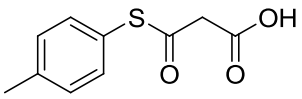
iii) 3-Oxo-3-(2-methoxyphenylthio)propanoic acid (47).[106]



47

Pale yellow solid (0.062 g, 51% yield) Mp 100 - 101 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.47-7.41 (m, 2H, Ar-*H*), 7.03-6.97 (m, 2H, Ar-*H*), 3.85 (s, 3H, OCH_3), 3.72 (s, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.99, 170.83, 159.31, 136.74, 132.50, 121.38, 114.74, 111.84, 56.17, 48.05 ppm. FT-IR [ATR] 1726, 1693, 1584, 1525, 1481, 1467, 1437, 1416, 1387, 1308, 1285, 1250, 1168, 1137, 1068, 1043, 1002, 945, 912, 833, 797, 750, 688 cm^{-1}. HRMS Calcd for $C_{10}H_{10}O_4S$ [M + Na]$^+$ 249.0198, found 249.0188.

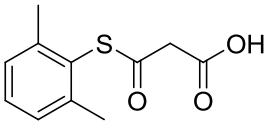
iv) 3-Oxo-3-(4-methylphenylthio)propanoic acid (48).[19]



48

White solid (0.047 g, 41% yield). Mp 108 - 110 °C, chloroform.^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.33 (d, 2H, *J* = 8 Hz, *part A system AB*), 7.24 (d, 2H, *J* = 8 Hz, *part B system AB*), 3.71 (s, 2H, C*H*$_2$), 2.39 (s, 3H, C*H*$_3$). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 190.46, 171.24, 140.38, 134.55, 130.72, 123.19, 48.38, 21.61 ppm. FT-IR (ATR) 1718, 1682, 1604, 1496, 1454, 1422, 1304, 1274, 1202, 1158, 1098, 1072, 994, 910, 800, 766, 700, 642, 610 cm^{-1}. HRMS Calcd for $C_{10}H_9O_3S$ $[M - H]^-$ 209.0278, found 209.0273 (FTMS-pNSI spectrometer used in this case).

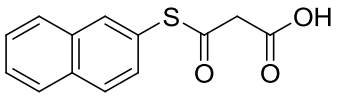
v) 3-(2,6-di-methylphenylthio)-3-oxopropanoic acid (49).



49

Pale yellow solid (0.028 g, 23% yield). Mp 84 - 86 ºC, chloroform. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (t, 1H, *J* = 7.5 Hz, Ar-*H*), 7.10 (d, 2H, *J* = 7.5 Hz, Ar-*H*), 3.67 (s, 2H, C*H*$_2$), 2.30 (s, 6H, C*H*$_3$). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 189.05, 171.45, 143.01, 130.59, 128.60, 125.98, 48.36, 21.73 ppm. FT-IR (ATR) 1711.5, 1681, 1461.5, 1423, 1395, 1375, 1302, 1288.5, 1275, 1150 cm^{-1}. MS (MALDI-TOF) calculated for $C_{11}H_{11}O_3S$ [M] 223.0, found 224.0. HRMS calculated for $C_{11}H_{11}O_3S$ $[M - H]^-$ 223.0434, found 223.0432 (FTMS-pNSI spectrometer used in this case).

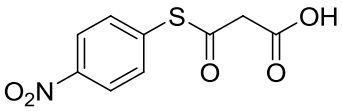
vi) 3-Oxo-3-(naphthalen-2-ylthio)propanoic acid (50).[107]



50

White solid (0.069 g, 52% yield). Mp 110 - 114ºC, chloroform. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H, Ar-*H*), 7.89 (d, 1H, *J* = 8.5 Hz, Ar-*H*), 7.86 (dd, 2H, *J* = 12, 7.5 Hz, Ar-*H*), 7.55 (dq, 2H, *J* = 7, 1.5 Hz, Ar-*H*), 7.47 (dd, 1H, *J* = 8.5, 1.5 Hz, Ar-*H*), 3.77 (s, 2H, C*H*$_3$). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 190.47, 170.47, 134.78, 133.73, 133.67, 130.68, 129.29, 128.22, 127.99, 127.68, 126.94, 123.78, 48.35 ppm. FT-IR (ATR) 1707, 1680, 1584, 1498, 1406, 1272, 1158, 1026, 940, 900, 866, 824, 796, 738, 654 cm^{-1}. HRMS Calcd for $C_{13}H_9O_3S$ $[M - H]^-$ 245.0278, found 245.0271 (FTMS-pNSI spectrometer used in this case).

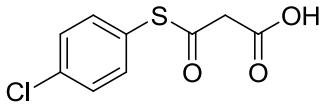
vii) 3-Oxo-3-(4-nitrophenylthio)propanoic acid (51).



51

White solid (0.068 g, 52% yield). Mp 117 - 119 ºC, chloroform. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.28 (d, 2H, *J* = 9 Hz, *part A system AX*), 7.65 (d, 2H, *J* = 9 Hz, *part X system AX*), 3.78 (s, 2H, C*H*$_2$). ^{13}C-NMR (126 MHz, CD_3CN) δ 189.66, 167.55, 149.60, 136.38, 136.09, 125.05, 49.63 ppm. FTIR [ATR] 1714, 1682, 1606, 1579, 1534, 1478, 1426, 1399, 1390, 1369, 1348, 1303, 1276, 1173, 1151, 1105, 1089, 1067, 1016, 990, 909, 852, 743, 729, 683, 639, 626, 608 cm^{-1}. HRMS Calcd for $C_9H_7NO_5S$ $[M - H]^-$ 239.9972, found 239.9970 (FTMS-pNSI spectrometer used in this case).

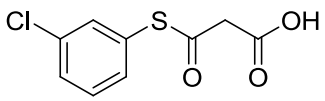
viii)3-Oxo-3-(4-chlorophenylthio)propanoic acid (52).[107]



52

Pale yellow solid (0.062 g, 50% yield). Mp 120 - 122 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.41 (d, 2H, *J* = 8 Hz, *part A system AB*), 7.37 (d, 2H, *J* = 8 Hz, *part B system AB*), 3.72 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.32, 170.50, 136.70, 135.82, 129.86, 124.97, 77.48, 77.16, 76.84, 48.40 ppm. FT-IR [ATR] 1713, 1683, 1572, 1477, 1417, 1390, 1304, 1280, 1177, 1156, 1112, 1096, 1083, 1017, 992, 909, 820, 786, 747, 703, 680 cm^{-1}. HRMS Calcd for $C_9H_6O_3{}^{35}ClS$ $[M - H]^-$ 228.9732, found 228.9737 (FTMS-pNSI spectrometer used in this case).

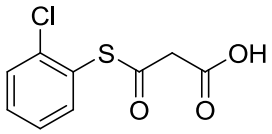
ix) 3-Oxo-3-(3-chlorophenylthio)propanoic acid (53).



53

Pale yellow oil (0.072 g, 58% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.46 (t, 1H, J = 2 Hz, Ar-*H*), 7.43-7.41 (m, 1H, Ar-*H*), 7.37 (t, 1H, J = 8 Hz, Ar-*H*), 7.34-7.32 (m, 1H, Ar-*H*), 3.73 (s, 2H, C*H*$_2$). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 188.90, 170.65, 135.14, 134.29, 132.70, 130.55, 130.40, 128.18, 48.48 ppm. FT-IR (ATR) 1700 (br), 1568, 1462, 1400, 1294, 1234, 1160, 994, 936, 876, 778, 678, 636 cm^{-1}. HRMS Calcd for $C_9H_6{}^{35}ClO_3S$ $[M - H]^-$ 228.9732, found 228.9727 (FTMS-pNSI spectrometer used in this case).

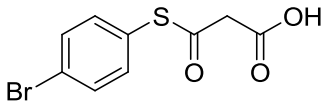
x) 3-Oxo-3-(2-chlorophenylthio)propanoic acid (54).



54

White solid (0.056 g, 45% yield). Mp 99 - 100 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.56-7.52 (m, 2H, Ar-*H*), 7.40 (td, 1H, *J* = 8.0, 1.6 Hz, Ar-*H*), 7.32 (td, 1H, *J* = 8.0, 1.2 Hz, Ar-*H*), 3.76 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 187.95, 170.94, 138.67, 137.18, 131.88, 130.56, 127.64, 126.14, 48.41 ppm. FT-IR [ATR] 1721, 1695, 1575, 1454, 1400, 1328, 1285, 1239, 1187, 1160, 1119, 1040, 1021, 942, 928, 905, 791, 759, 705 cm^{-1}. HRMS Calcd for $C_9H_7O_3NaS^{35}Cl$ [M + Na]$^+$ 252.9702, found 252.9706.

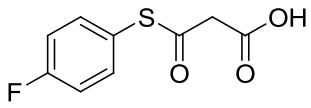
xi) 3-Oxo-3-(4-bromophenylthio)propanoic acid (55).



55

Pale yellow solid (0.061 g, 41% yield). Mp 123 - 126 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.57 (d, 2H, *J* = 8 Hz, *part A system AB*), 7.30 (d, 2H, *J* = 8 Hz, *part B system AB*), 3.72 (s, 2H, C*H*$_2$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.39, 169.73, 136.02, 132.82, 125.57, 124.98, 48.32. FT-IR [ATR] 1716, 1688, 1566, 1472, 1457, 1430, 1417, 1388, 1304, 1282, 1158, 1067, 997, 912, 817, 731 cm^{-1}. HRMS Calcd for $C_9H_6O_3{}^{79}BrS$ [M − H]$^-$ 272.9227, found 272.9219 (FTMS-pNSI spectrometer used in this case).

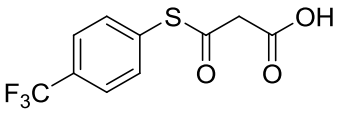
xii) 3-Oxo-3-(4-fluorophenylthio)propanoic acid (56).



56

Pale Yellow solid (0.075 g, 65% yield). Mp 72 - 75 ºC, chloroform. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.47 (s, 1H, br), 7.43-7.40 (m, 2H), 7.13 (dd, 2H, *J* = 8.5, 8.5 Hz), 3.72 (s, 2H) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$) δ 189.8, 171.1, 164.0 (d, J_{CF} = 251.65 Hz). 136.8 (d, J_{CF} = 8.8 Hz), 121.9, 116.8 (d, J_{CF} = 22.2 Hz), 48.4 ppm. FT-IR (ATR) 1712, 1684, 1588, 1490, 1418, 1388, 1304, 1280, 1220, 1156, 1092, 994, 908 cm^{-1}. MS (MALDI-TOF) calculated for $C_9H_7FKO_3S$ 253.0 $[M + K]^+$, found 252.8. HRMS Calcd for $C_9H_6O_3FS$ $[M - H]^-$ 213.0027, found 213.0025 (FTMS-pNSI spectrometer used in this case).

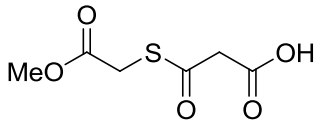
xiii) 3-Oxo-3-(4-trifluoromethylphenylthio)propanoic acid (57).



57

Pale yellow solid (0.071 g, 50% yield). Mp 83 - 84 °C, chloroform. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.71 (s, 1H, br), 7.69 (d, 2H, *J* = 8 Hz), 7.58 (d, 2H, *J* = 8 Hz), 3.76 (s, 2H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 188.4, 170.4, 134.7, 132.1 (q, J_{C-F} = 33.0 Hz), 131.1 (q, J_{C-F} = 1.3 Hz), 126.4 (q, J_{C-F} = 3.7 Hz), 123.8 (q, J_{C-F} = 273.70 Hz), 48.6 ppm. FT-IR [ATR] 1718, 1698, 1608, 1423, 1400, 1326, 1303, 1279, 1169, 1129, 1106, 1064, 1018, 993, 958, 928, 909, 837, 706 cm^{-1}. HRMS Calcd for $C_{10}H_7F_3O_3S$ $[M + H]^+$ 265.0141, found 265.0140.

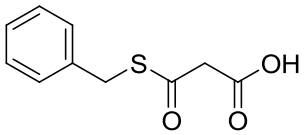
xiv) 3-(2-Methoxy-2-oxoethylthio)-3-oxopropanoic acid (58).



58

Pale yellow oil (0.031 g, 30% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.66 (s, 1H, COO*H*), 3.77 (s, 2H, CH_3OCOCH_2S), 3.74 (s, 3H, OCH_3), 3.68 (s, 2H, $SCOCH_2COOH$). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 189.65, 170.22, 168.85, 53.15, 48.50, 31.67 ppm. FT-IR (ATR) 2960, 2931, 1724 (br), 1688, 1438, 1386, 1302, 1156, 1002, 942, 886, 780, 638 cm^{-1}. MS (MALDI-TOF) calculated for $C_6H_8NaO_5S$ 215.0 $[M + Na]^+$, found 215.9. HRMS Calcd for $C_6H_7O_5S$ $[M - H]^-$ 191.0020, found 191.0015 (FTMS-pNSI spectrometer used in this case).

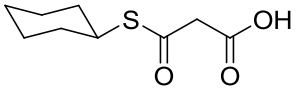
xv) 3-(Benzylthio)-3-oxopropanoic acid (59).[39]



59

White solid (0.062 g, 55% yield). Mp 54 - 56 ºC, chloroform (lit. 62 - 63 ºC, hexane/benzene). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.33-7.25 (m, 5H, Ar-*H*), 4.20 (s, 2H, $PhCH_2S$), 3.65 (s, 2H, $SCOCH_2COOH$). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 190.78, 171.35, 136.51, 129.01, 128.87, 127.72, 48.63, 34.05 ppm. FT-IR (ATR) 1718, 1682, 1422, 1304, 1274, 1158, 1098, 1072, 994, 910, 800, 766, 700, 642, 610 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{10}H_{10}NaO_3S$ 233.0 $[M + Na]^+$, found 233.5. HRMS Calcd for $C_{10}H_9O_3S$ $[M - H]^-$ 209.0278, found 209.0272 (FTMS-pNSI spectrometer used in this case).

xvi)3-(Cyclohexylthio)-3-oxopropanoic acid (60).[107]



60

Colourless oil (0.048 g, 44% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.92 (s, 1H, COO*H*), 3.58 (s, 2H, SCOCH_2COOH), 1.94-1,27 (m, 11H, *cyclohexyl*). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 191.49, 171.39, 49.04, 43.47, 32.78, 25.90, 25.54 ppm. FT-IR (ATR) 2930, 2854, 1718, 1680, 1448, 1400, 1298, 1264, 1160, 996, 912, 888, 858, 818, 732, 646 cm^{-1}. MS (MALDI-TOF) calculated for $C_9H_{14}NaO_3S$ $[M + Na]^+$ 225.0, found 225.5. HRMS Calcd for $C_9H_{13}O_3S$ $[M - H]^-$ 201.0591, found 201.0585 (FTMS-pNSI spectrometer used in this case).

4.2.5 NMR Deuterium Exchange Experiments.

^{2}D-^{1}H exchange experiments were carried out using a 500 MHz Bruker spectrometer. The internal temperature in the NMR probe was set at 20 °C and d_4-methanol 99.8 atom % D from Aldrich® was used as the deuterated solvent. The sample was weighed in a 5 mL glass vial and just before the experiment, dissolved in the required amount of d_4-methanol to afford a 70 μM solution. The solution was quickly transferred into a 5 mm NMR tube (Norell Standard Series) using a glass Pasteur pipette and the tube was introduced into the NMR spectrometer. After locking for d_4-methanol and shimming, a ^{1}H-NMR spectrum (16 scans) was acquired and processed to check the phase (this step took 4 minutes overall). Subsequently, ^{1}H-NMR spectrum (16 scans) were recorded every 5 minutes for a 90 minute experiment. After processing the spectrum, the peaks were integrated and normalized. The integral value of the peak for the methylene group was plotted against time.

4.3 Procedures and experimental data for decarboxylative aldol reactions.

4.3.1 General procedure for base-free decarboxylative aldol reaction catalysed by TBAB.

Malonic acid half thioester (1 equivalent) and 1 equivalent of the corresponding electrophile were mixed in toluene. One equivalent of *N,N,N,N*-tetrabutylammonium bromide and distilled water (same volume as toluene) were added to the solution. The resulting biphasic mixture was vigorously stirred for 24 hours at 40 ºC. After this time, the mixture was diluted with water (10 mL) and extracted with dichloromethane (3 x 10 mL). After drying over magnesium sulphate, filtration, and removing the solvent under reduced pressure a crude product was obtained as a pale yellow oil. The product was purified by column chromatography on silica gel (dichloromethane).

4.3.2 General procedure for base-free decarboxylative aldol reaction catalysed by dicyclohexano-18-crown-6.

Malonic acid half thioester (1 equivalent) and 1 equivalent of the corresponding electrophile were mixed in toluene (3 mL). 0.3 Equivalents of dicyclohexano-18-crown-6 and saturated KCl aqueous solution (3 mL) were added and the resulting biphasic mixture stirred for 24 hours at 40 ºC. After this time, the mixture was diluted with water (10 mL) and extracted with dichoromethane (3 x 10 mL). After drying over magnesium sulphate and removing the solvent under reduced pressure, a crude product was obtained which was purified by column chromatography on silica gel (dichloromethane).

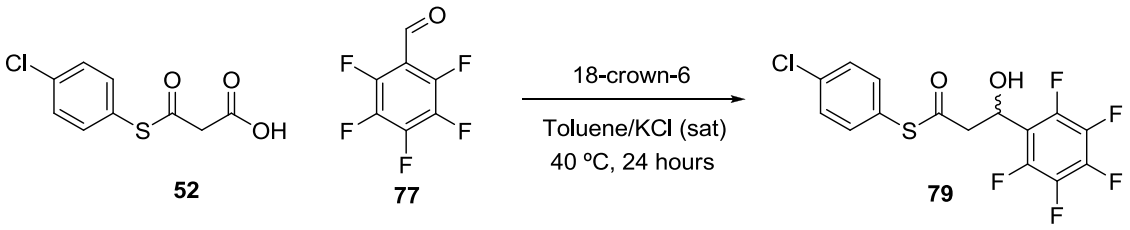
4.3.3 Procedure for base-free decarboxylative aldol reaction catalysed by valinomycin.

Malonic acid half thioester **55** (0.015 g, 0.055 mmol) and 4-nitrobenzaldehyde (0.008 g, 0.055 mmol) were mixed in toluene (0.4 mL) and saturated potassium chloride aqueous solution

(0.3 mL). Valinomycin (0.25 eq, 0.015 g, 0.014 mmol) was added to the reaction mixture and the biphasic mixture stirred for 3 hours at room temperature. After this time, more malonic acid half thioester **55** (0.008g, 0.029 mmol) was added and the mixture stirred for 3 additional hours. The reaction mixture was diluted with water (10 mL) and extracted with dichloromethane (3 x 10 mL). After drying over magnesium sulphate and removing the solvent under reduced pressure, a crude product was obtained which was purified by column chromatography (silica gel, dichloromethane) to afford 0.013 g of desired product as a white solid (62% yield).

4.3.4 Experimental data.

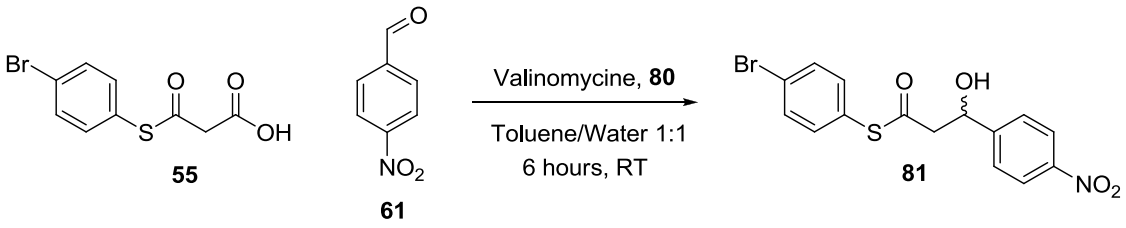
i) 4-Chlorophenyl 3-hydroxy-3-(pentafluorophenyl)propanethioate (79).[58]



3-(4-Chlorophenylthio)-3-oxopropanoic acid (0,010 g, 0,043 mmol) and pentafluorobenzaldehyde (0.008 g, 0.043 mmol) were mixed in toluene (0.25 mL). Dicyclohexano-18-crown-6 (0.005, 0.013 mmol) and saturated potassium chloride aqueous solution (0.25 mL) were added and the resulting mixture stirred at 40 ºC for 24 hours. After this time, the mixture was diluted with water (10 mL) and extracted with dichloromethane (3 x 10 mL). After drying over magnesium sulphate and removing the solvent under reduced pressure, a crude product was obtained which was purified by column chromatography on silica gel (dichloromethane). 0.014 g of desired product were isolated as a white solid (84% yield). Mp 121 - 122 ºC, chloroform. Rf = 0.52 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.41 (d, 2H, *J* = 9 Hz, *part A system AB*), 7.34 (d, 2H, *J* = 9 Hz, *part B system AB*), 5.61-5.57 (m, 1H, C*H*OHC_6F_5), 3.50 (dd, 1H, *J* = 16, 9 Hz, SCOC*H*$_2$C), 3.11 (dd, 1H, J = 16, 4 Hz, SCOC*H*$_2$C), 2.94 (d, 1H, J = 5.5 Hz, CHO*H*). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 195.5, 146.3-145.8 (m), 144.4-143.8 (m), 142.7-140.0 (m), 139.1-138.5 (m), 137.1-

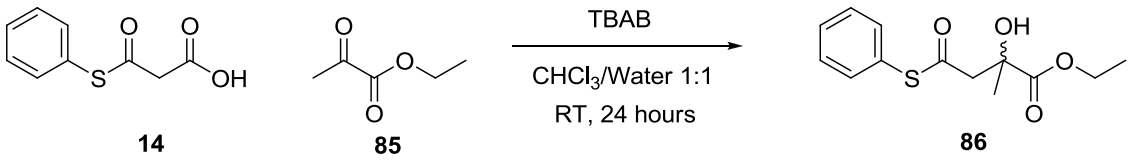
136.7 (m), 136.6, 135.8, 129.8, 125.0, 114.8 (t, *J*=17.4 Hz), 62.4, 48.9 ppm. FTIR [ATR] 3501, 1694, 1653, 1573, 1523, 1499, 1415, 1391, 1370, 1311, 1291, 1123, 1095, 1067, 1033, 999, 983, 935, 826, 655, 628 cm^{-1}. HRMS Calcd for $C_{15}H_9O_2Cl_1F_5S_1$ $[M + H]^+$ 382.9926, found 382.9917.

ii) 4-Bromophenyl 3-hydroxy-3-(4-nitrophenyl)propanethioate (81).



3-(3-Bromothiopheny)-3-oxopropanoic acid (0.015 g, 0.055 mmol) and 4-nitrobenzaldehyde (0.008 g, 0.055 mmol) were dissolved in toluene (0.4 mL) and potassium chloride saturated aqueous solution (0.4 mL). Valinomycine (0.015 g, 0.014 mmol) was added and the mixture stirred vigorously for 3 hours at room temperature. After this time, additional 3-(3-bromothiopheny)-3-oxopropanoic acid (0.007 g, 0.048 mmol) was added and the mixture stirred for an additional 3 hours at room temperature. The mixture was diluted with water (10 mL) and dichloromethane (10 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 10 mL). After drying over magnesium sulphate, filtration, and removing the solvent under reduced pressure the crude product was purified by column chromatography (silica gel, dichloromethane) to afford 0.013 g of desired product as a white solid (62% yield). Mp 110 - 111 ºC, chloroform. Rf = 0.40 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.23 (d, 2H, *J* = 9 Hz), 7.58-7.56 (m, 4H), 7.26 (d, 2H, *J* = 9 Hz), 5.33-5.30 (m, 1H), 3.18 (d, 1H, *J* = 4 Hz), 3.12-3.04 (m, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 196.36, 149.26, 147.73, 136.00, 132.79, 126.66, 125.70, 124.84, 124.04, 69.89, 51.81 ppm. FTIR [ATR] 3493, 1679, 1605, 1514, 1472, 1387, 1344, 1275, 1108, 1067, 1011, 981, 856, 818, 745, 731 cm^{-1}. HRMS Calcd for $C_{15}H_{13}O_4N_1Br_1S_1$ $[M + H]^+$ 381.9743, found 381.9736.

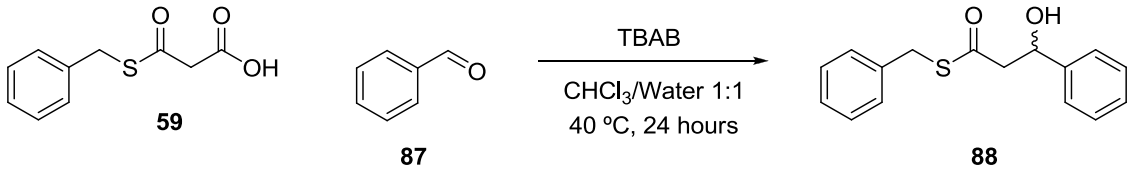
iii) Ethyl 2-hydroxy-2-methyl-4-oxo-4-(phenylthio)-butanoate (86).[24]



14 **85** **86**

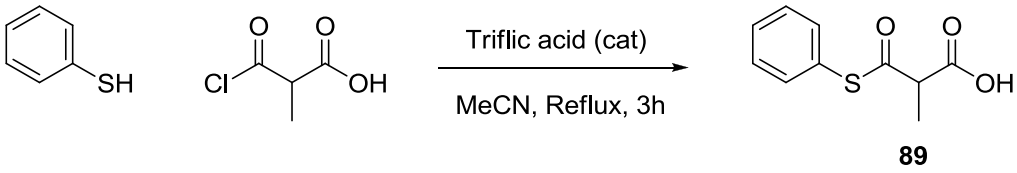
3-Oxo-3-phenylthiopropanoic acid **14** (0.022 g, 0.112 mmol) and ethyl pyruvate **85** (0.013, 0.112 mmol) were mixed in chloroform (0.22 mL). Phase transfer catalyst TBAB (tetrabutylammonium bromide, 0.036g, 0.112 mmol) and distilled water (0.22 mL) were added and the mixture stirred vigorously at room temperature for 24 hours. The mixture was diluted with water (10 mL) and extracted with dichloromethane (3 x 10 mL), dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to afford a crude product which was purified by column chromatography (silica gel, dichloromethane) to obtain 0.022 g of the desired product **86** as colourless oil (73% yield). Rf = 0.27 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.40 (s, 5H, Ar-*H*), 4.27-4.21 (m, 2H,OCH_2CH$_3$), 3.65 (s, 1H, CR_3OH), 3.28 (d, 1H, *J* = 16 Hz,SCOCH_2C), 3.06 (d, 1H, *J* = 16 Hz, SCOCH_2C), 1.45 (s, 3H, CH_3C), 1.28 (t, 3H, *J* = 7.5 Hz, OCH$_2$CH_3). ^{13}C-NMR (126 MHz, CDCl3) δ 195.66, 175.29, 134.60, 129.79, 129.42, 127.15, 73.00, 62.30, 52.47, 26.47, 14.23 ppm. FTIR [ATR] 3501, 3062, 2983, 2936, 2876, 1732, 1705, 1479, 1442, 1393, 1374, 1326, 1268, 1210, 1158, 1113, 1013, 962, 937, 782, 748, 706, 690, 624 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{13}H_{16}NaO_4S$ 291.07 $[M + Na]^+$, found 291.07.

iv) Benzyl 3-hydroxy-3-phenylpropanethioate (88).[38]



Following the general protocol, 0.006 g (8% yield) of desired product were obtained after column chromatography on silica gel (dichloromethane) as a colourless oil. Rf = 0.50 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.38-7.28 (m, 10H, Ar-*H*), 5.23 (dt, 1H, *J* = 9.0, 3.5 Hz, C*H*OHPh), 4.2 (d, 2H, *J* = 4.5 Hz, PhCH_2S), 3.08-3.03 (m, 1H, CHO*H*), 3.00-2.95 (m, 2H, SCOCH_2CHOHPh). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 198.23, 142.36, 137.22, 128.96, 128.82, 128.75, 128.08, 127.53, 125.79, 71.00, 52.41, 33.45 ppm. FTIR [ATR] 3445, 3087, 3063, 3031, 2961, 2918, 1683, 1495, 1455, 1410, 1323, 1289, 1243, 1200, 1184, 1086, 1057, 1028, 981, 762, 740, 700, 613 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{16}H_{16}NaO_2S$ 295.08 $[M + Na]^+$, found 295.12.

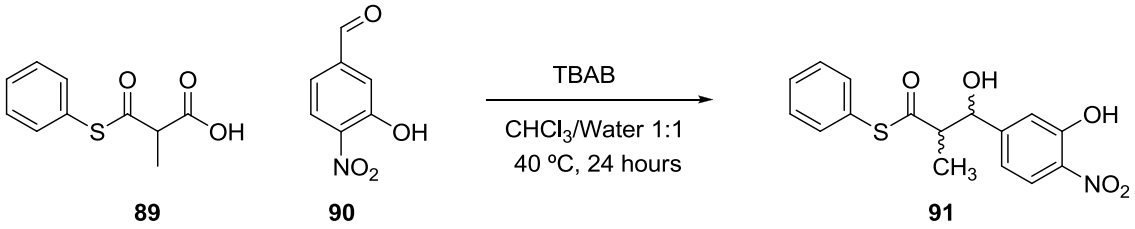
v) 2-Methyl-3-oxo-3-(phenylthio)-propanoic acid (89).[37]



To a solution of half methylmalonyl chloride (0.6 g, 4.39 mmol) and thiophenol (0.161 g, 1.465 mmol) in anhydrous acetonitrile (5 mL), triflic acid (0.013 mL, 0.022 g, 0.146 mmol) was added via syringe and the solution refluxed for 3 hours under nitrogen. After cooling under nitrogen, the solvent and volatiles were removed under reduced pressure. The crude residue was dissolved in dichloromethane and washed with distilled water (3 x 20 mL), dried over magnesium sulphate, filtered, and the solvent removed under reduced pressure to afford a crude product which was purified by column chromatography (silica gel, dichloromethane followed by dichloromethane / diethyl ether 9.5/0.5 V/V). 0.090 g of desired product were obtained as a colourless oil (29% yield).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.48-7.41 (m, 5H), 3.80 (q, *J* = 7.2 Hz, 1H), 1.55 (d, *J* = 7.2 Hz, 3H); FTIR [ATR] 2987, 2948, 1723, 1474, 1441, 1203 cm^{-1}.

vi) Phenyl β,3-dihydroxy-α-methyl-4-nitro-benzenepropanethioate (91).[37]

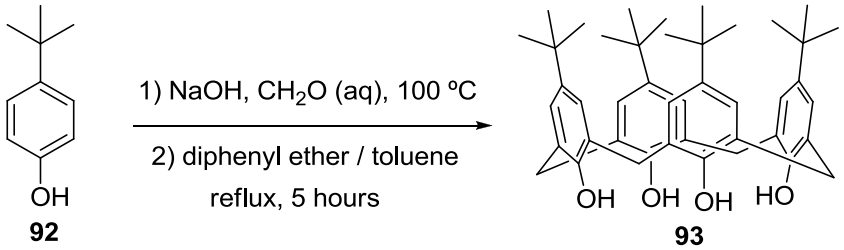


89 **90** **91**

Compound **89** (0.090 g, 0.428 mmol) and 4-nitro-3-hydroxybenzaldehyde (0,078 g, 0.428 mmol) were dissolved in chloroform (2 mL). Phase transfer catalyst TBAB (tetrabutylammonium bromide, 0.138 g, 0.428 mmol) and distilled water (2 mL) were added and the mixture was stirred vigorously at 40 ºC for 24 hours. The mixture was diluted with chloroform (10 mL) and water (10 mL) and the aqueous layer was extracted with chloroform (3 x 15 mL). The organic solution was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to afford a crude product which was purified by column chromaography on silica gel (dichloromethane). 0.069 g (*syn:anti* ratio = 1 / 3.6, determined by integration of peaks at 5.19 and 4.84 ppm) of desired compound were obtained as a yellow solid (48% yield). Rf = 0.26 (dichloromethane). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.61 (s, 1H, mixture of isomers), 8.10 (d, 1H, *J* = 8.5 Hz, *anti* isomer), 8.09 (d, 1H, *J* = 8.5 Hz, *syn* isomer), 7.35-7.45 (m, 6H, mixture of isomers), 7.19 (d, 1H, *J* = 1.5 Hz, *syn* isomer), 7.15 (d, 1H, *J* = 1.5 Hz, *anti* isomer), 6.98 (dd, 1H, *J* = 9.0, 2.0 Hz, *anti* isomer), 6.97 (dd, 1H, *J* = 8.5, 2 Hz, *syn* isomer), 5.19 (d, 1H, *J* = 3.5 Hz, *syn* isomer), 4.84 (d, 1H, *J* = 7.0 Hz, *anti* isomer), 3.10 (q, 1H, *J* = 7 Hz, *anti* isomer), 3.06-3.01 (m, 1H, *syn* isomer), 1.23 (d, 3H, *J* = 7.0 Hz, *anti* isomer), 1.22 (d, 3H, J = 7.5 Hz, *syn* isomer). MS (MALDI-TOF) Calculated for $C_{16}H_{15}NNaO_5S$ 356.06 $[M + Na]^+$, found 356.14.

4.4 Protocols and experimental data for cyclic scaffolds.

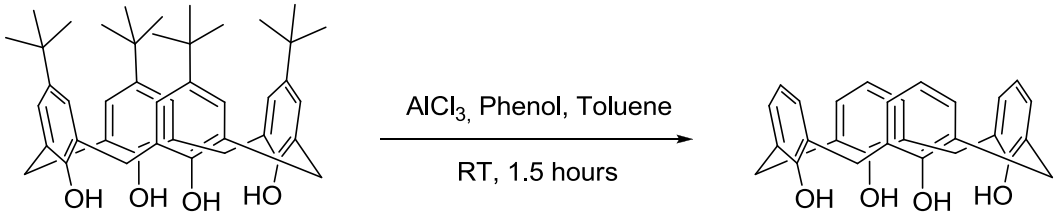
i) 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxy-calix[4]arene (93).[108]



92 **93**

A three-necked 3L round-bottom flask was charged with *p-tert*-butylphenol (250 g, 1.7 mol), formaldehyde (155 mL, 38% solution in water, 2.1 mol) and 10 M NaOH aqueous solution (3 mL, 3.0 mmol). The reaction mixture was heated at 100 ºC, using mechanical stirring and a nitrogen stream, until the solution became very viscous and white foam was formed. Heating and stirring were stopped and the reaction mixture was allowed to cool under nitrogen. Diphenyl ether (2L) and toluene (100 mL) were added and the resulting suspension was heated to 170 ºC, allowing water to evaporate. Once most of the water had been removed, the solution was refluxed for 5 hours. After cooling under nitrogen, the reaction mixture was transferred into a conical flask and ethyl acetate (1.5 L) was added. The solid formed was filtered by suction using a Buchner funnel and washed with acetone (500 mL). The obtained white solid was oven dried at 60 ºC under reduced pressure to afford 158 g of desired product **93**, used without further purification (59% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 10.34 (s, 4H), 7.05 (s, 8H), 4.25 (d, br, 4H, J = 13 Hz), 3.49 (d, br, 4H, *J* = 13 Hz), 1.21 (s, 36H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 146.82, 144.52, 127.84, 126.09, 34.16, 32.76, 31.55 ppm. FTIR [ATR] 3148, 3027, 2957, 2907, 2869, 1481, 1428, 1393, 1362, 1307, 1285, 1241, 1199, 1159, 872, 782, 739 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{44}H_{56}NaO_4$ 671.41 $[M + Na]^+$, found 671.58.

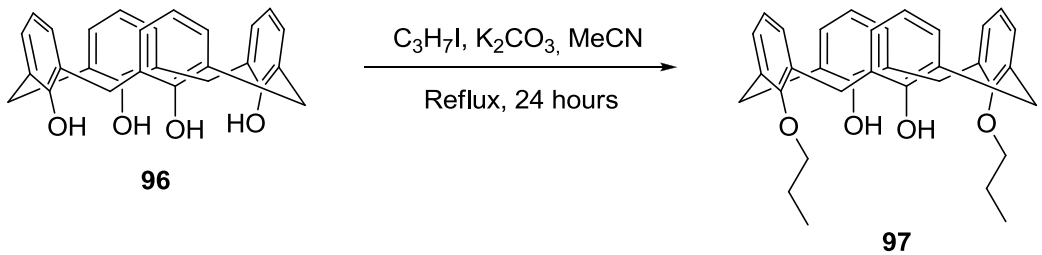
ii) 25, 26, 27, 28-Tetrahydroxycalix[4]arene (96).[108]



93 **96**

To a solution of calixarene **93** (15 g, 23.12 mmol) and phenol (10.44 g, 111.0 mmol) in toluene (150 mL), $AlCl_3$ (16.18g, 121.0 mmol) was added and the resulting mixture was stirred at rt for 1.5 hours. The solution was then poured into a beaker containing ice (200g) and the resulting biphasic mixture was separated. The aqueous phase was extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to afford a yellow oil. Trituration of the oil with cold diethyl ether (100 mL) afforded 6.80g of desired product **96** as a white solid (69% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 10.22 (s, 4H), 7.08 (d, 8H, *J* = 7.5 Hz), 6.75 (t, 4H, *J* = 7.5 Hz), *4*.29 (s, br, 4H), 3.57 (s, br, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 148.92, 129.12, 128.38, 122.39, 31.85 ppm. FTIR [ATR] 3152, 3094, 2984, 2935, 2869, 2779, 1594, 1470, 1449, 1416, 1381, 1267, 1246, 1197, 912, 834, 775, 754, 733, 650 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{28}H_{24}NaO_4$ 447.16 $[M+Na]^+$, found 447.25.

iii) 25, 27-Dipropyloxy-26,28-dihydroxycalix[4]arene (97).[109]

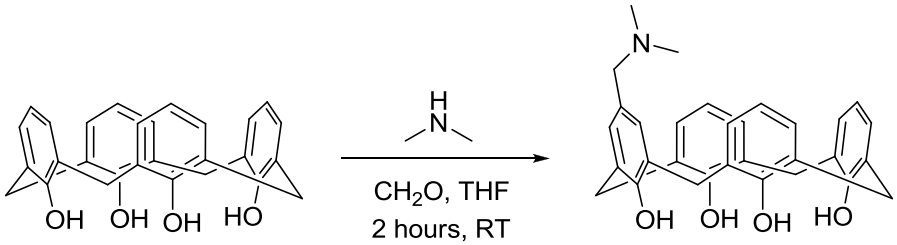


96 **97**

Calixarene **96** (5.69 g, 13.40 mmol), 1-iodopropane (5.13 g, 30.20 mmol) and potassium carbonate (2.08 g, 15.01 mmol) were dissolved in 55 mL of anhydrous acetonitrile under nitrogen. The mixture was refluxed for 24 hours. After cooling, a white solid precipitated which was filtered by suction in a Buchner funnel and washed with acetonitrile (3 x 10 mL) to afford 5.54 g of desired

product as a white solid (81% yield). Rf = 0.65 (petroleum ether / dichloromethane 1:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.31 (s, 2H), 7.06 (d, 4H, *J* = 7.5 Hz), 6.93 (d, 4H, *J* = 7.5 Hz), 6.75 (t, 2H, *J* = 7.5 Hz), 6.65 (t, 2H, *J* = 7.5 Hz), 4.33 (d, 4H, *J* = 13 Hz), 3.99 (t, 4H, *J* = 6.5 Hz), 3.39 (d, 4H, *J* = 13 Hz), 2.12-2.05 (m, 4H), 1.33 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 153.51, 152.03, 133.62, 129.04, 128.55, 128.29, 125.41, 119.08, 78.45, 31.57, 23.64, 11.06 ppm. FTIR [ATR] 3307, 3061, 3024, 2960, 2929, 2876, 1592, 1465, 1386, 1344, 1300, 1267, 1249, 1221, 1199, 1160, 1089, 1067, 1041, 1003, 963, 907, 837, 821, 757, 736 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{34}H_{36}NaO_4$ 531.25 $[M + Na]^+$, found 531.39.

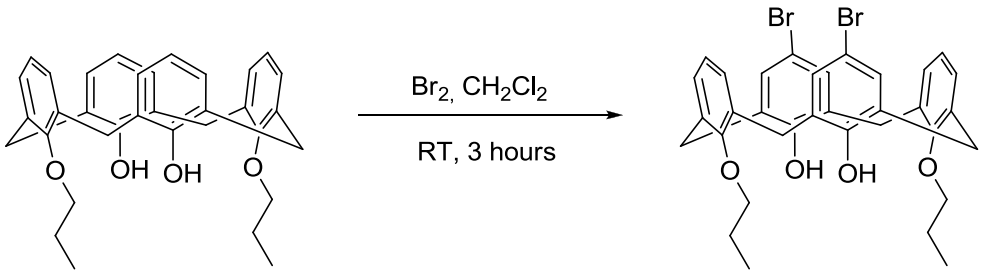
iv) 5-(*N,N*-dimethylaminomethyl)-25,26,27,28-tetra-hydroxycalix[4]arene (106).[96]



96 **106**

To a solution of calixarene **96** (4.56 g, 10.74 mmol) in tetrahydrofuran (90 mL), aqueous solutions of formaldehyde 37% (v/v) (1.74 g, 21.48 mmol) and of dimethylamine 40% (V/V) (2.48 g, 22.02 mmol) were added using a syringe. The solution was stirred for 2 hours at rt. The white solid precipitated was isolated by filtration on a Buchner funnel and washed with cold water, methanol and acetone respectively. The solid was vacuum dried to obtain 4.09 g of desired product as a white solid (79% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.05 (m, 6H), 6.98 (s, 2H), 6.72 (t, 3H, *J* = 7.5 Hz), 4.25 (s, br, 4H), 3.53 (s, br, 4H), 3.19 (s, 2H), 2.19 (s, 6H ppm). FTIR (ATR) 3043, 3012, 2951, 2921, 2856, 1729, 1702, 1653, 1591, 1467, 1435, 1362, 1292, 1253, 1236, 1206, 1161, 1131, 1082, 1064, 910, 762, 740 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{31}H_{31}NO_4$ 481.22 $[M]^+$, found 481.27.

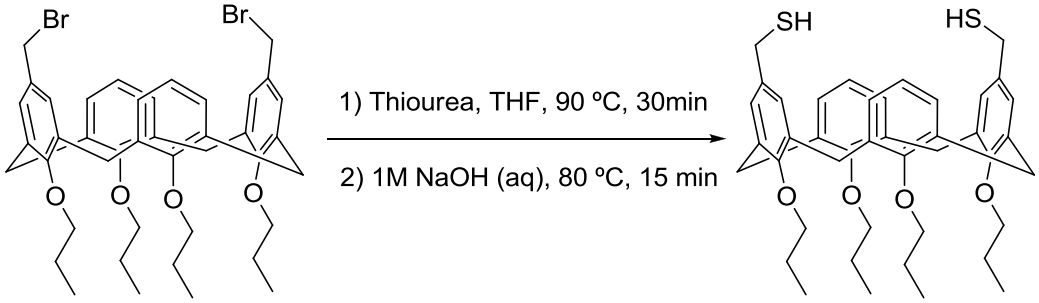
v) 11,23-Dibromo-26,28-dipropoxycalix[4]arene (107).[110]



97 **107**

To a solution of calixarene **97** (1.17 g, 2.30 mmol) in 30 mL of dry dichloromethane, bromine (0.29 mL, 0.92 g, 5.75 mmol) was added via syringe. The solution was stirred for 3 hours at room temperature and then the white precipitate formed was filtered by suction on a Buchner funnel and washed with dichloromethane (3 x 10 mL). The solid was dried under reduced pressure to afford 1.112 g of the desired compound **107** (72% yield) that was used without further purification. MS (MALDI-TOF) Calculated for $C_{34}H_{34}{}^{79}Br_2NaO_4$ $[M + Na]^+$ 689.07, found 689.61.

vi) 5,17-Bis(mercaptomethyl)-25,26,27,28-tetrapropoxycalix[4]arene (128).

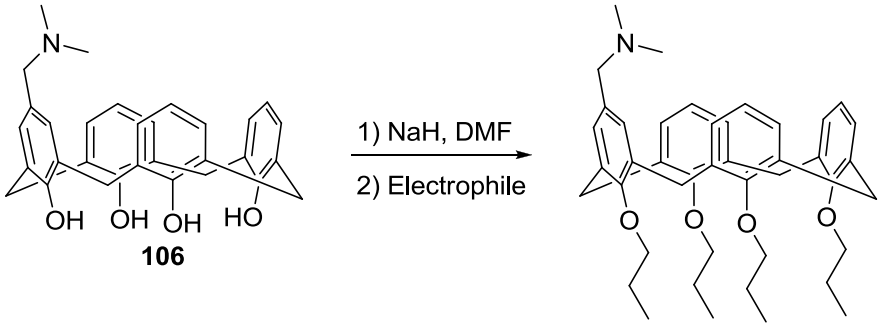


188 **128**

Calixarene **188** (0.096 g, 0.123 mmol) was dissolved in anhydrous tetrahydrofuran (2 mL) and thiourea (0.023 g, 0.308 mmol) was added. The mixture was microwave irradiated at 90 ºC for 30 minutes. The resulting cloudy solution was allowed to cool to room temperature and then 1M sodium hydroxide (2 mL, 0.080 g, 0.002 mmol) aqueous solution was added using a syringe. The reaction mixture was microwave irradiated for an additional 15 minutes at 80 ºC. After cooling to room temperature and quenching with 1M hydrochloric aqueous solution (3 ml), the aqueous layer

was extracted with dichloromethane (3 x 10 mL). After drying over magnesium sulfate, filtration, and removing the solvent under reduce pressure, the crude product was purified by column chromatography on silica gel (petroleum ether / dichloromethane 4:1, V/V) to afford 0.019 g of desired product as a white solid (24% yield). Rf = 0.7 (petroleum ether / dichloromethane 1.1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) 6.61-6.56 (m, 10H), 4.42 (d, 4H, *J* = 13 Hz), 3.84 (t, 4H, *J* = 7.5 Hz), 3.82 (t, 4H, *J* = 7.5 Hz), 3.41 (d, 4H, *J* = 6 Hz), 3.12 (d, 4H, *J* = 13 Hz), 1.96-1.87 (m, 8H), 1.56 (t, 2H, *J* = 6 Hz), 0.99 (t, 6H, *J* = 7.5 Hz), 0.98 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 156.57, 155.97, 135.49, 134.99, 134.20, 128.27, 127.79, 122.22, 76.84, 31.11, 28.83, 23.37, 23.35, 10.46, 10.43 ppm. FTIR [ATR] 2962, 2933, 2875, 1463, 1385, 1308, 1284, 1248, 1217, 1197, 1166, 1131, 1105, 1082, 1068, 1038, 1007, 966, 889, 871, 801, 760, 738, 704 cm^{-1}. HRMS Calcd for $C_{42}H_{56}NO_4S_2$ $[M + NH_4]^+$ 702.3645, found 702.3642.

vii) 5-(*N,N*-dimethylaminomethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene (149).

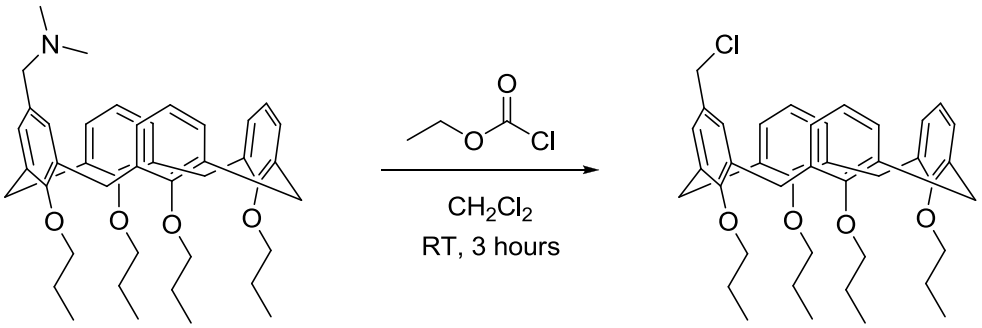


A. **Under thermal conditions**. To a stirred solution of calixarene **106** (2 g, 4.15 mmol) in anhydrous dimethyl sulphoxide (30 mL) under nitrogen was added sodium hydride (60% in mineral oil, 0.83 g, 20.76 mmol) at room temperature. The resulting suspension was stirred for 30 minutes and tetrabutylammonium chloride (3.46 g, 12.46 mmol) and *n*-propyl methanesulphonate (3.44 g, 24.92 mmol) were added. The reaction mixture was then stirred at 100 ºC for 3.5 hours. After cooling to room temperature, the reaction mixture was poured into water (100 mL). The aqueous layer was decanted off and the remaining brown oil was then washed with water. The oily residue was dissolved in chloroform and filtered through a silica plug flushing with chloroform (to remove the excess of *n*-propyl methanesulphonate)

followed by a mixture of chloroform / methanol 9:1, V/V to afford 1.727 g of compound **149** as a pale yellow foam (64% yield).

B. **Under microwave irradiation.** To a stirred solution of calixarene **106** (0.500 g, 1.038 mmol) in anhydrous *N*,*N*-dimethylformamide (17 mL) was added sodium hydride (60% in mineral oil, 0.21 g, 5.190 mmol) under nitrogen. The resulting suspension was stirred for 30 minutes at room temperature and a solution of *n*-propyl methanesulphonate (0.660 g, 4.780 mmol) in anhydrous *N*,*N*-dimethylformamide (3 mL) was then added using a syringe. The reaction mixture was heated at 90 ºC under microwave irradiation for 80 minutes. The reaction mixture was then poured into water (100 mL) and the aqueous phase was extracted with diethyl ether (3 x 30 mL). The combined organic phases were washed with an aqueous solution of potassium carbonate 1% (w/v), dried over magnesium sulphate and concentrated under reduced pressure to give a pale yellow oil. The oily residue was dissolved in dichloromethane (5 mL) and filtered through a silica plug flushing with dichloromethane to remove the excess of the electrophile, followed by a mixture of dichloromethane / methanol 9:1, V/V to yield 0.466 g of desired product as a pale yellow foam (69% yield). Rf = 0.60 (dichloromethane / methanol 9:1, V/V). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.67-6.50 (m, 11H), 4.44 (d, 2H, *J* = 12 Hz), 4.43 (d, 2H, *J* = 12 Hz), 3.89-3.79 (m, 8H), 3.22 (s, 2H), 3.15 (d, 2H, *J* = 12 Hz), 3.14 (d, 2H, *J* = 12 Hz), 2-1.87 (m, 14H), 1.02-0.96 (m, 12H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.86, 156.70, 156.31, 135.56, 135.51, 135.21, 135.02, 129.78, 128.47, 128.22, 122.27, 122.00, 77.04, 76.80, 44.17, 31.00, 30.95, 23.32, 23.24, 10.42, 10.40, 10.27 ppm. FTIR [ATR] 2961, 2934, 2874, 1455, 1384, 1246, 1212, 1194, 1087, 1067, 1037, 1006, 965, 908, 758, 730 cm^{-1}. Calcd for $C_{43}H_{56}NO_4$ $[M + H]^+$ 650.4204, found 650.4199.

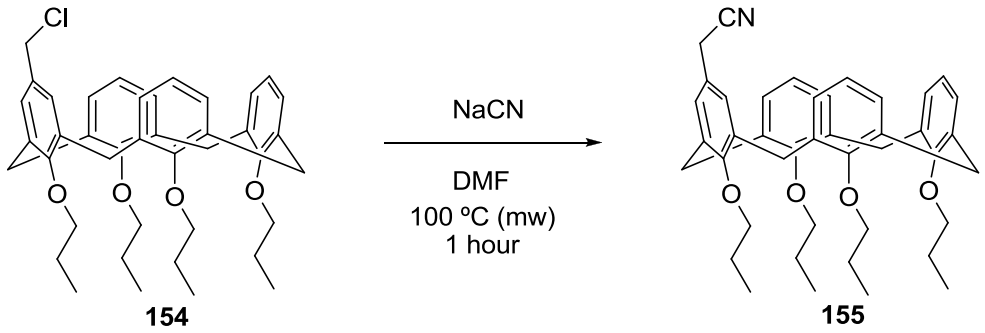
viii) 5-(chloromethyl)-25,26,27,28-*n*-propoxycalix[4]arene (154).[111]



149 **154**

A solution of **149** (0.346 g, 0.532 mmol) and distilled ethyl chloroformate (0.130 g, 1.198 mmol) in chloroform (5 mL) was stirred at room temperature for 3 hours. After this time, the solvent was removed under reduced pressure and the resulting pale yellow oil was filtered through a silica plug flushed with dichloromethane to afford 0.260 g of desired compound as a white foam (76% yield). Rf = 0.91 (petroleum ether / dichloromethane V/V 2:3). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.79-6.68 (m, 6H), 6.45-6.43 (m, 5H), 4.45 (d, 2H, *J* = 12.0 Hz), 4.44 (d, 2H, *J* = 12.0 Hz), 4.20 (s, 2H), 3.92-3.78 (m, 8H), 3.16 (d, 2H, *J* = 12 Hz), 3.15 (d, 2H, *J* = 12 Hz), 1.98-1.87 (m, 8H), 1.06-1.02 (m, 6H), 0.96 (t, 6H, *J* = 8 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 157.09, 156.65, 156.37, 135.80, 135.46, 135.18, 134.70, 130.64, 128.54, 128.42, 128.33, 127.86, 121.97, 121.59, 76.70, 76.67, 76.57, 46.79, 30.85, 23.19, 23.01, 10.32, 10.29, 10.00 ppm. FTIR [ATR] 2961, 2932, 2921, 2874, 1455, 1284, 1246, 1215, 1193, 1086, 1067, 1037, 1005, 966, 908, 758, 732 cm^{-1}. HRMS Calcd for $C_{41}H_{49}ClO_4$ $[M]^+$ 640.3314, found 640.3317 (FTMS-pAPCI spectrometer used in this case).

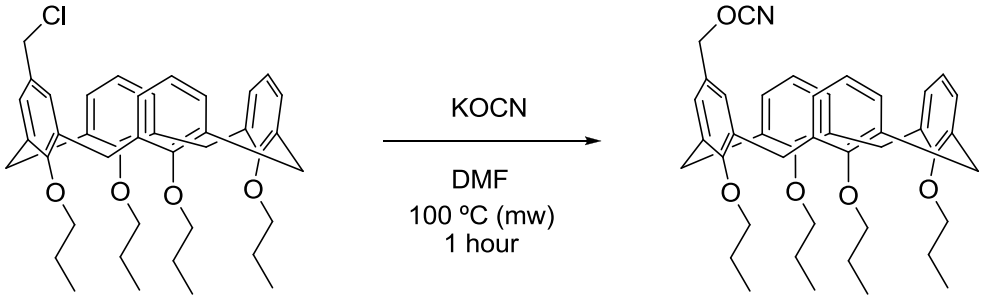
ix) 5-(Cyanomethyl)-25,26,27,28-*n*-propoxycalix[4]arene (155).



154 **155**

Calix[4]arene **154** (0.071 g, 0.111 mmol) was reacted with sodium cyanide (0.009 g, 0.183 mmol) in *N*,*N*-dimethylformamide (3 mL). The mixture was heated at 100 ºC under microwave irradiation for 1 hour. The mixture was allowed to cool to room temperature and dichloromethane (15 mL) was added. The organic layer was washed with water (3 x 15 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude product was further purified by preparative TLC (silica gel, petroleum ether (40-60) / chloroform 3:7, V/V) to afford 0.057 g of calix[4]arene **155** as a colorless oil (81% yield). Rf = 0.58 (petroleum ether / dichloromethane 2:3, V/V). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.85-6.71 (m, 6H), 6.46-6.40 (m, 3H), 6.27 (s, 2H), 4.44 (d, 4H, *J* = 12 Hz), 3.96-3.86 (m, 4H), 3.79-3.75 (m, 4H), 3.26 (s, 2H), 3.15 (d, 2H, *J* = 12 Hz), 3.13 (d, 2H, *J* = 12 Hz), 1.97-1.85 (m, 8H), 1.07-1.02 (m, 6H), 0.94 (t, 6H, *J* = 8 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 157.41, 156.45, 156.15, 136.23, 135.67, 134.67, 128.94, 128.67, 128.02, 127.48, 123.03, 122.24, 121.62, 118.52, 76.96, 76.94, 31.02, 23.41, 23.37, 23.16, 22.90, 10.56, 10.55, 10.12 ppm. FTIR [ATR] 2962, 2933, 2875, 1589, 1454, 1385, 1247, 1215, 1194, 1086, 1005, 965, 755, 387 cm^{-1}. HRMS Calcd for $C_{42}H_{53}N_2O_4$ $[M + NH_4]^+$ 649.4000, found 649.3997.

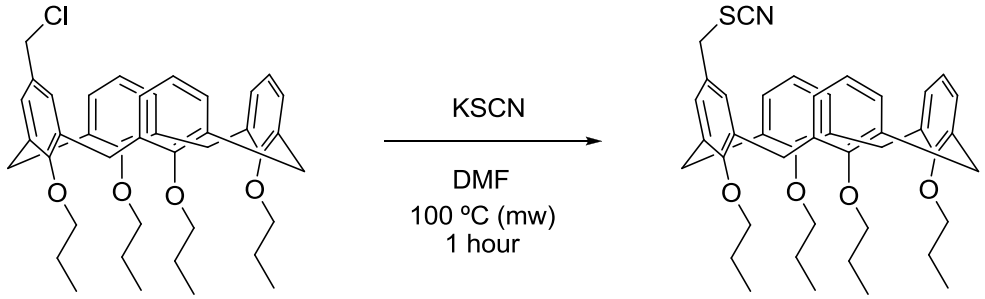
x) 5-(Cyanatemethyl)-25,26,27,28-*n*-propoxycalix[4]arene (156).



154 **156**

Following the protocol for the synthesis of calixarene **155**, derivative **154** (0.100 g, 0.156 mmol) was reacted with potassium cyanate (0.025 g, 0.312 mmol) to afford an impure compound which was purified by preparative TLC (dichloromethane / ethyl acetate 9.5:0.5, V/V) to yield 0.031 g of a colorless oil (30% yield). Rf = 0.81 (dichloromethane / diethyl ether 9:1, V/V). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.77-6.48 (m, 9H), 6.34 (s, 2H), 4.44 (d, 2H, *J* = 12 Hz), 4.43 (d, 2H, *J* = 12 Hz), 3.90-3.85 (m, 6H), 3.78 (t, 4H, *J* = 9 Hz), 3.13 (d, 2H, *J* = 15 Hz), 3.11 (d, 2H, *J* = 15 Hz), 1.98-1.84 (m, 8H), 1.01 (t, 6H, *J* = 9 Hz), 0.95 (t, 6H, *J* = 9 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.98, 156.45, 155.87, 135.68, 135.47, 135.08, 134.80, 128.44, 127.87, 127.15, 122.02, 121.50, 77.16, 76.76, 76.72, 44.43, 30.86, 23.17, 23.00, 10.29, 10.00, 0.83 ppm. FTIR [ATR] 2961, 2931, 2923, 2874, 1590, 1456, 1386, 1247, 1215, 1195, 1087, 1008, 967 cm^{-1}. MS (MALDI-TOF) *m/z* Calcd for $C_{42}H_{49}KNO_5$ $[M+K]^+$ 686.32, found 686.26.

xi) 5-(*S*-Thiocyanatemethyl)-25,26,27,28-*n*-propoxycalix[4]arene (157).



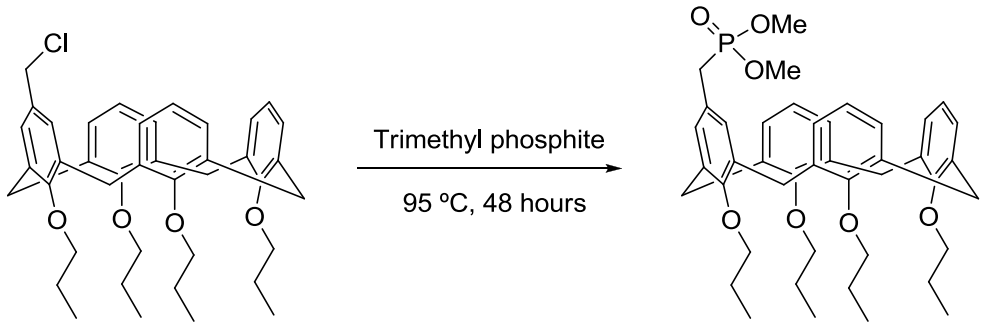
154 **157**

Potassium thiocyanate (0.038 g, 0.387 mmol) was added to a solution of calixarene **154** (0.124 g, 0.193 mmol) in 3 mL of *N*, *N*-dimethylformamide. The resulting suspension was heated at 100 ºC under microwave irradiation for 1 hour. The reaction mixture was then poured into distilled water (20 mL) and a white solid precipitated. The solid was collected by filtration, washed with water and dried under reduced pressure. The product was purified by preparative TLC on silica gel (petroleum ether (40-60) / dichloromethane 1:1, V/V) to afford 30 mg of desired compound **157** as a pale yellow oil (23% yield). Rf = 0.70 (petroleum ether (60-40) / dichloromethane 2:3, V/V). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.83-6.41 (m, 9H), 6.33 (s, 2H), 4.44 (d, 4H, *J* = 12 Hz), 3.94-3.74 (m, 10H), 3.15 (d, 2H, *J* = 12 Hz), 3.14 (d, 2H, *J* = 12 Hz), 1.98-1.83 (m, 8H), 1.04 (t, 3H, *J* = 6 Hz), 1.03 (t, 3H, *J* = 6 Hz), 0.94 (t, 6H, *J* = 6 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 157.14, 156.90, 156.28, 135.97, 135.52, 135.44, 134.55, 128.69, 128.58, 128.43, 127.77, 127.00, 122.08, 121.56, 112.58, 76.76, 38.54, 30.83, 30.81, 23.20, 22.97, 10.35, 10.31, 9.94 ppm. FTIR [ATR] 2962, 2931, 2920, 2875, 2155, 1588, 1457, 1385, 1284, 1215, 1195, 1087, 1007, 967 cm^{-1}. HRMS Calcd for $C_{42}H_{53}O_4N_2S$ $[M + NH_4]^+$ 681.3721, found 681.3716.

The same reaction also produced 5-(*N*-thiocyanatemethyl)-25,26,27,28-*n*-propoxycalix[4]arene (0.025 g, 20% yield) that was also isolated (white solid) and fully characterised. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.85-6.41 (m, 9H), 6.26 (s, 2H), 4.45 (d, 2H, *J* = 12 Hz), 4.44 (d, 2H, *J* = 12 Hz), 4.22 (s, 2H), 3.96-3.86 (m, 4H), 3.80-3.74 (m, 4H), 3.15 (d, 2H, *J* = 12 Hz), 3.14 (d, 2H, *J* = 12 Hz), 1.99-1.84 (m, 8H), 1.05 (t, 3H, *J* = 6 Hz), 1.03 (t, 3H, *J* = 6 Hz), 0.94 (t, 6H, *J* = 6 Hz). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 157.21, 156.26, 156.23, 136.04, 135.53, 135.27, 134.46, 128.74, 128.48, 127.80, 127.11, 126.38, 122.09, 121.46, 77.17, 76.76, 76.74, 48.16, 30.87, 30.84, 23.22, 23.20, 22.97,

10.37, 10.35, 9.93 ppm. FTIR [ATR] 2961, 2933, 2925, 2874, 2093, 1585, 1463, 1456, 1442, 1385, 1339, 1215, 1194, 1086, 1007, 967 cm^{-1}. HRMS Calcd for $C_{42}H_{53}O_4N_2S$ $[M + NH_4]^+$ 681.3721, found 681.3717.

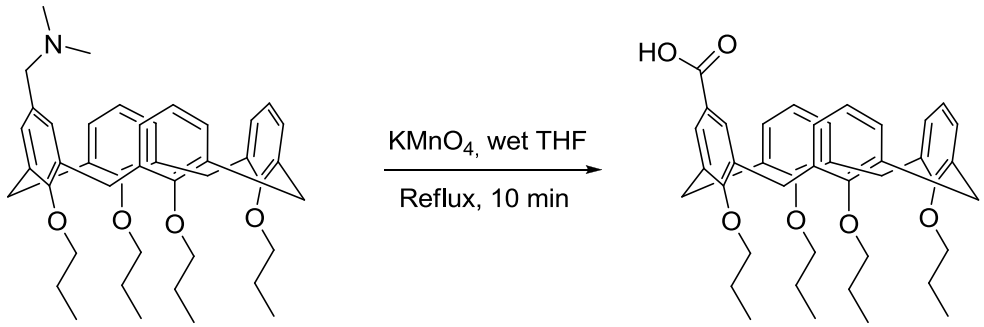
xii) 5-(Dimethylphosphonomethyl)-25,26,27,28-*n*-propoxycalix[4]arene (158).



154 **158**

A solution of compound **154** (0.118 g, 0.184 mmol) in trimethyl phosphite (1 mL) was stirred at 95 ºC under nitrogen for 48 hours. The excess of trimethyl phosphite was removed under reduced pressure and the product purified by preparative TLC (silica, chloroform / ethyl acetate 9.5:0.5, V/V) to afford 52 mg of the desired compound as a colorless oil (39% yield). Rf = 0.19 (petroleum ether / dichloromethane 2:3, V/V). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.66-6.52 (m, 11H), 4.46 (d, 2H, *J* = 16 Hz), 4.43 (d, 2H, *J* = 16 Hz), 3.87-3.81 (m, 8H), 3.55 (d, 6H, *J* = 12 Hz), 3.15 (d, 2H, *J* = 16 Hz), 3.14 (d, 2H, *J* = 16 Hz), 2.86 (d, 2H, *J* = 24 Hz), 1.96-1.88 (m, 8H), 1.02-0.98 (m, 12H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.94, 156.71, 156.10, 156.05, 135.65, 135.61, 135.49, 135.22, 135.02, 129.79, 129.70, 128.35, 128.32, 128.30, 124.13, 124.01, 122.09, 121.82, 76.84, 76.81, 52.93, 52.84, 33.13, 31.30, 31.00, 30.92, 23.26, 23.23, 10.34, 10.32, 10.28 ppm. FTIR [ATR] 2959, 2933, 2874, 1456, 1246, 1210, 1195, 1087, 1059, 1033, 1007, 966, 909, 886, 809, 761, 731 cm^{-1}. HRMS Calcd for $C_{43}H_{59}NO_7P$ $[M + NH_4]^+$ 732.4024, found 732.4019.

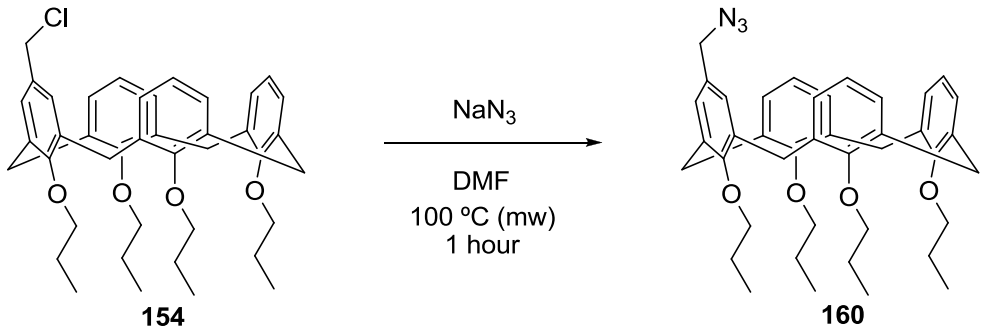
xiii) 5-(Carboxy)-25,26,27,28-tetra-*n*-propoxycalix[4]arene (159).[112]



149 **159**

To a solution of compound **149** (0.146 g, 0.225 mmol) in tetrahydrofuran (14 mL) was added potassium permanganate (0.426 g, 2.696 mmol) and distilled water (1 mL). The suspension was refluxed for 10 minutes and further diluted with tetrahydrofuran (30 mL) to be filtrated on a sintered funnel (porosity 1). The filtrates were concentrated under vacuum and the resulting pale brown oil was purified by preparative TLC on silica gel (dichloromethane / diethyl ether 4:1, V/V) to afford 0.037 g of calix[4]arene **159** as a colorless oil (27% yield). Rf = 0.67 (dichloromethane / diethyl ether 9:1, V/V). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.30 (s, 2H), 6.67-6.44 (m, 9H), 4.46 (d, 2H, *J* = 12 Hz), 4.43 (d, 2H, *J* = 12 Hz), 3.94-3.77 (m, 8H), 3.20 (d, 2H, *J* = 15 Hz), 3.14 (d, 2H, *J* = 15 Hz), 1.96-1.84 (m, 8H), 1.03-0.96 (m, 12H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 171.61, 161.93, 156.86, 156.75, 135.82, 135.63, 135.23, 134.71, 130.68, 128.82, 128.46, 128.36, 122.76, 122.40, 122.10, 77.37, 76.92, 76.85, 31.03, 23.39, 23.32, 23.28, 10.39, 10.36, 10.31 ppm. FTIR [ATR] 2962, 2916, 2875, 1682, 1455, 1288, 1210, 1197, 1107, 1087, 1037, 1006, 966, 758 cm^{-1}. HRMS Calcd for $C_{41}H_{52}NO_6$ $[M + NH_4]^+$ 654.3789, found 654.3788.

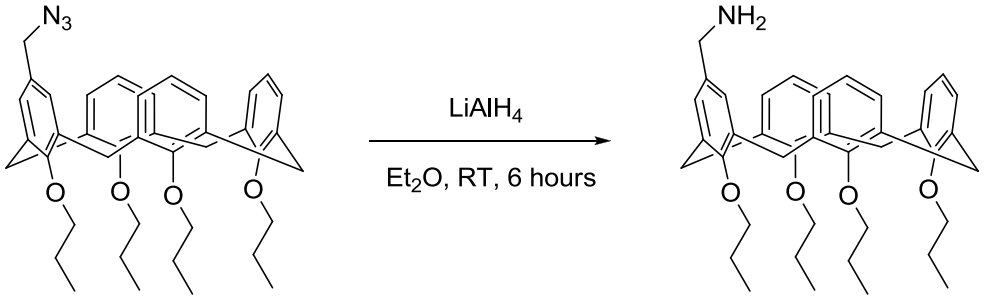
xiv) 5-(Azidomethyl)-25,26,27,28-*n*-propoxycalix[4]arene (160).



154 **160**

To a solution of compound **154** (0.125 g, 0.195 mmol) in *N*,*N*-dimethylformamide (3 mL) was added sodium azide (0.014 g, 0.214 mmol). The mixture was heated at 100 ºC under microwave irradiation for 1 hour. The mixture was allowed to cool to room temperature and dichloromethane (15 mL) was added. The organic layer was washed with water (3 x 15 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude product was further purified by column chromatography (silica gel, petroleum ether / dichloromethane 7:3 (V/V)) to afford 0.106 g of a pale yellow oil (84% yield). Rf = 0.81 (petroleum ether / dichloromethane 2:3, V/V). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.72-6.63 (m, 6H), 6.52-6.45 (m, 3H), 6.41 (s, 2H), 4.45 (d, 2H, *J* = 12 Hz), 4.44 (d, 2H, *J* = 12 Hz), 3.92 (s, 2H), 3.90-3.79 (m, 8H), 3.15 (d, 4H, *J* = 12 Hz), 1.97-1.87 (m, 8H), 1.02 (t, 3H, *J* = 8 Hz), 1.01 (t, 3H, *J* = 8 Hz), 0.97 (t, 6H, *J* = 8 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.02, 156.68, 156.59, 135.74, 135.50, 135.39, 135.01, 128.61, 128.45, 128.23, 128.11, 122.17, 121.72, 76.86, 58.49, 54.86, 31.17, 23.51, 23.39, 13.94, 10.64, 10.43 ppm. FTIR [ATR] 2961, 2932, 2920, 2875, 2092, 1455, 1384, 1301, 1282, 1246, 1214, 1194, 1129, 1086, 1067, 1038, 1006, 966, 759 cm^{-1}. HRMS Calcd for $C_{41}H_{53}N_4O_4$ $[M + NH_4]^+$ 665.4061, found 665.4060.

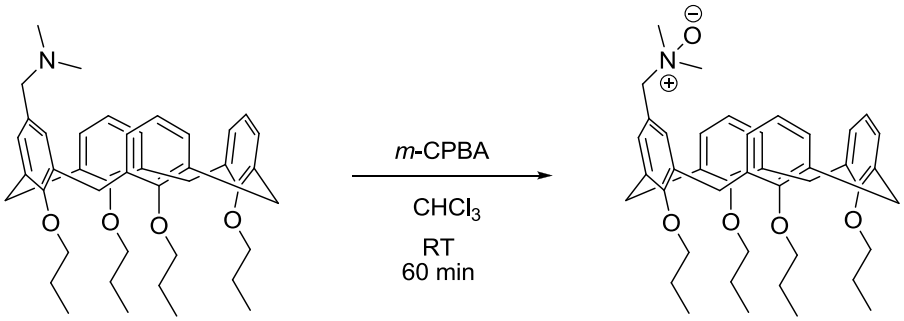
xv) 5-(Aminomethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene (161).



160 **161**

To a solution of calixarene **160** (0.149 g, 0.230 mmol) in anhydrous diethyl ether (6 mL), lithium aluminum hydride (0.052 g, 1.380 mmol) was added and the resulting suspension was stirred under nitrogen at room temperature for 6 hours. The mixture was quenched with water and washed with water (3 x 5 mL). After drying and removing the solvent under reduced pressure, a white solid was obtained, which was purified by preparative TLC on silica gel (dichloromethane / methanol 9:1, V/V) to afford 0.044 g of desired product as a colorless oil (31% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.75-6.64 (m, 6H), 6.51-6.44 (m, 3H), 6.38 (s, 2H), 4.44 (d, 2H, *J* = 12 Hz), 4.43 (d, 2H, *J* = 12 Hz), 3.91-3.87 (m, 4H), 3.79 (t, 4H, *J* = 8 Hz), 3.44 (s, 2H), 3.14 (d, 2H, *J* = 12 Hz), 3.13 (d, 2H, *J* = 12 Hz), 2.21 (s, 2H, br), 1.98-1.86 (m, 8H), 1.02 (t, 3H, *J* = 8 Hz), 1.01 (t, 3H, *J* = 8 Hz), 0.96 (t, 6H, *J* = 8 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.08, 156.55, 155.59, 135.77, 135.71, 135.03, 134.95, 128.60, 128.54, 128.04, 126.97, 122.13, 121.72, 77.01, 76.97, 76.83, 68.85, 58.49, 31.21, 31.16, 23.52, 23.36, 13.86, 13.82, 10.67, 10.40 ppm. FTIR [ATR] 2959, 2919, 2873, 1454, 1433, 1383, 1246, 1212, 1193, 1126, 1085, 1066, 1037, 1005, 965, 889, 842, 833, 757, 735, 702 cm^{-1}. HRMS Calcd for $C_{41}H_{52}O_4$ N $[M + NH_4]^+$ 622.3891, found 622.3883.

xvi) 5-(*N,N*-dimethylaminomethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene *N*-oxide (162).

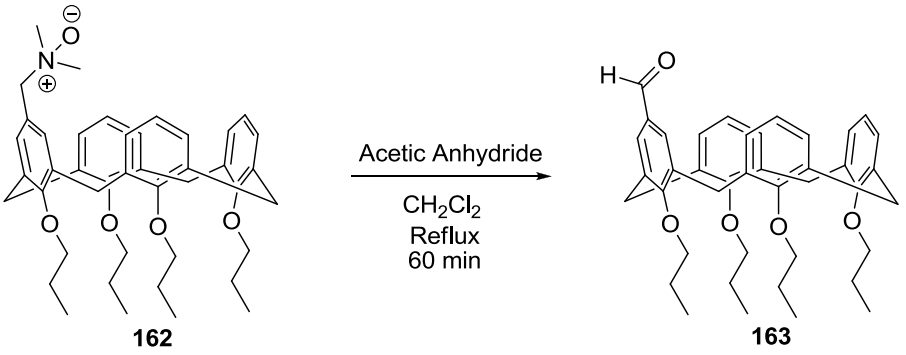


149 **162**

A solution of compound **149** (0.197 g, 0.303 mmol) and 3-chloroperbenzoic acid 77% (w/w) (0.149 g, 0.667 mmol) in chloroform (3 mL) was stirred for 1 hour at room temperature. The organic solution was washed with 1M sodium hydroxide (3 x 15 mL) and water (3 x 15 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, chloroform / methanol 9.5:0.5, V/V) to afford 0.141 g of a pale yellow oil (70% yield). Rf = 0.56 (dichloromethane / methanol 9:1, V/V). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.02-6.79 (m, 6H), 6.39-6.38 (m, 5H), 4.47 (d, 2H, *J* = 12 Hz), 4.42 (d, 2H, *J* = 12 Hz), 4.08-3.92 (m, 4H), 3.88 (s, 2H), 3.74-3.64 (m, 4H), 3.16 (d, 2H, *J* = 12 Hz), 3.15 (d, 2H, *J* = 12 Hz), 2.22 (s, 6H), 2.10-1.84 (m, 8H), 1.10-1.01 (m, 6H), 0.93 (t, 6H, *J* = 9 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 157.21, 156.87, 155.61, 136.71, 136.26, 134.74, 134.04, 131.80, 129.20, 128.74, 127.68, 123.68, 122.63, 122.43, 99.21, 77.71, 76.81, 76.52, 58.49, 56.30, 30.96, 23.63, 23.19, 13.87, 10.86, 10.84, 10.08 ppm. FTIR [ATR] 2960, 2932, 2874, 1455, 1384, 1300, 1282, 1247, 1218, 1208, 1195, 1171, 1131, 1105, 1087, 1066, 1037, 1005, 964, 942, 905, 885, 843, 836, 758 cm^{-1}. HRMS Calcd for $C_{43}H_{56}NO_5$ $[M + H]^+$ 666.4153, found 666.4151.

xvii) 5-Formyl-25,26,27,28-tetra-*n*-propoxycalix[4]arene (163).[113]

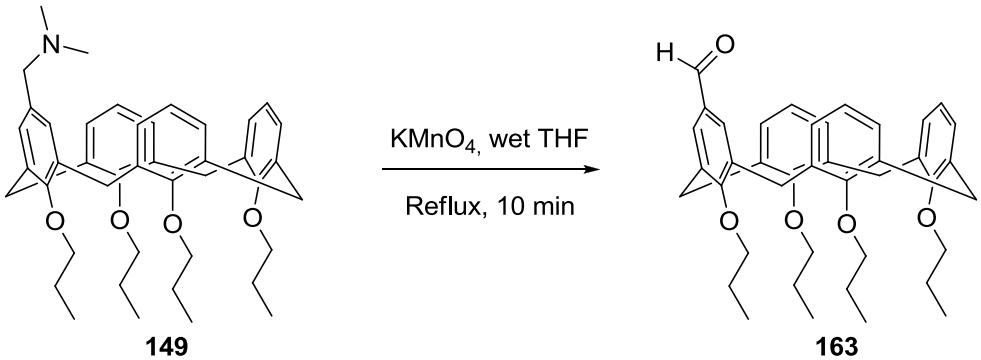
A. From compound 162.



162 **163**

A mixture of calixarene **162** (3.33 g, 5 mmol) and acetic anhydride (3.30 mL, 35 mmol) in dichloromethane (40 mL) was refluxed for 1 hour under nitrogen. After cooling, the reaction mixture was washed with a saturated aqueous solution of sodium hydrogen carbonate (3 x 25 mL) and water (3 x 25 mL), dried over magnesium sulphate and concentrated under reduced pressure to give a crude which was purified by column chromatography on silica gel (petroleum ether (40-60) / ethyl acetate 9:1, V/V) to afford 1.53 g of desired product as a white foam (49% yield).

B. From compound 149.[114]

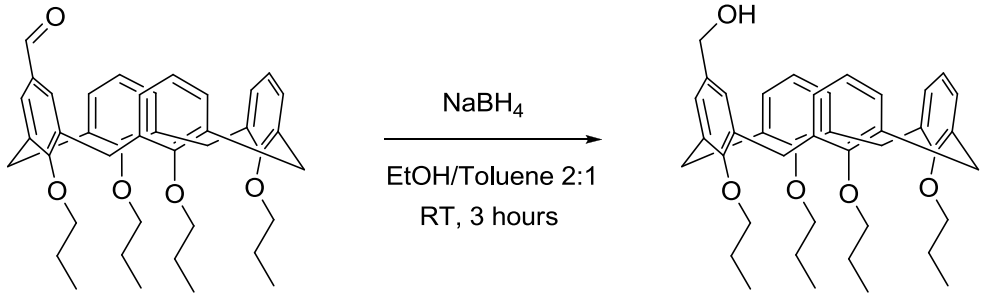


149 **163**

To a solution of compound **149** (0.146 g, 0.225 mmol) in tetrahydrofuran (14 mL) was added potassium permanganate (0.426 g, 2.696 mmol) and distilled water (1 mL). The suspension was refluxed for 10 minutes and further diluted with tetrahydrofuran (30 mL) to

be filtrated on a sintered funnel (porosity 1). The filtrates were concentrated under vacuum and the resulting pale brown oil was purified by preparative TLC on silica gel (dichloromethane / diethyl ether 4:1, V/V) to afford 0.027 g of desired product **163** as a colorless oil (20% yield). Rf = 0.53 (petroleum ether / dichloromethane 2:3, V/V). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.56 (s, 1H), 6.99 (s, 2H), 6.73-6.40 (m, 9H), 4.48 (d, 2H, *J* = 12 Hz), 4.43 (d, 2H, *J* = 12 Hz), 3.92-3.77 (m, 8H), 3.22 (d, 2H, *J* = 12 Hz), 3.15 (d, 2H, *J* = 12 Hz), 1.94-1.89 (m, 8H), 1.05-0.96 (m, 12H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 191.87, 162.11, 156.90, 156.31, 136.05, 135.85, 134.74, 134.71, 130.96, 130.01, 128.86, 128.34, 127.94, 122.23, 121.89, 76.84, 76.65, 76.62, 30.83, 23.24, 23.17, 23.02, 10.28, 10.22, 10.01 ppm. FTIR [ATR] 2962, 2932, 2924, 2875, 1691, 1588, 1455, 1433, 1383, 1277, 1246, 1209, 1193, 1121, 1086, 1066, 1036, 1004, 965, 909, 890, 759, 733 cm^{-1}. HRMS Calcd for $C_{41}H_{52}NO_5$ [M + NH_4]$^{+}$ 638.3840, found 638.3834.

xviii) 5-(Hydroxymethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene (164).[115]

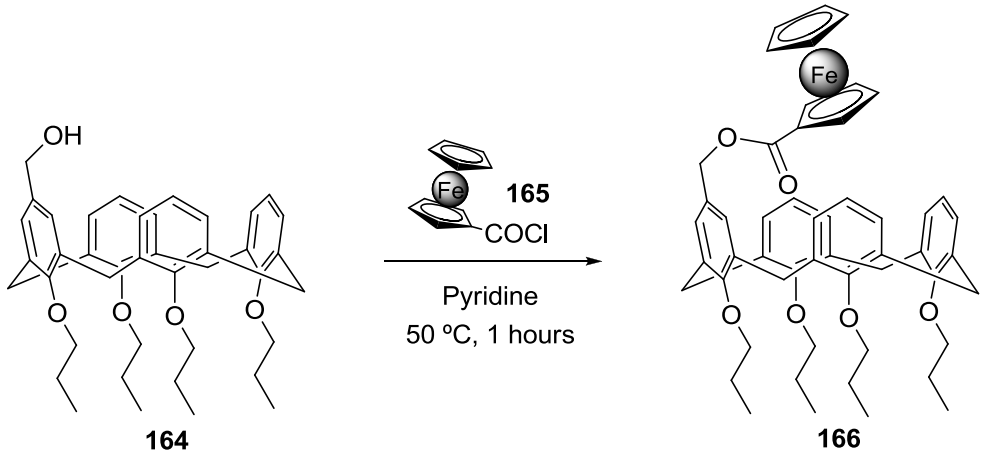


163 **164**

To a solution of calixarene **163** (0.281 g, 0.453 mmol) in toluene (4 mL) and ethanol (8 mL), sodium borohydride (0.021 g, 0.555 mmol) was added and the reaction was stirred at room temperature for 3 hours. The solvent was then removed under reduced pressure and the residue was dissolved in dichloromethane (10 mL), washed with water (15 ml), dried over magnesium sulfate and the solvent removed under vacuum to afford 0.019 g of desired product (67% yield). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.82-6.42 (m, 9H), 6.38 (s, 2H), 4.44 (d, 4H, *J* = 15 Hz), 4.19 (s, 2H), 3.94-3.88 (m, 4H), 3.77 (t, 2H, *J* = 9 Hz), 3.76 (t, 2H, *J* = 9 Hz), 3.14 (d, 4H, *J* = 12 Hz), 1.99-1.84 (m, 8H), 1.04 (t, 3H, *J* = 9 Hz), 1.03 (t, 3H, *J* = 9 Hz), 0.94 (t, 6H, *J* = 9 Hz). ^{13}C-NMR ($CDCl_3$, 75

MHz) δ 157.14, 156.41, 155.99, 135.84, 135.74, 134.81, 134.67, 134.22, 128.56, 128.54, 127.87, 127.07, 122.00, 121.38, 77.16, 76.78, 76.73, 65.30, 30.88, 30.83, 23.19, 22.97, 10.34, 9.94 ppm. FTIR [ATR] 2962, 2934, 2916, 2874, 1588, 1454, 1245, 1212, 1194, 1086, 1037, 1007, 967, 758, 521, 507 cm^{-1}. HRMS Calcd for $C_{41}H_{54}NO_5$ $[M + NH_4]^+$ 640.3997, found 640.3994.

xix) 5-(Ferrocenecarboxymethyl)-25,26,27,28-tetra-*n*-propoxycalix[4]arene (166).

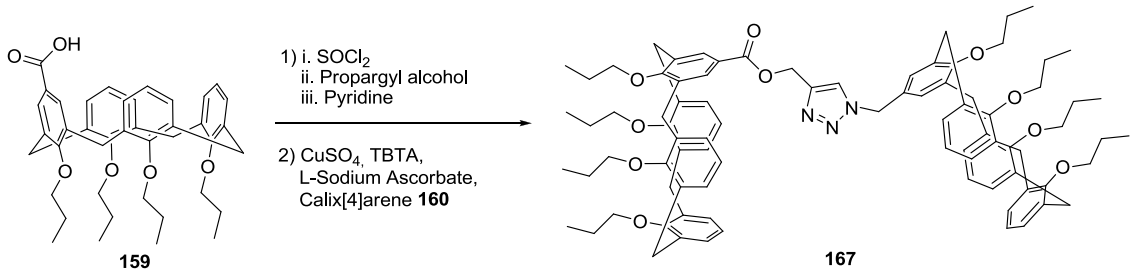


164 **166**

To a stirred solution of ferrocene monocarboxylic acid (8.42 mg, 0.037 mmol) under nitrogen, 2.5 equivalents of oxalyl chloride (0.012 g, 0.092 mmol) were added and the solution stirred for 2 hours at room temperature. The volatiles were removed under reduced pressure and the reaction crude was dissolved in anhydrous dichloromethane (2 mL) to which was added calixarene **164** (0.023 g, 0.037 mmol) and pyridine (6.0 µl, 5.84 mg, 0.074 mmol). The mixture was stirred for 1 hour at room temperature followed by 1 hour at 50 ºC. After removal of the solvent and volatiles under reduced pressure, the product was isolated by preparative TLC on silica gel (petroleum ether (40-60) / ethyl acetate 5:1, V/V) to afford 10 mg of an orange oil (32% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.75-6.49 (m, 11H), 4.84 (s, 2H), 4.79 (s, 2H), 4.45 (d, 2H, *J* = 12 Hz), 4.44 (d, 2H, *J* = 12 Hz), 4.40 (s, 2H), 4.13 (s, 5H), 3.88 (t, 4H, *J* = 8 Hz), 3.80 (t, 4H, J = 8 Hz), 3.17 (d, 2H, *J* = 16 Hz), 3.15 (d, 2H, *J* = 16 Hz), 1.97-1.85 (m, 8H), 1.03-0.94 (m, 12H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 171.59, 157.06, 156.50, 135.76, 135.51, 135.06, 134.90, 129.64, 128.53, 128.46, 128.07, 122.08, 121.75, 77.36, 76.79, 71.69, 71.61, 70.53, 70.03, 66.13, 31.17, 31.13, 23.45, 23.41, 23.33, 10.58, 10.57, 10.36, 1.17 ppm. FTIR [ATR] 2960, 2932, 2923, 2874, 1713,

1456, 1381, 1272, 1247, 1214, 1194, 1127, 1107, 1086, 1067, 1057, 1036, 1005, 966, 820, 759 cm^{-1}. HRMS Calcd for $C_{52}H_{62}FeNO_6$ $[M + NH_4]^+$ 852.3922, found 852.3924.

xx) 1-(5-methyl-25,26,27,28-n-propoxycalix[4]arene)-4-(5-methylcarboxy-25,26,27,28-n-propoxycalix[4]arene)-1H-1,2,3-triazole (167).



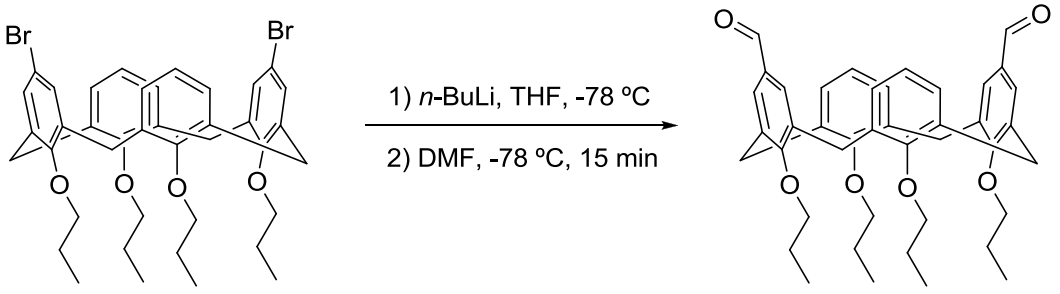
159 167

To a solution of calixarene **159** in anhydrous diethyl ether (5 mL), thionyl chloride (0.03 mL, 0.049 g, 0.410 mmol) was added under a nitrogen atmosphere using a syringe. The resulting solution was stirred under nitrogen at rt for 20 minutes and then the solvent and volatiles were removed under reduced pressure. The crude product was dissolved in anhydrous diethyl ether (5 mL) and propargyl alcohol (0.046 g, 0.82 mmol) was added, followed by pyridine (0.024 mL, 0.024 g, 0.301 mmol). The solution was stirred for 30 minutes at rt and then the solvent and volatiles were removed under reduced pressure. The crude product was purified by column chromatography on silica gel eluted with dichloromethane to afford 0.075 g of desired ester as a pale yellow oil (41% yield), which was used in the next step without further purification. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.38 (s, 2H), 6.65-6.54 (m, 9H), 4.84 (d, 2H, *J* = 3 Hz), 4.46 (d, 2H, *J* = 15 Hz), 4.44 (d, 2H, *J* = 15 Hz), 3.97-3.77 (m, 8H), 3.21 (d, 2H, *J* = 15 Hz), 3.15 (d, 2H, *J* = 15 Hz), 2.48 (t, 1H, *J* = 3 Hz), 1.98-1.84 (m, 8H), 1.03-0.96 (m, 12H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.87, 155.30, 134.76, 134.28, 134.02, 133.08, 129.08, 127.39, 127.19, 127.06, 121.71, 121.10, 120.88, 77.16, 76.31, 75.99, 75.72, 75.67, 75.57, 73.43, 50.82, 29.94, 29.91, 22.27, 22.25, 22.20, 9.35, 9.26, 9.23 ppm.

To 5-(azidomethyl)-25,26,27,28-n-propoxycalix[4]arene (0.096 g, 0.148 mmol), L-sodium ascorbate (2.35 mg, 0.012 mmol), copper sulfate pentahydrate (3 mg, 0.012 mmol) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (6.29 mg, 0.012 mmol) in *N*,*N*-dimethylformamide (3 mL), the propargyl ester (0.080 g, 0.119 mmol) was added and the resulting suspension was irradiated

at 80 ºC in a microwave reactor for 80 minutes. After quenching with water (10 ml), the crude product was extracted with diethyl ether (3 x 10 mL) and the combined organic fractions dried over magnesium sulfate. After filtering and removing the solvent under reduced pressure, the crude product was purified by column chromatography (eluting first with dichloromethane and then dichloromethane / ethyl acetate, 8:2 v/v) to afford 0.073 g of the desired compound **167** as a pale yellow oil (46% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.35 (s, 2H), 7.24 (s, 1H), 6.88-6.41 (m, 19H), 6.22 (s, 2H), 5.33 (s, 2H), 4.99 (s, 2H), 4.46-4.41 (m, 8H), 3.97-3.73 (m, 16H), 3.18-3.08 (m, 8H), 1.97-1.85 (m, 16H), 1.06-0.92 (m, 24H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 166.56, 161.45, 157.19, 156.77, 156.47, 156.29, 156.06, 136.15, 135.80, 135.63, 135.40, 135.23, 134.95, 134.33, 134.10, 130.06, 128.73, 128.52, 128.34, 128.24, 128.10, 127.82, 127.76, 127.30, 123.11, 122.15, 122.10, 121.76, 121.49, 77.26, 76.91, 76.88, 76.72, 76.62, 57.68, 54.06, 53.46, 30.97, 30.93, 23.40, 23.30, 23.28, 23.23, 23.10, 10.60, 10.56, 10.41, 10.28, 10.26, 10.08 ppm. IR [ATR] 2962, 2934, 2876, 1715, 1456, 1385, 1304, 1286, 1247, 1191, 1159, 1087, 1067, 1045, 1006, 967, 909, 891, 832, 760, 736 cm^{-1}. HRMS Calcd for $C_{85}H_{100}N_3O_{10}$ $[M + H]^+$ 1322.7403, found 1322.7369**.**

xxi) 5,17-Diformyl-25,26,27,28-tetrapropoxy-calix[4]arene (171).[116]

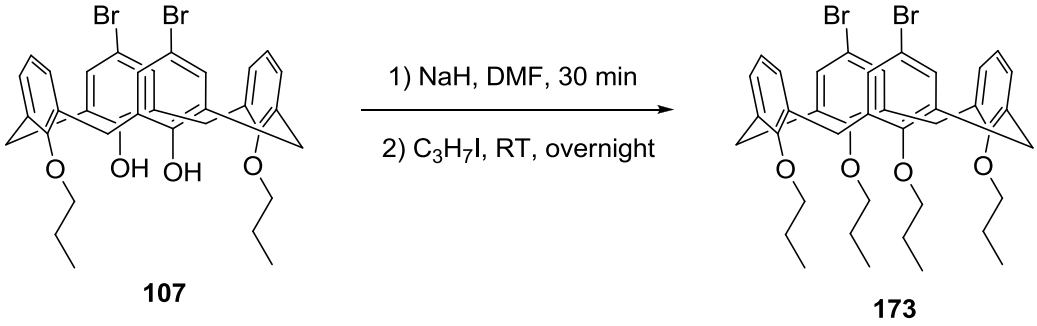


173 **171**

A solution of calixarene **173** (2.584 g, 3.44 mmol) in anhydrous tetrahydrofuran (30 mL) under nitrogen was cooled at −78 °C before adding *n*-butyllithium 2.5 M in hexanes (5.51 mL, 13.75 mmol) using a syringe. The solution was stirred at −78 °C for 30 minutes before adding anhydrous *N,N*-dimethylformamide (4.26 mL, 55.1 mmol) using a syringe. The reaction mixture was stirred at −78 ºC for further 30 minutes and then allowed to slowly reach room temperature. After quenching with water (30 ml) and removing the volatiles under reduced pressure, the aqueous layer was

extracted with diethyl ether (3 x 30 mL). The combined organic fractions were washed with water (3 x 20 mL), dried over magnesium sulphate, filtered, and the solvent was removed under reduced pressure to afford a crude product as colourless oil. The product was purified by column chromatography (silica gel, dichloromethane) to afford 0.978 g of desired product as a white solid (44% yield). Rf = 0.28 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.47 (s, 2H), 7.00 (s, 4H), 6.75-6.69 (m, 6H), 4.47 (d, 4H, *J* = 13 Hz), 3.91-3.86 (m, 8H), 3.23 (d, 4H, *J* = 13 Hz), 1.95-1.87 (m, 8H), 1.03 (t, 6H, *J* = 10 Hz), 0.97 (t, 6H, *J* = 10 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 191.81, 162.08, 156.70, 136.07, 134.92, 131.16, 129.98, 128.91, 122.79, 77.36, 77.13, 31.07, 23.51, 23.29, 10.51, 10.30 ppm. FTIR [ATR] 2964, 2934, 2876, 2793, 2728, 1694, 1597, 1456, 1434, 1384, 1279, 1248, 1217, 1161, 1123, 1082, 1065, 1036, 1004, 963, 936, 889, 839, 802, 759, 737, 701, 672, 650, 624, 604 cm^{-1}. HRMS Calcd for $C_{42}H_{49}O_6$ $[M + H]^+$ 649.3524, found 649.3518.

xxii) 5,17-Dibromo-25,26,27,28-tetrapropoxy-calix[4]arene (173).[117]

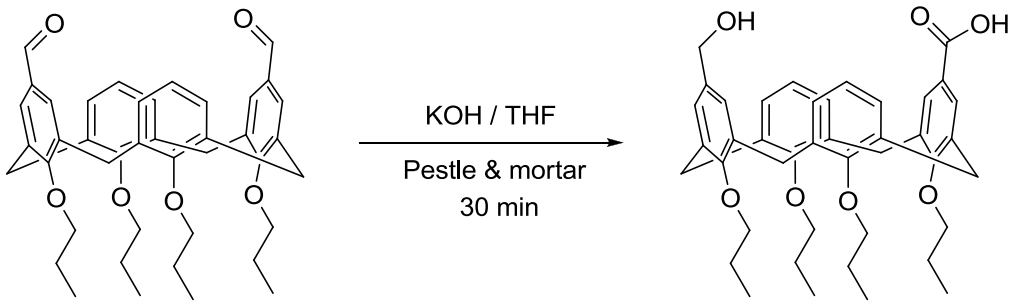


107 **173**

To a solution of calixarene **107** (2.68 g, 4.02 mmol) in 30 mL of anhydrous *N,N*-dimethylformamide was added NaH (60% in mineral oil, 0.80 g, 20.10 mmol) and the suspension stirred for 30 minutes at room temperature. 1-Iodopropane (2.35 mL, 4.10 g, 24.12 mmol) was added using a syringe and the solution was stirred overnight at room temperature. The reaction was quenched with water (50 mL) and the resulting precipitated solid was collected by filtration to afford 1.91 g of desired compound as a white solid (63% yield). Rf = 0.87 (petroleum ether / dichloromethane 1:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.77 (s, 4H), 6.64-6.63 (m, 6H), 4.40 (d, 4H, *J* = 13.5 Hz), 3.85-3.80 (m, 8H), 3.11 (d, 4H, *J* = 13.5 Hz), 1.94-1.85 (m, 8H), 0.99 (t, 6H, *J* = 10 Hz), 0.98 (t, 6H, *J* = 10 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 156.51, 155.84, 137.36, 134.51, 130.90, 128.55, 122.62, 114.84,

77.05, 76.94, 30.99, 23.32, 10.44, 10.40 ppm. FTIR [ATR] 2962, 2933, 2876, 1574, 1456, 1385, 1293, 1250, 1210, 1157, 1102, 1081, 1067, 1037, 1005, 965, 909, 865, 848, 760, 735 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{40}H_{46}{}^{79}Br_2NaO_4$ 771.17 $[M + Na]^+$, found 771.41.

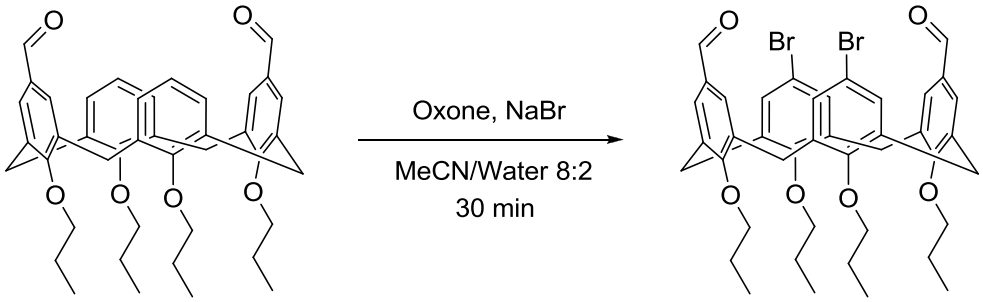
xxiii) 5-Carboxy-17-(hydroxymethyl)-25,26,27,28-tetrapropoxy-calix[4]arene (174).



171 **174**

Calixarene **171** (0.020 g, 0.031 mmol) was placed in a glass mortar and heated at 100 ºC. Potassium hydroxide (0.173 g, 3.08 mmol) was then added and the mixture ground at this temperature for 2 minutes. A few drops of tetrahydrofuran were added and the mixture was ground until it became dry. The process was repeated 5 times until no starting material was observed by TCL (dichloromethane). The paste was allowed to cool at room temperature and then quenched with 2M hydrochloric acid aqueous solution (5 ml) and extracted with diethyl ether (3 x 5 mL). After drying over magnesium sulphate, filtration, and removing the solvent under reduced pressure, 0.019 g of desired product were obtained as a white solid (92% yield). Rf = 0.22 (dichloromethane / diethyl ether 18:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.00 (s, 2H), 6.97-6.95 (m, 4H), 6.86-6.83 (m, 2H), 6.23 (s,2H), 4.44 (t, 4H, *J* = 13.0 Hz), 4.10 (s, 2H), 4.01-3.89 (m, 4H), 3.77 (t, 2H, *J* = 5 Hz), 3.70 (t, 2H, *J* = 5 Hz), 3.17 (d, 2H, *J* = 15 Hz), 3.13 (d, 2H, *J* = 15 Hz), 1.97-1.83 (m, 8H), 1.07 (t, 3H, *J* = 7.5 Hz), 1.06 (t, 3H, *J* = 7.5 Hz), 0.91 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.37, 160.84, 157.38, 155.37, 136.44, 135.74, 134.74, 134.21, 134.03, 130.13, 129.29, 128.86, 126.03, 122.68, 122.51, 77.00, 76.72, 64.39, 31.13, 31.05, 23.56, 23.19, 10.80, 10.73, 10.11 ppm. FTIR [ATR] 2962, 2932, 2875, 1683, 1599, 1543, 1516, 1456, 1432, 1385, 1305, 1283, 1247, 1211, 1197, 1129, 1106, 1082, 1066, 1036, 1006, 966 cm^{-1}. HRMS Calcd for $C_{42}H_{50}O_7Na_1$ $[M + Na]^+$ 689.3449, found 689.3439.

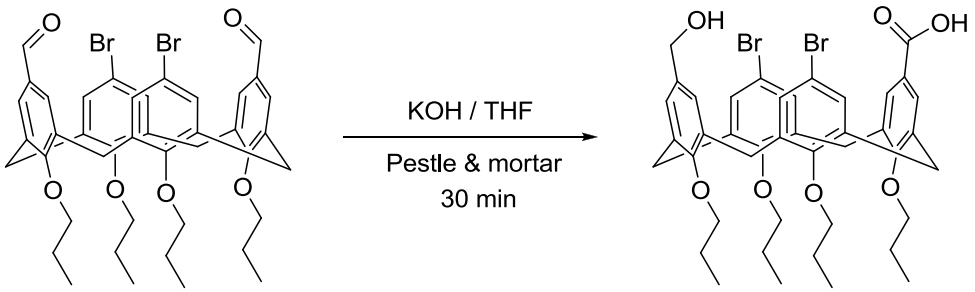
xxiv) 11,23-Dibromo-5,17-diformyl-25,26,27,28-tetrapropoxy-calix[4]arene (178).[118]



171 **178**

Calixarene **171** (0.131 g, 0.202 mmol) and sodium bromide (0.083 g, 0.808 mmol) were dissolved in acetonitrile (8 mL) and then a solution of oxone® (0.496 g, 0.808 mmol) in water (2 mL) was added to the solution using a syringe. The resulting orange solution was stirred at room temperature for 30 minutes and acetonitrile was then added until a white solid was formed. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford a crude product as pale yellow oil. The product was filtered through silica gel (dichloromethane / diethyl ether 9:1, V/V) to afford 0.157 g of desired compound as a white solid (96% yield). Rf = 0.45 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.60 (s, 2H), 7.14 (s, 4H), 6.82 (s, 4H), 4.42 (d, 4H, *J* = 13.5 Hz), 3.93-3.90 (m, 4H), 3.84-3.81 (m, 4H), 3.21 (d, 4H, *J* = 13.5 Hz), 1.92-1.85 (m, 8H), 1.01-0.97 (m, 12H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 191.61, 162.10, 155.67, 136.59, 135.70, 131.49, 131.38, 130.25, 115.48, 77.36, 77.28, 77.23, 30.97, 23.40, 23.25, 10.34 ppm. FTIR [ATR] 2963, 2933, 2876, 1740, 1694, 1598, 1457, 1431, 1384, 1307, 1281, 1240, 1202, 1160, 1124, 1108, 1065, 1035, 1001, 962, 865, 850, 773, 737 cm^{-1}. HRMS Calcd for $C_{42}H_{46}O_6Br_2Na_1$ $[M + Na]^+$ 827.1553, found 827.1545.

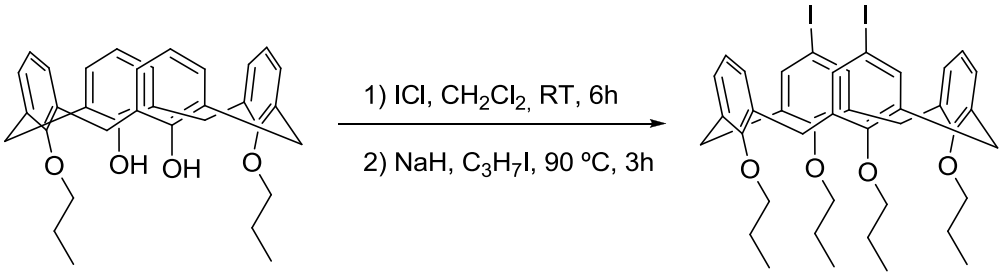
xxv) 11,23-dibromo-5-carboxy-17-(hydroxymethyl)-25,26,27,28-tetrapropoxy calix[4]arene (179).



178 **179**

The same protocol as described for the synthesis of calixarene **174** afforded 0.132 g of desired product as a pale yellow solid (82% yield). Rf = 0.30 (dichloromethane / diethyl ether 18:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.16 (s, 2H), 7.02-7.00 (m, 4H), 6.39 (s, 2H), 4.40 (d, 2H, *J* = 15 Hz), 4.37 (d, 2H, *J* = 15 Hz), 4.26 (s, 2H), 3.94-3.84 (m, 4H), 3.80 (t, 2H, *J* = 7.5 Hz), 3.72 (t, 2H, *J* = 7.5 Hz), 3.15 (d, 2H, *J* = 15 Hz), 3.10 (d, 2H, *J* = 15 Hz), 1.91-1.82 (m, 8H), 1.03 (t, 3H, *J* = 7.5 Hz), 1.01 (t, 3H, *J* = 7.5 Hz), 0.92 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 170.93, 161.01, 156.31, 155.58, 138.09, 137.36, 134.84, 134.41, 133.74, 131.64, 131.23, 130.41, 126.54, 123.19, 115.04, 64.44, 31.05, 30.95, 23.46, 23.43, 23.13, 10.63, 10.57, 10.15 ppm. FTIR [ATR] 2963, 2933, 2876, 1683, 1600, 1574, 1456, 1429, 1385, 1308, 1283, 1241, 1200, 1163, 1127, 1108, 1065, 1036, 1003, 964, 920, 908, 864, 804 cm^{-1}. HRMS Calcd for $C_{42}H_{48}O_7{}^{79}Br_2Na_1$ $[M + Na]^+$ 845.1659, found 845.1652.

xxvi) 5,17-Diiodo-25,26,27,28-tetrapropoxy-calix[4]arene (180).[119]



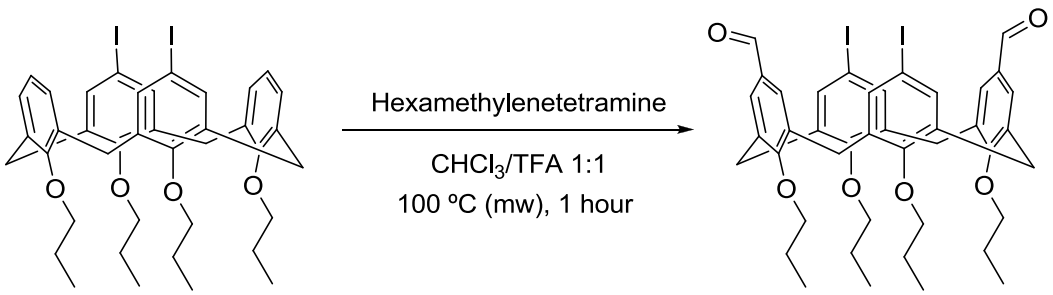
97 **180**

To a solution of calixarene **97** (1.0 g, 1.97 mmol) in dichloromethane (100 mL), iodide monochloride (1.60 g, 9.83 mmol) was added and the solution stirred for 6 hours at room temperature. After this time, the precipitated solid was collected by filtration and washed with dichloromethane to obtain 0.815 g (54% yield) of 11,23-diiodo-26,28-dipropoxycalix[4]arene as a pale yellow solid that was used without further purification. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.39 (s, 2H), 7.34 (s, 4H), 6.93 (d, 4H, *J* = 7.5 Hz), 6.81 (m, 2H), 4.22 (d, 4H, *J* = 13 Hz), 3.95 (t, 4H, *J* = 6 Hz), 3.30 (d, 4H, *J* = 13 Hz), 2.08-2.01 (m, 4H), 1.29 (t, 6H, *J* = 7.5 Hz). FTIR [ATR] 3348, 3322, 3303, 3190, 2957, 2921, 2873, 1583, 1456, 1438, 1427, 1386, 1346, 1287, 1269, 1217, 1197, 1149, 1105, 1071, 1041, 998, 956, 767 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{34}H_{34}I_2NaO_4$ 783.04 $[M + Na]^+$, found 783.39.

To a suspension of 11,23-diiodo-26,28-dipropoxycalix[4]arene (0.68g, 0.890 mmol) in anhydrous *N*,*N*-dimethylformamide (20 mL) was added sodium hydride (0.21 g, 5.34 mmol, 60% in mineral oil) and the mixture stirred for 30 minutes at 60 ºC. 1-Iodopropane (0.52 mL, 5.34 mmol) was added using a syringe and the reaction mixture was heated at 90 ºC under microwave irradiation for 3 hours. After cooling, the reaction mixture was quenched with water and extracted with diethyl ether (3 x 50 mL). After drying over magnesium sulphate, filtration, and removing the solvent under reduced pressure, 0.330 g of calix[4]arene **180** were obtained as a white solid (44% yield). Rf = 0.90 (petroleum ether / dichloromethane 1:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.12 (s, 4H), 6.54-6.47 (m, 6H), 4.36 (d, 4H, *J* = 13.5 Hz), 3.86 (t, 4H, *J* = 7.5 Hz), 3.77 (t, 4H, *J* = 7.5 Hz), 3.08 (d, 4H, *J* = 13.5 Hz), 1.92-1.86 (m, 8H), 1.00 (t, 6H, *J* = 7.5 Hz), 0.95 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 157.16, 156.06, 138.41, 137.19, 133.87, 128.30, 122.62, 85.64, 77.01,

76.99, 30.78, 23.40, 23.24, 10.56, 10.27, 1.16 ppm. FTIR [ATR] 2962, 2932, 2875, 1567, 1456, 1385, 1292, 1249, 1208, 1158, 1102, 1082, 1067, 1036, 1005, 965, 891, 862, 837, 819, 800, 759, 739 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{40}H_{46}I_2NaO_4$ 867.14 $[M + Na]^+$, found 867.56.

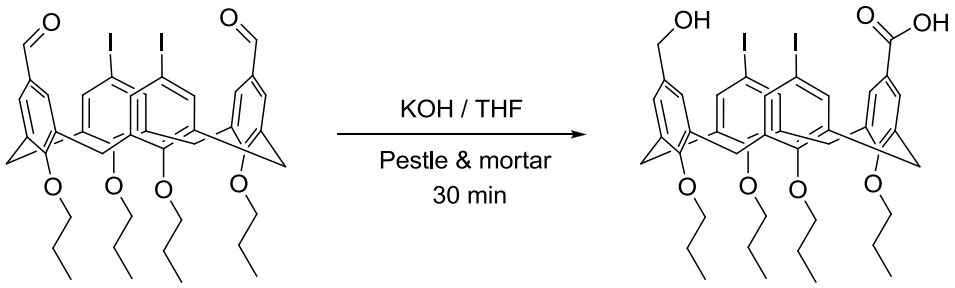
xxvii) 11,23-Diiodo-5,17-diformyl-25,26,27,28-tetrapropoxy-calix[4]arene (181).



180 **181**

Calixarene **180** (0.746 g, 0.883 mmol) was dissolved in chloroform (5 mL) and hexamethylenetetramine (0.74g, 5.30 mmol) was added followed by trifluoroacetic acid (13 mL) which was added using a syringe. The reaction mixture was then heated at 100 ºC under microwave irradiation for 1 hour. After cooling to room temperature, the reaction mixture was carefully quenched with 2M NaOH aqueous solution and extracted with dichloromethane (3 x 30 mL), dried over magnesium sulphate, filtered, and the was solvent removed under reduced pressure to afford a crude product that was purified by column chromatography on silica gel (dichloromethane followed by a mixture dichloromethane / diethyl ether 9:1, V/V) to afford 0.607 g of desired compound as a white solid (76% yield). Rf = 0.35 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.51 (s, 2H), 7.15 (s, 4H), 6.99 (s, 4H), 4.39 (d, 4H, *J* = 13.5 Hz), 3.87-3.84 (m, 8H), 3.18 (d, 4H, *J* = 13.5 Hz), 1.92-1.83 (m, 8H), 1.02 (t, 6H, *J* = 7.5 Hz), 0.95 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 191.64, 161.68, 156.87, 137.64, 137.52, 135.30, 131.54, 130.03, 86.31, 77.73, 77.30, 31.08, 30.77, 23.44, 23.18, 10.47, 10.20 ppm. FTIR [ATR] 2962, 2932, 2875, 1694, 1597, 1566, 1456, 1434, 1383, 1307, 1280, 1248, 1200, 1161, 1124, 1064, 1035, 1001, 960, 885, 863, 839, 738, 677 cm^{-1}. HRMS Calcd for $C_{42}H_{46}O_6I_2Na_1$ $[M + Na]^+$ 923.1276, found 923.1263.

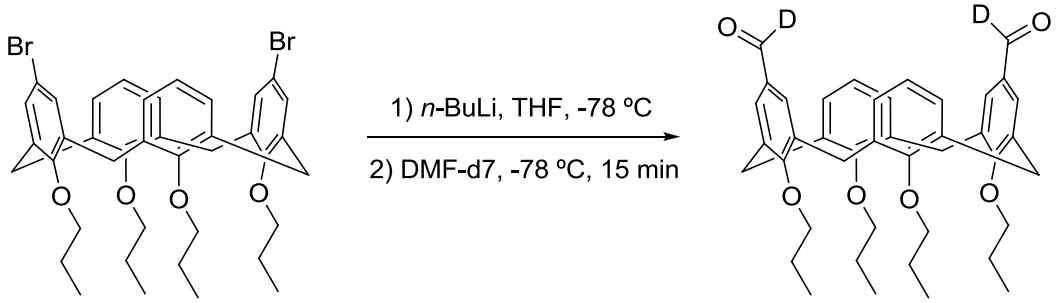
xxviii) 11,23-diiodo-5-carboxy-17-(hydroxymethyl)-25,26,27,28-tetrapropoxy calix[4]arene (182).



181 **182**

Same protocol as described for the synthesis of calixarene **174** afforded 0.047 g of desired product as a white solid (94% yield). Rf = 0.32 (dichloromethane / diethyl ether 18:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.33-7.31 (m, 4H), 7.03 (s, 2H), 6.26 (s, 2H), 4.37 (d, 2H, *J* = 12.5 Hz), 4.34 (d, 2H, *J* = 12.5 Hz), 4.18 (s, 2H), 3.99-3.87 (m, 4H), 3.74 (t, 2H, *J* = 7 Hz), 3.67 (t, 2H, *J* = 7 Hz), 3.12 (d, 2H, *J* = 15 Hz), 3.08 (d, 2H, *J* = 15 Hz), 1.91-1.82 (m, 8H), 1.05 (t, 3H, *J* = 7.5 Hz), 1.04 (t, 3H, *J* = 7.5 Hz), 0.89 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.26, 160.63, 157.49, 155.26, 138.98, 138.26, 137.84, 137.40, 134.76, 134.01, 133.28, 130.20, 126.35, 123.29, 85.94, 64.24, 30.87, 30.76, 30.46, 29.57, 23.49, 23.04, 10.75, 10.67, 10.01 ppm. FTIR [ATR] 2962, 2933, 2876, 1683, 1601, 1456, 1434, 1385, 1309, 1284, 1248, 1199, 1162, 1126, 1108, 1066, 1036, 1004, 964, 862, 839, 738 cm^{-1}. HRMS Calcd for $C_{42}H_{52}O_7N_1I_2$ $[M + NH_4]^+$ 936.1828, found 936.1820.

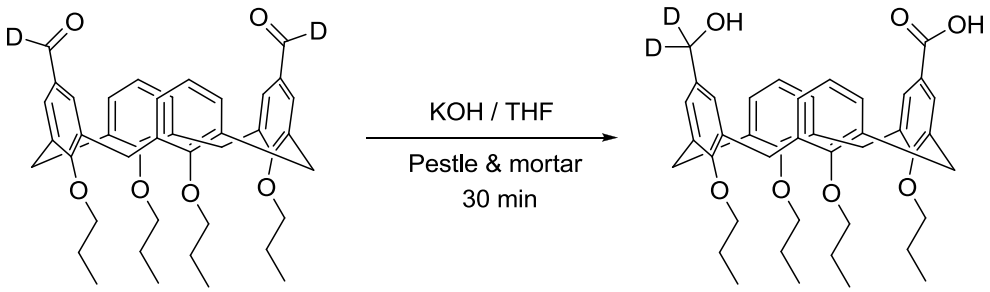
xxix) D_2-5,17-diformyl-25,26,27,28-tetrapropoxy-calix[4]arene (184).



173 **184**

Same protocol as described for the synthesis of calixarene **171**. *N*,*N*-dimethylformamide-d7 was used to quench the reaction instead. After purification by column chromatography on silica gel (neat dichloromethane) 0.041 g of desired calixarene were obtained as white solid (47% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.00 (s, 4H), 6.80-6.69 (m, 6H), 4.47 (d, 4H, *J* = 13.5 Hz), 3.91-3.86 (m, 8H), 3.23 (d, 4H, *J* = 13.5 Hz), 1.95-1.88 (m, 8H), 1.03 (t, 6H, *J* = 7 Hz), 0.97 (t, 6H, *J* = 7 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 191.52 (t, $J_{C\text{-}D}$ = 24.8 Hz), 162.09, 156.72, 136.07, 134.94, 131.08, 129.97, 128.92, 122.79, 122.64, 31.07, 23.51, 23.29, 10.51, 10.30. ppm. FTIR [ATR] 2962, 2933, 2875, 1683, 1668, 1597, 1456, 1385, 1283, 1249, 1218, 1163, 1133, 1036, 1004, 965, 925, 76 cm^{-1}. HRMS Calcd for $C_{42}H_{46}D_2O_6Na_1$ $[M + Na]^+$ 673.3469, found 673.3456.

xxx) D_2-5-carboxy-17-(hydroxymethyl)-25,26,27,28-tetrapropoxy-calix[4]arene (185).

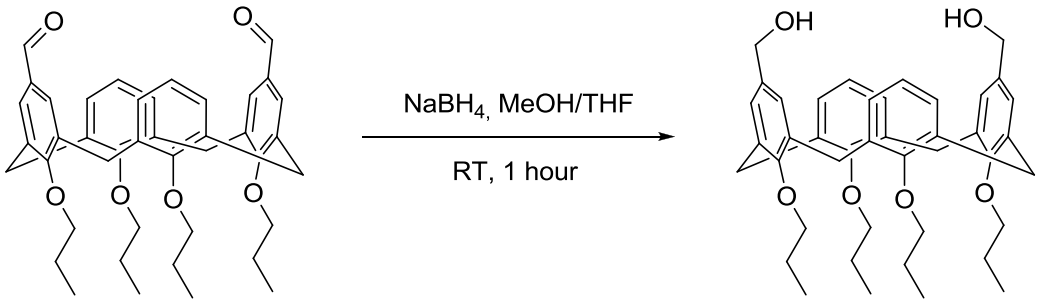


184 **185**

The protocol as described for the synthesis of calixarene **174** was employed. Purification of the crude product by column chromatography on silica gel (dichloromethane followed by a mixture dichloromethane / diethyl ether 4:1, V/V) afforded 0.018 g of desired product as a white solid (65% yield). Rf = 0.21 (dichloromethane / diethyl ether 18:1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.99-6.95 (m, 6H), 6.86-6.83 (m, 2H), 6.22 (s, 2H), 4.44 (d, 2H, *J* = 15.0 Hz), 4.42 (d, 2H, *J* = 15.0 Hz), 4.01-3.89 (m, 4H), 3.77 (t, 2H, *J* = 7.5 Hz), 3.69 (t, 2H, *J* = 7.5 Hz), 3.26 (d, 2H, *J* = 12.5 Hz), 3.22 (d, 2H, *J* = 12.5 Hz), 1.97-1.83 (m, 8H), 1.07 (t, 3H, *J* = 7.5 Hz), 1.05 (t, 3H, *J* = 7.5 Hz), 0.91 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.33, 160.80, 157.40, 155.36, 136.48, 135.79, 134.70, 134.07, 133.99, 130.11, 129.30, 128.88, 126.06, 122.71, 122.51, 77.11, 77.00, 77.00, 76.71, 76.71, 31.13, 31.05, 23.56, 23.19, 10.81, 10.74, 10.10 ppm. FTIR [ATR] 2962, 2934, 2876, 1683, 1600,

1456, 1428, 1385, 1306, 1284, 1247, 1197, 1140, 1106, 1082, 1067, 1037, 1006, 966, 920, 891, 759 cm^{-1}. HRMS Calcd for $C_{42}H_{52}D_2NO_7$ $[M + NH_4]^+$ 686.4018, found 686.4020.

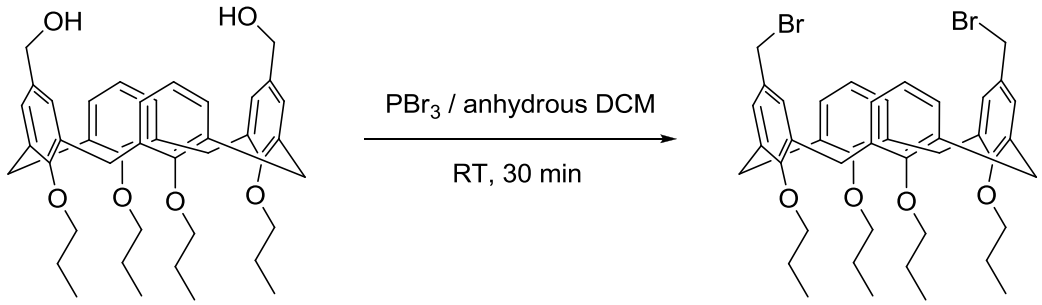
xxxi) 5,17-Bis(hydroxymethyl)-25,26,27,28-tetrapropoxycalix[4]arene (186).[120]



171 **186**

Calixarene **171** (0.111 g, 0.171 mmol) was dissolved in tetrahydrofuran (1 mL) and methanol (3 mL) was added. Sodium borohydride (0.026 g, 0.684 mmol) was added and the reaction mixture was stirred for 1 hour at room temperature. After this time, the solvent was removed under reduced pressure and the residue dissolved in dichloromethane and washed with 1M sodium hydroxide aqueous solution (3 x 10 mL). After drying over magnesium sulphate, filtration, and removing the solvent under reduced pressure 0.102 g of desired product were obtained as a white solid (91% yield). Rf = 0.32 (dichloromethane / diethyl ether, V/V = 18:1). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.92 (d, 4H, *J* = 7.5 Hz), 6.80-6.77 (m, 2H), 6.38 (s, 4H), 4.47 (d, 4H, *J* = 13.5 Hz), 4.17 (s, 4H), 4.00-3.96 (m, 4H), 3.74 (t, 4H, *J* = 7 Hz), 3.15 (d, 4H, *J* = 13.5 Hz), 2.02-1.87 (m, 8H), 1.07 (t, 6H, *J* = 7.5 Hz), 0.94 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 157.28, 155.69, 136.09, 134.49, 134.33, 128.85, 126.54, 122.29, 77.20, 76.72, 65.00, 31.16, 23.54, 23.17, 10.74, 10.15 ppm. FTIR [ATR] 3324, 3307, 2961, 2932, 2875, 1456, 1385, 1307, 1282, 1263, 1249, 1218, 1197, 1166, 1128, 1107, 1082, 1067, 1038, 1008, 968, 907, 889, 867, 836, 802, 760, 737, 703, 627 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{42}H_{52}Na_1O_6$ 675.37 $[M + Na]^+$, found 675.65.

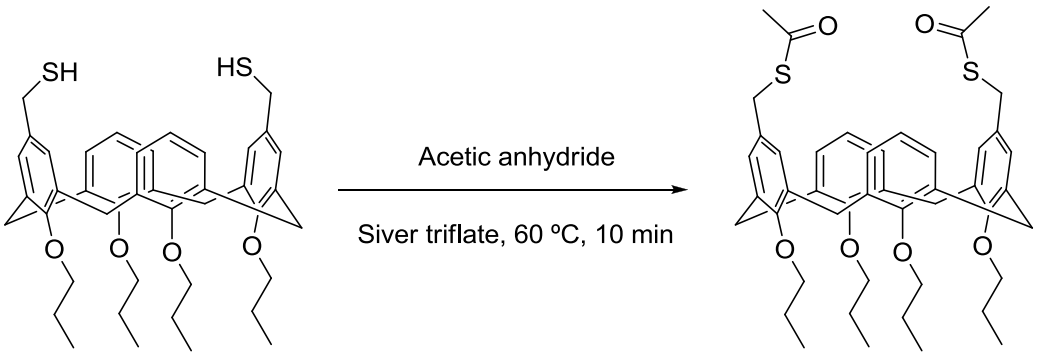
xxxii) 5,17-Bis(bromomethyl)-25,26,27,28-tetrapropoxycalix[4]arene (188).[121]



186 **188**

Calixarene **186** (0.102 g, 0.156 mmol) was dissolved in anhydrous dichloromethane (5 mL) under a nitrogen atmosphere. Phosphorous tribromide (15 µl, 0.043 g, 0.156 mmol) was added using a syringe and the solution stirred for 30 minutes at room temperature. After quenching with distilled water (5 ml) the organic phase was separated and washed with water (3 x 10 mL), dried over magnesium sulphate, filtered, and the solvent removed under reduced pressure to afford 0.099 g of desired product as a white solid (81% yield). Rf = 0.37 (petroleum ether / dichloromethane 1.1, V/V). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.67-6.61 (m, 6H), 6.60 (s, 4H), 4.42 (d, 4H, *J* = 13.5 Hz), 4.18 (s, 4H), 3.87-3.81 (m, 8H), 3.13 (d, 4H, *J* = 13.5 Hz), 1.96-1.87 (m, 8H), 1.00 (t, 6H, *J* = 7.5 Hz), 0.98 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 157.12, 156.66, 135.58, 135.03, 130.91, 128.95, 128.44, 122.36, 77.01, 76.84, 34.88, 31.04, 23.41, 23.30, 10.46, 10.38 ppm. FTIR [ATR] 2962, 2932, 2875, 1588, 1464, 1385, 1308, 1285, 1249, 1208, 1168, 1133, 1082, 1067, 1037, 1006, 966, 890, 760, 739 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{42}H_{50}{}^{79}Br_2Na_1O_4$ 799.20 [M + Na^+], found 799.51.

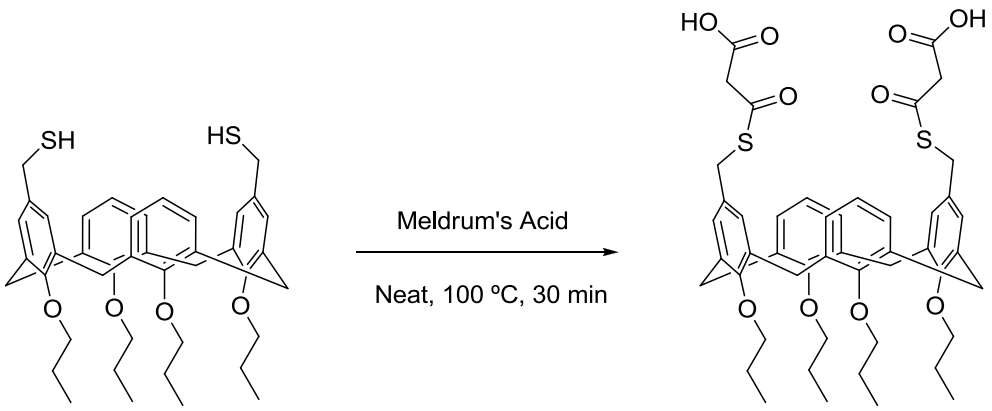
xxxiii) 5,17-Bis(acetylthiomethyl)-25,26,27,28-tetrapropoxycalix[4]arene (189):



128 **189**

To a solution of calixarene **128** (0.012g, 0.018 mmol) in toluene (0.1 mL), acetic anhydride (0.036 g, 0.350 mmol) and silver triflate (0.2 mg, 0.876 µmol) were added and the mixture stirred at 60 ºC for 10 minutes. The solvent and volatiles were removed under reduced pressure and the crude product purified by column chromatography on silica gel (dichloromethane) to afford 0.008 g of desired product as a white solid (59% yield). Rf = 0.68 (dichloromethane). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.62 (s, 4H), 6.53-6.48 (m, 6H), 4.39 (d, 4H, *J* = 13.0 Hz), 3.90 (s, 4H), 3.85-3.77 (m, 8H), 3.09 (d, 4H, *J* = 13.0 Hz), 2.33 (s, 6H), 1.93-1.86 (m, 8H), 0.99 (t, 6H, *J* = 7.5 Hz), 0.95 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 195.58, 156.27, 156.17, 135.66, 134.47, 130.25, 129.99, 128.64, 128.00, 122.14, 33.44, 30.91, 30.38, 23.26, 23.19, 10.42, 10.21 ppm. FTIR [ATR] 2961, 2930, 2874, 1692, 1456, 1385, 1284, 1248, 1216, 1196, 1132, 1106, 1083, 1067, 1039, 1007, 965, 759, 627 cm^{-1}. HRMS Calcd for $C_{46}H_{60}O_6N_1S_2$ $[M + NH_4]^+$ 786.3857, found 786.3850.

xxxiv) 5,17-Bis(carboxyacetylthiomethyl)-25,26,27,28-tetrapropoxycalix[4]arene (190):



128 **190**

A mixture of calixarene **128** (0.131 g, 0.191 mmol) and Meldrum's acid (0.276 g, 1.912 mmol) was stirred at 100 ºC for 30 minutes. After cooling, dichloromethane (10 mL) was added and the formed solid removed by filtration. The filtrates were concentrated under reduced pressure to afford a crude product which was purified by column chromatography on silica gel (dichloromethane followed by a mixture dichloromethane / diethyl ether 4:1, V/V) to afford 0.062 g of desired product as a white solid (38% yield). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.72 (s, 4H), 6.54-6.52 (m, 4H), 6.46-6.44 (m, 2H), 4.39 (d, 4H, *J* = 13.0 Hz), 3.93 (s, 4H), 3.86-3.76 (m, 8H), 3.60 (s, 4H), 3.10 (d, 4H, *J* = 13.0 Hz), 1.98-1.87 (m, 8H), 1.00 (t, 6H, *J* = 7.5 Hz), 0.97 (t, 6H, *J* = 7.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 191.45, 170.57, 156.31, 156.21, 135.46, 134.67, 129.13, 129.01, 128.39, 122.42, 77.02, 48.45, 34.27, 30.90, 23.40, 23.35, 10.45 ppm. FTIR [ATR] 2964, 2935, 2877, 1728, 1683, 1464, 1435, 1385, 1307, 1285, 1248, 1216, 1197, 1168, 1037, 1008, 966, 910, 759, 734 cm^{-1}. MS (MALDI-TOF) Calculated for $C_{48}H_{56}NaO_{10}S_2$ 879.32 $[M + Na]^+$, found 879.64.

- Chapter 5-

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