SYNTHESIS OF PHENYLCALIXARENES AND ANALOGUES FOR USE AS TUBERCULOSIS THERAPIES

By

Kerry Joanne Goodworth

A thesis submitted in partial fulfilment for the degree of Doctor of Philosophy

University College London
2003
Acknowledgements

I wish to express my sincere thanks to Dr Helen Hailes, firstly for giving me the opportunity to work on this project, but mostly for her support, ideas and unending enthusiasm for this work. I would also like to thank Dr Gordon Weingarten (GSK) for support, advice and ideas throughout the project, and to GlaxoSmithKline for funding for this Ph.D. through the Action TB programme. I would also like to take this opportunity to remember the late Dr Jo Colston (MRC), who carried out most of the biological testing in this Ph.D. himself. An inspiring man who will be sadly missed by all who had the pleasure of working with him.

Thanks also to the people within the Dept. of Chemistry (UCL) whose technical expertise is greatly appreciated, John Hill, and Dave Butler especially.

Huge thanks to all in the Helen Hailes Research Group past and present, my second family! The lads, Kason, Chris, Prakash (the Squirrel), Dirk (who taught me a lot in my first year and had endless patience!), and Jon. Special thanks to Cec qui, who made the lab great fun and was always there to hold my hand if I needed it, you’re a great friend. Also to Valerie, Mike, and Isabelle, thanks for the chats and the gossip!

Finally, I would like to dedicate this thesis to my wonderful husband Toby, and my fabulous parents. I would be nothing without you, so thank you for your endless patience, love and encouragement through the dark times!
Abstract

Initially the literature in the area is reviewed. The first chapter details current TB therapies and trends in TB, whilst the second chapter reviews the synthesis of macrocyclic TB therapies, and the synthesis and reactions of calixarenes.

In Chapter 3 the synthesis of phenylcalixarenes is discussed, and a templating effect upon product distribution established. Synthesis of the tert-butyl octamer and hexamer, and the nude calix[8]arene and calix[6]arene is also discussed. Sulfonation and acylation of the upper rim of the nude calixarenes is also discussed.

In Chapter 4 the synthesis of PEG-chains via protection and activation strategies is discussed. In Chapter 5 PEGylation of phenylcalix[8]arene and phenylcalix[7]arene is described, and a difference in reactivity of the octamer and heptamer towards PEG-chains is discussed. Derivatisation of the octamer and heptamer with alkyl nitrile chains is also described, as is derivatisation of the octamer with active electrophiles.

In Chapter 6 the synthesis of a series of small PEGylated analogues, and fluorescently labelled compounds is discussed. In Chapter 7 the results of biological testing of compounds synthesised are presented, and Chapter 8 concludes the thesis and presents ideas for future work. The final chapter describes the experimental methods employed.
### Abbreviations

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Disease Syndrome</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette Guerin</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>Isotope of carbon with nuclear spin</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOTS</td>
<td>Directly Observed Treatment, Short-Course</td>
</tr>
<tr>
<td>ETH</td>
<td>Ethambutol</td>
</tr>
<tr>
<td>$^1$H</td>
<td>An isotope of hydrogen with nuclear spin</td>
</tr>
<tr>
<td>HBC</td>
<td>Higher melting tert-butyl compound</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HOC</td>
<td>Higher melting tert-octyl compound</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>INH</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>IR</td>
<td>Infra Red</td>
</tr>
<tr>
<td>$J$</td>
<td>Coupling in Hertz</td>
</tr>
<tr>
<td>LBC</td>
<td>Lower melting tert-butyl compound</td>
</tr>
<tr>
<td>LD$_{50}$</td>
<td>Half Lethal Dose</td>
</tr>
<tr>
<td>LOC</td>
<td>Lower melting tert-octyl compound</td>
</tr>
<tr>
<td>m</td>
<td>medium intensity signal (IR Spectroscopy)</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>$M.$</td>
<td>Mycobacterium</td>
</tr>
<tr>
<td>MDR</td>
<td>multi-drug resistant</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
</tbody>
</table>
MRC Medical Research Council

MS mass spectroscopy

m/z mass to charge ratio

$^{31}\text{P}$ Isotope of phosphorous with nuclear spin

PAS $p$-aminosalicylic acid

PCR polymerase chain reaction

PEG polyethylene glycol

PEGylated A compound with attached PEG-chain (precedented in gene therapy)

PZA Pyrazinamide

q Quartet

RCT randomised controlled trial

RD relaxation delay

RFLP restriction fragment length polymorphism

s singlet

s strong intensity signal (IR Spectroscopy)

t triplet

TB Tuberculosis

THF tetrahydrofuran

THP tetrahydropyran

UV Ultra Violet

w weak intensity signal (IR Spectroscopy)

WHO World Health Organisation

$\sigma$ standard deviation

$<x>$ mean of $x$

$\nu_{\text{max}}$ maximum intensity peaks (IR Spectroscopy)

$\lambda_{\text{max}}$ maximum intensity peaks (UV Spectroscopy)
# Contents

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7.1</td>
<td>Isoniazid</td>
<td>18</td>
</tr>
<tr>
<td>1.7.2</td>
<td>Ethambutol</td>
<td>20</td>
</tr>
<tr>
<td>1.7.3</td>
<td>Rifampicin</td>
<td>21</td>
</tr>
<tr>
<td>1.7.4</td>
<td>Pyrazinamide</td>
<td>21</td>
</tr>
<tr>
<td>1.7.5</td>
<td>Other TB Drugs</td>
<td>22</td>
</tr>
<tr>
<td>1.8</td>
<td>Multi-Drug-Resistant TB</td>
<td>22</td>
</tr>
<tr>
<td>1.9</td>
<td>The Mycobacterial Cell Wall</td>
<td>24</td>
</tr>
<tr>
<td>1.10</td>
<td>Summary</td>
<td>25</td>
</tr>
<tr>
<td>2.0</td>
<td>Calixarenes and Tuberculosis</td>
<td>26</td>
</tr>
<tr>
<td>2.1</td>
<td>Synthesis of Calixarenes</td>
<td>38</td>
</tr>
<tr>
<td>2.1.1</td>
<td>tert-Butyl Calixarenes</td>
<td>38</td>
</tr>
<tr>
<td>2.1.2</td>
<td>Mechanism of Calixarene Formation</td>
<td>43</td>
</tr>
<tr>
<td>2.1.3</td>
<td>Other Calixarenes</td>
<td>45</td>
</tr>
<tr>
<td>2.1.4</td>
<td>Stepwise Synthesis of Calixarenes</td>
<td>48</td>
</tr>
<tr>
<td>2.2</td>
<td>Derivatisation of Calixarenes</td>
<td>49</td>
</tr>
<tr>
<td>2.3</td>
<td>Summary</td>
<td>57</td>
</tr>
<tr>
<td>3.0</td>
<td>Phenylcalixarenes</td>
<td>58</td>
</tr>
<tr>
<td>3.1</td>
<td>Synthesis of the (p)-Phenylcalixarene Series</td>
<td>65</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Purification of Phenylcalixarenes</td>
<td>71</td>
</tr>
</tbody>
</table>
Chapter 7  Biological Results  141
  7.0  Introduction  141
  7.1  Summary  152

Chapter 8  Conclusions and Future Work  153
  8.0  Conclusions  153
  8.1  Future Work  154

Chapter 9  Experimental  155
  9.0  Materials and Methods  155
  9.1  Synthesis of Calixarenes  156
  9.2  Synthesis of Small Analogues  190
  9.3  Synthesis of PEG-Chains  211
  9.5  Synthesis of Fluorescently Labelled Compounds  230

Bibliography  235

Appendices  243
1.0 Tuberculosis

Tuberculosis (TB) is a disease which is known to have existed since ancient times. In ancient Hindu texts TB is referred to as Rogaraj, the king of disease, and Rajayakshma, the disease of kings.\(^1\) The first of these names refers to the fact that TB was, and in the twenty first century still is, a prodigious killer. The second name refers to the fact that TB is an infectious disease which indiscriminately kills both peasants and nobility alike. Evidence of tuberculosis disease has been identified in stone-age skeletons and Egyptian mummies.\(^2\) In 1882 Robert Koch first identified and cultured the tuberculosis bacilli (*Mycobacterium Tuberculosis*), the infectious particle which spreads the disease in air.\(^3\) Inhalation of these particles is the main cause of TB infection.

In 1962 the British Office of Health Economics noted that the use of the new anti-tuberculosis drugs opened up the possibility for the final defeat of the disease in Britain within fifteen years.\(^4\) Forty one years later there are approximately seven thousand TB notifications in the UK annually.\(^5\) Since the early 1990s incidence of TB has been steadily increasing.

The global problem is acute and the World Health Organisation (WHO) has declared a TB epidemic. The WHO estimates that the current incidence of TB is eight million and mortality rates are high with an estimated 3 million people dying from the disease annually. Although it is estimated that one third of the world’s population are infected with the tuberculosis bacillus, the majority of infected people will never develop symptomatic disease.\(^5\) Only 5-10% of infected people will develop clinical TB.\(^6\) Of this group 1-5% will die from the disease.\(^7,8\)
1.1 Tuberculosis Infection

Tuberculosis infection is most commonly caused by inhalation of TB bacilli. *Mycobacterium tuberculosis* was the first mycobacteria to be identified, and the name mycobacterium, literally meaning fungus-bacterium, refers to the fungal like pellicle which is produced when the bacteria are cultured on liquid media. In 1889 Theobold Smith divided Koch’s tubercle bacilli into human and bovine types (*M. tuberculosis, M. bovis*) due to small but constant cultural differences between the two types. A third type, vole bacillus (*M. microti*), was added later by Wells. The three types are often regarded as separate species though they are actually variants of a single species.

Tuberculosis infection is diagnosed when a patient has TB bacilli present within the body, but has no symptomatic evidence of disease. TB disease is classed as a patient who has at least one organ of the body shown by diagnostic procedures to be diseased. TB disease can take many forms, which usually conform to a sequential timetable following infection.

The initial phase of the infection, known as primary TB is usually pulmonary as the bacilli are inhaled, and accounts for 70-90% of all TB patients. Most primary infections heal rapidly and leave no trace, however if the immune system cannot rapidly contain the disease a lesion, known as a tubercle, may occur on the lung. For children under ten years of age and adults over seventy five primary infection may be followed by Miliary TB. It is also found in patients who are immunosuppressed such as those with HIV. It occurs as a result of dissemination of bacterial bacilli through the body via the bloodstream.

The most common form of TB disease is known as post-primary TB, and occurs either from reactivation of a primary lesion, progression of a primary lesion, or from re-infection. Reactivation of a primary lesion can occur many decades after initial infection, and is usually caused by suppression of the immune system either due to ageing or the presence of secondary diseases.
1.2 Factors Affecting Infectivity and Transmission of TB

One reason for the resurgence of TB in recent years is that HIV infection increases the risk of developing TB disease from initial infection by a factor of ten.\textsuperscript{11,12} Diabetes mellitus also increases the risk of developing the disease, and the use of steroids such as prednisolone and other immunosuppressants has been shown to cause reactivation of TB.\textsuperscript{13,14}

The relationship between TB and poor housing conditions is well known, as overcrowding leads to greater spread of the infection.\textsuperscript{15-18} It has also been shown that malnutrition predisposes an individual to TB.\textsuperscript{8} This partly explains why TB is more prevalent in developing countries. Spence \textit{et al} demonstrated that a greater prevalence of TB infection exists among lower social classes in the UK for similar reasons.\textsuperscript{19} In Africa and the Middle East one in three TB patients will die from the disease, whereas in the UK and the USA that figure is only one in every fifteen patients.

1.3 Diagnosis of Tuberculosis

The first problem facing clinicians in dealing with tuberculosis is the ability to make a correct diagnosis of the disease in a patient. Many of the diagnostic tests which are available cannot unequivocally confirm the presence of disease. Tuberculin skin tests, which measure a patient's hypersensitivity upon direct exposure to tubercle bacilli are only an indicator of infection or exposure to the bacilli, not necessarily the presence of the disease. The tuberculin skin tests are performed world-wide prior to administration of the TB vaccine BCG (Bacille Calmette-Guerin).

In developed countries X-rays are widely used in TB diagnosis. They enable visualisation of TB lesions, and successive X-rays allow determination of whether the lesion is actively spreading. The cornerstone of TB diagnosis however remains the examination and culture of patient sputum. Sputum cultures are also used to test for sensitivity to \textit{anti}-TB medicines.
Modern techniques for TB diagnosis use PCR (polymerase chain reaction) and RFLP (restriction fragment length polymorphism). These methods are particularly useful for investigation of the spread of particular strains of TB, as each strain can be identified by its unique DNA fingerprint. In 2003 a new rapid test for TB was announced in the UK which identifies T-lymphocytes present in the blood. The rapidity of the test could help prevent the spread of disease.

1.4 The BCG Vaccine

Various strains of the BCG vaccine are in use today, but all originate from the strain of *Mycobacterium bovis* isolated by Calmette and Guerin early in the last century. The efficacy of TB vaccination is widely disputed, but at best it produces the same level of immunity in a patient which is normally acquired by primary infection, with a lower risk of progression to the disease. BCG does not confer lifelong protection against TB as the immune response decreases over time, but secondary vaccination has no benefit.

Between 1927 and 1968 twenty one controlled clinical trials of BCG vaccine efficacy were carried out in ten countries. The protective efficacy shown (against an unvaccinated control group) ranged from -56% to +80% in the eight randomised controlled trials. Findings of little or no protection from the BCG vaccine in two trials in southern states of the USA are a major contributory factor to the USA not introducing a community wide vaccination programme. However the BCG remains the most widely used vaccine in the world today, and in 1993 immunisation using BCG was carried out in 172 countries.

In the UK the Medical Research Council (MRC) began the largest randomised controlled trial of the BCG to date in 1950. The protective efficacy of the vaccine declined over the twenty years of the trial, but equated to +77% over the whole trial period. The annual cost of administering the BCG vaccine to schoolchildren in the UK is estimated at £3 million, and in recent years as supplies of the vaccine have fallen short of the requirements, there has been public debate about the validity of its continued use. Efforts to produce a new vaccine are being undertaken worldwide, but it is generally recognised that it will be twenty five years or more before there is a
possibility of having a new vaccine for worldwide use, and in the meantime fifty million lives will be lost to TB. There is therefore a need to identify new chemotherapeutic approaches, including the development of drugs with novel modes of action.

1.5 The Human Immune System

The human immune system is extremely complex, however an area of major importance to tuberculosis is the role of macrophages and T cells within the immune system.

Tubercle bacilli possess hundreds of antigens that upon entering the body cause activation of the immune system. For the immune system to be able to invoke a bactericidal effect, the antigen must be processed by accessory cells such as macrophages and B-lymphocytes which are present in the blood and lymph fluid. The processing of antigen is necessary because helper T-lymphocytes, which co-ordinate the immune response, can only recognise antigen that is presented bound to Major Histocompatibility Complex (MHC) glycoproteins on the surface of macrophages. The first processes of the immune response involve capture and processing of the antigen by macrophages and subsequent presentation of the processed antigen to helper T-lymphocytes.

Upon entering the body antigens such as bacterial bacilli enter the bloodstream and become coated with an immunoglobulin IgG, which is made by B-lymphocytes. IgG has two sites for binding antigen, and a region which binds to specific receptors on macrophages, known as the Fc (constant) region. A diagram of a bacillus coated with IgG is shown below (fig 1.5.1).
In order for a macrophage to be able to process the antigen to present it to helper T-lymphocytes, the macrophage must first phagocytose the antigen. Phagocytosis is activated via the macrophage surface receptors binding to the Fc region of IgG, which is coating the antigen as shown above (Fig 1.5.1). The binding of macrophage surface receptors and initiation of phagocytosis is shown below (Fig 1.5.2).

Once inside the macrophage the bacilli are stored in a compartment known as a phagosome. The pH inside the phagosome is low and there are also enzymes present which partially destroy the digested bacilli. As peptide fragments are broken off from the bacilli they associate with Class II Major Histocompatibility Complex (MHC-II) molecules inside the macrophage to form an immunogenic complex. The MHC-II molecules are transported to the surface of the macrophage and are responsible for presenting the antigen (bacilli) to helper T-Lymphocytes. The macrophage also produces a soluble protein mediator, Interleukin-1 (IL-1) which also binds to helper T-Lymphocytes.

Binding of both IL-1 and the MHC-II-antigen complex causes activation of the helper T-Lymphocyte. The activated helper T-Lymphocytes themselves produce an interleukin, IL-2, which has two main functions:
- IL-2 binds to macrophages causing upregulation of phagocytosis;
- IL-2 binds to cytotoxic T-Lymphocytes causing their activation.

Cytotoxic T-Lymphocytes (T\(_c\) cells) destroy cells which are presenting antigen bound to MHC-I glycoproteins. All nucleated cells of the body have MHC-I glycoproteins, and in this way the immune system can act on cells of the body which do not have a specialised immune function.

T\(_c\) cells do not however destroy infected macrophages. Instead macrophages are capable of digesting phagocytosed material themselves. The phagosome containing newly ingested material is transported inside the cell towards the Golgi apparatus. This is the site of formation of lysosomes, vacuoles full of digestive enzymes. Lysosomes contain unique membrane proteins and a wide-range of hydrolytic enzymes which work best at pH 5, the internal pH of lysosomes. This pH is maintained by an ATP-driven proton pump in the membrane of the lysosome. Usually the phagosome fuses with a lysosome, allowing complete digestion of the material within the phagosome, and formation of a residual body. This process is summarised below (Fig 1.5.3).

![Fig 1.5.3. Diagram showing the processes involved in digestion of phagocytosed material by a macrophage](image-url)
1.6 The Latent Phase

Bacilli of *M. tuberculosis* are able to survive inside macrophages, and will continue to divide and multiply, eventually rupturing the macrophage and allowing the bacilli to escape and spread throughout the body. However if the immune system is strong enough the bacilli are prevented from growing and dividing, and enter a dormant or latent state inside the macrophage, from which they may be re-activated if the immune system is subsequently weakened.

The TB strain Bacillus Calmette-Guerin is also known to survive in a dormant state within macrophages. Patients with no known exposure to active-TB, who in later life become immunocompromised through HIV infection, have been shown to develop the active disease with bacilli of the strain BCG.\(^\text{32}\)

It has been hypothesised by Hasan *et al* that a reduction in the number of endosomal/lysosomal markers such as the lysosome-associated glycoproteins (LAMP-1 and 2) and $\beta$-hexoaminindase prevents fusion of lysosomes with the phagosomes.\(^\text{33}\) However, in order for bacilli to continue to survive within the phagosome, which have an acidic, anaerobic internal environment, *M. tuberculosis* must be able to adapt, and it does so by entering what is commonly described as a latent phase. Mycobacteria which are contained within phagosomes but are not actively growing and dividing are described as latent or dormant. By processes which are not fully understood they may be re-activated at any time during a person’s lifetime, most often if the person becomes immunocompromised.

In order for bacilli to survive in a dormant state inside macrophages the normally aerobic bacteria must adapt to anaerobic conditions. Cunningham and Spreadbury cultured *M. tuberculosis* under aerobic, microaerobic and anaerobic conditions.\(^\text{34}\) They found that if bacilli were transferred suddenly from aerobic to anaerobic conditions nearly all the bacteria died. However if the oxygen tension was slowly reduced the bacilli would no longer grow and divide, but if re-aerated after six months they would become active again.
Using electron microscopy Cunningham and Spreadbury demonstrated that a remarkable thickening of the outer layer of the cell wall occurred when bacilli adapted to anaerobic conditions. Through the use of transverse sections of the cell they also demonstrated that the diameter of the cell is reduced concomitantly with the cell wall thickening. It is believed that this thickened cell wall helps the bacilli to survive in oxygen deficient conditions, and that it may also protect it against low pH. It is believed that inside macrophages oxygen-tension depletes at a slow enough rate to allow this adaptation to occur. Cunningham and Spreadbury demonstrated that a 16 kDa small heat shock protein (smHSP) was upregulated in response to low-oxygen tension. Yuan et al concurred that the 16 kDa protein is upregulated in \textit{M. tuberculosis} during the latent phase or under reduced oxygen tension.\textsuperscript{35} It is believed that the role of the small heat shock protein is to stabilise the intracellular structure during dormancy. It is also known that the family of smHSPs can act in an ATP-independent manner, which is crucial during dormancy as ATP will not be available. It is not known what causes re-activation at the cellular level, but it is believed that the immune system maintains the dormant state, as compromising or weakening of it allows re-activation to occur.

\subsection*{1.7 Current Tuberculosis Therapies}

The British and American Thoracic Societies currently recommend the following treatment for TB.\textsuperscript{36-38} For two months;

1. Ethambutol 15 mg/Kg daily
2. Pyrazinamide 2.0 g daily
3. Isoniazid 300 mg daily
4. Rifampicin 600 mg daily

Isoniazid and rifampicin are then continued for a further four months. In 1970 the MRC published the results of an East-African study which showed that the efficacy of some treatments was significantly lower than had been demonstrated in clinical trials, largely because few patients completed the entire course of treatment.\textsuperscript{39} Studies such as this led to the WHO recommendation that treatment is carried out under a system known as DOTS (Directly-Observed-Treatment-Short-course). The main aim of DOTS is to
improve patient compliance to help prevent occurrence of resistance. Under the DOTS system health care workers are responsible for ensuring that every patient takes the correct medication for the entire treatment period. Cure rates of patients in countries which have implemented DOTS are consistently around 90%, compared with rates of around 40% for non-DOTS patients.

To further aid patient compliance combined formulations of the recommended TB drugs have been developed. Current preparations include: isoniazid-rifampicin; isoniazid-ethambutol; isoniazid-rifampicin-pyrazinamide; isoniazid-rifampicin-pyrazinamide-ethambutol. However it has been discovered that for some formulations the bioavailability of rifampicin is significantly lower than for the single drug, and therefore these formulations must meet stringent control standards.

The first synthetic TB medicine was streptomycin. In 1947 the first randomised controlled trials (RCTs) of streptomycin began, and one year later its remarkable ability to reduce death as a result of TB was reported. However the use of streptomycin alone rapidly led to resistance to the drug among patients, whose ultimate fate was little better than those without treatment. A second drug, p-aminosalicylic acid (PAS) was found to prevent resistance if given concurrently with streptomycin, and the chemotherapeutic era of TB treatment began.

The next major advance in treatment was the discovery of isoniazid (INH) in 1952, which was shown to be very effective in several RCTs in the USA. During the years that followed its discovery several studies were undertaken in which combinations of INH, PAS and streptomycin were evaluated. In 1964 it was reported that a regime using all three drugs initially, followed by INH and PAS alone was highly effective. This regime became the standard treatment in the developed world, but was too expensive to be implemented in the developing world where TB was rife.

In 1958 the MRC began to investigate cheaper alternatives to the standard treatment regime which could be used in the developing world, especially Africa. It established that PAS could be replaced with thiacetazone which, due to its low cost and high
efficacy, became part of the treatment regime in Africa. The discovery of ethambutol led to the replacement of PAS in the standard treatment protocol in 1962. During the AIDS epidemic ethambutol has also replaced thiacetazone in developing countries, as HIV positive patients sometimes experience fatal side effects from thiacetazone.  

The era of six-month therapeutic regimes (known as short-course) began in 1966 when it was demonstrated that the addition of pyrazinamide (PZA) and/or rifampicin to a streptomycin-INH regime substantially reduced relapse rates. The replacement of streptomycin with ethambutol led to the current recommended short-course treatment regime.

1.7.1 Isoniazid

Isoniazid (INH) 1 (fig. 1.7.1.1) is the oldest and most widely prescribed TB drug in use today. It is a narrow spectrum agent, only active against mycobacteria and not other forms of bacteria. Although INH was first used in the 1960s its mechanism of action was only fully elucidated by examination of TB strains which became resistant to the drug. Winder and Collins had demonstrated in 1970 that INH has a profound effect on mycolic acid biosynthesis, thus inhibiting synthesis of the bacterial cell wall.

However it was known that INH was not acting in the form in which it was administered, it was a pro-drug. In the 1990s it was discovered that INH-resistant isolates of *M. tuberculosis* had attenuated catalase-peroxidase activity, and that the catalase-peroxidase enzyme, known as KatG, activated the pro-drug to its oxidised, forms (3-6) shown in fig. 1.7.1.2.
It was known that none of the stable forms shown above in figure 1.7.1.2 are toxic to bacteria, and it was surmised that the active form of the drug must be a transient reactive intermediate, though additional effects could be due to a reactive oxygen species produced during the reaction.\textsuperscript{51,52} The gene which codes for the KatG enzyme, known as the \textit{katG} gene, has been shown in the majority of INH-resistant isolates to have deletions or mis-sense mutations.\textsuperscript{53,54} The mechanism of action of ethionamide 2 (fig. 1.7.1.1), whose structure is similar to INH, is also believed to involve the activation of a pro-drug.\textsuperscript{55}

The synthesis of an \textsuperscript{15}N-labelled version of INH was published in 1998, in order to allow study of the mechanism of resistance to the drug (scheme 1.7.1.1).\textsuperscript{56}
1.7.2 Ethambutol

Ethambutol 14, (fig. 1.7.2.1) is a potent TB drug which is also found to suppress the growth of most INH and streptomycin resistant strains of TB. The mechanism of action of ethambutol relies upon its inhibition of mycobacterial arabinosyl transferases, which are used to transfer D-arabinose into cell wall arabinogalactams, an important feature of the mycobacterial cell wall.

In 2002 a novel efficient synthesis of ethambutol was published by Stauffer and Datta (scheme 1.7.2.1). The four step synthesis from the readily available amino acid L-methionine affords the enantiomerically pure product in good yield.

Scheme 1.7.2.1. Scheme showing synthesis of ethambutol from L-methionine
1.7.3 Rifampicin

The only therapeutically useful rifamycins are semi-synthetic derivatives of rifamycin B which itself has poor activity. These are among the most important of all antimicrobial agents. Rifampicin 18, fig. 1.7.3.1, is included in the drug regimes for treatment not only of TB, but also leprosy and other mycobacterial infections in patients with HIV. The mechanism of action involves binding to the β-subunit of bacterial RNA-polymerase, which prevents initiation of transcription. Patient isolates which are resistant to rifamycin almost invariably have mutations within the β-subunit of the RNA-polymerase gene.

1.7.4 Pyrazinamide

Pyrazinamide (PZA) 19, (fig. 1.7.4.1), forms the cornerstone of the short-course TB treatment regimes. It has found favour due to its high activity against the semi-dormant TB bacilli which often remain following treatment with other TB drugs. If not treated it is these bacilli which can cause reactivation of the disease.

Little is known of the mechanism of action of PZA, though it is only active at acidic pH or inside macrophages. Again it is a pro-drug, pyrazinoic acid being its activated bactericidal form. It is converted to its active form by a pyrazinamidase/nicotinamidase enzyme, and loss of activity of this enzyme is a simple test to detect resistance to PZA.
1.7.5 Other TB Drugs

Several other TB drugs have been reported, and those most commonly used are shown below (fig. 1.7.5.1). Streptomycin, 20, works by binding irreversibly to the bacterial ribosome unit, thereby preventing protein synthesis. p-Aminosalicylic acid (PAS, 21) prevents iron assimilation by bacterial bacilli and thiacetazone, 22, is known to inhibit mycolic acid biosynthesis.

1.8 Multi-Drug-Resistant-TB

Inadequate and incomplete treatment of TB can lead to the development of multi-drug resistant TB (MDR-TB). This is defined as being resistant to INH and rifampicin, with or without resistance to any other anti-TB drugs. MDR-TB is initially treated with at least three medicines to which isolates have been shown to be sensitive, until a negative sputum culture is achieved. Patients then continue taking two of the three drugs for a further nine months and reserve or second line medicines may also be co-administered.

Data from the US in 1998 showed that approximately 13% of all new TB cases are resistant to at least one first-line drug (known as primary resistance), and a further 1.6%
High rates of acquired MDR-TB have been reported around the world, including 48% in Nepal, 34% in India and 30% in New York City at the start of the 1990s. Research published in 2002 showed that 6% of TB cases in the UK during the period 1993 to 1999 were resistant to one or more drugs and 1% were MDR-TB.

The mechanisms of resistance by tuberculosis bacilli to the most common TB drugs have been studied by molecular biologists and are well-known. For example, virtually all isolates resistant to rifampicin and other rifamycins have a mutation altering a twenty seven amino acid region of the β-subunit of RNA-polymerase, impeding the binding of the drug to the enzyme, the site of action of rifampicin. Resistance to isoniazid is more complex. Many isolates have mutations in the katG gene encoding a catalase-peroxidase enzyme that leads to an altered enzyme structure which prevents activation of the pro-drug. However loss of this enzyme does lead to bacteria which have significantly lower virulence. The exact nature of the contribution of the enzyme to virulence is not known. Pyrazinamide resistance in the majority of organisms is caused by mutations in the gene (pncA) encoding pyrazinamidase that leads to diminished enzyme activity, meaning that the pro-drug cannot be activated.

It is unclear whether MDR-TB arises as a consequence of a series of sequential mutations, each conferring resistance to a single drug, or whether a single step process occurs, possibly via acquisition of an MDR element, or whether a single mutation occurs which alters, for example, cell wall structure. The issue of virulence of MDR-TB has been contested since the first publication in 1953 which reported that strains resistant to isoniazid had significantly reduced virulence in guinea pigs. Mouse studies employing a panel of fifteen human isolates of M. tuberculosis, including several resistant to one or more TB drugs, found no simple relationship between the degree of drug resistance and relative virulence. It is now widely accepted that katG is a TB virulence factor, and that mutation significantly reduces the virulence of mutated strains. It remains unknown whether mutations which confer resistance to other anti-TB drugs are also virulence factors.
The treatment of MDR-TB is considerably more expensive than that of susceptible TB. Mahmoudi et al showed in 1993 that in the US treatment of an MDR-TB patient costs $180,000 compared with $2,000 for non-resistant patients. This is partly due to the longer treatment regime for patients with MDR-TB, typically eighteen to twenty-four months.

1.9 The Mycobacterial Cell Wall

The mycobacterial cell wall is extremely complex, and will not be discussed in detail here. The cell walls of mycobacteria are unique among bacteria, and have been adapted over time to allow the mycobacteria to survive inside cells of the body, where they are often found at the interface of water and air due to their aerobic nature. The mycobacterial cell wall is hydrophobic in nature and has a high lipid content. The role of the mycobacterial cell wall is to protect the bacilli from attack by the body’s defence mechanisms whilst interacting with the host as necessary.

The bulk of the cell wall is made up of mycolic acids, which are long chain branched fatty acids and often contain cyclopropane rings. Mycolic acids can contribute to up to 60% of the dry weight of the bacilli. They are responsible for forming a lipid coating around the bacilli and can be used to distinguish different types of mycobacteria. The arabinogalactan portion of the cell wall consists of a galactose backbone with arabinose branches. Adjacent to the cell membrane is the peptidoglycan layer which consists of alternating units of N-acetylg glucosamine and modified muramic acid. A diagram of a mycobacterial cell wall is shown in figure 1.9.1 below.
1.10 Summary

Tuberculosis is a disease which has been prevalent since ancient times, and remains the greatest infectious disease killer in the world today. Although the pathology of the disease is well known, the majority of TB drugs in use today were developed several decades ago, and the search for a new more effective vaccine has thus far proved to be challenging. Treatment for TB, although described as short-course, is six months and the drugs have many side effects such as causing potentially fatal liver damage. Patient compliance rates can therefore be low, and this is one of the causes of the rise of MDR-TB. MDR-TB has so far been relatively contained, largely due to the fact that many of the drug-resistance factors are also virulence factors. However should TB develop the ability to acquire drug resistance whilst also maintaining high virulence, deaths from the disease would rapidly escalate. The development of new TB treatments, which could shorten the course of treatment regimes, without the rapid development of resistance, is a significant challenge in the twenty first century.
2.0 Calixarenes and Tuberculosis

In 1951 Cornforth, D’Arcy Hart, Rees and Stock reported that a commercially available surfactant, Triton A20 had a remarkable *anti*-tuberculosis effect on TB in mice. The effect was shared with a series of surface active compounds which the group synthesised from a condensation reaction between *p*-tert-octylphenol and formaldehyde under acidic conditions, followed by a polymerisation reaction with ethylene oxide. They proposed that the general structure of the products of the reaction would be 23 as shown below (fig. 2.0.1), however due to the mechanism of extension it is also possible that some molecules of general structure 24 (fig. 2.0.1) could also exist.

![Figure 2.0.1](image)

These compounds, a mixture where \( n = 0,1,2,3 \) and higher integers, and \( x = \text{av. 10, 20 or 60} \), also exhibited their *anti*-TB effect in the guinea pig, in which a substantial curative action was shown on an established infection of three weeks duration. Phagocytosis of the bacilli was not increased either *in vivo* or *in vitro* by these compounds, and their mechanism of action therefore remained obscure.

In 1953 a third paper in the series detailed the stepwise synthesis of these linear compounds, so that compounds of known structure (\( n \), see fig. 2.0.1) rather than a
mixture could be synthesised, along with the first synthesis of the macrocyclic condensation products of the same series.\textsuperscript{85} Tert-octylphenol itself was condensed with ethylene oxide to give products of the general formula 25 (fig. 2.0.2), and the dimer, 26, was also readily accessed from the reaction of an excess of tert-octylphenol with formaldehyde and an acid catalyst, followed by condensation with ethylene oxide.

\[ \text{Fig. 2.0.2} \]

The reaction with ethylene oxide produced polymers of random chain length where for both 25 and 26 \( x \) equalled an average length of 10, 20 or 60 units of ethylene oxide were present. Synthesis of the linear trimer 29 required a different starting material, 2,6-bishydroxymethyl-4-tert-octylphenol, 27, as shown below in scheme 2.0.1.

\[ \text{Scheme 2.0.1. Synthesis of a phenolic trimer via acid catalysed condensation and polymeric condensation with ethylene oxide} \]
Synthesis of higher homologues in the series caused significant problems due to contamination of the products with smaller homologues, however the linear tetramer and pentamer were also prepared and condensed with ethylene oxide.

As a result of these purification problems, Comforth was attracted to the synthesis of macrocyclic compounds from tert-octylphenol and tert-butylphenol, which Zinke had reported could be obtained as crystalline compounds. Comforth repeated the procedures of Zinke and isolated two products from the condensation of tert-butylphenol, and two products from the same reaction with tert-octylphenol. For both phenols the two products appeared to be chemically similar, but had different melting points. The use of a basic rather than acidic catalyst was the main difference in the procedure used compared to that for the linear compounds.

The presence of a macrocyclic rather than linear structure was confirmed by a negative reaction of the four compounds with bromine, and also with para-nitrobenzenediazonium chloride, indicating blocking of the ortho position of the phenol. This series of macrocyclic compounds, whose general structure 31 is shown in figure 2.0.3, was later given the name calixarene.

![Fig. 2.0.3. General structure of tert-butyl and tert-octyl calixarenes](image)

The Rast method was used to determine the number of units in the macrocycle, and surprisingly indicated a tetrameric structure for both the higher and lower melting compounds, which were assigned the names HBC and LBC for the higher and lower
melting tert-butyl compounds, and HOC and LOC for the higher and lower melting tert-octyl compounds.\textsuperscript{85} However results for the acetates of the compounds were inconsistent, leading to X-ray crystallographic investigations by Dorothy Hodgkin.\textsuperscript{85} Unfortunately her data was also inconsistent, due to the very complex crystal structures of the compounds. However, she was able to report that HOC-acetate was most likely a tetramer, though it may also be an octamer or even a heptamer. LOC and LBC both appeared to be tetramers, though LOC could have been a polymer with a two-fold centre of symmetry, implying that it too could be an octamer. Comforth concluded that all four compounds were most likely tetramers and pairs of stereoisomers. Stereoisomerism is possible with calixarene tetramers, as the annulus of the calixarene is small enough to prevent free rotation about the methylene bridges, and Comforth hypothesised that four configurations, shown in figure 2.0.4, were possible for tetramers, which were not readily convertible. Comforth assigned the conformation of LBC to be either the cone or 1,3-alternate, based on the molecular symmetry which had been demonstrated by X-ray crystallography, although the nomenclature of the different conformations was not assigned until much later by Gutsche.\textsuperscript{89}

Polymerisation of the macrocyclic compounds with ethylene oxide was carried out in an autoclave with catalytic amounts of sodium hydroxide in benzene or toluene. The
rationale was that phenoxide reaction with ethylene oxide would occur more readily than alkoxide, leading to derivatisation at all phenolic positions. However more recent reports have indicated that steric hindrance can inhibit phenoxide reaction.\(^{88}\)

Reaction of ethylene oxide with the calixarenes was carried out with known quantities of ethylene oxide, and the reaction only halted once the pressure in the autoclave ceased to drop, indicative of the full consumption of ethylene oxide. However it is also possible that polymerisation of the ethylene oxide may occur, and that the amount of ethylene oxide reacting with the calixarene could be significantly lower. Obviously, reaction of ethylene oxide with the calixarene is unlikely to produce PEG chains of equal length. Therefore the length of the PEG chains denoted by Cornforth's nomenclature would in reality form only a tiny proportion of the heterogeneous products of the reaction. Mass spectra of two compounds synthesised by Cornforth, Macrocyclon (Fig. 2.0.5) and HOC-60 (Fig. 2.0.6) were taken more recently using MALDI-TOF methods, and show the heterogeneous nature of the compounds.

![Fig. 2.0.5. MALDI-TOF mass spectra of Macrocyclon](image)
The compounds synthesised by Cornforth were tested on albino mice infected with the TB strain H37Rv, and were administered in three doses over a period of 34 days. All the compounds had a very high LD$_{50}$, except for four of the linear compounds. However all the LOC compounds (lower melting tert-octyl) and some of the linear compounds caused gross lipaemia and removal of lipids from the adrenal cortex of the mice, which has been shown to lead to a two-fold increase in levels of cholesterol in the blood\textsuperscript{83}. Lipaemia did not occur for the majority of the HOC compounds (higher melting tert-octyl), which also exhibited the highest activity against TB, therefore the lipaemia is not related to the chemotherapeutic effect.

Anti-TB activity was demonstrated by the linear mixture (Li-mix), HBC, LOC and HOC with PEG chains of less than twenty units length on average. The highest activity achieved (Li-mix-10.5, Di-20, LOC-15, LOC-20, HOC-15, HOC-20) was equivalent to a daily dose of 2mg of streptomycin, at the time the best therapy for TB. A single dose of these compounds was found to be as effective as the full course of treatment. Some compounds were found to exacerbate the infection, i.e. were pro-TB in nature, all of
which had PEG chain lengths longer than forty five units on average, (Li-mix-60, Li-mix-90, LOC-45, LOC-60, LOC-90, HOC-45, HOC-60, and HOC-75). When the average chain length was between twenty and forty five units no activity was found. Therefore it was concluded that as the lipophilic/hydrophilic ratio decreases, activity of the compounds passes from anti-TB to inactive to pro-TB.

In 1973 Comforth et al reported a new synthesis of the PEGylated calixarenes, in which the PEG chains were pre-assembled prior to reaction with the calixarene, such that the length of chain could be accurately established. Compounds derived from cholesterol 32, and 2,2-bisoctadecylpropane 33, (fig. 2.0.7) were also PEGylated and tested for activity against TB in mice.

The most active compounds synthesised by Comforth previously had 'just enough PEG to become water soluble'. For HOC to be water soluble this involves reaction with forty to fifty equivalents of ethylene oxide, therefore if HOC is a tetramer the most active compound is HOC-12.5, which Comforth called Macrocyclon.

Comforth synthesised PEG chains using the methods of Perry and Hibbert. This method, shown in scheme 2.0.2, involved the reaction of a dichlorinated glycol 34, with two equivalents of a metallic mono-anionic salt of a second glycol 35. The glycol, for example hexaethyleneglycol was then mono-tosylated, and protection with tetrahydropyran was carried out at the second terminus. Purification of these compounds by solvent partition methods would probably however not have afforded pure compounds.
Scheme 2.0.2. Synthesis of PEG-chains using the Perry and Hibbert method

Reaction of the mono-tosyl-hexaethyleneglycol $37$ (scheme 2.0.3) with HOC, $38$, gave a product which still exhibited free phenolic hydroxyls $39$. Therefore Comforth protected the second terminus of the hexaethyleneglycol $37$ with a THP group, but reaction of this compound with HOC gave a product which was still not fully derivatised.

Scheme 2.0.3. Partial derivatisation of tert-octylcalixarenes with PEG-6

Comforth then used PEG chains with mesyl activation (scheme 2.0.4). The presence of free phenolic hydroxyls in the calixarene was detected using UV spectroscopy. In methanolic potassium hydroxide free phenolic hydroxyls absorb strongly at approximately 300 nm, whereas the phenolic ethers have a characteristic double peak of absorption at approximately 270-280 nm. Comforth believed that this method of
characterisation could be applied to calixarenes, and hence characterised his PEGylated calixarenes using UV spectroscopy.

Reaction of the hexaethyleneglycol 40 (scheme 2.0.4) with HOC was carried out using an overnight distillation of four equivalents of PEG and four equivalents of potassium tert-butoxide to one equivalent of HOC, with the addition of four further equivalents of base and PEG over the course of a second day. For decaethyleneglycol 41 the reaction was carried out over the course of five days. In order to try to drive the reaction towards completion an apparatus which allowed the removal of tert-butanol (which formed during the reaction) by co-distillation with dry benzene was used. UV spectroscopy of the products 42 and 43 suggested fully derivatised compounds. Cholesterol reacted more readily with the PEG and required only an overnight reaction with five equivalents of both base and PEG.

![Scheme 2.0.4. Derivatisation of tert-octylcalixarenes with PEG-6 and PEG-10](image)

Cornforth also re-synthesised HOC-12.5 (Macrocyclon) using the random polymerisation method, and methylated the termini of the PEG chains. HOC-12.5 with carboxy termini instead of hydroxyls were synthesised, and these compounds were
tested on mice to demonstrate the effect of the functionality of the terminus upon biological activity.

Results of testing in mice against the H37Rv strain of TB again showed that the product of a random condensation of HOC, HOC-12.5 was highly active. HOC-6, 42 and HOC-10, 43 had activities slightly lower than HOC-12.5, though comparable, suggesting that a wide range of products in HOC-12.5 were probably responsible for the overall biological effect. The wide range of PEG chain lengths which gave some activity (4 to 12 units) suggested to Cornforth that “physical rather than chemical properties are responsible for the biological activity.”

The HOC-12.5-monomethylether, and HOC-12.5-carboxy had activity similar to HOC-12.5, suggesting that small alterations in the functional termini of the PEG chain had little significance. Cholesterol-10-monomethylether 44 also exhibited some activity, though other PEGylated cholesterol products, cholesterol-10, 45 and 20, 46 showed no activity, suggesting that the macrocyclic structure was preferable (fig. 2.0.8). 2,2-dioctadecylpropane-1,3-diol-PEG-8, 47, had some activity against TB, whilst with PEG-64, 48, pro-TB activity was observed (fig. 2.0.8). Again for the HOC compounds HOC-60 exhibited pro-TB activity.

![Chemical structures](image)

Fig. 2.0.8.
Metabolic studies were carried out using HOC-12.5 labelled with $^{14}$C at both the calixarene and the PEG chain, which showed that the compound had a long persistence after injection, as no radioactivity was detected in expired carbon dioxide. This indicated that there was no significant degradation of the compounds \textit{in vivo}.

In summary Cornforth demonstrated that the use of a homogeneous product had no chemotherapeutic advantage over the product of random polymerisation, that the biological activity was tolerant of small changes in the functionality of the termini of the PEG chain, and that the presence of a macrocyclic structure was not essential to achieve activity.

The mechanism of action of these compounds remains unclear, though some publications have provided insights. In 1968 D'Arcy Hart reported the effect of Macrocyclon and HOC-60 upon macrophages \textit{in vitro}. HOC compounds were able to enter macrophages in living animals, and thus if the compounds had no effect upon isolated macrophages then they will act \textit{via} a host-mediated mechanism. Macrocyclon and HOC-60 were added to the culture medium of macrophages which had been infected with TB. If Macrocyclon was added immediately after infection of the macrophages bacterial multiplication was markedly inhibited for up to two weeks, though the effect could be extended by reintroduction of Macrocyclon to the culture medium, (see Fig. 2.0.9). In contrast HOC-60 caused a striking acceleration of bacterial growth, and caused gross deterioration of infected cell layers within eight to twelve days of administration.

![Fig. 2.0.9. Graph showing effect of Macrocyclon and HOC-60 on TB in isolated macrophages](image-url)
The action of Macrocyclon *in vitro* has been characterised as bacteriostatic rather than bactericidal, which is in contrast to the results in the guinea pig where Macrocyclon was shown to have a significant curative action upon an established infection. Therefore it is possible that Macrocyclon works *via* more than one mechanism, or that the effect which is observed *in vitro* is not that by which it works *in vivo*, or it may be due to the heterogeneous nature of the compound.

The macrocyclic nature of Macrocyclon was finally established by Ungaro *et al* in 1982. The HOC calixarene thought by Comforth to be a tetramer, was in fact a mixture of oligomers of which the major compound was the octamer. Ungaro followed the same procedures used by Comforth to synthesise HOC and LOC. An X-ray structure of LOC confirmed that it was the tetramer, but the structure of HOC was more difficult to elucidate by X-ray. Molecular weight determination supported the hypothesis that HOC was an octamer, though confirmation was *via* a derivative of HOC. Complete removal of the tert-octyl groups afforded a compound which was identical to that achieved by complete removal of the tert-butyl groups from tert-butylcalix[8]arene, whose structure had been unequivocally established. These compounds displayed identical $^{13}$C NMR spectroscopy and infra red data. The octa-acetyl derivatives of both compounds also exhibited identical chromatographic behaviour (hplc and tlc) and identical spectrographic behaviour (IR, $^1$H NMR and $^{13}$C NMR). In addition the $^{13}$C NMR of both tert-butylcalix[8]arene and HOC when derivatised with short-PEG chains showed similar data which implied free rotation of the macrocycle which is found in large ring calixarenes, in comparison to that of tert-butylcalix[4]arene which has a more complex spectra due to the more rigid conformations in which the compound is held due its smaller annulus.

The fact that HOC and LOC are different compounds as opposed to a pair of stereoisomers was also supported by their toxicological effects, LOC causes gross lipaemia which HOC does not, though different pharmacological effects are not impossible from pairs of stereoisomers.
2.1 Synthesis of Calixarenes

2.1.1 tert-Butyl Calixarenes

Although calixarenes with different functionalities at the \textit{para} position of the phenol, known as the upper rim in calixarene nomenclature, have been synthesised, by far the most studied are those with \textit{tert}-butyl groups in that position. The most common sizes of the macrocycle, the major calixarenes, are the tetramer $50$, the hexamer $51$, and the octamer $52$ (scheme 2.1.1.1). For \textit{tert}-butylcalixarenes three different procedures are most commonly used to access the different ring sizes.

The procedure used to access the tetramer is known as the Zinke-Cornforth procedure, an adaptation of the original Zinke procedure.$^{85,86}$ This method involves heating the phenol with aqueous formaldehyde solution and 0.045 equivalents of sodium hydroxide at 110 °C for two hours. The original Zinke procedure involved trituration of the product of this stage of the reaction to remove the majority of remaining base, however this is no longer used as the final product of the reaction has been shown to be extremely sensitive to the quantity of base which remains, leading to possible formation of different ring sizes.$^{97}$ The Zinke procedure then involved heating the mixture in linseed oil, whereas the modified procedure heats the mixture at reflux in diphenyl ether for a further two hours. Filtration of the crude mixture affords the tetramer $50$, as the major product, with small amounts of larger ring sizes.

Scheme 2.1.1.1. Synthesis of the major \textit{tert}-butylcalixarenes
Synthesis of the larger ring sizes was originally carried out by the Petrolite Corporation of Missouri. The Petrolite procedure is still in use today to access the octamer 52, and a summary of the procedure is shown below (scheme 2.1.1.2). The reaction is carried out at reflux temperature for four hours with the removal of water into a Dean-Stark Trap. The octamer is the major product of the reaction but smaller ring sizes also form in small quantities.

A modified Petrolite procedure is used to access the hexamer 51. It was discovered by Gutsche et al in the early 1980s that if ten times the quantity of base present in the original procedure were used, the main product of the reaction was the hexamer rather than the octamer. Further modifications to the aldehyde source and reaction time led to the publication of the modified Petrolite procedure, which is summarised below (scheme 2.1.1.3).
In 1986 Gutsche published a further paper in which the synthesis of the tert-butyl series of calixarenes was examined in greater detail.\textsuperscript{97} It was reported that the Zinke-Cornforth procedure used to synthesise the tert-butylcalix[4]arene was very susceptible to variations in the quantity of base used, with larger concentrations of base (>0.15 eq.) changing the main product of the reaction from tetramer to hexamer. Altering the base to other alkali metals also affected the yields of the reaction. Sodium hydroxide consistently gave the best yields of tetramer (55%), with the yields becoming increasingly lower as the size of the cation increased (KOH, 48%; RbOH, 36%; CsOH, 22%) as well as smaller cation sizes giving lower yields (LiOH, 41%). However variation in the length of time of the reaction had very little effect on the yield, as did a change in the ratio of reagents.

It was also reported that the tert-butylcalix[8]arene could be accessed by the Zinke-Cornforth procedure as well as the Petrolite procedure, however the simplicity of the Petrolite procedure favours its use.\textsuperscript{97} An investigation of the optimum base for the reaction revealed a greater tolerance to the nature of the cation than for the tetramer, with sodium hydroxide again giving the highest yield of octamer (57%) (KOH, 55%; RbOH, 37%; LiOH, 35%).\textsuperscript{97} The crude product of the reaction was found to be contaminated with the other major ring sizes, particularly the hexamer.

In 1981 Gutsche reported that synthesis of the hexamer was affected by the nature of the base used in the reaction, and that it too could be synthesised using the Petrolite procedure, though higher yields were obtained through use of the Modified Petrolite procedure above (88%).\textsuperscript{99,100} When using the Petrolite procedure the highest yield was obtained with rubidium hydroxide (74%), with the yield decreasing rapidly with different sizes of cation (KOH, 56%; CsOH, 40%).

The larger size tert-butylcalixarenes (nine to twenty) have also been reported (scheme 2.1.1.4). Although present in small amounts in the crude mixtures with the major calixarenes (1-3%), they have also been prepared by direct synthesis.\textsuperscript{101} Synthesis of calixarenes using acid catalysis was initially thought to afford linear oligomers, with insignificant amounts of macrocyclic products.\textsuperscript{102} However the use of a refluxing acetic
acid solution with tert-butylphenol, an excess of paraformaldehyde and a catalytic amount of hydrochloric acid was found to afford a mixture of the larger ring calixarenes which accounted for approximately 25-30% of the crude mixture. The procedure was refined leading to a 95% conversion of phenol to a crude mixture of calixarenes, from which small amounts of the larger ring sizes could be isolated as well as approximately 26% of the octamer.

Separation of the large calixarenes from the crude mixture was carried out by the combined processes of trituration, chromatography and recrystallisation, which afforded the ring sizes 9 to 16, and 20, in greater than 95% purity, and sizes 17 to 19 in greater than 75% purity. Yields were significantly lower than for the major calixarenes (0.2% to 9%) and decreased as the size of the calixarene ring increased.

The minor tert-butylcalixarenes with five and seven units have also been synthesised. The synthesis of tert-butylcalix[5]arene 65 was first reported in 1982 by Ninagawa (Scheme 2.1.1.5) in 3 to 5% yield. This was followed by the much higher yielding synthesis (15-20%) of Gutsche and Shinkai in 1993 and 1994 respectively.
The first synthesis of tert-butylcalix[7]arene by Nakamoto and Ishida in 1982 afforded the compound in only 6% yield, though later the use of lithium hydroxide produced it in 11-17% yield.  The heptamer is also generated by the acid catalysis method used to access the larger calixarenes (scheme 2.1.1.4), which utilised acid catalysis to access the larger calixarenes in 24% yield.

### 2.1.2 Mechanism of Calixarene Formation

The mechanism of calixarene formation is not fully understood, however for base catalysed condensations the mechanism shown below (fig. 2.1.2.1) is widely accepted for the initial synthesis of linear precursors, although the mechanism of ring closure remains the subject of much discussion.
It is possible that a linear oligomer forms which then ring closes between the two terminal moieties to afford the macrocyclic product. However, mechanistic studies have indicated, at least for the octamer, that this may not be the case and that a linear tetramer forms, which then forms a hemicalixarene by hydrogen bonding to a second linear tetramer (see Fig. 2.1.2.2). The termini in this hemicalixarene are in close proximity and therefore are correctly oriented for bond formation to generate the cyclic octamer, the kinetic product of the reaction. Under more strenuous conditions it has been demonstrated that the cyclic octamer can revert back to the cyclic tetramer, the product of thermodynamic control. If the synthesis of tert-butylcalix[4]arene using diphenylether, discussed previously, is halted early in the reaction the product is the octamer alone, indicating that the tetramer forms from a contraction of the octamer under harsh heating conditions. Indeed a sample of pure tert-butylcalix[8]arene was contracted to the tetramer under basic conditions in refluxing diphenylether. The mechanism for this molecular 'mitosis' is not well understood.

![Fig. 2.1.2.2. Proposed formation of hemi-calixarenes](image)

Analysis of the reaction intermediates in the formation of tert-butylcalix[6]arene do show the presence of the linear trimer, however the linear hexamer is present in far greater quantity. When the same group examined the reaction intermediates in the synthesis of the octamer they found no linear octamer present, supporting Gutsche’s theory of hemicalixarenes.
It is believed that the alkali metal cation from the base may play a key role in the ring
closure, which requires high reaction temperatures. Different sizes of cation have
been reported to influence the formation of different ring sizes via a templating effect.
For example the synthesis of tert-butylcalix[6]arene, which has an annulus of between
2.0 and 2.9 Å, is favoured as the major product from the Petrolite procedure using
potassium hydroxide (2.66 Å) and rubidium hydroxide (2.94 Å).

2.1.3 Other Calixarenes

Calixarenes with groups other than tert-butyl at the upper rim have also been
synthesised, though in general in much lower yield. Cornforth synthesised the tert-
nature of these compounds was confirmed when re-synthesised by the group of Ungaro
in 1982, who reported the synthesis of the tert-octylcalix[4]arene in 10% yield and the
octamer of the same series in 30% yield, which were both isolated from the crude
mixture of a Zinke-Cornforth procedure.

The benzylcalixarene series is also well known, with Vicens reporting the first synthesis
of the pentamer, hexamer and octamer in 1992. The condensation reaction was
carried out using a Zinke-type procedure with 14N aqueous potassium hydroxide. The
reaction afforded the octamer as a precipitate, the pentamer, and also the hexamer.

The benzyloxy-calixarene series was synthesised in 1997 (scheme 2.1.3.1). Using a
Petrolite procedure the octamer, was isolated from the reaction mixture in 48% yield. They were able to isolate the heptamer and hexamer from the mixture only
via acetylation of the crude reaction mixture.
Calixarenes have been prepared from \( p \)-cresol \( \text{70} \) and \( p \)-ethylphenol \( \text{71} \), as well as from a range of long alkyl chain phenols \( \text{72} \) where \( n = 7,9,13,15, \) and \( 17 \) (fig. 2.1.3.1).\textsuperscript{113,114}

The long chain alkylphenols afforded the hexamer and octamer of their respective series via a Zinke-Cornforth procedure, though yields are only given for decylphenol in the paper (octamer, 10\%, hexamer 12\%).\textsuperscript{114}

Removal of the \textit{tert}-butyl groups from \textit{tert}-butylcalixarenes has also afforded the opportunity for the attachment of novel groups to the upper rim of calixarenes, although the so-called ‘nude’ calixarenes have proven to be difficult to derivatise at the \textit{para}-position (scheme 2.1.3.2). The aluminium catalysed reaction removes the \textit{tert}-butyl groups from phenols within the calixarene which are not derivatised at the lower rim. The yield of the reaction is typically quantitative for the octamer \( \text{52} \), with yields decreasing concomitantly with the size of the calixarene (tetramer, 66\%).\textsuperscript{115,116}
Much work has concentrated on the solubilisation of calixarenes via upper rim functionalisation. The group of Shinkai first published sulfonation of the calixarene upper rim in 1984, with a full paper following in 1987 (scheme 2.1.3.3).

They initially described procedures for sulfonation of the 'nude' calix[6]arene in 75% yield, later improving the synthesis with careful control of the time of the reaction and temperature achieving simultaneous de-tert-butylation and sulfonation of the tert-butylicalix[6]arene in 50% overall yield.

In 2001 Raston published sulfonation on the phenylcalixarene series, using both the Shinkai method and chlorosulfonic acid, although no yields are given for either method.
2.1.4 Stepwise Synthesis of Calixarenes

Calixarenes have also been synthesised using a stepwise approach. Although these procedures are typically low yielding, they do have the advantage that a variety of groups can be introduced at the upper rim of the calixarene. The only size of calixarene synthesised via these methods is the tetramer. The original method for stepwise synthesis (now known as the non-convergent method) involved the very low yielding step-wise synthesis of a linear oligomer, followed by the higher yielding cyclisation step which was carried out at high dilution, for example scheme 2.1.4.1.\textsuperscript{121,122}
A more recent method for the stepwise synthesis of calixarenes is known as the fragment condensation method, and involves either the condensation of a trimer and monomer (the 3+1 method) or of two dimers (2+2 method). The ring closing step is usually carried out in refluxing dioxane with TiCl₄ catalyst and a long reaction time (scheme 2.1.4.2).<sup>123,124</sup>

![Scheme 2.1.4.2. Utilisation of fragment condensation of calixarenes](image)

A variety of \( \text{para} \)-substituents have been employed in the reaction shown in scheme 2.1.4.2, including methyl, \( \text{tert} \)-butyl and phenyl<sup>123</sup> chloro, \( \text{CO}_2\text{Et} \) and \( \text{NO}_2 \) with yields varying between 15-38%.<sup>124</sup>

### 2.2 Derivatisation of Calixarenes

The most readily and commonly derivatised calixarenes are the tetramers, since their fixed conformation and small number of reactive sites make the most tractable starting materials. Likewise the most commonly used tetramer is the \( \text{tert} \)-butylicalix[4]arene, since it is the most readily available, though in principle for the tetramers the reactions discussed below are also possible with other \( \text{para} \) substituents.

When a calixarene tetramer is fully derivatised at the lower rim four possible conformations of product can be generated, as shown in Fig. 2.0.4. It has been demonstrated that a templating effect can be exerted by the base used in the alkylation reaction. For example the use of a sodium base favours a cone formation in the product, whereas a larger cation (\( \text{K}^+ \), \( \text{Cs}^+ \)) leads to the formation of 1,3-alternate isomers.<sup>125 - 128</sup> In 1992 a thorough investigation into the effect of base, time of reaction and upper rim
substituent was carried out.\textsuperscript{129} It was found that the exhaustive alkylation of \textit{tert}-butylcalix[4]arene with PicCl was strongly influenced by the nature of the base (scheme 2.2.1).

When sodium hydride was used as the base the product formed from both \textit{tert}-butylcalix[4]arene and calix[4]arene was exclusively in the cone conformation in high yield, whereas both caesium carbonate and potassium carbonate gave a mixture of cone, partial cone and 1,3-alternate conformations. Caesium carbonate gave predominantly the partial cone, whilst potassium carbonate gave predominantly 1,3-alternate conformation.

When an excess of alkylating agent and a limiting amount of weak base is used for the alkylation it is possible to achieve monoalkylation not only of tetramers, but also of the less readily derivatised larger ring sizes.\textsuperscript{125} In 2000 Santoyo-Gonzalez reported the monoalkylation of \textit{tert}-butylcalix[4]arene and \textit{tert}-butylcalix[6]arene, using a limiting amount of both good alkylating agents and base (scheme 2.2.2).\textsuperscript{130}
Compounds of the type 87 and 88 were synthesised via a long procedure in which the calixarene (50,51) was refluxed for four days with 1.1 equivalents of bis(tributyltin)oxide with a Dean-Stark separator attached, after which the alkylating agent (1.1 equivalent) was added to the reaction mixture along with 1.1 equivalents of tetrabutylammonium iodide. The reaction mixture was then refluxed for one to five hours. A variety of alkylating agents were tested, some of which are shown in scheme 2.2.2. Yields were generally higher for the tetramer than the hexamer, although a wide range was shown across the different alkylating agents (23% to 80%).

Monoalkylation of a calix[8]arene has been reported by the group of Neri. They achieved monoalkylation of tert-butylcalix[8]arene 52 using a limiting amount of the weak base caesium fluoride (1.2 equivalents), and an excess (10 equivalents) of the alkylating agent p-methylbenzyl bromide (scheme 2.2.3). The forty hour reflux reaction resulted in a 25-50% conversion of the calixarene 52 to the mono-alkylated product 89.
Etherification of the calix[4]arenes can generally be effected at all positions, provided that sufficiently reactive reagents are employed. In 1983 Gutsche reported the formation of methyl, ethyl, allyl and benzyl ethers on tert-butylcalix[4]arene in high yields using an excess of sodium hydride. For the larger ring sizes full derivatisation can be achieved provided that large excesses of strong bases (NaH, BaO/Ba(OH)$_2$), reactive electrophiles and long reaction times are employed.

Neri et al also demonstrated that full alkylation, in near quantitative yield (80-95%), of tert-butylcalix[8]arene was possible using 16 equivalents of strong base (sodium hydride) with 8 equivalents of benzyl bromide at reflux in THF/DMF for 8 hours. The fully alkylated product was also formed with a weaker base (K$_2$CO$_3$), however a greater number of equivalents, a more active electrophile ($p$-CNBnBr) and a longer reaction time (12 hours) were required. Use of caesium fluoride can also afford the fully alkylated product, however the reaction time needed to be increased to 96 hours. After 16 hours the product of the reaction was the tetra-substituted compound 90, exhibiting a symmetrical 1,3,5,7-substitution pattern as shown in scheme 2.2.4.

Scheme 2.2.4. Partial derivatisation of tert-butylcalix[8]arene to produce a symmetrical 1,3,5,7-substituted product

The 1,3,5,7-tetra-benzylated compounds could be readily formed, albeit in lower yield than the fully alkylated compounds, when limiting amounts of a weak base and
electrophile were used.\textsuperscript{132} It was reported that for a range of benzyl electrophiles, when using 16 equivalents of a weak base the tetra-substituted product was achieved in 20 to 49\% yield. For the more active electrophiles possessing electron withdrawing groups on the aromatic ring (shown below in Fig. 2.2.1), very complex mixtures resulted when 16 equivalents of base were used. The tetra-substituted product was only achieved when a limiting amount of both base (8 equivalents) and electrophile (4 equivalents) were used.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig221.png}
\caption{Fig. 2.2.1}
\end{figure}

The use of electron donating groups on the aromatic ring increased the selectivity of the reaction by reducing the reactivity of the electrophile. Neri reported that use of the weaker base caesium fluoride should lead to greater regioselectivity, indeed it did lead to an increase in the yields of the tetra-substituted products over potassium carbonate.\textsuperscript{132} This effect was demonstrated most effectively in the reactions with the most reactive electrophiles (91 and 92 shown above) where yields increased from 10-15\% to 20-45\%. Also tert-butyl bromoacetate and 2-chloro-\textit{N},\textit{N}-dimethylacetamide which afforded only intractable mixtures with potassium carbonate gave readily separable tetra-substituted products with caesium fluoride.

Neri proposed that the tetra-substituted product was formed as a result of the formation of a mono-anion in the presence of weak bases, which was followed by a mono-alkylation, and further formation of mono-anions and alkylations, rather than any templating effect.\textsuperscript{131} From the mono-alkylated species four possible monoanion species may be formed by deprotonation at positions two to five (see below, Fig. 2.2.2). However if the mono-anion forms at position two it can only be stabilised by hydrogen bonding from the adjacent position 3. Whereas for the other positions (3,4,5) the anion can be stabilised by hydrogen bonding from two adjacent positions. Thus the concentration of anion at position two in the reaction mixture would be negligible.\textsuperscript{131}
In 1995 Neri reported the results of a systematic study of a series of monoalkylations of \textit{tert}-butylcalix[8]arene with the electrophile \textit{p}-methylbenzyl bromide, using caesium fluoride.\textsuperscript{13} Neri was able to isolate and characterise the partially alkylated derivatives which ranged from the mono-substituted to the octa-substituted product. To the mono-alkylated product previously reported, a further monoalkylation was carried out using 1.2 equivalents of CsF and 10 equivalents of \textit{p}-methylbenzyl bromide. This afforded the 1,3; 1,4; and 1,5-disubstituted compounds. As expected none of the 1,2-disubstituted compound was formed, which supports the proposed mechanism of alternate alkylation. The 1,3-disubstituted compound was formed in the greatest yield (22\%). Mono-alkylation of the 1,3-disubstituted compound under the same conditions afforded the 1,3,5-trisubstituted compound, and a small amount of the 1,3,6-trisubstituted compound. Mono-alkylation of the 1,5-disubstituted compound also afforded the 1,3,5-trisubstituted compound, in relatively high yield, 41\%. In this way it is possible for two separate pathways in the reaction to give rise to the 1,3,5-trisubstituted compound, as is shown below in Fig. 2.3.7.
Neri also carried out a time-course study on the alkylation reaction, using sixteen equivalents of CsF and eight equivalents of the electrophile. After one hour the reaction mixture contained mainly the starting calixarene, with the mono and di-alkylated (1,3) compound, and small amounts (less than 1%) of the other di- and tri-substituted compounds. After the second hour the tetra-substituted 1,3,5,7-derivative was detected, and after three hours the starting material calixarene had disappeared. After its appearance the tetra-substituted compound steadily accumulated in the reaction mixture, and reached its highest concentration between the seventh and tenth hours.

The penta-substituted 1,2,3,5,7 compound became detectable after formation of the tetra-substituted compound, and at thirteen hours its concentration was equal to that of the tetra-substituted compound. At this point the hepta-substituted compound was also detected. A steady decrease in the concentration of the tetra-substituted compound was then observed, concurrent with an increase in the presence of the hexa and hepta-substituted derivatives. The fully alkylated compound was not detected even after a long reaction time (96 hours), therefore Neri concluded that as he had previously
reported, a greater number of equivalents of both base and electrophile (32) are required to obtain the fully alkylated compound.

The accumulation of the tetra-substituted material was attributed to the fact that further anion formation can only occur through the formation of less stable, unstabilised anions, and therefore their formation and subsequent reaction are less likely to occur. The ready formation of the tetra-substituted compound does afford the opportunity for differential alkylation at the remaining four positions, by exposure of the tetra-substituted compound to a second electrophile and a strong base. The principle of this was demonstrated by Reinhoudt, who partially alkylated tert-butylcalix[4]arene using the weak base potassium carbonate, and fully alkylated the compound with the strong base sodium hydride (scheme 2.2.5).133

Neri also demonstrated the possibility of fully alkylating a partially alkylated calixarene through use of a stronger base, when he fully alkylated compound 95 with sodium hydride (scheme 2.2.6).134 This established the use of differential alkylation for the calixarene octamer.
2.3 Summary

The field of calixarene chemistry is very diverse, with most work focusing on derivatisation of the calixarene tetramers with active electrophiles. Some work has however focused on the larger major ring size, the octamer, again using active electrophiles. Cornforth derivatised an octamer via a polymerisation reaction with ethylene oxide, which afforded a compound with remarkable anti-TB activity, Macrocyclon. Neri has fully derivatised tert-butylicalix[8]arene using an active electrophile and weak base, and established a mechanism of alternate alkylation for the procedure.

2.4 Aims and Objectives

The initial aim of the project was to synthesise the major size rings of the phenyl series of calixarenes, the octamer, hexamer and tetramer. Subsequent biological testing of the tert-butylicalixarene series within our group revealed that the octamer was the most active ring size of the series, and therefore our synthetic studies focused initially on this size of phenylcalixarene.
We next turned our attention to the synthesis of PEG-chains, as the longest commercially available homogeneous PEG-chain is hexaethyleneeglycol. The work of Comforth had indicated that the PEG-chains needed to be protected with a base stable protecting group at one terminus, with activation at the second terminus to allow coupling to the calixarenes. Our aim was to synthesis a PEG-12 unit which was differentially protected, as the most active compound synthesised by Comforth, Macrocyclon, was believed to contain predominantly an octamer coupled to PEG-12 units. We also aimed to synthesise differentially protected smaller PEG units in order to test the conditions necessary to couple PEG-chains to the calixarenes.

Due to the large mass of PEGylated phenylcalixarenes, we also had an interest in synthesising a range of small PEGylated phenolic monomers and dimers, which would come closer to obeying the mass criteria of the Lipinski Rule of 5. We also wanted to be able to synthesise fluorescently labelled PEGylated calixarenes in order to probe the mechanism of action of the compounds. Throughout the project future synthetic strategies were developed in accordance with the results of biological testing of compounds.
Results and Discussion

Chapter 3 Calixarenes

3.0 Phenylcalixarenes

Previous work in the area of phenylcalixarenes includes the first reported synthesis of *p*-phenylcalix[4]arene, 75, by Gutsche, using the non-convergent stepwise method, developed by Hayes and Hunter. Zinke had also reported the synthesis of the *p*-phenyl tetramer using the Zinke procedure (see Section 2.1.1), but the melting point reported by Zinke differed from that reported by Gutsche, suggesting problems with the method.

The Gutsche procedure, shown below (scheme 3.0.1), involves a stepwise synthesis of the linear tetramer, followed by acid catalysed cyclisation to afford the calixarene.

![Scheme 3.0.1. Stepwise synthesis of phenylcalix[4]arene](image-url)

---

58
The procedure resulted in an overall yield of just 0.24\%.\textsuperscript{135} The procedure also had very limited scale-up possibilities, as a large excess of \textit{\textit{p}}-phenylphenol 97 was used in the acid catalysed condensation steps (40 eq.), and removal of this excess by flash column chromatography was required.

Two other products were obtained from the final acid catalysed cyclisation step, which Gutsche assigned to structures 103 and 104 shown below (fig. 3.0.1). These compounds resulted from a cyclisation occurring at the 2' and 4' positions of the upper phenyl ring.

Gutsche assigned the structure of these compounds all with the same m/z of 728 based on differences of their infra red spectra and melting points. 75 melts sharply at 407-409 °C, whereas 103 melts at 256-258 °C, and 104 melts at the even lower temperature of 193-195 °C. The $^1\text{H}$ NMR spectra for 75 showed the calixarene tetramer to have a singlet resonance for the methylene bridges at temperatures above 60 °C, whilst below 0 °C a well resolved pair of doublets were observed. Compound 103 however displayed four singlets for the methylene bridge resonances, whilst 104 displayed a multiplet in the same region.

A second paper by Gustche and No was published in the same journal issue, dealing with the synthesis of phenylcalix[4]arene by a more convergent stepwise synthesis.
This synthesis also suffered from a lack of scalability due to the large excesses of phenylphenol which were required, but this shorter route had advantages with an overall yield of 20% c.f. 0.24%.

Scheme 3.0.2. Stepwise synthesis of phenylcalix[4]arene


Although this synthesis is more rapid than either published by Gutsche, no synthesis of the starting material 106 was described. Presumably it is synthesised from the tert-butylcalix[4]arene, via de-tert-butylation, methylation at the lower rim and then bromination at the upper rim.
In 1985 Gutsche had attempted to synthesise phenylcalix[4]arene via a similar method, starting from the calix[4]arene-tetra-methylether 108 (scheme 3.0.4). Treatment of this compound with phenylzinc chloride in the presence of diisopropylaluminium hydride and Cl₂Pd[PPh₃]₂ in THF/ether afforded only starting material however.

![Scheme 3.0.4. Failed synthesis of phenylcalix[4]arene](image)

The synthesis of phenylcalix[6]arene 76 and phenylcalix[8]arene 77 were also reported using both a modified Zinke-Cornforth procedure and the Petrolite Procedure. Formation of the tetramer was not observed, although the phenyl-dihomooxa-calix[4]arene 109 was available from methylation of the crude reaction mixture of the Petrolite Procedure (Fig. 3.0.2).

![Fig. 3.0.2](image)

The modified Zinke-Cornforth Procedure involved heating p-phenylphenol with sodium hydroxide and formaldehyde in methanol followed by acidification, drying overnight,
and further heating of the residue in diphenyl ether with sodium hydroxide for 18h, followed by trituration of the precipitate. Phenylcalix[8]arene 77 was extracted from the crude mixture into boiling pyridine, and recrystallised from pyridine/dioxane in 14% yield. Repetition of this procedure, but with a 24-fold increase in the quantity of base, and extraction into chloroform instead of pyridine, followed by flash chromatography, afforded phenylcalix[6]arene 76 in 10% yield.

The Petrolite Procedure afforded the octamer in 4.4% yield, and by methylation of the crude reaction mixture Gutsche identified the octamer, hexamer, homooxacalix[4]arene 109, a compound he believed may be the heptamer, and a mixture of linear oligomers. Gutsche characterised the phenylcalix[6]arene 76 from the Zinke-Cornforth Procedure by melting point, IR spectroscopy and $^1$H and $^{13}$C NMR, although the integration for the $^1$H NMR was not consistent with the hexamer but the tetramer (4H for the OH, 28H, ArH, and 8H for the methylene bridges). Also only seven resonances were reported in the $^{13}$C NMR spectrum not the expected eight, though overlap is possible. The octamer was characterised by melting point, IR spectroscopy and $^1$H NMR spectroscopy. No mass spectra were presented for the compounds.

A second paper detailing the synthesis of the phenylcalix[8]arene 77 and phenylcalix[6]arene 76 was published in 2001, at the end of the first year of this Ph.D. Raston used a modified Petrolite Procedure, shown below (scheme 3.0.5), which afforded the tetramer, pentamer, hexamer and octamer as a mixture. Separation of the mixture was effected by precipitation of the chloroform soluble material using an acetone/methanol mixture to give the hexamer, followed by evaporation and trituration in acetone/dichloromethane, to afford the pentamer. Further evaporation and trituration with acetone gave the tetramer. However this process of fractional crystallisation did not afford the octamer, rather, repeated triturations of the chloroform insoluble material with an acetone/chloroform mixture gave the octamer 77. This is the first reported one-step synthesis of the phenylcalix[4]arene 75, which was achieved in 10% yield as a mixture of ring sizes.
Raston varied both the base and quantity of base in the reaction, using sodium and potassium hydroxide in a range of equivalents from 0.045 to 0.75. The highest yields of octamer 77 and tetramer 75, 38% and 10% respectively, were achieved with 0.45 eq. of potassium hydroxide. The highest yields of hexamer 76 and pentamer 110, 11% and 15%, were also achieved with potassium hydroxide, though with only 0.045 equivalents present. This is in direct contrast to the findings of Gutsche for the tert-butyl series of calixarenes, where a ten-fold increase in base was needed to switch the major product of the Petrolite Procedure from the octamer to the hexamer (see Section 2.1.1).

When using large quantities of base (0.75 eq.) no products were obtained. Although products were obtained when using 0.045 eq. of base no products were obtained when sodium hydroxide was used in 0.18 equivalents, and only small yields of the pentamer and hexamer (2%, 8%) were obtained when potassium hydroxide was used in the same number of equivalents. Overall Raston demonstrated the extremely sensitive nature of these reactions.

No characterisation of the phenylcalix[8]arene is provided, only of its sulfonated derivative 80 which is discussed in Section 2.1.3. Interestingly the data Raston provided for the tetramer differed from that reported by Gutsche in 1982 in that; for the proton NMR Raston reports that the methylene bridge gives rise to two doublets which resonate at δ 3.67 and δ 4.38. Gutsche reported that the methylene bridge in the same solvent affords a singlet at δ 4.14, though at temperatures below 0 °C he does report that a well resolved pair of doublets are observed. It may be that the tetramer synthesised by Raston is in the partial cone conformation, whilst the tetramer synthesised by Gutsche is
in the 1,3alternate conformation, as the different conformations of tetr...due to its small annulus.

The phenylcalix[7]arene 111 was reported by the Hitachi Chemical Company in 1984. They reported the synthesis of the heptamer from a modified Petrolite Procedure utilising 0.2 equivalents of base and two equivalents of paraformaldehyde. The reaction was carried out in refluxing dioxane for 48 hours (scheme 3.0.6).

\[
\begin{align*}
\text{C}_{12}H_{10} + \{\text{HCHO}\}_n & \xrightarrow{\text{KOH}} \text{Dioxane} \quad 48\text{h} \\
\text{111} & \text{Heptamer}
\end{align*}
\]


The heptamer 111 was afforded from fractional crystallisation of the crude reaction mixture together with a linear oligomer, and flash column chromatography generated the heptamer, though no yield is provided. The heptamer was characterised by its melting point (>310 °C), a mass spectrum which recorded the molecular ion at 1274, and by \(^1\text{H} \) NMR and infra red spectroscopy. In the 1985 paper by Gutsche he refers to this patent, and to similar patents on the hexamer and octamer of the phenyl series, indicating that in his opinion the characterisation data was sparse and inconclusive.
3.1 Synthesis of the $p$-Phenylcalixarene Series

$p$-Phenylcalixarenes possess deep cavities within the ring, particularly compared to the tert-butyl analogues. If the calixarene exerts its biological activity by residing within a membrane, a deeper ring cavity could, with PEG-chains attached, more readily span the membrane. We therefore initially investigated the synthesis of the phenylcalixarene series via a direct route utilising the Petrolite Procedure.\textsuperscript{98} Initiation of the project preceded the Raston paper, which was published at the end of the first year of this Ph.D., therefore at that time no one-step synthesis of the tetramer or hexamer had been reported, and the octamer had only been reported in 4.4% yield from the one-step Petrolite Procedure.

Initial reactions to synthesise the octamer 77 were carried out using the Petrolite Procedure, with two molar equivalents of paraformaldehyde, and 0.05 molar equivalents of sodium hydroxide (50% aqueous solution, 50g in 100 ml). The reaction was carried out in refluxing xylene for four hours, with removal of water into a Dean-Stark Trap (scheme 3.1.1).

\begin{center}
\includegraphics{scheme311.png}
\end{center}


TLC analysis of the crude reaction mixture revealed the presence of starting material $p$-phenylphenol, and three other products, with $R_f$ values of 0.24, 0.84 and 0.95.
(hexane/ethyl acetate 2:1). Separation of these compounds was not however straightforward, as the crude material was sparingly soluble in most organic solvents. Separation was eventually achieved using flash column chromatography and eluting with a hexane/ethyl acetate mixture (3:1). Mass spectral analysis revealed that the three compounds were the phenylcalix[8]arene 77, phenylcalix[6]arene 76, and phenylcalix[4]arene 75, respectively, in 16%, 4% and 7% yield respectively (see Table 3.1.1). At that time this was the first synthesis of the hexamer and tetramer via the one-step Petrolite Procedure.

Alternative conditions were used, similar to those used by Gutsche in the synthesis of the alkylcalixarenes, which utilised a 10 M (approximately 40% aqueous for NaOH) solutions of base. Therefore the reaction was repeated using a 10 M solution of sodium hydroxide, in 0.03 equivalents. This reaction again produced the three major ring sizes of the phenylcalixarene series, though in different yields: the octamer 77 in 13% yield; the hexamer 76 in 4%; and the tetramer 75 in only 0.6% yield.

Given that a templating effect can be exerted both upon the reactions of calixarenes with electrophiles, and upon their synthesis, the base was changed to potassium hydroxide to produce a higher yield of the octamer. Therefore the reaction was carried out using a 10 M solution of potassium hydroxide. The results were very interesting, the yield of the octamer increased significantly from 13% to 27%, whilst the hexamer yield decreased to 0.2% and no tetramer was formed.

Potassium hydroxide was then used as a 50% aqueous solution, since the 50% aqueous solution of sodium hydroxide had resulted in a higher yield than the 10 M solution. However the use of a 50% aqueous potassium hydroxide solution in 0.075 equivalents, produced a pronounced result. Initially it was believed that the octamer had been formed in very high yield, 60%, along with the hexamer in 8% yield, and 2% of the tetramer. However mass spectral analysis revealed that the compound we had isolated by flash chromatography was not the octamer, but was instead the heptamer 111 (scheme 3.1.2).
This was an extremely surprising result as the heptamer \textbf{111} had only been mentioned by Gutsche in his 1985 paper, when he had observed by TLC a small amount of a compound which he thought may be the heptamer, and in a Japanese Patent which Gutsche discounts in the same paper.\textsuperscript{140,141} The synthesis of an odd numbered calixarene \textit{via} a one-pot synthesis is not known, and the heptamer is also formed in extremely high yield, 60\%, compared to the other ring sizes of the phenylcalixarene series. The heptamer was only formed from this one set of reaction conditions, though the conditions stated above produced the heptamer as the main product, no octamer was generated.

It was decided that an increase in the number of equivalents of base used in the reaction may further increase the yield of the heptamer, so potassium hydroxide was used in 0.15 equivalents instead of 0.075. However this reaction produced no cyclic compounds, indicating how sensitive the calixarene series is to the conditions used.

As it was believed that a templating effect may be playing a role in the synthesis of the differing ring sizes, caesium hydroxide was also used in the reaction, initially in 0.075 equivalents (50\% aqueous solution). This reaction afforded the octamer \textbf{77} in 28\% yield, the hexamer \textbf{76} in 1\% yield, and no tetramer \textbf{75} or heptamer \textbf{111}. A decrease in the number of equivalents of base (to 0.05) afforded the octamer in its highest yield, 34\%, 1.5\% of the hexamer, and 0.5\% of the tetramer. A second decrease in the number
of equivalents, this time to 0.015, afforded the octamer in lower yield, 21%, the hexamer in 0.7% and the tetramer in 3% yields. A large increase in the number of equivalents to three afforded no cyclic products at all.

A further increase in the size of the cation of the base, by using rubidium hydroxide in 0.05 equivalents, afforded the octamer 77 in 14% yield, the hexamer 76 in 1.2% and tetramer 75 in 0.6% yield. An increase in the number of equivalents to 0.15 afforded only the octamer, in 8% yield. The divalent bases calcium and barium hydroxide did not produce any cyclic products, which was not surprising since this has not been preceded in the literature.

Therefore, the highest yield of the octamer via a standard Petrolite Procedure used 0.05 equivalents of a 50% aqueous solution of caesium hydroxide. Synthesis of the heptamer used 0.075 equivalents of a 50% aqueous solution of potassium hydroxide. The same reaction also gave rise to the highest yield of the hexamer, 8%. It was believed that a templating effect was occurring, with the larger cation sizes giving rise to the larger ringed calixarenes. For example, the hexamer was only formed in very minimal yield when the ionic radius of the cation was greater than 1.33 Å (K⁺). The highest yield of the tetramer was achieved with sodium hydroxide (0.05 eq. 50% aq.). Although one reaction with caesium hydroxide did afford the tetramer in 3% yield, most reactions using potassium hydroxide and larger size bases did not afford the tetramer at all.

A further investigation into the possible templating effect upon synthesis of the tetramer was then carried out. Use of lithium hydroxide, in 0.075 and 0.15 equivalents, as a 50% aqueous solution, afforded only the tetramer (0.2% and 1% respectively), and no other ring sizes. It is believed that the small cationic radius of lithium favours the synthesis of the smallest calixarene, and that the cation is too small to hold the two hemi-calixarenes involved in synthesis of the octamer (see Section 2.2.2 ) in place to allow the ring closure reaction to occur.
In order to examine the effect of the concentration of bases upon the reaction, both sodium and potassium hydroxide were used as 2% aqueous solutions (by mass). Both bases were used in 0.075 equivalents, and neither reaction produced any phenylcalixarenes.

<table>
<thead>
<tr>
<th>Base</th>
<th>% Aq. Soln</th>
<th>Eq</th>
<th>Aldehyde</th>
<th>Aldehyde</th>
<th>% 77</th>
<th>% 76</th>
<th>% 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0.2%</td>
</tr>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>NaOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>(10 M)</td>
<td>0.03</td>
<td>[HCHO]n</td>
<td>2</td>
<td>13%</td>
<td>4%</td>
<td>0.6%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]n</td>
<td>2</td>
<td>16%</td>
<td>4%</td>
<td>7%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>33%</td>
<td>0.3%</td>
<td>1%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>4</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>(10 M)</td>
<td>0.03</td>
<td>Trioxane</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>27%</td>
<td>0.6%</td>
<td>0.3%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>4</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.015</td>
<td>[HCHO]n</td>
<td>2</td>
<td>21%</td>
<td>0.7%</td>
<td>3%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>3</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>28%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]n</td>
<td>2</td>
<td>34%</td>
<td>1.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>RbOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]n</td>
<td>2</td>
<td>14%</td>
<td>1.2%</td>
<td>0.6%</td>
</tr>
<tr>
<td>RbOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>8%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Ba(OH)₂</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 3.1.1. Yields of synthesis of the major ring sizes of phenylcalixarenes
The Petrolite Procedure utilised paraformaldehyde as the source of aldehyde in the reaction. Most of the reactions discussed above used two equivalents of paraformaldehyde. Formaldehyde (37% aq. solution) was also tested in two reactions, and again, no products resulted. The alternative formaldehyde source, 1,3,5-trioxane was also tested in four reactions. None of the reactions using 1,3,5-trioxane afforded phenylcalixarenes, presumably because an acid is needed to break up the trioxane ring.

\[ p\text{-Phenylcalix}[6]\text{arene} \, 76 \text{ was also synthesised using the Modified Zinke-Cornforth procedure which had been reported by Gutsche in 1985.} \]

Our yields compare favourably to those of Raston for synthesis of the phenylcalixarenes from his modified Petrolite Procedure.\(^{118}\) He used 15 M solutions of sodium and potassium hydroxide, which are significantly more concentrated than ours, and although he did not observe the heptamer as we did, he isolated the pentamer which was not generated under the conditions we used. Several of our reactions are similar to those reported by Raston, notably his reaction using 0.045 equivalents of sodium hydroxide, and ours using 0.05 equivalents of the same base. Raston reported a 30% yield for the octamer, whilst we observed only a 16% yield, and for the hexamer he reported a 10% yield whilst our yield was 4%, though he reported no yield of the tetramer, which we observed in 7% yield. Likewise for the potassium hydroxide reaction (ours in 0.075 equivalents) we found that the heptamer was formed exclusively instead of the octamer, in very high yield, 60%, whereas Raston reported that the octamer was generated in 18% yield. However yields for the hexamer and tetramer are very similar, 8% and 11% for the hexamer from our findings and Raston’s respectively, and 2% and 3% for the tetramer.

At greater numbers of equivalents we used sodium hydroxide in 0.15 equivalents and Raston used a more concentrated solution in 0.18 equivalents. Raston generated no
products from this reaction, whereas we synthesised the octamer in 33% yield with less significant amounts of the hexamer and tetramer (see Table 3.1.1).

### 3.1.1 Purification of Phenylcalixarenes

The phenylcalixarenes are poorly soluble white solids, which caused problems when purifying using conventional methods of flash column chromatography. Although it was possible to purify the calixarenes using flash column chromatography, the crude mixture has to be loaded onto the column as a solid bound to silica, and the procedure was slow as retention times on the column were long. Also the procedure often needed to be repeated due to streaking of the material. Therefore a more convenient method of separating the octamer and heptamer from the smaller ring sizes in the crude mixture was sought.

Various solvents were examined in the hope of allowing separation of the octamer or heptamer from the smaller ring sizes due to their differing solubilities. However ethyl acetate, diethylether, ethanol, acetone, hexane, dichloromethane, chloroform and an ethyl acetate/hexane mixture (1:1, 2:1, 1:2, 1:3), and a variation in the length of time of stirring from thirty minutes to two days, did not afford separation of the products. Neither did variation in the temperature used, from room temperature to the reflux temperature of the solvent. Separation was eventually achieved by stirring the crude mixture from a reaction in a large excess of methanol at room temperature for thirty minutes, removal of the remaining solid by filtration and stirring of this solid under the same conditions in fresh methanol. Repetition of this procedure (up to five times) afforded the pure octamer 77 or heptamer 111.

### 3.1.2 Characterisation of Phenylcalixarenes

The phenylcalixarenes have extremely high melting points (>400 °C). The best method for determining which ring size had been formed was mass spectrometry. However, all ring sizes do suffer from extensive fragmentation using FAB, resulting in a small molecular ion peak. Another feature of these calixarenes was that they could form host-
guest complexes with both solvent (when using ES method, for example methanol/water) and ions, most commonly in our case sodium ions.

Obtaining good NMR spectroscopy data of the phenylcalixarenes was not trivial due to their poor solubility and poor relaxation. Gutsche reported both $^1$H and $^{13}$C NMR spectra for the phenylcalix[4]arene 75, proton and carbon NMR for the phenylcalix[6]arene 76, though these were incomplete (see above), and only $^1$H NMR data for the octamer. Raston also did not report NMR spectral data for the phenylcalix[8]arene 77, only for its sulfonated derivative 80. The Japanese patent for the phenylcalix[7]arene 111 only contained $^1$H NMR, and therefore no $^{13}$C NMR spectral data for the heptamer or octamer have ever been published.

Our initial efforts focused on a full characterisation of the heptamer 111. Using acetone and chloroform solvents an initial $^{13}$C NMR spectrum were obtained with eight signals, including the methylene bridge resonance at $\delta$ 32.68. Use of solid state NMR afforded the missing C-1 shift. The data, together with calculated shifts, are presented in Table 3.1.2.1, the spectra are presented in the appendix.
<table>
<thead>
<tr>
<th>Carbon #</th>
<th>Calculated Shift</th>
<th>Observed Shift (CD$_3$)$_2$CO</th>
<th>Observed Shift CDCl$_3$</th>
<th>Observed Shift Solid State</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154.5</td>
<td>126.58</td>
<td>126.88</td>
<td>149.11</td>
</tr>
<tr>
<td>2</td>
<td>126.3</td>
<td>126.95</td>
<td>128.47</td>
<td>128.19</td>
</tr>
<tr>
<td>3</td>
<td>127.8</td>
<td>133.51</td>
<td>135.58</td>
<td>127.03</td>
</tr>
<tr>
<td>4</td>
<td>141.5</td>
<td>141.60</td>
<td>140.79</td>
<td>134.07</td>
</tr>
<tr>
<td>5</td>
<td>127.4</td>
<td>126.82</td>
<td>127.03</td>
<td>134.07</td>
</tr>
<tr>
<td>6</td>
<td>129</td>
<td>129.29</td>
<td>128.70</td>
<td>134.07</td>
</tr>
<tr>
<td>7</td>
<td>127.5</td>
<td>127.73</td>
<td>128.12</td>
<td>134.07</td>
</tr>
<tr>
<td>8</td>
<td>30-34</td>
<td>32.63</td>
<td>32.75</td>
<td>32.74</td>
</tr>
</tbody>
</table>

Table 3.1.2.1. Comparison of calculated and observed $^{13}$C NMR shifts in different solvents for phenylcalix[7]arene

Good correlation between the calculated and observed chemical shifts was observed. C-1 was only observed via solid state NMR spectroscopy due to its extremely poor relaxation. Long experiments were required in order to obtain NMR spectra, up to 13,000 scans. (See appendices 1 and 2 for details of machine parameters.)

Carbon-proton correlation spectra were obtained (see appendix), confirming the methylene bridges, at δ 4.10, in the $^1$H spectrum and in the $^{13}$C NMR spectrum at δ 32.63. The phenolic proton was only observed at δ 10.54 when a saturated solution of the heptamer was prepared in chloroform.

It has been reported by Mendoza et al that for calixarenes with aromatic rings in the syn position the methylene bridge carbon resonance is observed in the region δ 30-34, whilst for anti symmetry the resonance is higher at δ 35-38. The shift for the methylene bridge of the heptamer was observed within the δ 30-34 region under all conditions, therefore suggesting that the aromatic rings are in a syn orientation.

To acquire data for the phenylcalix[8]arene 77 similar machine parameters were used as for the heptamer. However the spectra of the octamer were significantly less well
defined, and no signals were observed for any of the quaternary carbons. We therefore concluded that relaxation of the aromatic quaternary carbons of the octamer was significantly slower than for the heptamer, and increased the relaxation delay to six seconds and the number of scans to 30,000. This afforded a spectrum where the aromatic region was well defined (figure 3.1.2.2), including the C-1 resonance which had only been observed for the heptamer by solid state NMR.

Again good correlation was observed between the calculated and observed shifts with the methylene bridge at $\delta$ 33.51, a slightly higher shift than for the heptamer, but still within the syn range as described by Mendoza. The shift observed for the C-1 carbon was significantly higher for the octamer than for the heptamer, $\delta$ 154.01 compared with $\delta$ 149.11, however this is closer to the calculated figure of $\delta$ 154.5.

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>Calculated Shift</th>
<th>Observed Shift (CD$_3$)$_2$CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154.5</td>
<td>154.01</td>
</tr>
<tr>
<td>2</td>
<td>126.3</td>
<td>126.79</td>
</tr>
<tr>
<td>3</td>
<td>127.8</td>
<td>129.38</td>
</tr>
<tr>
<td>4</td>
<td>134.2</td>
<td>132.81</td>
</tr>
<tr>
<td>5</td>
<td>141.5</td>
<td>142.31</td>
</tr>
<tr>
<td>6</td>
<td>127.4</td>
<td>127.18</td>
</tr>
<tr>
<td>7</td>
<td>129</td>
<td>130.35</td>
</tr>
<tr>
<td>8</td>
<td>127.5</td>
<td>127.85</td>
</tr>
</tbody>
</table>

Table 3.1.2.2. Comparison of calculated and observed $^{13}$C NMR shifts for phenylcalix[8]arene
A $^1$H NMR spectrum of the octamer was obtained in deuterated DMSO, and showed a single resonance for the methylene bridges, at $\delta$ 4.01. The phenolic proton was not observed.

$^{13}$C NMR spectra of the phenylcalix[6]arene 76 were also obtained in acetone, with a long relaxation delay and 31,000 scans. Again excellent correlation was shown between the observed and calculated shifts, and the C-1 carbon was observed at a value closer to that seen for the heptamer, at $\delta$ 150.23. The aromatic region of the NMR is shown below in figure 3.1.2.3. The methylene bridge resonance was again within the $\text{syn}$ region, at $\delta$ 33.43, a value between that for the octamer and heptamer.

![Fig. 3.1.2.3. Aromatic region of $^{13}$C NMR of phenylcalix[6]arene](image)

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>Calculated Shift</th>
<th>Observed Shift $(\text{CD}_3)_2\text{CO}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154.5</td>
<td>153.95</td>
</tr>
<tr>
<td>2</td>
<td>126.3</td>
<td>126.67</td>
</tr>
<tr>
<td>3</td>
<td>127.8</td>
<td>129.23</td>
</tr>
<tr>
<td>4</td>
<td>134.2</td>
<td>132.71</td>
</tr>
<tr>
<td>5</td>
<td>141.5</td>
<td>142.23</td>
</tr>
<tr>
<td>6</td>
<td>127.4</td>
<td>127.08</td>
</tr>
<tr>
<td>7</td>
<td>129</td>
<td>130.26</td>
</tr>
<tr>
<td>8</td>
<td>127.5</td>
<td>127.75</td>
</tr>
</tbody>
</table>

Table 3.1.2.3. Comparison of calculated and observed $^{13}$C NMR shifts for phenylcalix[6]arene
$^{13}$C NMR data for the tetramer was obtained in acetone, again with a relaxation delay of six seconds. However it was much easier to obtain a carbon NMR of the small size calixarene and the number of scans was reduced to 7,500 scans. The aromatic region is shown below in figure 3.1.2.4, and the methylene bridge signal was at δ 32.91. The tetramer had a lower value resonance for the C-1 carbon, at δ 149.61, close to that observed for the heptamer.

![Fig. 3.1.2.4. Aromatic region of $^{13}$C NMR of phenylcalix[4]arene](image)

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>Calculated Shift</th>
<th>Observed Shift (CD$_3$)$_2$CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154.5</td>
<td>149.61</td>
</tr>
<tr>
<td>2</td>
<td>126.3</td>
<td>126.16</td>
</tr>
<tr>
<td>3</td>
<td>127.8</td>
<td>128.72</td>
</tr>
<tr>
<td>4</td>
<td>134.2</td>
<td>132.19</td>
</tr>
<tr>
<td>5</td>
<td>141.5</td>
<td>141.72</td>
</tr>
<tr>
<td>6</td>
<td>127.4</td>
<td>126.56</td>
</tr>
<tr>
<td>7</td>
<td>129</td>
<td>129.74</td>
</tr>
<tr>
<td>8</td>
<td>127.5</td>
<td>127.23</td>
</tr>
</tbody>
</table>

Table 3.1.2.4. Comparison of calculated and observed $^{13}$C NMR shifts for phenylcalix[4]arene

As expected there is a large degree of similarity between the $^{13}$C NMR data for the phenylcalixarene series, however there are also marked difference between the differing
ring sizes, notably with the C-1 resonance. However mass spectrometry affords the easiest confirmation of the size of calixarene which has been formed. Comparison of spectral data for our phenylcalixarenes with published data reveals that the majority of carbon signals for the hexamer are in close agreement with those published by both Gutsche (although one signal is missing in that data) and Raston, although both report the shift for the C-1 signal as around δ 149, which is significantly lower than the shift we observed.\(^{120,140}\) However our data were recorded in acetone, and both that of Gutsche and Raston were recorded in chloroform. It is also possible that concentration effects can alter a chemical shift. For the tetramer all signals are in good agreement with the data published by both Gutsche and Raston, including the C-1 signal. Methylene bridge shifts for both compounds are in agreement with literature data.

<table>
<thead>
<tr>
<th>Phenylcalixarene</th>
<th>C-1 Shift</th>
<th>Raston(^{130})</th>
<th>Gutsche(^{140})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octamer</td>
<td>154.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heptamer</td>
<td>149.11 (Solid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>State)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexamer</td>
<td>153.95</td>
<td>149.35</td>
<td>149.23</td>
</tr>
<tr>
<td>Tetramer</td>
<td>149.61</td>
<td>148.69</td>
<td>148.8</td>
</tr>
</tbody>
</table>

Table 3.1.2.5. Comparison of recorded \(^{13}\)C NMR shifts of the phenolic carbon of different phenylcalixarene ring sizes

3.2 Synthesis of Other Unsubstituted Calixarenes

In order to investigate the possibility of novel upper rim functionality in calixarenes, we synthesised the octamer and hexamer of the tert-butyl calixarene series, with the aim of utilising the de-tert-butylation reaction developed by Gutsche, to access the so-called nude calixarenes.\(^{115,116}\) Therefore tert-butylcalix[8]arene 52 was synthesised following the standard Petrolite Procedure, in 48% yield (scheme 3.2.1).
Synthesis of tert-butylcalix[6]arene 51 was also carried out as it was hoped that the smaller number of reactive sites would make for a more tractable compound once the de-tert-butylation reaction had been carried out. We chose the Modified Petrolite Procedure, using rubidium hydroxide to synthesise the hexamer, as reported by Gutsche in 1981.\textsuperscript{99} The hexamer was generated in 48\% yield (scheme 3.2.2).

Purification of the tert-butylcalixarenes was far more straightforward than for the phenyl series; both the octamer and hexamer were recrystallised from chloroform. With the tert-butylcalixarenes in hand, we turned our attention to the removal of the tert-butyl groups. Gutsche had reported in 1986 the removal of the tert-butyl groups, as discussed in Section 2.1.3.\textsuperscript{115,116} We followed Gutsche's procedure for the reaction, but
obtained only partial removal of the tert-butyl groups using the reaction time prescribed by Gutsche, one hour. Re-exposure of the partially de-tert-butylated calixarene to the reaction conditions afforded a compound which was then shown by mass spectrometry to have between one and three tert-butyl groups remaining. A third exposure of this compound to the reaction conditions afforded the fully de-tert-butylated calix[8]arene. In order to improve the efficiency of the reaction we increased the reaction time of the first exposure of the tert-butylcalix[8]arene to 72 hours, and full de-tert-butylation was achieved in one-step in near quantitative yield.

Removal of the tert-butyl groups from the hexamer was considerably lower yielding. Gutsche had reported a decrease in the yield of the hexamer which was achieved (89%), however in our hands, using the same conditions which we had applied to the octamer, we achieved only a 19% yield of the nude calix[6]arene 112 (scheme 3.2.3).115,116

Scheme 3.2.3. Synthesis of calix[6]arene

With the nude calixarenes in hand we turned our attention to functionalisation of the upper rim. In order to investigate whether the biological activity which we had observed (see Chapter 7) for our calixarenes, which was enhanced when the calixarenes were PEGylated, was due to increased water solubility or other effects, we needed to confer water solubility to the calixarene via a different functionality. Therefore we
synthesised the water soluble sulfonato-calix[8]arene, using the procedure of Shinkai (scheme 3.2.4).\textsuperscript{117,118}

\begin{center}
\begin{tikzpicture}
\node at (-1,0) {73};
\node at (1.5,0) {113};
\node at (0,0.5) {$\text{c}_2\text{H}_5\text{SO}_4$};
\end{tikzpicture}
\end{center}

Scheme 3.2.4. Synthesis of sulfonatocalix[8]arene

Exposure of the nude calix[8]arene 73 to concentrated sulphuric acid at 80 °C for four hours afforded the fully sulfonated calixarene 113 in high yield 90\% (scheme 3.2.4). This compound was completely water soluble.

We were also interested in the biological effects of acetyl groups attached to the upper rim of the calixarene, as the phenylcalix[8]arene with eight acetyl groups at the lower rim had shown marked pro-tuberculosis effect (see Chapter 7). Shinkai had fully acylated the upper rim of the nude calixarene (sizes 4,6 and 8) series using a one-step procedure, with hexanoyl chloride and dodecanoyl chloride, whilst we applied the procedure using the much smaller acetyl chloride.\textsuperscript{141} The reaction was heated at 70 °C for two days, whereas the Shinkai procedure stirred the reaction at 50 °C for ten hours, followed by warming to 70 °C for a further three hours. Shinkai reported that the lower temperature afforded the \( O \)-acylated product, and the increase in temperature was necessary for a Fries Rearrangement to occur to afford the \( C \)-acylated compound. However we found that the \( O \)-acylated product was exclusively generated using the Shinkai conditions, as observed by $^{13}$C NMR signals at $\delta$ 169 (C=O) and $\delta$ 21 (Me). Extension of the reaction by heating at 70 °C for a further day afforded a mixture of both \( O \)-acylated and \( C \)-acylated compounds. We achieved the fully \( C \)-acylated compound only when the reaction was carried out for the whole period at 70 °C (scheme 3.2.5).
3.3 Summary

We have synthesised the phenylcalixarene series in reasonable yield, from the one-pot Petrolite Procedure. The phenylcalix[8]arene has been fully characterised using $^{13}$C NMR for the first time, as has the phenylcalix[7]arene. We have investigated the use of a number of different bases in the reaction, and have demonstrated a templating effect upon the formation of the phenylcalixarenes.

We have also synthesised the tert-butylcalix[8]arene and calix[6]arene, and the nude calix[8]arene and calix[6]arene. Sulfonation of the nude calix[8]arene was carried out to afford a fully water soluble compound. Acylation of the calix[8]arene has also been carried out, and adaptations to the procedures of Gutsche for synthesis of the nude calix[8]arene, and of Shinkai for synthesis of the acylated calixarenes are described.
Chapter 4

Polyethyleneglycol Chains

4.0 Introduction

Polyethyleneglycol (PEG) chains are widely utilised in the chemical industry as solubilising agents and to aid drug delivery. They are often found as polymers of average molecular weight, and there are very few directed syntheses of defined PEG-units. As discussed in Section 2.0, one of the first reported manipulations of PEG was by Perry and Hibbert in 1936. They reported the coupling of two n-mers of PEG, to give non-functionalised PEG-chains, as shown below in scheme 4.0.1.

\[
\begin{align*}
\text{Cl} & \quad (\text{O})_x \quad \text{Cl} + 2 \text{HO} (\text{O})_y \quad \text{OH}^- \\
& \quad + 2 \quad \text{HO} \quad (\text{O})_y \quad \text{OH}^- \\
& \quad \text{HO} \quad (\text{O})_{x+2y+2} \quad \text{OH} \\
\end{align*}
\]

Scheme 4.0.1. Synthesis of PEG-chains using the Perry and Hibbert method

In a later report by Hibbert, Lovell and Fordyce this method was utilised to synthesise PEG chains of length six, eighteen and forty two units. They reported the synthesis of hexaethylene glycol in 48% yield, with purification of the product via high vacuum distillation. Synthesis of PEG-18 was reported in 50% yield, this time with purification of the product via recrystallisation, likewise for PEG-42, reported in 47% yield. However further selective reaction of these diols is not reported, particularly desymmetrisation reactions.

A more recent procedure for PEG-chain extension was reported in 1989 by Bartsch, et al. Reactions were carried out using the phase transfer catalyst tetrabutylammonium
hydrogen sulphate, with one equivalent of tetraethyleneglycol 115, and six equivalents of the mono-THP-mono-chloro-ethyleneglycol 116 at 65 °C for three days, and afforded the di-THP protected hexaethyleneglycol 117 in 80% yield (scheme 4.0.2).

![Scheme 4.0.2. Synthesis of di-THP protected hexaethyleneglycol](image)

More recently the synthesis of PEG-chains of up to twenty eight units in length by use of an iterative procedure which is outlined below (scheme 4.0.3) has been described.

![Scheme 4.0.3. Iterative method of coupling PEG-chains to synthesis PEG-diols](image)

Reactions were carried out for three days at room temperature, and purification of the product was achieved by use of gel filtration, which had to be repeated at least ten times. The dodecaethyleneglycol 120 was afforded in 82% yield from 118, with 122 afforded in 37% yield from 120.

Desymmetrisation via mono-activation or mono-protection of PEG-chains is a vital step if they are to be useful synthetic building blocks. In 1990 a method for the mono-tosylation of PEG-chains was reported. The method utilised a 1:1 mixture of THF and water as solvent, with sodium hydroxide solution. Mono-tosylation of mono-
methyl PEG chains was reported in 88-90% yield. Mono-tosylation of PEG-3 diol was reported in 1997 using sodium hydride in THF, to afford the mono-tosyl-PEG-3 in 80% yield.\textsuperscript{152}

4.1 Synthesis of PEG-Chains

In our approach to the synthesis of PEG-chains our aims were to desymmetrise the PEG-chain through synthesis of mono-activated and mono-protected PEG-chains of varying length.

4.1.1 Protection of PEG-Chains

In our synthetic work a base stable protecting group was required, that would also not migrate under basic conditions. The initial choice of protecting group was a benzyl moiety (scheme 4.1.1.1).

![Scheme 4.1.1.1. Synthesis of benzyl-protected PEG-chains](image)

Mono-selectivity was achieved using 0.9 equivalents of base, one equivalent of benzyl bromide, a large solvent volume and addition of reaction components at low temperature. The initial reaction conditions were tested on triethylene glycol and tetraethylene glycol, where yields of 85% (123) and 82% (124) were achieved. However when the reaction was carried out using hexaethylene glycol, compound 125 was afforded in significantly lower yield, 62%. The lower yield for PEG-6 compared to either PEG-4 or PEG-3 is a pattern which is repeated throughout our PEG investigations. The addition of one ethylene glycol unit to PEG-3 does not appear to
greatly affect its reactivity, but a doubling of the length of the PEG chain has a noticeable effect. One of the reasons for this is likely to be the enhanced folding of a longer PEG chain, leading to reduced accessibility of reactants to the reactive termini of the chain. The yield achieved for the synthesis of 125 was however acceptable for a mono-protection strategy, particularly because any starting material glycol was readily removed by an aqueous wash, and the small amount (<2%) of di-protected compound 128 was readily removed via flash chromatography to afford the mono-benzyl-hexaethyleneglycol 125.

In order to test deprotection of the PEG chain, dibenzyl-tetraethyleneglycol 127 (scheme 4.1.1.2) was synthesised in quantitative yield using an excess of both sodium hydride and benzyl bromide. Hydrogenation was the method of choice for removal of the benzyl protecting groups, as this avoided the use of acids which can degrade the PEG chain.\textsuperscript{153} Hydrogenation using Pd/C resulted in no de-benzylation. However full deprotection was achieved when palladium hydroxide on carbon (Pearlman’s Catalyst) was used, in near quantitative yield (98%).\textsuperscript{153} Unfortunately removal of the protecting group when the PEG-chain was coupled to the calixarene could not be achieved.\textsuperscript{154} It was rationalised that the termini of the PEG chains could fold up into the annulus of the calixarene, resulting in very low accessibility of the protecting group.

\begin{equation}
\begin{array}{c}
\text{HO} \\
\text{Pd(OH)}_2/C \\
\text{H} \_2 \\
\text{NaH, THF}
\end{array}
\end{equation}

\begin{equation}
\begin{array}{c}
\text{115} \\
\text{127}
\end{array}
\end{equation}

Scheme 4.1.1.2. Synthesis of di-benzyl protected PEG-4, and deprotection using hydrogenation

Therefore an alternative protecting group strategy was sought, to more readily afford deprotected products once the PEG chains were coupled to the calixarene. THP
protection was selected due to its base stability, and standard removal conditions involving the use of dilute acids.

A test reaction on tetraethyleneglycol (scheme 4.1.1.3) afforded the mono-THP compound 129 in 46% yield. Again mono-selectivity was achieved by the use of 0.9 equivalents of the protecting group, with a catalytic amount of acid, a large volume of solvent and the addition of reactants at 0 °C. Purification of 129 was achieved by removal of the starting glycol with an aqueous wash, then flash column chromatography which revealed only a trace amount of the diprotected compound 131 (4%), had been formed. Flash column chromatography was not therefore frequently performed as the diprotected compound would remain unreactive in any future work and was only present in trace quantities. The analogous reaction using hexaethyleneglycol gave 130 in 59% yield. To confirm that the compounds could be readily deprotected 130 was treated with 1 M HCl in methanol and dichloromethane for one hour at room temperature, and afforded hexaethyleneglycol in quantitative yield.

However removal of the protecting group once the PEG-chain was coupled to the calixarene again proved to be problematic. Under standard conditions (1 M HCl) deprotection could not be achieved, though it was successfully carried out when the concentration of the acid was increased to 18 M from 1 M, and for some compounds the time was increased from one hour to eighteen hours (see Chapter 5). Fortunately no degradation of the PEG-chains was observed even in the presence of 18 M acid.

With a successful protection and deprotection strategy achieved we turned our attention to activation of the PEG-chains, for coupling of the PEG-chains together, and attachment to calixarenes.
4.1.2 Activation of PEG-Chains

Activation of PEG-chains was initially carried out via conversion of the hydroxyl termini to sulfonic esters, as they are readily formed and are extremely good leaving groups. Cornforth activated PEG-chains for coupling to calixarenes both as mesylates and tosylates, though he found the tosyl activated PEG-chain was not capable of fully derivatising the calixarene. However for PEG-chain to PEG-chain couplings and attachment to the calixarene, both mesyl and tosyl groups were considered.

Mono-tosyl-hexaethyleneglycol 37 was formed in 52% yield by reaction of the diol 133 with triethylamine and tosyl chloride in dichloromethane (scheme 4.1.2.1). High reaction selectivity was achieved again through use of limited equivalents of both base and electrophile. The formation of no di-tosylated product was observed.

![Scheme 4.1.2.1. Mono-tosylation of PEG-6](image)

Mono-mesylation of triethyleneglycol 134 gave 135 in 35% yield (scheme 4.1.2.2). When 0.9 equivalents of mesyl chloride were used 135 was formed together with the dimesy1 136, which could be separated via flash chromatography. However when the number of equivalents were reduced to 0.5, compound 135 was generated as the sole product in 35% yield. Therefore for ease of purification the reaction was carried out with 0.5 equivalents of mesyl chloride.

![Scheme 4.1.2.2. Mesylation of triethyleneglycol](image)

Mesylation of mono-benzyl-hexaethyleneglycol 125 afforded compound 137 in 88% yield (scheme 4.1.2.3).
Scheme 4.1.2.3. Mesylation of mono-benzyl-PEG-6

Dimesyl-hexaethyleneglycol 138 was synthesised for further reaction in PEG chain extensions (scheme 4.1.2.4). The yield of the reaction was 78%, despite the use of three equivalents of both base and mesyl chloride, due to loss of product during the extensive washing which was required to remove the excess mesyl chloride. An excess of mesyl chloride is required as two equivalents led to the formation of a mixture of mono and dimesylated products.

Scheme 4.1.2.4. Dimesylation of hexaethyleneglycol

PEG chains were also synthesised with halide activation. Mono-benzyl-hexaethyleneglycol 125 was chlorinated using thionyl chloride and pyridine acting as both solvent and base, in 75% yield (scheme 4.1.2.5).

Scheme 4.1.2.5. Chlorination of mono-benzyl-PEG-6

Dichlorination of triethyleneglycol was achieved in quantitative yield, and the same procedure afforded the dichlorinated PEG-6 compound, 140, in 95% yield (scheme 4.1.2.6). These dichlorinated units were of interest for the synthesis of longer PEG chains.

Scheme 4.1.2.6. Di-chlorination of hexaethyleneglycol
Mono-chlorination of hexaethyleneglycol 133, was achieved in 31% yield (scheme 4.1.2.7), again by use of limiting amounts of base and thionyl chloride, though in this case as pyridine was acting as both the base and solvent a large solvent volume could not be used. Therefore it was necessary to separate the dichlorinated and mono-chlorinated products using flash chromatography. The mono-chlorinated compound 141 was then protected with a THP protecting group in 62% yield, rather than protection prior to activation since the acidic aqueous wash necessary to remove pyridine in the earlier procedure may have affected the THP protecting group.

Bromination of PEG-chains was initially carried out using carbon tetrabromide and triphenylphosphine, as shown in scheme 4.1.2.8. However it was subsequently discovered that triphenylphosphine was able to complex to the PEG-chains, but was not detectable by $^1$H NMR spectroscopy, perhaps due to folding of the PEG around the triphenylphosphine. Once the brominated PEG-chain was exposed to sodium hydride, which was used to add the PEG-chain to the calixarene, displacement of the triphenylphosphine was observed, rendering it ‘visible’ by NMR spectroscopy, see Chapter 5.

Therefore compounds which had been synthesised using the triphenylphosphine method had to be re-synthesised using an alternative method. Chong has recently reported near total selectivity for the mono-bromination of a range of aliphatic diols using 50%
aqueous hydrobromic acid in toluene, at reflux for three days. The reaction was tested initially using mono-methylether-triethyleneglycol 143 (R = Me), and compound 144 was afforded in 53% yield (scheme 4.1.2.9). Mono-bromination of hexaethyleneglycol afforded compound 145 in 56% yield, which was then protected with THP, to afford 146 in 59% yield.

\[
\begin{align*}
&\text{RO}\bigg\{\begin{array}{c}O \\
h_n\end{array}\bigg\}_{n}OH \xrightarrow{HBr, \text{Toluene}} \text{RO}\bigg\{\begin{array}{c}O \\
h_n\end{array}\bigg\}_{n}Br \\
\end{align*}
\]

\[n = 2, R = \text{Me}, 53\%, 144\]
\[n = 5, R = \text{H}, 56\%, 145\]
\[n = 5, R = \text{THP}, 59\%, 146\]

Scheme 4.1.2.9. Synthesis of brominated PEG-chains using hydrobromic acid

4.1.3 Extension of PEG chains

The controlled synthesis of longer PEG-chains, as opposed to random condensation reactions, is extremely challenging. Therefore in order for longer chain PEGs to be homogeneous compounds they needed to be synthesised via a protection and activation strategy.

In general as the length of the PEG-chain increases the reactivity of the diol decreases, due to greater coiling and wrapping effects, and long reaction times were often necessitated to synthesise long chains. It was also necessary that the starting materials for the synthesis of longer PEG-chains were themselves readily available, in high yield and purity, to avoid undesired side reactions.

Purification of longer PEG-chains was also challenging, as it was not possible to use normal phase flash chromatography as the difference in polarity of longer PEG-chains on normal phase chromatography does not afford good separation. Therefore a reverse phase flash chromatography system was developed in which compounds were columned using reverse phase silica, eluting with a gradient from 100% water to 100% acetonitrile. Recycling of the reverse phase silica reduced the cost of this expensive procedure, which afforded a clean, efficient separation of the PEG-chains.
4.1.3.1 Synthesis of PEG-6

In order to test reaction conditions for the synthesis of long PEG-chains, a synthesis of PEG-6 was initially undertaken (scheme 4.1.3.1.1). Mono-benzyl-triethyleneglycol 123, and dichloro-triethyleneglycol 147 were reacted 1:1, with one equivalent of sodium hydride, in refluxing THF for two days. Reverse phase chromatography (acetonitrile/water) afforded compound 139 in 62% yield, and a small amount of compound 148 which resulted from the coupling of 123 to both termini of 147.

![Scheme 4.1.3.1.1. Coupling of PEG-chains using sodium hydride](image)

4.1.3.2 Synthesis of PEG-9

The conditions applied to the synthesis of PEG-6 were then used to prepare PEG-9. Mono-benzyl-triethyleneglycol 123 was reacted with one equivalent of dichloro-hexaethyleneglycol 140 and one equivalent of sodium hydride (scheme 4.1.3.2.1). However the product 149 was only afforded in only low yield (12%) when using the same reaction conditions as for PEG-6. A doubling of the reaction time to four days however afforded the desired product in 53% yield. Reverse phase chromatography (acetonitrile/water) was necessary to purify 149, and again a small amount of the product from the coupling of 123 to both termini of 140, compound 150 was seen (<5%).
Scheme 4.1.3.2.1. Coupling of PEG-chains using sodium hydride

A second route was also investigated for the synthesis of PEG-9, using the PEG-chain activated as a mesyl (scheme 4.1.3.2.2).

Scheme 4.1.3.2.2. Coupling of PEG-chains using sodium hydride

However the yield of 151 was low, only 12%, presumably due to 135 self-reacting. It is of course also possible that 139 may react with any of the random polymerisation reaction products, leading to further complication. Therefore the route shown in scheme 4.1.3.2.1 was preferred for the synthesis of PEG-9.

4.1.3.3 Synthesis of PEG-12

Synthesis of PEG-12 was initially investigated using two different strategies. The first involved the reaction of 139 with 37 (1:1 ratio). This reaction also suffered from the self- reaction of 37, and the yield of product 152 was extremely low, 8% (scheme 4.1.3.3.1).
The synthesis of PEG-12 was also explored by reacting 149 and 135 (scheme 4.1.3.3.2). Again self-reaction of 135 occurred leading to a very low yield of compound 153, 5%.

A successful route to PEG-12 was devised using mono-THP-hexaethyleneglycol, 130, and dimesyl-hexaethyleneglycol, 138 (scheme 4.1.3.3.3). Two equivalents of 138 were used to try to minimise the formation of undesired PEG-18, 154. The product 153 was formed in 50% yield following a four day reflux. With a shorter reaction time the yield rapidly decreased, to 14% (2 days), but a longer reaction time (7 days) did not lead to an increase in yield of either 153 or the undesired PEG-18 product, which was not seen in significant quantities (<5%).
The purification of 153 was achieved by reverse phase chromatography (acetonitrile/water). However although the PEG-12 was activated, mesyl activation was not sufficient to react a PEG-chain with the calixarene (see Chapter 5), therefore a Finkelstein reaction was carried out to afford the iodinated PEG-12, compound 155.\textsuperscript{157} Though more usually a halide exchange reaction in which either the bromide or chloride are exchanged for the iodide or fluoride, we successfully applied the reaction to the mesylate (scheme 4.1.3.3.4).

Iodination was achieved in near quantitative yield, however partial removal of the THP protecting group occurred during the reaction to afford a mixture of 155 and 156. It is possible that small amounts of water may remain bound to the PEG-12 153, which are not removed even following drying under high vacuum conditions for several days. The water may then lead to the formation of small amounts of hydrogen iodide or mesic acid, resulting in partial removal of the THP group. Reprotection of 156 with THP was
effected on the mixture of 155 and 156, under standard conditions, in quantitative yield (scheme 4.1.3.3.4).

Towards the end of this Ph.D. however problems were encountered with the synthesis of 153, and no PEG-12 was present in the crude reaction mixture. Many factors of the synthesis were investigated, and will be discussed briefly here. Initially it was considered that the mono-THP species 130 was not fully deprotonating under reaction conditions (60% NaH), and therefore reactions were carried out with 95% NaH, however none of the PEG-12 compound 153 was generated.

As PEG-chains can readily complex ions and water, leading to folding of the PEG-chain around the ion or solvent molecule, the method of drying the PEG-chains 130 and 138 was also investigated. As the exact nature of the hydrophobic frit in phase separators is not disclosed by the manufacturers, drying of the PEG-chains with magnesium sulphate and sodium sulphate was also investigated. However no 153 was generated from reactions using 130 and 138 which had been dried in this way. A second source of ions was considered to be the drying of THF in a still, therefore anhydrous THF was purchased from Aldrich and used, again no 153 was observed.

Longer reaction times were also investigated in the synthesis of 153, however an extension of the reaction time to fourteen days also resulted in no generation of 153. Finally an alternative source of hexaethyleneglycol 133 was considered, as the Aldrich compound is only 97% pure, we considered that the nature of the 3% impurity may have changed, leading to inhibition of our reaction. Hexaethyleneglycol purchased from Lancaster was used in the synthesis of 130 and 138, and compound 153 was isolated from the reaction of these two compounds, though in lower yield than had previously been obtained (<15%). However we believe that as a thorough investigation of all other causes of the failure of the synthesis had been carried out, the source of the problem was an impurity in the hexaethyleneglycol 133 purchased from Aldrich.
4.1.4 Characterisation of PEG Chains

All PEG-chains synthesised were characterised by both $^1$H and $^{13}$C NMR spectroscopy, infra red spectroscopy and mass spectroscopy. In general different length PEG-chains are fairly indistinguishable by $^1$H NMR spectroscopy, due to the extensive overlap of signals in the central region of the chain (δ 3 - 4) and poor integration. $^{13}$C NMR is also ineffective at distinguishing different length PEG-chains due to overlap of signals. Therefore mass spectrometry was heavily relied upon to distinguish the different number of polymeric units present.

Electrospray was the method of choice for mass spectroscopy, as it resulted in lower levels of fragmentation of the PEG-chain. However fragmentation did still occur, and to determine whether a peak was due to fragmentation or a mixture of compounds the voltage used in the experiment was decreased and the sample repeated. If the peak was still visible and did not significantly decrease in size following the change in voltage it was unlikely to be as a result of fragmentation.

PEG-chains also readily complex ions and molecules (such as triphenylphosphine) within the mass spectrometer. Most commonly sodium ions complexed to the PEG-chain, and multiply charged peaks were occasionally observed for PEG chains. PEG-chains also complex solvent molecules, typically methanol and water.

4.2 Summary

PEG-chains of six, nine and twelve units have been synthesised in good yield. Methods of mono-protection have been developed for two protecting groups, benzyl and tetrahydropyran. Methods for mono-activation and di-activation with sulfonic esters and halides have also been developed. Purification of PEG-chains was achieved using reverse phase chromatography (acetonitrile/water).
Chapter 5

Calixarene Reactions

5.0 Introduction

The reported reactions of calixarenes with active electrophiles were discussed in Section 2.2. Our initial studies on the derivatisation of the phenylcalixarene series focused on the use of active electrophiles in order to assess the overall reactivity of the octamer ring system. Gutsche reported the full $O$-acetylation of $p$-phenylcalix[8]arene 157 in 1985, using pyridine as both base and solvent, in 30% yield.\textsuperscript{138} He also $O$-methylated the octamer 77 using 30 equivalents of sodium hydride and 60 of methyl iodide to afford the fully methylated product in 46% yield. These yields were significantly lower than the quantitative yields reported for reaction of tert-butylcalixarenes with active electrophiles (Section 2.2), highlighting that fact that the reactivity of the phenyl series is severely diminished. More recently Raston reported sulfonation of the phenylcalixarenes and mentioned difficulties in obtaining a pure product, though the yield for the conversion was not given.\textsuperscript{120}

5.1 $p$-Phenylcalix[8]arene and Active Electrophiles

As discussed above, the use of active electrophiles to derivatise the calixarenes has been extensively explored for the tert-butyl series, and a couple of derivatives for the phenylcalixarene series have been reported. We initially investigated the reaction of phenylcalix[8]arene 77 with three active electrophiles, acetyl chloride, allyl bromide, and ethyl bromoacetate. We used the procedure which Gutsche used for methylations since this had produced a higher yield of the fully substituted product than for the synthesis of the acetyl derivative. The compounds 157, 158, and 159 were obtained when using 30 equivalents of sodium hydride and 16 equivalents of the electrophile.
The number of equivalents of electrophile was reduced from 60 to 16 due to problems encountered when removing a large excess of the material.

![Figure 5.1.1](image-url)

**Fig. 5.1.1**

Compound 157 was synthesised in 46% yield, whilst 158 and 159 were isolated in 33% yield. The use of weaker bases had been reported for the addition of benzyl groups, and therefore the use of both caesium carbonate and potassium carbonate were explored. The reactions were carried out in DMF at 60 °C, with twelve equivalents of base and electrophile. The results are summarised below in table 5.1.1.

<table>
<thead>
<tr>
<th>Electrophile</th>
<th>NaH (30 eq.)</th>
<th>Cs$_2$CO$_3$ (12 eq.)</th>
<th>K$_2$CO$_3$ (12 eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetyl chloride</td>
<td>46%</td>
<td>26%</td>
<td>40%</td>
</tr>
<tr>
<td>allyl bromide</td>
<td>33%</td>
<td>21%</td>
<td>28%</td>
</tr>
<tr>
<td>ethyl bromoacetate</td>
<td>33%</td>
<td>0%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Table 5.1.1. Yields of reactions between phenylcalix[8]arene and active electrophiles using a variety of bases

Almost comparable yields were obtained when using potassium carbonate and acetyl chloride (compared to NaH), particularly when considering that fewer equivalents were utilised. For the least active of the electrophiles, ethyl bromoacetate, no product was afforded when using caesium carbonate, and only 18% yield was achieved with
potassium carbonate. Potassium carbonate afforded higher yields in all cases than caesium carbonate, which was expected as it is a stronger base. The isolated products were fully derivatised at all positions, however mixtures of partially derivatised products were also observed.

5.2 Phenylcalixarenes and PEG-chains

The reaction of calixarenes with PEG-chains has received little attention in the literature, however a few examples do exist for short PEG-chains (< 4 PEG units). The work of Cornforth with his PEGylated calixarenes was the first report (see Chapter 2), however he initially used a polymerisation reaction using ethylene oxide rather than reaction with pre-formed PEG-chains. Though later work did involve reaction with pre-formed PEG-chains, the characterisation data of these products is sparse. Ungaro reacted both the tert-octylcalix[8]arene and tert-butylcalix[8]arene with mono-tosyl-mono-methyl-PEG-chains (10 eq., up to 3 PEG-units), using potassium tert-butoxide (10 eq.) in refluxing benzene. He carried out the reactions until no free phenolic positions could be observed via UV spectroscopy. Although Ungaro reported that the calixarenes fully PEGylated under these conditions, no mass spectra are presented for the compounds, and a large degree of overlap of $^{13}$C NMR signals is presented. Therefore it is possible that the compounds are not fully derivatised.

PEGylated calixarenes have been referred to as 'octopus molecules', due to their hydrophobic hydrocarbon core (body) and the polyether ligands (tentacles) extending outwards from the body. One of the earliest reports of the synthesis of octopus molecules was in 1988, by Taniguchi and Nomura. Tert-butylcalix[6]arene 51 was fully PEGylated using 16 equivalents of a tosyl activated PEG-3 and 38 equivalents of potassium hydroxide, in refluxing dichloromethane for two days, with a catalytic amount of benzyltrimethylammonium chloride (scheme 5.2.1). The fully alkylated material 160, was reported to be synthesised in 94% yield, however no NMR or MS data were presented, therefore the exact nature of the compound is not proven.
A more thorough investigation into the PEGylation of tert-butylcalix[6]arene 51 and the tert-butyl tetramer 50, together with the nude hexamer 112 and tetramer 161, was published in 1991 (scheme 5.2.2).\textsuperscript{159} Brominated methoxy protected PEG-chains were added to calixarenes using sodium hydride, in a THF/DMF (10:1) mixture. Reaction of the nude hexamer 112 with PEG-2 (n=1) was carried out with 15 equivalents of PEG-2 and 18 equivalents of sodium hydride and a 24 hour reaction time to afford the compound 162 (where n=1) in 56% yield (scheme 5.2.2). When the PEG-chain length was increased to PEG-3 (n=2, 163) the yield decreased to 49%, and when it was increased still further to PEG-4 (n=3, 164), despite the use of 30 equivalents of PEG the yield decreased to a negligible 3%. The drastic drop in yield can be attributed to several factors. Firstly the reactivity of the PEG-chain decreases as the length increases, and secondly due to steric factors. The longer the PEG-chain the greater the amount of coiling, wrapping and aggregation that occurs, which also lowers the reactivity of the electrophile. Reactions between calixarenes and longer PEG-chains were not reported. It is possible that the PEG-chain may complex to the sodium ion present from sodium hydride, as shown in figure 5.2.1, leading to very limited accessibility of the PEG-chain, and that this effect is greater for longer PEG-chains.
For the reaction between tert-butylcalix[6]arene 51 with PEG-2 an increase in reaction time to 53 hours afforded a half PEGylated compound 165 (n=0), in 25% yield (scheme 5.2.3). With further equivalents of PEG-3 the half PEGylated compound 166 was formed in only 19% yield, though 4% of the fully PEGylated compound was observed. No comment is made as to the symmetrical nature of the half-PEGylated compounds 165 and 166, and as the compounds are only characterised by $^1$H NMR where broad signals are reported it is impossible to deduce the exact nature of the compounds.
For calix[4]arene 161 and tert-butylicalix[4]arene 50 fully PEGylated compounds were observed in all reactions (scheme 5.2.4). Reaction with 15 equivalents of PEG-2 and PEG-3 afforded the fully PEGylated nude calix[4]arenes (167 and 168) in 20% yield, from reaction times of 28 and 39 hours respectively. The tert-butylicalix[4]arene 50 afforded the fully PEGylated PEG-2 and PEG-3 compounds (169 and 170) in 30% and 40% yields respectively, from reaction times of 40 and 45 hours.

In general the nude calixarenes proved to be more reactive towards PEG-chain addition than the tert-butylicalixarenes, possibly due to increased conformational mobility,
enabling ring inversion so that PEGylated positions are oriented away from the non-PEGylated ones. The phenylcalixarenes possess a large group at the upper rim which could lead to enhanced steric hindrance to inversion compared to the tert-butylcalixarenes.

Overall the reactions of calixarenes with PEG-chains are not well precedented in the literature, and the data which has been published has focused on the derivatisation of the smaller ringed calixarenes, rather than a heptamer or octamer. The length of PEG-chains which has been reported is also very limited, with most work focusing on the use of short PEG-chains of less than four units length. Reported PEGylation reactions are also much lower yielding than reactions with active electrophiles (see Section 2.2). However we had an interest in synthesising the fully PEGylated octamer, ideally with a PEG-chain of twelve units in length. This represented an interesting synthetic challenge, not only in the synthesis of the PEG-12 unit with suitable activation (see Chapter 4), but also in reaction of such a long PEG-chain with a calixarene.

5.2.1 Phenylcalixarenes and PEG-3

In order to test the reactivity of the \( p \)-phenylcalix[8]arene 77 towards PEG-chains we initially reacted 77 with PEG-3, as this is commercially available as a mono-methyl ether. One terminus of the PEG-chain required protection to prevent polymerisation reactions occurring during the addition. Using mono-bromo-PEG-3-mono-methylether, 144, the PEGylation of 77 was carried out using a variety of different solvents, reaction times, and numbers of equivalents of both base and PEG-chain (table 5.2.1.1, scheme 5.2.1.1). Initial reactions using sodium hydride afforded complex, inseparable mixtures of compounds, although mass spectral data of the crude reaction mixture indicated that the fully PEGylated octamer was present in the mixture. However the use of potassium carbonate exclusively afforded the tetra-PEGylated octamer.
Table 5.2.1.1. Yields of reactions between phenylcalix[8]arene and PEG-3

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Reaction Time</th>
<th>K₂CO₃ Eq.</th>
<th>PEG Eq.</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>THF/DMF (10:1)</td>
<td>7 days</td>
<td>16</td>
<td>8</td>
<td>No product</td>
</tr>
<tr>
<td>THF/DMF (10:1)</td>
<td>7 days</td>
<td>16</td>
<td>16</td>
<td>No product</td>
</tr>
<tr>
<td>DMF</td>
<td>7 days</td>
<td>16</td>
<td>16</td>
<td>No product</td>
</tr>
<tr>
<td>THF</td>
<td>7 days</td>
<td>16</td>
<td>16</td>
<td>No product</td>
</tr>
<tr>
<td>MeCN</td>
<td>7 days</td>
<td>16</td>
<td>16</td>
<td>171, 68%</td>
</tr>
</tbody>
</table>

Only when acetonitrile was used was a product isolated, the half-PEGylated octamer 171 in 68% yield. Having established that we could successfully achieve the tetra-substitution of phenylcalix[8]arene 77, we attempted to optimise the yield of the reaction (table 5.2.1.2). The number of equivalents of PEG-chain used were considered to potentially be a limiting factor in the reaction, since the synthesis of longer PEG-chains was synthetically challenging, and time consuming, and therefore we had a desire to limit the number of equivalents of PEG-chain used.
<table>
<thead>
<tr>
<th>PEG Eq.</th>
<th>K₂CO₃ Eq.</th>
<th>Reaction Time</th>
<th>Yield 171</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>16</td>
<td>7 days</td>
<td>68%</td>
</tr>
<tr>
<td>16</td>
<td>32</td>
<td>7 days</td>
<td>64%</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>7 days</td>
<td>66%</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>7 days</td>
<td>61%</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>4 days</td>
<td>71%</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>2 days</td>
<td>22%</td>
</tr>
</tbody>
</table>

Table 5.2.1.2. Yields of reactions between phenylcalix[8]arene and PEG-3

The use of a greater number of equivalents of base had no effect on the outcome of reaction, as did halving the number of equivalents of both base and PEG-chain. A decrease in reaction time to four days afforded the highest yield, 71%, whereas a further decrease in reaction time to two days afforded a complex mixture of products, from which 22% of 171 was finally isolated.

Although broadening of the NMR signals was observed for the PEGylated calixarenes, we believe that the tetra-PEGylated octamer exhibited a symmetrical 1,3,5,7-alkylation pattern. Examination of the $^{13}$C NMR of the aromatic region of the PEGylated calixarenes (section 5.2.5) reveals that although broadening of signals is observed, as expected for the tetra-PEGylated compounds a splitting of some signals can be seen. If the tetra-PEGylated compound exhibited an unsymmetrical alkylation pattern a greater complexity of signals would be probably be observed. In a parallel study reactions of tert-butylcalix[8]arene 52 afforded a 1,3,5,7-tetra-PEGylated compound under the same conditions, with very clear symmetry observed in the $^{13}$C NMR of the compounds. Therefore we believe that by analogy with the tert-butyl series, the octamer of the phenyl series also exhibits a 1,3,5,7-tetra-substitution pattern.

For all PEGylation reactions with calixarenes the products were purified via flash column chromatography, which was repeated several times to afford the pure compounds. With a procedure for the tetra-PEGylation of phenylcalix[8]arene 77, it was hoped that full PEGylation of the tetra-PEGylated calixarene would be possible by
exposure of the half-PEGylated product to a strong base such as sodium hydride, as Neri had demonstrated (Section 2.2).  

PEGylation of phenylcalix[7]arene 111 was also explored as biological testing of the heptamer had shown that it had anti-TB activity at similar levels to the octamer 77 (see Chapter 7). The PEGylation of 111 was carried out under the same conditions used to give the half PEGylated octamer 171, 16 equivalents of potassium carbonate and eight equivalents of brominated PEG-3, in refluxing acetonitrile for four days (scheme 5.2.1.2). The product of the reaction was very surprising, the fully PEGylated heptamer 172 was afforded in 31% yield. The formation of no partially PEGylated compounds was observed.

![Scheme 5.2.1.2. Synthesis of phenylcalix[7]arene-PEG-3](image)

This result was extremely surprising since the full PEGylation of phenylcalix[8]arene 77 with potassium carbonate had not been observed, nor for the parallel studies with tert-butylcalix[8]arene 52. Repetition of the procedure, but using sodium hydride instead of potassium carbonate, afforded an inseparable mixture of compounds, however as with the octamer the crude mixture was shown to contain the fully PEGylated compound by mass spectrometry. Although the reason why the heptamer affords a fully PEGylated compound when using potassium carbonate while the half-PEGylated octamer is generated is not clear, one reason may be that the heptamer
(albeit of the tert-butylcalixarene series) has been reported to be the most flexible of all the calixarenes, as calculated from temperature dependent NMR. By analogy this suggest that the phenyl heptamer ring is significantly more flexible than the octamer, permitting ring inversion so that free hydroxyls are not as sterically hindered by adjacent PEG-chains as they are in the octamer. For the tert-butyl series, the octamer is also considered to be very flexible, although the attachment of large groups to the lower rim can prevent rotation via the oxygen through the annulus route. Therefore the greater flexibility of the heptamer may be key to its higher reactivity in the PEGylation reaction.

5.2.2 Phenylcalixarenes and PEG-6

Reactions were then carried out using PEG-6 chain lengths, the longest commercially available PEG-chain as a homogeneous compound. Building upon our previous studies, and using phenylcalix[7]arene 111, the use of several leaving groups was explored. Three reactions were carried out with PEG-6, one with chloro activation 142, a second with mesyl 137 activation, and a third with bromo activation, 146.(scheme 5.2.2.1). When using 142 the fully PEGylated compound 173 was isolated in only 18% yield, however when using 137 no additions were observed. The use of a mono-THP-mono-bromo-PEG-6, 146, afforded 173 in significantly higher yield, 49% (scheme 5.2.2.1).
Initial PEGylation of phenylcalix[8]arene was carried out under the same conditions described above which afforded 171 (scheme 5.2.2.2). Again the tetra-PEGylated compound 174 resulted, and no fully PEGylated product was observed. The tetra-PEGylated compound 174 was afforded in 20% yield, reflecting the difficulty in adding longer PEG-chains to the calixarene.

Full PEGylation of tetra-PEG6-phenylcalix[8]arene 174 was then carried out (scheme 5.2.2.3). The use of 16 equivalents of sodium hydride, and 8 equivalents of PEG-chain, in refluxing THF for four days afforded the fully PEGylated compound 175 also in 20% yield. In a parallel study the use of the same procedure afforded the fully PEGylated tert-butylcalix[8]arene (PEG-6) from the 1,3,5,7-tetra-substituted compound in 55% yield. The lower yield of 175 again reflects the lower reactivity of the phenylcalixarenes in the PEGylation reactions, when compared to the tert-butylcalixarenes. By analogy we believe that compound 174 also exhibits a symmetrical 1,3,5,7-substitution pattern (figure 5.2.2.1).
At this point in the Ph.D. we discovered that our PEGylated calixarenes were contaminated with triphenylphosphine, present from the activation of the PEG-chains (see Chapter 4). Therefore all PEGylated calixarenes to this point were resynthesised.
using PEG-chain which was not activated using triphenylphosphine, and yields reported in this Chapter are for compounds synthesised without triphenylphosphine.

5.2.3 p-Phenylcalixarenes and PEG-12

PEGylation of calixarenes with PEG-12 (for synthesis see Chapter 4) was synthetically challenging as very large quantities of PEG-12 needed to be synthesised for the PEGylation, as eight equivalents of PEG-chain were needed (typically 1 g of PEG-chain for 200 mg of calixarene). The PEGylation of phenylcalix[8]arene 77 with PEG-12 was carried out under the same conditions as for PEG-3 and PEG-6, except the reaction time was increased to seven days. The increase in reaction time afforded an increase in yield of the tetra-PEG-12-phenylcalix[8]arene 176 from 14% to 25% (scheme 5.2.3.1). This is probably as a result of slower reaction rates due to the extensive coiling and wrapping of the longer PEG-chain. Due to time constraints and problems encountered in the later stages of the Ph.D. generating sufficient quantities of PEG-12 (see Chapter 4) the fully PEGylated phenylcalix[8]arene was not synthesised.

Scheme 5.2.3.1. Synthesis of tetra-PEG-12-phenylcalix[8]arene
The addition of PEG-12 to Phenylcalix[7]arene 111 once again directly afforded a fully PEGylated product using eight equivalents of PEG-chain and 16 equivalents of potassium carbonate, in refluxing acetonitrile for seven days (scheme 5.2.3.2). Again an increase in reaction time was necessary to increase the yield of 177 to 22% (four days, 14%). A further increase in reaction time from seven days to 14 days however did not improve the yield.

Scheme 5.2.3.2. Synthesis of phenylcalix[7]arene-PEG-12

5.2.4 Deprotection of PEGylated Calixarenes

The deprotection of PEGylated calixarenes was investigated in the earlier stages of the project for benzyl protected PEG-chains (see Chapter 4 for details) and was not achieved when benzyl protected PEG-chains were attached to calixarenes. In order to remove the THP protecting group from isolated PEG-chains a one hour reaction in a dichloromethane/methanol with 1 M HCl at room temperature was required. Attempts to deprotect the tetra-PEG-6-phenylcalix[8]arene 174 under the same conditions were unsuccessful. Therefore the concentration of HCl solution used was increased until full deprotection was achieved, which was using 18 M HCl and a two hour reaction to give 178 in 85% yield (scheme 5.2.4.1). Deprotection of the fully PEGylated octamer (PEG-
6), 175, was achieved under the same conditions to afford 179 in 86% yield (scheme 5.2.4.1).

Scheme 5.2.4.1. Synthesis of deprotected tetra-PEG6-phenylcalix[8]arene

The fully PEGylated heptamer (PEG-6,) 173, was deprotected under the same conditions to afford 180 in 77% yield (scheme 5.2.4.2).

Scheme 5.2.4.2. Synthesis of deprotected phenylcalix[7]arene-PEG-6
For the PEG-12 calixarenes 176 and 177, a reaction time of two hours produced only partial deprotection. Therefore an increase in reaction time to 18 hours was required probably due to coiling of the longer PEG-chain decreasing accessibility to the THP group. The deprotected compounds 181 and 182 were afforded in 61% and 50% yields respectively (figure 5.2.4.1). The lower yield observed was due to loss of the highly water soluble deprotected compounds during the aqueous work-up of the reaction. Surprisingly, exposure of the PEGylated calixarenes to concentrated acids for a prolonged time did not result in degradation of the PEG-chain.

Purification of the PEGylated calixarenes was carried out by flash column chromatography. However, due to the size of the compounds significant amounts of streaking were observed, and in most solvents the compounds had long retention times on the column. In general for all PEGylated calixarenes the procedure was repeated between three and five times before pure compounds were achieved.

5.2.5 Characterisation of PEGylated Calixarenes

Characterisation of the PEGylated calixarenes was not straightforward, and provided even greater difficulties than the characterisation of the p-phenylcalixarenes themselves.
Initially no mass spectral data for the compounds with PEG-6 and PEG-12 could be provided, as their high molecular mass prohibited use of the mass spectrometers available at UCL. However for the PEG-3-phenylcalix[8]arene 171 and PEG-3-phenylcalix[7]arene 172, MS data were obtained using an electrospray (positive) mass spectrometer at UCL. A characteristic feature of the mass spectra of PEGylated calixarenes was their ability to complex sodium, as both the calixarene and the PEG-chains can complex ions. Therefore often multiply charged signals were observed. Tetra-PEG-3-phenylcalix[8]arene 171, gave rise to no molecular ion peak by ESMS, instead affording a doubly charged peak due to complexing two sodium ions at \( m/z \) 1041 ([M+2Na]^{2+}/2). The PEGylated calixarenes were observed to also complex solvent molecules, and as the electrospray sample was run in methanol signals were observed in the mass spectra of 171 with methanol complexes ([M+2Na+MeOH]^{2+}/2). PEG-3-phenylcalix[7]arene 172, gave rise to a molecular ion signal with one sodium ion complexed, at \( m/z \) 2320 (M+Na^+). A multiply charged peak was also observed ([M+2Na]^{2+}/2) at \( m/z \) 1172. Initially compound 172 was synthesised using a PEG-chain which had been prepared using triphenylphosphine and carbon tetrabromide (see Chapter 4), however MS data of this compound revealed a peak of strong intensity which corresponded to the molecular ion with one triphenylphosphine molecule complexed to it. Displacement of the triphenylphosphine was achieved by stirring the compound overnight in NaCl (sat.), and this gave rise to a signal at (M+Na^+) by ESMS. Although the triphenylphosphine could not be visualised by either \(^1\)H or \(^{13}\)C NMR, it could be observed with \(^{31}\)P NMR spectra. This discovery led to the re-synthesis of all PEGylated calixarenes using PEG-chain which was activated without the use of triphenylphosphine.

ESMS data were also obtained for the phenylcalix[7]arene-PEG-6, 173. A molecular ion peak was observed at \( m/z \) 3711 (M+Na^+), with 100% intensity.

We were able to obtain MALDI-TOF MS for some of our PEGylated calixarenes. For the half PEGylated phenylcalix[8]arene (PEG-6), 174, M^+ was observed at 2849 and (M+Na^+) at 2872. Loss of the THP protecting groups was also observed. For example
for compound 174, signals corresponding to the loss of two THP protecting groups were observed.

For the fully PEGylated phenylcalix[8]arene (PEG-6), 175, the MALDI-TOF MS gave rise to a signal with 100% intensity corresponding to [(M+4Na-4THP)^4+/4).

For the half PEGylated phenylcalix[8]arene (PEG-12) 181, MALDI-TOF MS showed a signal corresponding to the molecular ion with two sodium ions complexed ([M+2Na]^2+/2). However the highest intensity peak (50%) again corresponded to the molecular ion with the loss of four THP protecting groups and four sodium ions complexed ([M+4Na-4THP]^4+/4).

The heptamer with PEG-12, compound 182, was analysed by MALDI-TOF MS and showed the highest degree of complexation to sodium ions of any of the PEGylated calixarenes synthesised. This is most likely due to the greater number of PEG units present. The molecular ion was observed complexed to five sodium ions, at m/z 1133.6, ([M+5Na]^5+/5).

NMR analysis of the PEGylated calixarenes also presented many problems. The ^1H NMR spectra were extremely broad, often with poorly defined peaks leading to difficulties in obtaining accurate integration ratios. The ^13C NMR spectra suffered from very poor relaxation, and many different relaxation delays were explored in order to achieve well defined signals, as with a two second relaxation delay (RD) the PEG region was clearly defined, but the aromatic calixarene region was not observable. One advantage when obtaining ^13C NMR for the PEGylated calixarenes in comparison to the unsubstituted calixarenes was their far greater solubility.

A doubling of the RD to four seconds afforded a first glimpse of the aromatic region of the tetra-PEG-6-phenylcalix[8]arene, 174, however the peaks were still poorly defined. A further increase to six seconds afforded the more clearly defined peaks (figure 5.2.5.1). Splitting of the signals at δ 126, δ 133 and δ 140 can be seen, confirming that two different sets of phenolic nuclei are present. The simplicity of the signals also
indicates that the compound is symmetrical and displays a 1,3,5,7-tetra-substitution pattern, as does the fact that only one signal for the methylene bridge was observed, at δ 30.91.

Fig. 5.2.5.1 Aromatic region of $^{13}$C NMR of tetra-PEG-6-phenylcalix[8]arene

A large number of scans (up to 90,000) were also required, which coupled with the increased relaxation delay led to long experiment times (up to four days). The phenylcalix[8]arene which was fully PEGylated with PEG-6, 175, was also analysed using an RD of six seconds and the aromatic region is shown in figure 5.2.5.2. However despite increasing the number of scans the large size of the molecule led to significant line broadening.

Fig. 5.2.5.2. Aromatic region of $^{13}$C NMR of tetra-PEG-6-phenylcalix[8]arene
For tetra-PEG-12-phenylcalix[8]arene, compound 176, an RD of six seconds did afford a clear picture of the aromatic region, however a further extension of the relaxation delay to ten seconds was tested (figure 5.2.5.3). As is shown below, this did not improve the signal to noise ratio, and therefore an RD of 6s was preferred.

![RD 6 seconds](image)

![RD 10 seconds](image)

Fig. 5.2.5.3. Aromatic regions of two $^{13}$C NMR spectra of tetra-PEG-12-phenylcalix[8]arene

Again clear splitting of the signal at $\delta$ 126 into two signals is observed, indicating that 176 has a symmetrical 1,3,5,7-substitution pattern, as does the presence of only one methylene bridge signal at $\delta$ 30.61. The phenylcalix[7]arene-PEG-3 compound 172, did not require an extension in the RD, and an increase to four seconds led to a decrease in clarity of the spectra. However a very large number of scans, 90,000, were required to obtain the spectra shown below, which exhibits a single peak for the signal at $\delta$ 126 (figure 5.2.5.4).
For the heptamer with PEG-6, compound 173, an increase in RD to six seconds was necessary to visualise the aromatic region, figure 5.2.5.5.

This was also the case for the heptamer with PEG-12, compound 177, which showed a significant broadening of signals compared to the PEG-6 spectra shown above, again due to the greater size of the compound (figure 5.2.5.6).
In general the carbon NMR spectra of the PEGylated calixarenes show significant broadening in comparison to the NMR of the parent calixarenes (see Chapter 3). The methylene bridge of the PEGylated calixarenes was not observed for the tetra-PEG-6-phenylcalix[8]arene, but for other compounds was within the δ 30-34 region described by Mendoza for calixarenes with syn orientation. For the p-phenylcalix[8]arene a decrease in the shift of the methylene bridge was observed concurrent with an increase in PEG-chain length (table 5.2.5.1). For the phenylcalix[7]arene series however an increase in shift of the methylene bridge resonance was observed concurrent with an increase in the length of the PEG-chain (table 5.2.5.2).

<table>
<thead>
<tr>
<th>δ Shift (ArCH₂Ar)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.51</td>
<td>Octamer</td>
</tr>
<tr>
<td>31.31</td>
<td>Ph[8]4xPEG3OMe</td>
</tr>
<tr>
<td>30.91</td>
<td>Ph[8]8xPEG6OTHP</td>
</tr>
<tr>
<td>30.61</td>
<td>Ph[8]4xPEG12OTHP</td>
</tr>
</tbody>
</table>

Table 5.2.5.1. Methylene bridge carbon NMR shifts of PEGylated phenylcalix[8]arene

<table>
<thead>
<tr>
<th>δ Shift (ArCH₂Ar)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.63</td>
<td>Heptamer</td>
</tr>
<tr>
<td>30.07</td>
<td>Ph[7]7xPEG3OMe</td>
</tr>
<tr>
<td>30.31</td>
<td>Ph[7]7xPEG6OH</td>
</tr>
<tr>
<td>32.16</td>
<td>Ph[7]7xPEG12OTHP</td>
</tr>
</tbody>
</table>

Table 5.2.5.2. Methylene bridge carbon NMR shifts of PEGylated phenylcalix[7]arene
5.3 Phenylcalixarenes and Alkyl Nitrile Chains

We were interested in fully derivatising \( p \)-phenylcalix[8]arene 77 and \( p \)-phenylcalix[7]arene 111 with bromobutyronitrile, with the possibility of further functionalisation of the nitrile component (for example via reduction). Initially the reactions were carried out with 16 equivalents of sodium hydride and eight equivalents of electrophile to one equivalent of 77 or 111. However for both the octamer and heptamer this produced an inseparable mixture of partially alkylated compounds. We therefore considered the possibility of applying our two-step procedure, developed for the PEGylation of calixarenes. However, since the electrophile is a commercially available compound, we decided to investigate whether a one-step direct procedure was possible using a weak base.

We used potassium carbonate as this had proven to be the most successful weak base, and systematically increased both the number of equivalents of base and electrophile, and reaction time, until a fully derivatised product was achieved. The fully alkylated octamer 183 was generated in 9% yield when 64 equivalents of both base and electrophile were used, with a very long reaction time of 14 days (scheme 5.3.1).

![Scheme 5.3.1. Synthesis of bromo-nitrile-phenylcalix[8]arene](image)

The phenylcalix[7]arene 111 did not fully alkylate under the conditions which gave the fully PEGylated heptamer, and an inseparable mixture of compounds resulted.
Therefore an increase in equivalents of both base, electrophile (32 equivalents) and reaction time, were used to afford the fully alkylated compound 16 in 18% yield (scheme 5.3.2).

It is interesting that these conditions did not afford the fully alkylated octamer, rather, more reagents were required, confirming that the heptamer appears to be more reactive towards electrophiles. The difference in reactivity of the calixarenes towards these electrophiles compared to PEG-chains is most likely due to differing polyanion stability in the presence of the bromo-alkynitrile chains.

An attempt was made to reduce the nitrile functionality in compound 183, with lithium aluminium hydride. Twenty equivalents of lithium aluminium hydride in THF (1 M solution) were used, and the reaction was carried out at reflux temperature for 14 days. However no reduction of the nitriles was observed.

5.4 Reactions on the Nude Calixarenes

We were also interested in the synthesis of straight chain alkyl groups at the upper rim calixarenes, in order to investigate the possible change in biological activity, as compounds with an alkyl chain, especially one with an unsaturated moiety are known to incorporate into biological membranes, a possible site of activity of our compounds.
Although the synthesis of some alkyl upper rim calixarenes was reported by Vicens (see Section 2.2.2), a personal communication revealed that the procedure was not robust.\textsuperscript{111, 162} We therefore attempted a synthesis starting from the nude calix[8]arene 73 and calix[6]arene 112.

We initially investigated allylation at the lower rim of the nude calix[6]arene 112, as its smaller number of reactive sites made for a more tractable compound. Full allylation of the calix[6]arene to afford 185, was achieved from reaction with 30 equivalents of sodium hydride and 12 equivalents of allyl bromide, in 62% yield (scheme 5.4.1).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {112};
\node (b) at (2,0) {185};
\draw (a) -- (b);
\node at (1,-0.5) {$\text{NaH, THF}$};
\end{tikzpicture}
\end{center}


Likewise for the calix[8]arene 73, the fully allylated compound 186 was afforded using 30 equivalents of both sodium hydride and allyl bromide (scheme 5.4.2). The greater number of equivalents of allyl bromide were necessary to afford the full allylation of the octamer, and reflected the lower reactivity of calix[8]arene in comparison to calix[6]arene.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {73};
\node (b) at (2,0) {186};
\draw (a) -- (b);
\node at (1,-0.5) {$\text{NaH, THF}$};
\end{tikzpicture}
\end{center}

Scheme 5.4.2. Synthesis of allyl-calix[8]arene

It has been reported that a Claisen rearrangement can afford an upper rim allyl calixarene for calix[4]arenes in high yield, and in modest yield for a calix[6]arene
which is partially derivatised. The reaction was carried out in refluxing diethylaniline for two hours. It was hoped that if the upper rim allyl calixarenes could be formed a cross metathesis reaction would afford an upper rim alkyl chain with an unsaturated moiety (scheme 5.4.3).

![Scheme 5.4.3. Claisen rearrangement of lower rim allyl-calix[4]arene](image)

We applied these conditions to the rearrangement of both calix[6]arene-hexa-allyl 185, and calix[8]arene-octa-allyl 186, however no reliable rearrangement was observed in either case. An increase in reaction time to 16 hours did not afford any rearrangement, though loss of starting material was observed. For the larger ring calixarenes it has been reported that rearrangement to the upper rim affords a compound which readily rearranges to the O-allyl compound, and it is believed that this may have been the case with the attempted rearrangements of 185 and 186.

However in the hope of obtaining a reliable rearrangement of the lower rim allyl compounds, test cross metathesis reactions were carried out, initially using styrene, 189 and allyl-TMS, 190, with Grubbs catalyst and the Shrock molybdenum catalyst in order to optimise the conditions. Two main products were isolated from the reactions, stilbene 191, and the desired cross metathesis compound 192, scheme 5.4.4.
It was found that when the reaction was run in dilute conditions (~10 mg/ml) the only product isolated from the reaction was 191. However under more concentrated conditions (~100 mg/ml) the compound 192 was isolated in variable yields, shown in table 5.4.1.

The Shrock molybdenum catalyst 194 failed to afford any products from the reaction (fig. 5.4.1). The greatest yield of 192 was obtained with the Grubbs catalyst 193, in refluxing DCM for 24 hours, 45%. These conditions were then applied to the cross metathesis of a small analogue which was synthesised via allylation and Claisen rearrangement as shown in scheme 5.4.5.

<table>
<thead>
<tr>
<th>Styrene (Eq.)</th>
<th>AllylTMS (Eq.)</th>
<th>Catalyst</th>
<th>Mol%</th>
<th>Solvent</th>
<th>Conc.</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>2</td>
<td>THF</td>
<td>Dilute</td>
<td>191</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>2</td>
<td>DCM</td>
<td>Dilute</td>
<td>191</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Shrock</td>
<td>2</td>
<td>DCM</td>
<td>Dilute</td>
<td>191</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>2</td>
<td>DCM</td>
<td>Dilute</td>
<td>191</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Shrock</td>
<td>2</td>
<td>DCM</td>
<td>Conc.</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Grubbs</td>
<td>2</td>
<td>Toluene</td>
<td>Conc.</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>2</td>
<td>DME</td>
<td>Conc.</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>10</td>
<td>DCM</td>
<td>Conc.</td>
<td>192</td>
<td>45%</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>10</td>
<td>DME</td>
<td>Conc.</td>
<td>192</td>
<td>23%</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Grubbs</td>
<td>10</td>
<td>Toluene</td>
<td>Conc.</td>
<td>192</td>
<td>18%</td>
</tr>
</tbody>
</table>

Table 5.4.1. Yields of cross-metathesis reactions between styrene and allyl-TMS
Cross metathesis between 195 and 190 was achieved successfully in 41% yield, to afford 196 (scheme 5.4.6). However, as the Claisen rearrangement was not achieved on either the calix[6]arene-hexa-allyl 185, or the octamer 186, cross metathesis on the calixarene was not attempted.
The synthesis of sulphonato-calix[8]arene was discussed in section 3.2, but we also were interested in synthesising a calixarene in which the hydrophilic/hydrophobic nature of the octopus molecules was reversed, with a hydrophilic group at the upper rim, and the hydrophobic moiety at the lower rim. The calix[8]arene was therefore fully alkylated at the lower rim with a dodecyl-chain, prior to sulfonation at the upper rim. The alkylation was carried out prior to sulfonation to avoid any complications of further reaction at the upper rim.

Alkylation at the lower rim was carried out using 30 equivalents of sodium hydride and 16 equivalents of bromododecane, in refluxing THF for three days. The extremely insoluble product 197, was afforded in 68% yield, and characterisation was only possible using deuterated pyridine as the NMR solvent (scheme 5.4.7).
Sulfonation of the upper rim of 20 was then carried out under the same conditions which afforded the sulfonato-calixarene in Section 3.2, refluxing concentrated sulphuric acid for four hours. The novel water soluble derivative 198 was afforded in 76% yield (scheme 5.4.8).

\[
\text{c. } \text{H}_2\text{SO}_4 \xrightarrow{\text{reflux, 4 h}} 76\% \\
197 \quad 198
\]

Scheme 5.4.8. Sulfonation of dodecane-calix[8]arene

5.5 Summary

A method of partial alkylation has been established for PEGylation of phenylcalix[8]arene with a weak base. The tetra-substituted compound probably exhibited a symmetrical 1,3,5,7-substitution pattern. Full derivatisation of the octamer was possible via a second step using sodium hydride. The phenylcalix[7]arene was fully PEGylated in one step from the same conditions which afford a half PEGylated octamer. The \(p\)-phenylcalixarenes were derivatised with PEG-3, PEG-6 and PEG-12, and deprotections afforded the free hydroxyl termini of the PEG-chains.

The phenylcalixarenes were also derivatised with short alkyl nitrile chains. These compounds were generated only when using a large excess of weak base and electrophile, and a very long reaction time. The difference in reactivity of the calixarenes towards these electrophiles compared to PEG-chains is most likely due to differing polyanion stability in the presence of the bromo-alkynitrile chains.

The nude calix[8]arene was reacted with a long alkyl chain to afford an insoluble compound 197, which was made fully water soluble by sulfonation at the upper rim. The nude calix[6]arene and calix[8]arene have been fully alkylated at the lower rim
with allyl groups, and the planned Claisen rearrangement was observed not to readily occur. A small analogue was O-allylated, and the Claisen rearrangement successfully carried out. Cross metathesis of this compound using the Grubbs catalyst was also successful.
6.0 Introduction

Due to the very high formula weight of the PEGylated calixarenes (often >4000), a series of PEGylated monomers and dimers were prepared, as these were closer to obeying the mass criteria of the Lipinski Rule of 5, a set of parameters which an ideal drug candidate will obey. As reviewed in Chapter 2, Cornforth synthesised a PEGylated tert-octylphenol, and a series of linear PEGylated analogues of tert-octylphenol (up to a pentamer) were also synthesised. Although these compounds did exhibit anti-tuberculosis activity, many of them were also toxic to the mice. We therefore wanted to investigate a series of PEGylated monomers and dimers to investigate their possible biological activity and toxicity.

6.1 PEG-6 Compounds

Four compounds were PEGylated with brominated PEG-6, which was protected with a THP group, 146. Application of the potassium carbonate reaction conditions which were developed for the PEGylation of p-phenylcalixarenes afforded the PEG-6 small analogues in moderate yields.

Bisphenol-PEG-6 199, was afforded in 51% yield from a four day reflux reaction utilising four equivalents of base and three equivalents of PEG-chain (scheme 6.1.1). The numbers of equivalents of base are the same per hydroxyl as for PEGylation of the calixarenes, but a slightly greater number of equivalents of PEG were used in the reaction.
Application of the same procedure to bis-2-phenylmethane afforded compound 200, figure 6.1.1, in 41% yield. The bis-4-phenylmethane compound 201 was generated in 43% yield.

A PEGylated monomer 202 was also synthesised (scheme 6.1.2). n-Octylphenol was investigated as a straight chain analogue and was prepared in 29% yield, using three equivalents of potassium carbonate and two of PEG-chain.
6.2 PEG-9 Compounds

Two compounds were also attached to PEG-9, which had been activated as a mesyl group and protected with a benzyl group, 151. The coupled products were synthesised by reaction in THF/DMF in order to investigate the effect (if any) of a different solvent system.

As shown in scheme 6.2.1 the PEGylated phenylphenol monomer 203 was synthesised in 23% yield, using two equivalents of PEG-chain and base. This yield was lower than for any of the PEG-6 compounds and was most likely a reflection of the lower reactivity of the longer PEG-chain. It is interesting that the use of a mesyl activating group was sufficient for coupling of PEG-chains to these small analogues, but not to the phenylcalixarenes.
A second PEG-9 compound, 204, was also synthesised (scheme 6.2.2) in 18% yield, despite an increase in the number of equivalents of base (three equivalents per hydroxyl) and PEG (four per hydroxyl). For both the PEG-9 compounds an increase in reaction time to six days was employed, compared to the four day reaction time used for the PEG-6 compounds.
6.3 PEG-12 Compounds

Four compounds were synthesised with PEG-12, using an even longer reaction time of between six and eleven days. The three PEG-12 dimers were synthesised by reaction with four equivalents of base per hydroxyl and two equivalents of PEG-12. Compound 205, shown in scheme 6.3.1 was synthesised in 78% yield.

Scheme 6.3.1. PEGylation of 4,4'-bisphenol

The two dimers 206 and 207 shown below in figure 6.3.1 were also synthesised in 57% and 22% yields respectively, using reaction times of 10 days.

Fig. 6.3.1
The PEG-12 monomer 208, was synthesised in 72% yield from the use of three equivalents of PEG-12 and four equivalents of base, and an 11 day reaction time (scheme 6.3.2). As with the PEG-6 compounds the higher yielding small analogues were those with the least steric hindrance, the bisphenol and the phenylphenol.

![Scheme 6.3.2. PEGylation of phenylphenol](image)

6.4 Deprotection of Monomers and Dimers

The removal of benzyl protecting groups from the two PEG-9 compounds 203 and 204 was not carried out due to problems encountered during the removal from PEGylated calixarenes. Removal of the THP protecting groups was carried out with 18 M hydrochloric acid in dichloromethane. A much shorter reaction time afforded the deprotected compounds than was required for the PEGylated calixarenes, as shown in table 6.4.1 and figure 6.4.1.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction Time (h)</th>
<th>Deprotected Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>199</td>
<td>3</td>
<td>209</td>
</tr>
<tr>
<td>200</td>
<td>3</td>
<td>210</td>
</tr>
<tr>
<td>201</td>
<td>3</td>
<td>211</td>
</tr>
<tr>
<td>202</td>
<td>3</td>
<td>212</td>
</tr>
<tr>
<td>203</td>
<td>10</td>
<td>213</td>
</tr>
<tr>
<td>204</td>
<td>10</td>
<td>214</td>
</tr>
<tr>
<td>205</td>
<td>10</td>
<td>215</td>
</tr>
<tr>
<td>206</td>
<td>10</td>
<td>216</td>
</tr>
</tbody>
</table>

Table 6.4.1. Reaction times which afforded deprotected monomers and dimers

The PEG-6 monomer and dimers 199 to 202, were deprotected in three hours, whereas the PEG-12 monomer and dimers 203 to 206, required a longer reaction time of ten hours.

Fig. 6.4.1
6.5 Synthesis of Labelled Compounds

In order to allow confocal microscopy work to be carried out we wished to synthesise a series of compounds labelled with the fluorescent label Texas Red (Molecular Probes). The synthesis was designed so that only one fluorescent moiety was attached to each molecule. Therefore we initially investigated reactions using the PEGylated small analogues due to their smaller number of reactive sites and greater ease of identification. Initial investigations were carried out with a pyrene label which was activated in the same way as Texas Red, as an N-hydroxysuccinimide-ester, due to cost considerations, and also to allow the biologists to assess the confocal system.

Initially deprotected bisphenol-PEG-6, 209, was reacted with one equivalent of triethylamine and one equivalent of pyrene-butyric-acid-N-hydroxysuccinimide-ester, 218 (scheme 6.5.1). The reaction was carried out in dry dichloromethane at room temperature for six days.

Flash chromatography afforded 5% of the mono-labelled compound, as shown by mass spectroscopy and UV spectroscopy. The same conditions were then applied using Texas Red, and 220 (fig. 6.5.1) was afforded as a mixture with the unlabelled
compound. Purification using flash column chromatography led to the removal of any unreacted Texas Red. When testing the labelled compounds they are diluted with unlabelled small analogues, and therefore the mixture isolated did not present a problem, provided that any unreacted Texas Red was removed.

An increase in reaction time was required to label 209 with Texas Red, nine days compared to the six days which was used with the label, compound 218. Again the compound was characterised by both mass spectroscopy and UV spectroscopy. Initially MS data of the Texas Red compound 220 could not be obtained with ESMS with the compound in a methanol/water mixture, however it was found that the addition of a very small amount of sodium chloride to the sample revealed the molecular ion signal.

Labelling of the PEG-12 dimer 215 with Texas Red was then carried out. Compound 215 was reacted with the same number of equivalents of triethylamine and Texas Red as had afforded the mono-labelled compound 220. However a further increase in reaction time to 12 days was found to be necessary. Compound 221, (scheme 6.5.2), was afforded again as a mixture with the unlabelled compound and was characterised by UV spectroscopy.
Labelling of a PEGylated calixarene was also carried out on the deprotected phenylcalix[7]arene-PEG-6, compound 180. Using the same conditions which had afforded mono-labelled dimers, one equivalent of base and activated fluorescent label, the phenylcalix[7]arene was labelled with the pyrene label 218 initially, as shown below in scheme 6.5.3. A further increase in reaction time to 14 days was necessary to obtain compound 222.
Scheme 6.5.3. Synthesis of mono-labelled-(PEG-6)-phenylcalix[7]arene

The pyrene labelled phenylcalix[7]arene 222 was synthesised in 7% yield, purified by flash column chromatography, and characterised by UV spectroscopy. The same conditions were then applied to synthesise the Texas Red labelled phenylcalix[7]arene 223, (figure 6.5.2), which was isolated by flash column chromatography as a mixture with the unlabelled calixarene 180. Separation of the unreacted Texas Red was again possible, and therefore the presence of a mixture did not present a problem for the future testing of the compound via confocal microscopy.
6.6 Summary

A series of PEGylated phenolic monomers and dimers have been synthesised in moderate yields using mild reaction conditions. Labelling of a selection of compounds with the fluorescent species, pyrene butyric acid and Texas Red have been carried, in order that confocal microscopy may be used to gain information on the location of the PEGylated calixarenes and small analogues within a cell.
7.0 Introduction

Biological testing was carried out at the MRC laboratories at Mill Hill, London, by the late Dr. Jo Colston and his group. Compounds were selected for testing in the context of previous data accumulated, rather than testing all of the compounds synthesised, due to consideration for animal welfare.

Compounds were tested on nude mice, also known as athymic mice, which are bred to have no T-lymphocytes in their immune system. This has the advantage of both reducing the time required for testing, and providing a greater dynamic range of activities. Furthermore, it provided some information on how the compounds were operating, since if a compound displayed activity in an athymic mouse it could not be acting via the immune system. Compounds were administered to the mice in saline solution, (12.5% w/v), adjusted to pH 7 and autoclaved. 0.2 ml of the solution was injected intraperitoneally, according to the dosing schedule shown below (fig. 7.0.1). Bacterial loads in the lung and spleen were recorded, the lower the load, the more effective the compound.

![Fig. 7.0.1. Dosing schedule for biological testing](image-url)
Compounds are tested against a blank saline control, and the original Macrocyclon synthesised by Cornforth which had been stored at the MRC. Each compound was tested on a group of four mice, and the results are presented in graphical form below. Each black triangle represents the bacterial count from a different mouse, and the coloured squares are the mean count for each compound. The use of a mean is important as some compounds displayed a wide spread of activity. The percentage figures at the top of the graph are the ratio of standard deviation to the mean ($\sigma/\langle x \rangle$) (where $\langle x \rangle$ is the mean). This provided a straightforward method for comparing the spread of activities of different compounds. The ideal compound would display a very low bacterial count and a low spread. Macrocyclon consistently displayed a very large range of activity (spread), probably because it is a mixture of compounds which have very different biological activities. The phenylcalix[8]arene 77, and phenylcalix[7]arene 111, were the first compounds tested and results for the lung and spleen counts are shown below (graph 7.0.1).
Graphs 7.0.1. Bacterial TB counts from lungs and spleens of mice treated with phenylcalixarenes
Both compounds exhibited activity in the lung, and the phenylcalix[7]arene 111 appeared to be more active than the phenylcalix[8]arene 77. Also the average value for the heptamer, shown as a coloured square, was lower than that for Macrocyclon. However in the spleen the heptamer, 111, was far less active than Macrocyclon, perhaps due to its lower solubility leading to reduced transport properties. The octamer was also less active in the spleen than the lung, though both compounds did exhibit low level activity in the spleen. The spread of activity for the phenylcalix[8]arene remains consistently low (20-30%) in both the lung and spleen, whereas the heptamer displayed a spread of activity in the lung which was greater than that for Macrocyclon. Again this may be due to solubility issues causing variable absorption.

The calixarenes which were synthesised by reaction with good electrophiles, and by reaction with PEG-6 were the next compounds tested (graph 7.0.2). Phenylcalix[8]arene-octa-acyl, 157, appeared to be pro-TB in the lung, the bacterial count was much higher than in the control. It also has a very small spread of activity (19-31%) indicating that this is a statistically consistent result. In the spleen however it had no activity, the average was the same as that of the control, and therefore it is possibly not transported well around the body. Phenylcalix[8]arene-octa-allyl, 158, displayed moderate activity in both the lungs and spleen, and again has a very small spread of activity (18-36%, graph 7.0.2).

The tetra-PEGylated phenylcalix[8]arene-PEG-6-OH, 178, is active in both the lung and spleen at a very low level. The fully PEGylated phenylcalix[7]arene-PEG6-OH, 180, was more active in the lung, though it had similar activity to 178 in the spleen. Hexaethyleneglycol, 133, was also tested to confirm that it had little effect, and displayed a low level of activity in both the lung and spleen, though not of the order of activity which was demonstrated by the calixarenes above, indicating that the calixarene is the most active component in the lead compound Macrocyclon. Hexaethyleneglycol also displayed a large spread of activity in the lung (109%), indicating that the activity displayed was not consistent.
Graphs 7.0.2. Bacterial TB counts from lungs and spleens of mice treated with PEGylated calixarenes
The fully PEGylated octamer with PEG-6 179, was then tested and a comparison between the half PEGylated octamer 178, and the fully alkylated compound (179) revealed very little difference in activity (graph 7.0.3). However the half PEGylated octamer 178 displays a large spread of activity (65-81%) whereas the fully PEGylated octamer 179 displays a very small spread of activity (5-15%). This indicated that although the level of activity as shown by the mean is not markedly different, the likelihood of that activity occurring is far greater in the fully PEGylated octamer than the half PEGylated compound. The PEG-3OMe-phenylcalix[7]arene, 172, also displayed little activity, certainly at a much lower level than its PEG-6 analogue compound 180, although the spread of activity of the PEG-3 compound is also markedly low, 12-14%.

Two PEG-12 phenylcalixarenes have been tested. The half PEG-12-phenylcalix[8]arene 181, and the fully PEGylated heptamer 182, both had an average activity in the lung which was significantly higher than that shown by Macrocyclon (graph 7.0.3). This does indicate that perhaps the most active component of the Macrocyclon mixture is a PEG-12 octamer. In the spleen both compounds had very good activity, though at a lower level than that shown by Macrocyclon. However both compounds also exhibited a very small spread of data in both the lungs and spleen. The half-PEG-12-phenylcalix[8]arene 181 has a spread of only 32-37% compared to that of Macrocyclon at 77-116%. The PEG-12-phenylcalix[7]arene 182 has a spread of only 29-45%. Therefore both these compounds match the criteria of having high activity with a low spread of activity from the mean.
Graphs 7.0.3. Bacterial TB counts from lungs and spleens of mice treated with PEGylated calixarenes
Testing of some of the small molecule PEGylated monomers and dimers has been carried out. No toxicity was observed in the testing of the compounds, in contrast to the results observed by D'Arcy Hart for Comforth's linear PEGylated tert-octyl compounds\(^{85,90}\). Bisphenol-PEG-9-OBz, 204, displayed some activity in both the lung and spleen, with a low spread of activity in the lung (28%) though this was higher in the spleen (62%) (graph 7.0.4). The difference may be due to solubility issues as the compound was not deprotected, or the presence of a free hydroxyl may be necessary for biological activity. Bisphenol-PEG-6-OH 209, displayed very similar average activity to Macrocyclon in the lung, though in the spleen its activity was much lower, though the bacterial count was significantly lower than the control. It was interesting to note that for the bisphenol, the PEG-6 compound was more active than the PEG-9 compound, though that may be due to reasons discussed above. The spread of activity of the PEG-6 compound 209 was also much smaller than that shown by Macrocyclon, (50-67%) compared to (77-116%), and so this compound is also better than Macrocyclon at achieving a high average activity with a low spread of activity from the mean.

The bis-2-phenylmethane-PEG-12-OH, 215, displayed good activity in the lung and moderate activity in the spleen (graph 7.0.4). In general the small linear molecules display reasonable anti-TB activity, indicating that the presence of a macrocyclic structure is surprisingly not essential to biological activity, though it may be possible for the linear compounds to arrange into a pseudo-macrocyclic structure \textit{in situ}. The spread of activity in the lung was fairly large (94%), however in the spleen it was much lower at 43%.

Two labelled compounds were also tested for activity, as a large change in activity compared to the unlabelled version could possibly affect the validity of the confocal microscopy studies. The bisphenol-PEG-6-pyrene 219 displayed very good activity in both the lung and spleen, with a very small spread. The pyrene labelled phenylcalix[7]arene-PEG6 222 displayed the same level of activity as its unlabelled version 180, again with a low spread. Therefore the activities of the compounds do not appear to be greatly altered by the attachment of a single fluorescent moiety.
Graphs 7.0.4. Bacterial TB counts from lungs and spleens of mice treated with PEGylated monomers and dimers
Dosage studies were also carried out with Macrocyclon and the fully PEGylated tert-butylecalix[8]arene-PEG-12-OH, 224, which was synthesised by Dr A.C. Hervé within our group and is shown below in figure 7.0.2.

![Chemical Structure](https://example.com/structure.png)

Fig. 7.0.2

The compounds were tested concurrently at the standard dose, and at a dose one tenth that level (graph 7.0.5). When the dose of Macrocyclon was reduced to one tenth of the original dose a significant decrease in activity was observed in both the lung and spleen. For 224 a much smaller decrease in activity was observed compared with Macrocyclon. This indicates that our compounds are able to maintain activity at much lower doses than Macrocyclon, though the dosage levels of these compounds compared with many drugs are already low (<1 mg/kg). It is interesting to note that the spread of activity for both Macrocyclon and 224 decreases significantly with the decrease in dosing level, for Macrocyclon in the spleen from 138% to 43%. This agrees with the hypothesis that the activity of Macrocyclon is decreased significantly when the dosage decreases.

It should also be noted that the spread of data for the control group, who are administered only saline, is also variable. Although it is usually low (7-30%), it is occasionally high (up to 73%), which is a reflection that the experiments are carried out on live animals which can be affected by the disease to a different degree. This supports the use of the ratio of standard deviation to the mean as a measure of the spread of results, as an active compound would ideally be active to the same degree in all mice tested.
Graphs 7.0.5. Bacterial TB counts from lungs and spleens of mice treated with PEGylated calixarenes
7.1 Summary

All compounds synthesised exhibited anti-TB activity at some level, apart from one pro-TB compound (157). The phenylcalix[8]arene 77 and phenylcalix[7]arene 111, displayed good activity in the lung, but poor activity in the spleen, possibly due to their very low solubilities. The octamer and heptamer with PEG-6 178 and 180 displayed a greater level of activity in the lungs and spleen, with the heptamer displaying more activity than either the half or fully PEGylated octamer in the lungs. The spread of data for the fully PEG-6 octamer 179 compared to the half-PEGylated was significantly lower. Both the octamer (half PEGylated, compound 181) and heptamer with PEG-12 182 had an average activity in the lung which was higher than that shown by Macrocyclon, and a much smaller spread of activity. The smaller analogues displayed moderate activity in both the lung and spleen. Dosage studies indicate that the compounds retain activity at a very low dose, whilst the activity of Macrocyclon begins to decrease significantly as the dose is decreased. The spread of activity for Macrocyclon is consistently large which is probably due to its heterogeneous nature.

Overall we have synthesised a series of compounds of increasing PEG-chain length on the calixarenes, which display increasing activity, culminating in the two PEG-12 compounds which have an average activity significantly greater than that of our lead compound, Macrocyclon. We have synthesised a series of small analogues which also display good biological activity, and a series of labelled compounds which exhibit activity at the same level as their unlabelled analogues, allowing confocal microscopy work to be carried out.
8.0 Conclusion

The $p$-phenylcalix[8]arene series has been synthesised in good yield from the one-pot Petrolite Procedure. The phenylcalix[8]arene and phenylcalix[7]arene have been fully characterised by $^{13}$C NMR for the first time, and this is also the first reported fully characterised synthesis of the phenylcalix[7]arene, which has been synthesised in high yield. The use of different bases in the Petrolite Procedure has been examined, and a templating effect has been demonstrated.

We have also synthesised the nude calix[8]arene and calix[6]arene from the tert-butyl octamer and hexamer. Sulfonation at the upper rim of the octamer afforded a fully water soluble derivative, and a novel water soluble calixarene with a sulfonated upper rim and a dodecyl chain lower rim has been synthesised. An upper rim acyl calixarene has also been synthesised, and reactions of the nude octamer and hexamer have afforded lower rim allyl derivatives. An attempted Claisen rearrangement on these compounds to afford upper rim ally derivatives failed, though rearrangement of a small analogue readily occurred. Cross metathesis was successfully carried out on the rearranged small analogue using Grubbs’ catalyst.

$p$-Phenylcalix[8]arene has been fully derivatised at the lower rim with active electrophiles using a strong base and the octamer and heptamer have been fully derivatised with alkyl nitrile chains using a large excess of both electrophile and weak base. The phenylcalix[8]arene and phenylcalix[7]arene have also been derivatised at the lower rim with PEG-chains, which were synthesised via protection and activation strategies. Under the weakly basic reaction conditions the heptamer fully PEGylated at all positions, whilst a half-PEGylated octamer was afforded, which is believed to exhibit a symmetrical substitution pattern. It is thought that the greater conformational
flexibility of the heptamer allows free rotation of phenolic positions away from PEGylated ones, lessening steric hindrance and thereby increasing reactivity of the heptamer towards PEG-chains. Full PEGylation of the octamer was achieved by exposure of the half-PEGylated compound to strong base conditions.

A series of small PEGylated analogues has also been synthesised, which are closer to obeying the mass criteria of the Lipinski Rule of 5 than the PEGylated calixarenes. Biological testing of the compounds has revealed that the phenylcalix[8]arene and phenylcalix[7]arene display moderate activity, which increases with increasing PEG-chain length. The octamer and heptamer derivatised with PEG-12 displayed higher average activity than our lead compound, Macrocyclon. The small analogues also display reasonable biological activity, surprisingly indicating that a macrocyclic functionality is not essential to biological activity.

8.1 Future Work

Further biological testing of compounds synthesised, including confocal microscopy and activity testing of compounds such as the water soluble sulfonated calixarenes, will provide guidance for future studies. However, solubilisation of calixarenes via functionalities other than PEG-chains may provide compounds with even greater biological activity, for example the use of polyamine chains at the lower rim may afford novel water soluble calixarenes. Further derivatisation of the upper rim of the nude calixarenes could focus on reaction to afford an aldehyde group at the upper rim which could be further derivatised to afford an amine.

The PEGylated small analogues displayed good biological activity and are readily accessible in comparison to the PEGylated calixarenes. Therefore future work with the small analogues could examine further derivatisation of them with alternative solubilising agents. Also, the synthesis of longer linear analogues could be examined as a greater chain length may provide even greater biological activity.
Chapter 9
Experimental

9.0 Materials and Methods

Chemicals were purchased from the Aldrich Chemical Company Ltd or from Lancaster Chemicals Ltd and were used as received. Unless otherwise stated, water refers to the use of deionised water. Solvents were purchased from BDH Ltd apart from anhydrous dimethylformamide (Aldrich) and were used as received. Anhydrous reaction solvents were HPLC grade and were distilled over potassium hydroxide (pyridine), calcium hydride (DCM), phosphorous pentoxide (acetonitrile) or sodium with benzophenone (THF). All anhydrous reactions were performed under nitrogen. Ether refers to diethyl ether, ethanol refers to absolute ethanol (>99.7%) and was used as received.

$^1$H NMR experiments were carried out using a Bruker instrument at 300 MHz, 400 MHz or 500 MHz. $^{13}$C NMR experiments were performed at 75 MHz, 100 MHz or 125 MHz on a Bruker instrument. Residual protic solvent was taken as the internal standard using deuterated chloroform ($\delta_{\text{H}}$ 7.24) unless otherwise stated. Mass spectrometry (ES, FAB) was performed either at the University College London mass spectrometry service, or at the London School of Pharmacy using a ZAB-SE instrument (FAB). High resolution mass spectrometry (FAB) was performed at the London School of Pharmacy. Infrared spectra (IR) were recorded on a Perkin Elmer 983 G or FT-IR spectrometer using potassium bromide discs or as thin films using sodium chloride plates. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected.

Analytical thin layer chromatography was performed on precoated glass backed plates (Merck Kieselgel 60 F$_{254}$ or RP-6 F$_{254}$). TLC plates were visualised using UV (254 nm) and solutions of potassium permanganate or anisaldehyde. Flash chromatography was performed using 220-400 mesh silica as stationary phase, reverse phase flash chromatography was performed using Si60 Silanised silica gel as the stationary phase (BDH).
9.1 Synthesis of Calixarenes


Optimum Procedure

A mixture of p-phenylphenol (8.50 g, 0.05 mol), paraformaldehyde (3.00 g, 0.10 mol) and caesium hydroxide (0.85 ml, 0.003 mol, 50% aq. soln w/v), in xylene (50 ml) was heated at 130 °C for 4 h, under nitrogen, with the azeotropic removal of water. The resulting precipitate was collected by filtration and washed with water (20 ml), ethyl acetate (20 ml) and acetone (20 ml). Flash chromatography (hexane/ethyl acetate, 3:1) yielded the title compound as a white powder (12.0 g, 34%).

mp >400°C (ethyl acetate/hexane) (421-423 °C);
νmax / cm⁻¹ (KBr Discs) 3200 br, 2920 s, 2960-2850 s; 751 s;
δH (300 MHz; DMSO- d₆) 4.01 (16H, br s, ArCH₂Ar), 7.20-7.71 (56H, m, ArH);
δC (100 MHz; Acetone- d₆) 33.51 (C-2), 126.79 (C-3), 127.18 (C-7), 127.85 (C-9), 129.38 (C-4), 130.35 (C-8), 132.81 (C-5), 142.31 (C-6), 154.01 (C-OH);
m/z (FAB⁺) 1479 (M⁺ + Na⁺, 5%);
\( \lambda_{\text{max}}/\text{nm} \) \((\text{KOH}/\text{MeOH})\) 230, 290.

Other Reactions Carried Out

Reactions carried out as optimum procedure, except differences in base and aldehyde source as indicated below.

<table>
<thead>
<tr>
<th>Base</th>
<th>% Aq. Sol$^\text{a}$</th>
<th>Eq</th>
<th>Aldehyde</th>
<th>Aldehyde Eq</th>
<th>% [8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH (10 M)</td>
<td>0.03</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>16%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>33%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>KOH (10 M)</td>
<td>0.03</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>[7]</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.015</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>21%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>3</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>28%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>34%</td>
</tr>
<tr>
<td>RbOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>14%</td>
</tr>
<tr>
<td>RbOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>Ca(OH)$_2$</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>Ba(OH)$_2$</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]$_n$</td>
<td>2</td>
<td>0%</td>
</tr>
</tbody>
</table>

Direct Method

A mixture of p-phenylphenol (8.50 g, 0.05 mol), paraformaldehyde (3.00 g, 0.10 mol) and potassium hydroxide (0.40 ml, 0.004 mol, 50% aq. sol" , w/v), in xylene (50 ml) was heated at 130 °C for 4 h, under nitrogen, with the azeotropic removal of water. The crude product was isolated by filtration and washed with water (20 ml), ethyl acetate (20 ml) and acetone (20 ml). Flash chromatography (hexane/ethyl acetate, 3:1) yielded the title compound as a white powder (0.73 g, 8%).

Highest Yielding Procedure

A mixture of p-phenylphenol (34.0 g, 0.20 mol), formaldehyde (46.0 ml, 37% aqueous solution, 46 ml) and sodium hydroxide (80.0 ml, 3N), was heated at reflux under nitrogen for 1 h. Heating was continued for a further 2 h (without condenser), and the mixture was cooled to room temperature. Hydrochloric acid was added (2N, 250 ml) and the solid collected by filtration. To this was added diphenylether (100 ml) and sodium hydroxide (50% aq. solution, 1.5 ml). The resulting solution was heated at 215 °C for 2 h, diethylether (100 ml) was added and the mixture stirred at room
temperature overnight. The resulting precipitate was isolated by filtration, the precipitate dissolved in acetic acid (2M) and heated at reflux for 1 h. Flash chromatography (hexane/ethyl acetate, 4:1) yielded the title compound as a white powder (4.30g, 14%).

mp > 400 °C (ethyl acetate/hexane) (408-410 °C)

ν\text{max} / cm^{-1} (KBr disc) 3068 br, 3040 m, 754 s;

δ\text{H} (300 MHz; DMSO-d_6) 4.10 (12H, s, ArCH₂Ar), 7.21-7.64 (42H, m, ArH);

δ\text{C} (100 MHz; acetone-d₆) 33.43 (C-2), 126.67 (C-3), 127.08 (C-7), 127.75 (C-9), 129.23 (C-4), 130.26 (C-8), 132.71 (C-5), 142.23 (C-6), 153.95 (C-OH);

m/z (ES) 1093 (M⁺, 0.5%).

Other reactions carried out using direct procedure

Reactions carried out as direct procedure, except differences in base and aldehyde source as indicated below.

<table>
<thead>
<tr>
<th>Base</th>
<th>% Aq. Sol*</th>
<th>Eq</th>
<th>Aldehyde</th>
<th>Aldehyde Eq</th>
<th>% [6]</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>(10 M)</td>
<td>0.03</td>
<td>[HCHO]n</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]n</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0.3%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>(10M)</td>
<td>0.03</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0.6%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]n</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.015</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[HCHO]n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------</td>
<td>-----</td>
<td>---------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>CsOH 50%</td>
<td>0.075</td>
<td>2</td>
<td>0.075</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>CsOH 50%</td>
<td>0.05</td>
<td>2</td>
<td>[HCHO]n</td>
<td>2</td>
<td>1.5%</td>
</tr>
<tr>
<td>RbOH 50%</td>
<td>0.05</td>
<td>2</td>
<td>[HCHO]n</td>
<td>2</td>
<td>1.2%</td>
</tr>
<tr>
<td>RbOH 50%</td>
<td>0.15</td>
<td>2</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>Ca(OH)₂ 50%</td>
<td>0.075</td>
<td>2</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>Ba(OH)₂ 50%</td>
<td>0.075</td>
<td>2</td>
<td>[HCHO]n</td>
<td>2</td>
<td>0%</td>
</tr>
</tbody>
</table>


Optimum Procedure

A mixture of p-phenylphenol (8.50 g, 0.05 mol), paraformaldehyde (3.00 g, 0.10 mol) and sodium hydroxide (0.20 ml, 0.003 mol, 50% aq. sol⁹), in xylene (50 ml) was heated at 130 °C, under nitrogen for 4 h with the azeotropic removal of water. The resulting precipitate was isolated by filtration and washed with water (20 ml), ethyl acetate (20 ml) and acetone (20 ml). Flash chromatography (hexane/ethyl acetate, 3:1) yielded the title compound as a white powder (0.64 g, 7%).
mp > 400 °C (ethyl acetate/hexane) (407-409 °C)

ν<sub>max</sub> / cm<sup>-1</sup> (KBr disc) 3084 br, 3045 m 748 s;

δ<sub>H</sub> (300 MHz, DMSO-d<sub>6</sub>) 4.91 (8H, s, ArCH<sub>2</sub>Ar), 7.38-7.72 (28H, m, ArH);

δ<sub>C</sub> (100 MHz; acetone-d<sub>6</sub>) 32.91 (C-2), 126.16 (C-3), 126.56 (C-7), 127.23 (C-9), 128.72 (C-4), 129.74 (C-8), 132.19 (C-5), 141.72 (C-6), 149.61 (C-OH);

m/z (FAB<sup>+</sup>) 728.2899 (M<sup>+</sup>, 5%, C<sub>52</sub>H<sub>40</sub>O<sub>4</sub> requires 728.2927).

Other Reactions Performed

Reactions carried out as optimum procedure, except differences in base and aldehyde source as indicated below.

<table>
<thead>
<tr>
<th>Base</th>
<th>% Aq. Sol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Eq</th>
<th>Aldehyde</th>
<th>Aldehyde Eq</th>
<th>% [4]</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0.2%</td>
</tr>
<tr>
<td>LiOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>NaOH</td>
<td>2%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>NaOH</td>
<td>(10 M)</td>
<td>0.03</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0.6%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.05</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>7%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>NaOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>2%</td>
<td>0.075</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>HCHO</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>(10M)</td>
<td>0.03</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0.3%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>KOH</td>
<td>50%</td>
<td>0.15</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>0.6</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.05</td>
<td>Trioxane</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.015</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>3</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>CsOH</td>
<td>50%</td>
<td>0.075</td>
<td>[HCHO]&lt;sub&gt;n&lt;/sub&gt;</td>
<td>2</td>
<td>0%</td>
</tr>
</tbody>
</table>

|     |     |     | [HCHO]n |     |  
|-----|-----|-----|---------|-----|-----
| CsOH| 50% | 0.05| [HCHO]n| 2   | 0.5%|
| RbOH| 50% | 0.05| [HCHO]n| 2   | 0.6%|
| RbOH| 50% | 0.15| [HCHO]n| 2   | 0%  |
| Ca(OH)\(_2\) | 50% | 0.075| [HCHO]n| 2   | 0%  |
| Ba(OH)\(_2\) | 50% | 0.075| [HCHO]n| 2   | 0%  |

A mixture of \(p\)-phenylphenol (8.50 g, 0.05 mol), paraformaldehyde (3.00 g, 0.10 mol) and potassium hydroxide (0.40 ml, 0.004 mol, 50% aq. sol\(^{\circ}\) w/v ), in xylene (50 ml) was heated at 130 °C for 4 h, under nitrogen, with the azeotropic removal of water. The resulting precipitate was collected by filtration and washed with water (20 ml), ethyl acetate (20 ml) and acetone (20 ml). Flash chromatography (hexane/ethyl acetate, 3:1) yielded the title compound as a white powder (5.46 g, 60%).

m.p. >400 °C (ethyl acetate/hexane);

\(\nu_{\text{max}}/\text{cm}^{-1}\) (KBr Disc) 3190 br and 2980 s, 744 s;

\(\delta_H\) (300 MHz; DMSO-\(d_6\)) 4.65 (14H, br s, ArCH\(_2\)Ar), 7.10-7.58 (49H, m, ArH);
δC (125 MHz; Acetone-d6) 32.63 (C-2), 126.58 (C-3), 126.82 (C-7), 127.73 (C-9), 128.95 (C-4), 129.29 (C-8), 133.51 (C-5), 141.60 (C-6);  
δC (125 MHz; CDCl3) 32.63 (C-2), 126.58 (C-3), 126.82 (C-7), 127.73 (C-9), 128.95 (C-4), 129.29 (C-8), 133.51 (C-5), 141.60 (C-6);  
δC (75 MHz; Solid State) 32.74 (C-2), 128.19 (C-4), 134.07 (C-5), 140.05 (C-6), 149.11 (C-OH);  
m/z (FAB⁺) 1275.5235 (M⁺, 15%, C₉₁H₇₁O₇ requires 1275.5235);  
λ_max/nm (KOH/MeOH) 275.


To a suspension of tert-butylphenol (10.0 g, 0.07 mol) in xylene (60 ml), at room temperature under a nitrogen atmosphere, paraformaldehyde (3.20 g, 0.11 mmol) was added. Sodium hydroxide (0.20 ml, 2.00 mmol, 50% aq. sol¹⁰) was added and the mixture stirred at 140 °C for 4 h, with the azeotropic removal of water. The resulting precipitate was collected by filtration, washed with diethyl ether (30 ml), acetone (30 ml) and water (30 ml). The crude product was recrystallised (chloroform) to afford the title compound as a white solid (5.20 g, 48%).

mp > 400 °C (chloroform) (411-412 °C);  
ν_max/cm⁻¹(KBr Disc) 3250 br, 1490 s and 1250 s, 749 s;  
δH (300MHz; CDCl3) 1.24 (72H, s, Me), 3.50 (8H, br s, ArCH₂Ar), 4.33 (8H, br s, ArCH₂Ar), 7.16 (16H, s, ArH) and 9.58 (8H, s, OH);

The reaction was carried out under anhydrous conditions. To a suspension of 52 (2.00g, 1.54 mmol) in toluene (40 ml) at 0 °C, aluminium chloride (2.47g, 18.5 mmol) was added in one portion. Phenol (1.16g, 12.3 mmol) was added and the reaction mixture was stirred at room temperature for 72 h. The reaction mixture was quenched by addition of iced water (40 ml). Separation and evaporation in vacuo afforded the crude product as an orange solid. Washing with acetone (3 x 10 ml), diethyl ether (3 x 10 ml), water (2 x 10 ml) and recrystallisation (methanol) afforded the title compound as a pale cream solid. (1.25g, 95%).

$\delta_C$ (75MHz; CDCl$_3$) 31.46 (CH$_3$), 32.19 (C-2), 34.00 (C-CH$_3$), 125.53 (C-4), 128.70 (C-3), 144.73 (C-5) and 146.64 (C-OH);

$m/z$ (FAB') 1296 (M$^+$, 10%).

A slurry of tert-butylphenol (10.0 g, 0.07 mol), paraformaldehyde (3.99 g, 0.13 mol), and rubidium hydroxide (1.96 ml, 0.03 mol, 50% aq. soln) was heated at reflux in xylene (100 ml) for 4 h, under a nitrogen atmosphere, with the azeotropic removal of water. The resulting precipitate was collected by filtration in vacuo and washed with water (40 ml), chloroform (30 ml) and methanol (40 ml). The crude product was recrystallised from chloroform to afford the title compound as a white powder (5.21 g, 48%).

mp 378-382 °C (chloroform) (380-381 °C);

$\nu_{\text{max}}$ /cm$^{-1}$ (KBr Disc) 3410 s, 3156 s, 1490 s and 1250 s, 749 s;

$\delta_H$ (300 MHz; CDCl$_3$) 1.24 (54H, s, Me), 3.84 (12H, br s, ArCH$_2$Ar), 7.10 (12H, s, ArH), 9.58 (6H, s, OH);

$\delta_C$ (75 MHz; CDCl$_3$) 31.25 (C(CH$_3$)$_3$), 32.76 (C(CH$_3$)$_3$), 33.78 (C-2), 125.30 (C-4), 125.80 (C-3), 144.68 (C-5), 147.32 (C-OH);

$m/z$ (FAB') 972 (100%, M$^+$).

165
Synthesis of $37,38,39,40,41,42$-hexahydroxycalix[6]arene (112)$^{116}$

The reaction was carried out under anhydrous conditions. To a suspension of 51 (2.50 g, 2.57 mmol) in toluene (30 ml) at 0 °C, aluminium chloride (2.75 g, 21.1 mmol) was added in two portions. Phenol (1.45 g, 15.4 mmol) was added and the reaction mixture stirred at room temperature for 3 d. The reaction was quenched by the addition of water (40 ml) separation and evaporation in vacuo afforded the crude product as a yellow solid. The product was recrystallised from methanol to afford the title compound as a pale cream solid (310 mg, 19%).

$\nu_{\text{max}}$/cm$^{-1}$ (KBr Disc) 3389 br, 3148 s, 1475 s and 1243 s, 745 s;
$\delta_H$ (300 MHz; CD$_2$Cl$_2$) 3.91 (12H, br s, ArCH$_2$Ar), 7.07 (18H, s, ArH);
$\delta_C$ (75 MHz; CD$_2$Cl$_2$) 31.99 (C-2), 121.63 (C-5), 127.19 (C-4), 129.27 (C-3), 149.45 (C-OH);
$m/z$ (FAB') 635 (100%, M$^+$);
$\lambda_{\text{max}}$/nm (MeOH/KOH) 270.

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (720 mg, 0.02 mol) in THF/DMF (4:1, 40 ml), 77 (874 mg, 0.60 mmol) was added in portions. Following stirring for 15 min, acetyl chloride (750 mg, 9.60 mmol) was added dropwise. The resulting solution was heated at 70 °C for 24 h. The reaction mixture was quenched with iced water (2 x 10 ml), the crude product extracted into chloroform (3 x 50 ml) and washed with saturated lithium chloride solution (2 x 20 ml). Drying (MgSO₄), filtration and evaporation in vacuo afforded a crude oil which upon flash chromatography (hexane/ethyl acetate, 3:1) afforded the title compound as colourless crystals (490 mg, 46%).

mp 235-249 °C (ethyl acetate/hexane) (230-265 °C);
νmax/cm⁻¹ (KBr Discs) 2954 s, 2854 m, 1759 m, 1658 w, 1597 w, 1555 w, 1460 m and 1376 m;

δH (300MHz; CDCl3) 1.85 (24H, s, Me), 3.78 (16H, s, ArCH₂Ar), 7.23-7.81 (56H, m, ArH);
δC (100 MHz; CDCl3) 21.24 (CH₃), 30.98 (ArCH₂Ar), 125.49 (C-3), 126.19 (C-6, C-8), 127.85 (C-7), 133.38 (C-2), 140.95 (C-4, C-5), 152.91 (C-1);
m/z (FAB⁺) 1793 (0.2%, M⁺).

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (720 mg, 0.02 mol) in THF/DMF (4:1), 77 (874 mg, 0.60 mmol) was added in portions. Following stirring for 45 min, allylbromide (0.83 ml, 9.60 mmol) was added dropwise. The resulting solution was heated at 70 °C for 24 h. The mixture was quenched with iced water (2 x 10 ml), the crude product was extracted into chloroform (3 x 50 ml) and washed with saturated lithium chloride solution (2 x 20 ml). Drying (MgSO₄), filtration and evaporation in vacuo afforded a crude oil which upon flash chromatography (hexane/ethyl acetate, 3:1) afforded the title compound as colourless crystals (350 mg, 33%).

mp 249-257 °C (ethyl acetate/hexane)

ν_max/cm⁻¹ (KBr Discs) 2958 s, 2854 m, 1671 m, 1597 w, 1469 m;

δ_H (300 MHz; CDCl₃) 3.98-4.18 (32H, m, 3'-CH₂, ArCH₂Ar), 4.98-5.03 (16H, m, 1'-CH₂), 5.54-5.78 (8H, m, 2'-CH), 6.91-7.48 (56H, m, ArH);

δ_C (100 MHz; CDCl₃) 74.99 (C-3'), 116.45 (C-1'), 125.23, 126.94, 127.49, 134.26, 141.38, 151.68;

m/z (FAB⁺) 1777 (M⁺, 0.1%).

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (720 mg, 0.02 mol) in THF/DMF (4:1), 77 (874 mg, 0.60 mmol) was added in portions. Following stirring for 45 min. ethylbromoacetate (1.60 g, 9.60 mmol) was added dropwise. The resulting solution was heated at 70 °C for 24 h. The reaction mixture was quenched with iced water (2 x 10 ml), the crude product extracted into chloroform (3 x 50 ml) and washed with saturated lithium chloride solution (2 x 20 ml). Drying (MgSO₄), filtration and evaporation in vacuo yielded a crude oil which upon flash chromatography (hexane/ethyl acetate 3:1) afforded the title compound as colourless crystals (400 mg, 33%).

mp 229-241 °C (ethyl acetate/hexane)

νₘₐₓ / cm⁻¹ (KBr Discs) 2853 s, 1744 m, 1658 m, 1598 w, 1462 s and 1376 s;

δ_H (300 MHz; CDCl₃) δ 1.18 (24H, s, Me), 4.23 (32H, m, 1'-CH₂, ArCH₂Ar), 4.49 (16H, m, 1'-CH₂), 4.91 (16H, s, 3'-CH₂), 7.10-7.45 (56H, m, Ar);

δ_C (100 MHz; CDCl₃) 13.74 (Me), 59.81 (C-1'), 75.78 (C-3'), 125.71, 125.93, 126.89, 128.86, 134.81, 141.78, 158.91 (C-1), 171.35 (C-2');

m/z (FAB⁺) 2145 (M⁺, 0.3%).
General Procedure using Caesium Carbonate (acetylation/allylation)

The reaction was carried out under anhydrous conditions. To a solution of \( p \)-phenylcalix[8]arene (1.5 g, 1 mmol) in DMF, caesium carbonate (12 eq) was added in one portion. The electrophile (12 eq) was added dropwise and the resulting solution stirred at 60 °C for 24 h. The reaction mixture was quenched with iced water (2 x 10 ml) and product extracted into chloroform (3 x 50 ml) and washed with saturated lithium chloride solution (2 x 20 ml). Drying (MgSO\(_4\)) and evaporation in vacuo yielded a crude oil which upon flash chromatography (hexane/ethyl acetate, 3:1) afforded the title compound as colourless crystals.

General Procedure using Potassium Carbonate

The reaction was carried out under anhydrous conditions. To a solution of \( p \)-phenylcalix[8]arene (1.5 g, 1 mmol) in DMF, potassium carbonate (12 eq) was added in one portion. The electrophile (12 eq) was added dropwise and the resulting solution stirred at 60 °C for 16 h. The reaction was quenched with iced water (2 x 10 ml) and the product extracted into chloroform (3 x 40 ml) and washed with saturated lithium chloride solution (2 x 20 ml). Drying (MgSO\(_4\)) and evaporation in vacuo yielded a crude oil which upon flash chromatography (hexane/ethyl acetate, 3:1) afforded the title compound as colourless crystals.

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (425 mg, 10.6 mmol) in THF (40 ml), 73 (300 mg, 0.35 mmol) and bromododecane (1.36 ml, 5.70 mmol) were added. The reaction mixture was stirred at 70 °C for 3 d. The reaction was quenched with water (40 ml), and the precipitate recovered by filtration to afford the title compound as a white powder (530 mg, 68%).

mp 248-265 °C

$\nu_{\text{max}}$ /cm$^{-1}$ (KBr Disc) 2849 s, 1462 m and 1376 m;

$\delta_H$ (300 MHz; Pyridine-d$_6$) 0.85 (24H, t, $J$ 5.9, Me), 1.19-1.31 (144H, m, 2 to 10-CH$_2$), 1.69-1.78 (16H, m, 2-CH$_2$), 3.39 (16H, t, $J$ 6.8, 12-CH$_2$), 4.17 (16H, s, ArCH$_2$Ar), 7.19 (24H, s, ArH);

$\delta_C$ (100 MHz, RD 6s; Pyridine-d$_6$) 14.15 (Me), 20.76 (C-2), 29.20-30.91 (C-2 to C-9, ArCH$_2$Ar), 33.30 (C-10), 70.74 (C-12), 118.91 (C-4'), 128.68 (C-3'), 129.55 (C-2'), 155.03 (C-1');

$m/z$ (FAB$^+$) 2192 (M$^+$, 1%), 848 (M$^+$ - 8 x (CH$_2$)$_{11}$Me, 12), 192 ([CH$_2$)$_{11}$Me + Na$^+$], 4).


Calix[8]arene (450 mg, 0.53 mmol) was suspended in concentrated sulfuric acid (10 ml; 18M), and heated at 80 °C for 4 h. The reaction was quenched with water (30 ml) and neutralised by the addition of potassium carbonate. Following filtration in vacuo, sodium carbonate was added to the filtrate, and evaporation in vacuo afforded a pale yellow solid which was dissolved in water (30 ml), and the remaining precipitate removed by filtration in vacuo. Following evaporation in vacuo the crude solid was
recrystallised from water and methanol to afford the title compound as a white solid (712 mg, 90%).

\[ \nu_{\text{max}} / \text{cm}^{-1} (\text{KBr Disc}) 2858 \text{ s}, 1454 \text{ m}, 1051 \text{ s}; \]

\[ \delta \text{H} (400 \text{ MHz}; \text{D}_2\text{O}) 3.84 \text{ (8H, s, ArCH}_2\text{Ar)}, 3.86 \text{ (8H, s, ArCH}_2\text{Ar), 7.18 \text{ (16H, s, ArH)}; \]

\[ \delta \text{C} (100 \text{ MHz}; \text{D}_2\text{O}) 33.31 \text{ (ArCH}_2\text{Ar), 125.78 \text{ (C-3), 133.66 \text{ (C-2), 139.2 \text{ (C-4), 157.69 \text{ (C-1);}})}} \]

\[ m/z \ (\text{ES}^+) 1504 \text{ (M + Na}^+, 14\% \).


195 (450 mg, 0.20 mmol) was suspended in concentrated sulfuric acid (10 ml; 18M), and heated at 80 °C for 4 h. The reaction was quenched with water (30 ml) and neutralised by the addition of potassium carbonate. Following filtration in vacuo, sodium carbonate was added to the filtrate, and evaporation in vacuo afforded a pale yellow solid which was dissolved in water (30 ml), and the remaining precipitate removed by filtration in vacuo. Following evaporation in vacuo the crude solid was recrystallised from water and methanol to afford the title compound as a white solid (430 mg, 76%).

\[ \nu_{\text{max}} / \text{cm}^{-1} (\text{KBr Disc}) 2858 \text{ s}, 1454 \text{ m}, 1376 \text{ m}, 1051 \text{ s}; \]

\[ ^{13}\text{C} (100 \text{ MHz}; \text{D}_2\text{O}) 14.18 \text{ (Me), 20.79 \text{ (C-2), 29.21-30.97 \text{ (C-2 to C-9, ArCH}_2\text{Ar), 33.31 \text{ (C-10), 72.65 \text{ (C-12), 118.95 \text{ (C-4), 126.47 \text{ (C-3'), 128.35 \text{ (C-2'), 156.18 \text{ (C-1');}})}} \]

\[ m/z \ (\text{ES}^+) 1439 \text{ ([M + 2 x Na}^+)\text{/2, 18%}. \]

The reaction was carried out under anhydrous conditions. To a suspension of 77 (500 mg, 0.34 mmol) in acetonitrile (60 ml), potassium carbonate (758 mg, 5.50 mmol) and 144 (624 mg, 2.75 mmol) were added and the reaction was stirred at 80 °C for 4 d. The reaction mixture was quenched with water (30 ml), the crude product extracted into dichloromethane (40 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Flash chromatography (ethyl acetate/hexane 2:1, then ethyl acetate/methanol 10:1) afforded the title compound as a yellow oil (536 mg, 71%).

υ max /cm⁻¹ (film) 2848 s, 1453 m;
δ H (300 MHz; CDCl3) 3.28 (12H, s, 4 x Me), 3.45-3.64 (64H, m, 8 x 9-CH₂, 4 x 1" to 8"-CH₂), 7.18-7.86 (56H, m, ArH);
δ C (100 MHz; CDCl3) 31.31 (C-9), 58.77 (Me), 69.82-72.21 (C-1" to C-9"), 126.55, 126.69, 127.49, 127.69, 128.37, 133.97, 140.25, 141.05, 153.68 (C-1);
m/z (ES⁺) 1041 ([M + 2 x Na⁺]/2, 45%), 1057 ([M + 2 x Na⁺ + MeOH]/2, 40), 1073 ([M + 2 x Na⁺ + 2 x MeOH]/2, 30).

The reaction was carried out under anhydrous conditions. To a suspension of 77 (500 mg, 0.34 mmol) and potassium carbonate (758 mg, 5.49 mmol) in acetonitrile (60 ml) at room temperature, 146 (1.18 g, 2.75 mmol) in acetonitrile (10 ml) was added and the reaction stirred at reflux for 4 d. The reaction was quenched by the addition of water (30 ml), the crude product extracted into dichloromethane (50 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded the title compound as a viscous yellow oil (196 mg, 20%).

$\nu_{\text{max}}$ / cm$^{-1}$ (film) 2868 s, 1456 m, 1122 s; 

$\delta_{\text{H}}$ (300 MHz; CD$_2$Cl$_2$) 1.67–1.98 (24H, m, 4 x 3"-CH$_2$ to 5"-CH$_2$), 3.55–4.00 (129H, m, 4 x 6"-CH$_2$, 8 x 9-CH$_2$, 4 x 1"-CH$_2$ to 17"-CH$_2$), 4.76 (4H, br, 4 x 2"-CH), 7.45–7.77 (21H, m, ArH);
\( \delta_c \) (100 MHz, d1 6s; CDCl₃) 18.88 (C-5"), 24.92 (C-4"), 29.81 (C-9), 30.01 (C-3"), 61.00 (C-17"), 66.06 (C-6"), 69.96-70.79 (C-1" to C-16"), 98.25 (C-2"), 126.21, 126.68, 127.13, 127.46, 128.09, 133.25, 140.17, 140.38, 157.86 (C-1);

\( m/z \) (MALDI-TOF⁺) 2872 (M⁺ + Na⁺, 40%), 2849 (M⁺, 30), 2786 (M⁺ - THF + Na⁺, 45), 2680 (M⁺ - 2x THF, 35);

\( \lambda_{max}/\text{nm} \) (KOH/MeOH) 230, 255.


The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (44.0 mg, 1.10 mmol) in THF (40 ml), 174 (196 mg, 0.07 mmol) in THF (10 ml) and 146 (236 mg, 0.55 mmol) in THF (10 ml) was added and the reaction stirred at reflux for 4 d. The reaction was quenched with iced water (10 ml), the crude product extracted into dichloromethane (40 ml), dried (phase separator), and evaporated in vacuo to afford the crude product as a yellow oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded the title compound as a viscous yellow oil (58 mg, 20%).
\( v_{\text{max}} \) /\( \text{cm}^{-1} \) (Film) 2849 s, 1451 m;
\( \delta_H \) (300 MHz; CDCl\(_3\)) 1.44-1.66 (48H, m, 8 x 3'-CH\(_2\) to 5'-CH\(_2\)), 3.41-3.83 (176H, m, 8 x 6'-CH\(_2\), 8 x 9-CH\(_2\), 8 x 1''-CH\(_2\) to 17''-CH\(_2\)), 4.58 (8H, t, J 2.6, 8 x 2'-CH), 7.24-7.78 (8H, m, ArH);
\( \delta_C \) (100 MHz, RD 6s; CDCl\(_3\)) 19.18 (C-5'), 25.12 (C-4'), 30.12 (C-3'), 61.92 (C-17''), 66.90 (C-6'), 70.22 to 70.39 (C-1'' to C-16''), 98.64 (C-2'), 126.8, 127.6, 128.5, 134.2, 136.3, 140.7, 154.3 (C-1);
\( m/z \) (MALDI-TOF) 1001.6 ([M + 4xNa\(^+\) - 4 x THP]/4) 100%;
\( \lambda_{\text{max}} /\text{nm} \) (KOH/MeOH) 230, 262.


174 (50.0 mg, 0.02 mmol) was added in portions to a solution of hydrochloric acid (5 ml, 18M sol\(^b\)) and dichloromethane (5 ml) at room temperature. The resulting solution was stirred at room temperature for two hours. Evaporation afforded a brown oil which was suspended in an aqueous solution of sodium hydroxide (pH 14, 10 ml) and stirred vigorously for thirty minutes. The product was extracted into dichloromethane (5 ml),

\( 178 \) (50.0 mg, 0.02 mmol) was added in portions to a solution of hydrochloric acid (5 ml, 18M sol\(^b\)) and dichloromethane (5 ml) at room temperature. The resulting solution was stirred at room temperature for two hours. Evaporation afforded a brown oil which was suspended in an aqueous solution of sodium hydroxide (pH 14, 10 ml) and stirred vigorously for thirty minutes. The product was extracted into dichloromethane (5 ml),

176
dried (MgSO₄), filtration and evaporation in vacuo afforded the title compound as a yellow viscous oil (43 mg, 85%).

\[ \nu_{\text{max}} / \text{cm}^{-1} \] (Film) 2868 s, 1456 m, 1122 s;

\[ \delta_H \] (300 MHz; CD₂Cl₂) 3.55-4.00 (129H, m, 8 x 9-CH₂, 4 x 1"-CH₂ to 17"-CH₂), 7.45-7.77 (21H, m, ArH)

\[ \delta_C \] (100 MHz, RD 6s; CDCl₃) 62.17(C-17"), 69.63-73.16 (C-1" to C-16"), 125.38, 126.18, 127.73, 127.91, 128.03, 134.17, 140.19, 156.49(C-1).


To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 175 (50.0 mg, 0.01 mmol) was added and the resulting solution stirred at room temperature for 2 h. Evaporation in vacuo afforded a brown oil which was suspended in an aqueous solution of sodium hydroxide (10%; 10 ml) and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 ml), dried (MgSO₄), filtered and evaporated in vacuo to afford the title compound as a viscous yellow oil (36 mg, 86%).

\[ \nu_{\text{max}} / \text{cm}^{-1} \] (Film) 2908 s, 14546m, 1118 s;
\( \delta_H (300 \text{ MHz}; \text{CDCl}_3) 3.52-4.08 \text{ (208H, m, 8 x 9-CH}_2, 4 x 1"-\text{CH}_2 \text{ to 17"-CH}_2), 7.45-7.77 \text{ (56H, m, ArH).} \)


The reaction was carried out under anhydrous conditions. To a suspension of 77 (200 mg, 0.14 mmol) and potassium carbonate (303 mg, 2.19 mmol) in acetonitrile (50 ml) at room temperature, 155 (813 mg, 1.09 mmol), in acetonitrile (10 ml), was added dropwise. The resulting solution was heated at 80 °C for 7 d. The reaction was quenched by addition of water (20 ml), the crude product extracted into dichloromethane (40 ml), dried (phase separator) and evaporated *in vacuo* to afford the crude product as brown oil. Flash chromatography (ethyl acetate/hexane, 2:1 then ethyl acetate/methanol, 10:1) afforded the *title compound* as a viscous brown oil (133 mg, 25%).

\( \nu_{\text{max}}/\text{cm}^{-1} \) (Film) 2868 s, 1469 s, 1119 s, 1033 m;
δ_H(400 MHz; CDCl_3) 1.38–1.79 (4H, m, 3″ to 5″-CH_2), 3.21-5.28 (75H, m, 2″-CH_2, 6″-CH_2, 1″-CH_2 to 35″-CH_2), 7.13-7.93 (56H, m, ArH);
δ_C (100 MHz, RD 6s; CDCl_3) 19.48 (C-5″), 25.45 (C-4″), 29.66 (C-3″), 30.61 (C-9), 62.18 (C-35″), 66.66 (C-6″), 70.56-73.85 (C-1″-C-34″), 98.95 (C-2″), 125.45, 126.67, 126.91, 127.69, 128.59, 134.10, 137.01, 137.55, 140.59, 154.13 (C-1′), 155.27 (C-1);
m/z (MALDI-TOF) 1975 ([M+ + 2Na]/2, 40%), 1807.5 ([M - 4xTHP + 2Na^+]/2, 50).


To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 176 (51 mg, 0.01 mmol) was added and the solution stirred at room temperature for 2 h. Evaporation in vacuo afforded a brown oil which was suspended in an aqueous solution of sodium hydroxide (10 %; 10 ml), and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 ml), dried and evaporated in vacuo to afford the title compound as a brown viscous oil (28 mg, 61%).

ν_max/cm⁻¹ (Film) 2849 s, 1451 s, 1118 s;
δ_H (300 MHz; CDCl₃) 3.31-4.21 (208H, m, 1" to 35"-CH₂), 7.12-7.76 (56H, m, ArH).


The reaction was carried out under anhydrous conditions. To a solution of 111 (500 mg, 0.40 mmol) in acetonitrile (100 ml) at room temperature, potassium carbonate (879 mg, 6.30 mmol) was added and the reaction mixture stirred at 40 °C for 1 h. 144 (709 mg, 3.10 mmol) in acetonitrile (10 ml) was added dropwise and the reaction mixture was stirred at 80 °C for 4 d. The reaction was quenched by addition of water (10 ml) and the crude product extracted into dichloromethane (30 ml). Filtration and evaporation in vacuo afforded the crude product as a brown oil. Flash chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded a brown solid which was stirred in brine (20 ml) overnight. Extraction into dichloromethane (10 ml), drying (hydrophobic frit) and evaporation in vacuo afforded the title compound as an orange oil (281 mg, 31%).

ν_max /cm⁻¹ (Film) 2870 s, 2322 w, 1438 m, 1119 s, 722 m;
δ_H (300 MHz; CDCl₃) 3.38 (21H, s, Me), 3.48-3.93 (98H, m,7 x 1' to 8'-CH₂ and 7 x 9-CH₂), 7.25-7.60 (ArH);
δ_C (100 MHz; CDCl₃) 30.07 (C-9), 58.83 (Me), 70.30-71.70 (C-1 to C-8), 126.64 (C-3, C-6), 128.41 (C-7,C-8), 133.61 (C-2), 136.61 (C-4), 140.31 (C-5), 154.51 (C-1)
$m/z$ (ES$^+$) 2320 ($M^+ + Na$, 30%), 1172 ($[M + 2xNa^+]$)/2, 60;

$\lambda_{max}$ (KOH/MeOH)/nm 230, 260.


The reaction was carried out under anhydrous conditions. To a suspension of 111 (127 mg, 0.10 mmol) in acetonitrile (40 ml) at room temperature, potassium carbonate (224 mg, 1.60 mmol) was added and the reaction mixture stirred at 40 °C for 15 min. 146 (342 mg, 0.80 mmol) in acetonitrile (10 ml) was added dropwise and the resulting solution stirred at 80 °C for 4 d. The reaction mixture was quenched with water (10 ml) and extracted into chloroform (2 x 30 ml). Drying (MgSO$_4$), filtration and evaporation in vacuo afforded the crude compound as yellow oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded the title compound as a viscous yellow oil (181 mg, 49%).

$\nu_{max}$/cm$^{-1}$ (Film) 2869 s, 1456 m, 1123 s, 872 m;
δ_H (400 MHz; Acetone-d_6) 1.64-1.70 (42H, m, 7 x 3' to 5'-CH₂), 3.62-3.93 (196H, m, 7 x 1" to 17"-CH₂, 7 x 6'-CH₂, 7 x C-9), 4.78 (7H, t, J 3.3, 7 x 2'-CH), 7.47-7.89 (49H, m, ArH);

δ_C (100 MHz, DCl_3) 19.11 (C-5'), 25.07 (C-4'), 30.20 (C-3'), 61.30 (C-17''), 66.28 (C-6'), 69.37 to 72.19 (C-1" to C-16''), 98.56 (C-6'), 127.08 (C-3), 127.12 (C-6), 128.51 (C-8), 129.08 (C-7), 134.47 (C-2), 137.58 (C-4), 141.01 (C-5), 155.18 (C-1);

m/z (ES') 3711 (M+, 55%), 3734 (M⁺ + Na, 100);

λ_max / nm (KOH/MeOH) 232, 258.


To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 173 (60.0 mg, 0.02 mmol) was added and the resulting solution stirred at room temperature for 2 h. Evaporation in vacuo afforded a brown oil which was suspended in an aqueous solution of sodium hydroxide (10 %; 10 ml) and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 ml), dried (MgSO₄),
filtered and evaporated in vacuo to afford the title compound as a viscous yellow oil (48 mg, 77%).

\[ \nu_{\text{max}}/\text{cm}^{-1} \text{ (Film)} 2848 \text{ s}, 1447 \text{ m}, 1117 \text{ s}, 875 \text{ m}; \]

\[ \delta_{\text{H}} (300\text{MHz}; \text{CDCl}_3) 3.43-3.99 \text{ (182H, m, 7 x 1' to 17'-CH}_2, 7 \times 9-\text{CH}_2), 6.97-7.89 \text{ (49H, m, ArH);} \]

\[ \delta_{\text{C}} (100\text{MHz, RD 6s; CDCl}_3) 30.13 \text{ (C-9), 61.46 (C-17'), 69.51-72.39 (C-1 to C-16),} \]

\[ 126.47 \text{ (C-3), 126.68 (C-6), 127.85 (C-8), 128.47 (C-7), 133.64 (C-2), 137.00 (C-4),} \]

\[ 140.53 \text{ (C-5), 155.05 (C-1).} \]


The reaction was carried out under anhydrous conditions. To a suspension of 111 (200 mg, 0.16 mmol) and potassium carbonate (347 mg, 2.51 mmol) in acetonitrile (50 ml) at room temperature, 155 (929 mg, 1.26 mmol) in acetonitrile (10 ml) was added dropwise. The resulting solution was stirred at 80 °C for 7 d. The reaction was
quenched by the addition of water (20 ml) and the crude product extracted into dichloromethane (40 ml). Drying (phase separator) and evaporation in vacuo afforded the crude product as a brown oil. Flash chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded the title compound as a viscous oil (190 mg, 22%).

$\nu_{\text{max}}$/cm$^{-1}$ (Film) 2873 s, 1442 m, 1182 s;

$\delta_H$ (400 MHz, CDCl$_3$) 1.40 (7H, m, 7 x 3'-CH$_2$ to 5''-CH$_2$), 3.14-4.69 (70H, m, 7 x 1' to 35''-CH$_2$, 7 x 9-CH$_2$), 5.11 (7H, br m, 2''-CH) 7.15-7.91 (ArH);

$\delta_C$ (100 MHz, CDCl$_3$) 19.46 (C-5''), 25.38 (C-4''), 30.69 (C-3''), 32.16 (C-9), 62.19 (C-35''), 67.69 (C-6''), 69.64-71.89 (C-1' to C-34''), 98.70 (C-2'') 124.78, 126.53, 126.65, 128.29, 133.99, 136.44, 140.37 (C-5), 154.79 (C-1);

$m/z$ (MALDI-TOF) 1133.6 ([M + 5Na$^+$]/5, 30%).


\begin{center}
\includegraphics[width=\textwidth]{image.png}
\end{center}
To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 177 (50 mg, 0.01 mmol) was added and the solution stirred at room temperature for 2 h. Evaporation in vacuo afforded a brown oil which was suspended in an aqueous solution of sodium hydroxide (10 %; 10 ml), and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 ml), dried and evaporated in vacuo to afford the title compound as a brown viscous oil (23 mg, 50%).

\[ \nu_{\max}/\text{cm}^{-1} \text{ (Film)} \quad 2885 \text{ s}, 1437 \text{ m}, 1165 \text{ s}; \]

\[ \delta_{\text{H}} \text{ (300 MHz, CDCl}_3\) \quad 3.21-5.22 \text{ (74H, m, 1' to 35'-CH}_2\), 7.10-7.94 \text{ (49H, m, ArH).} \]


The reaction was carried out under anhydrous conditions. To a suspension of 111 (100 mg, 0.08 mmol) in acetonitrile (50 ml) at room temperature, potassium carbonate (345 mg, 2.50 mmol) was added and the reaction mixture stirred for 15 min. at 40 °C. 4-Bromobutyronitrile (0.25 ml, 2.50 mmol) was added and the reaction mixture stirred vigorously for 14 d at 80 °C. The reaction was quenched by addition of water (10 ml), dried (MgSO\(_4\)), filtered and evaporated in vacuo to afford the crude product as a yellow oil. Distillation under reduced pressure then flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a yellow oil (24 mg, 18%).

\[ \nu_{\max}/\text{cm}^{-1} \text{ (film)} \quad 2910 \text{ s}, 2329 \text{ m}, 1846 \text{ m}, 1076 \text{ w}; \]
The reaction was carried out under anhydrous conditions. To a solution of 77 (100 mg, 0.07 mmol) in acetonitrile (50 ml) at room temperature, potassium carbonate (606 mg, 4.39 mmol) was added and the reaction mixture stirred at 40 °C for 15 min. 4-Bromobutyronitrile (0.44 ml, 4.39 mmol) was added and the reaction mixture stirred at 80 °C for 14 d. Following cooling to room temperature the reaction mixture was quenched by addition of water (10 ml), dried (MgSO₄), filtered and evaporated in vacuo to afford the crude product as a yellow oil. Distillation under reduced pressure then flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a yellow oil (12 mg, 9%).

\( \nu_{\text{max}} / \text{cm}^{-1} \) (film) 2950 s, 2890 s, 2356 w, 2246 w, 1076 s;
\( \delta_{\text{H}} \) (300 MHz; CDCl₃) 1.84-2.02 (16H, m, 8 x 2'-CH₂), 2.38-2.51 (16H, m, 8 x 3''CH₂), 3.43-3.75 (32H, m, 8 x 1'-CH₂, ArCH₂Ar), 7.18-7.72 (56H, m, ArH);
δ_c (100 MHz; CDCl₃) 14.78 (C-3'), 25.84 (C-2'), 71.29 (C-1'), 117.76 (CN), 126.75, 128.94, 129.37, 134.65, 136.38, 141.94 (C-5), 157.05 (C-1);
m/z (FAB⁺) 1993 (M⁺, 15%), 1745 (M⁺ - (CH₂)₃CN x 4 + Na⁺, 4).


![Synthesis reaction diagram]

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (566 mg, 14.2 mmol) in THF (40 ml) at 0 °C, 112 (300 mg, 0.47 mmol) and allylbromide (0.5 ml, 5.66 mmol) were added and the reaction stirred at 70 °C for 3d. The reaction was quenched with iced water (10 ml), the crude product was extracted into dichloromethane (20 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Flash chromatography (ethyl acetate/hexane, 3:1) and recrystallisation (methanol/chloroform) afforded the title compound as pale cream solid (258 mg, 62%).

mp 283-293 °C

ν_max/cm⁻¹ (KBr Disc) 2949 s, 2847 m, 1670 m, 1581 w, 1449 m;

δ H (300 MHz; CDCl₃) 3.17 (12H, d, J 6.6, 6 x 3-CH₂), 3.73 (12H, s, ArCH₂Ar), 4.83-5.03 (12H, m, 6 x 1-CH₂), 5.67-5.84 (6H, m, 6 x 2-CH), 6.80-6.97 (18H, m, ArH);

δ C (75 MHz; CDCl₃) 30.62 (ArCH₂Ar), 73.90 (C-3), 117.54 (C-1), 123.57 (C-7), 129.19 (C-6), 133.67 (C-5), 134.49 (C-2), 155.06 (C-4);

m/z (FAB⁺) 876 (M⁺, 16%), 835 (M⁺ - allyl, 13%).

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (708 mg, 17.7 mmol) in THF (50 ml) at 0 °C, 73 (500 mg, 0.59 mmol) was added in one portion followed by allylbromide (1.53 ml, 17.7 mmol). The reaction mixture was stirred at 0 °C for 1 h and 70 °C for 16 h. The reaction was quenched by addition of water (20 ml), the crude product was extracted into dichloromethane (20 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Recrystallisation (methanol/chloroform) afforded the title compound as pale yellow solid (367 mg, 57%).

mp 295-308 °C

\[ \nu_{\text{max}}/\text{cm}^{-1} (\text{KBr Disc}) \ 2927 \ s, \ 2843 \ m, \ 1663 \ m, \ 1595 \ w, \ 1458 \ m; \]

\[ \delta_H (300 \text{ MHz; } \text{CDCl}_3) 3.97 (16H, s, ArCH_2Ar), 4.06 (16H, d, 3-CH_2, J 5.5), 5.09-5.17 (16H, m, 1-CH_2), 5.78-5.89 (8H, m, 2-CH), 6.73-6.90 (24H, m, ArH); \]

\[ \delta_C (75 \text{ MHz; } \text{CDCl}_3) 76.96 (\text{C-3}), 117.25 (\text{C-1}), 124.33 (\text{C-7}), 129.37 (\text{C-6}), 134.37 (\text{C-5}), 134.56 (\text{C-2}), 155.81 (\text{C-4}); \]

\[ m/z (\text{FAB}) 1168 (M^+, 50\%); \]


188
The reaction was carried out under anhydrous conditions. Calix[8]arene-octa-allyl (50.0 mg, 0.042 mmol), dissolved in diethylaniline (5 ml) was stirred at 220 °C for 16 h. The solution was poured into a rapidly stirring solution of ice/conc. hydrochloric acid (10:1, 50 ml). The precipitate formed was collected by filtration under reduced pressure. Only starting materials were recovered from the reaction.


The reaction was carried out under anhydrous conditions. To a suspension of 73 (200 mg, 0.24 mmol) in nitrobenzene (10 ml), acetyl chloride (0.27 ml, 3.77 mmol) and aluminium chloride (504 mg, 3.77 mmol) were added. The reaction was stirred at 70 °C for 2 d. The reaction mixture was quenched by addition to 1M HCl (50 ml), and the resulting precipitate was recovered by filtration *in vacuo*. Recrystallisation from chloroform and methanol afforded the title compound as a brown solid (31 mg, 11%).

$\nu_{\text{max}}$ /cm$^{-1}$ 2856 s, 1720 m;

$\delta_H$ (300 MHz; CDCl$_3$) 2.31 (24H, s, Me), 4.16 (16H, s, ArCH$_2$Ar), 7.40 (16H, s, ArH);

$\delta_C$ (75 MHz; CDCl$_3$) 25.71 (Me), 30.87 (ArCH$_2$Ar), 123.40 (C-2), 129.24 (C-3), 134.50 (C-4), 148.19 (C-1), 195.99 (C-5);

$m/z$ (FAB$^+$) 1184 (M$^+$, 23%).
9.2 Synthesis of Small Analogues

Biphenyl-4-yloxy-26-benzyloxy-3,6,9,12,15,18,21,24-octaoxahexacosyloxy (203)

The reaction was carried out under anhydrous conditions. To a solution of p-phenylphenol (170 mg, 1.00 mmol) in THF/DMF (10:1), potassium carbonate (276 mg, 2.00 mmol) was added. 151 (1.16 g, 2.00 mmol) was added dropwise at 40 °C. The reaction mixture was stirred at 60 °C for 6 d, quenched with iced water (2 x 5 ml), and washed with saturated lithium chloride solution (3 x 50 ml). The crude product was extracted into chloroform (3 x 40 ml) dried (NaSO₄), filtered and evaporated in vacuo to afford the crude product as an orange oil. Flash chromatography (hexane/ethyl acetate, 3:1) afforded the title compound as a viscous yellow oil (187 mg, 23%).

\[ \nu_{\text{max}} / \text{cm}^{-1} \text{ (Film)} \ 2870 \text{ s}, 1450 \text{ s}, 1103 \text{ s}; \]
\[ \delta_{\text{H}} \text{ (300 MHz; CDCl}_3\) 3.64 (36H, m, 1 to 26-CH₂), 5.05 (2H, s, 1'-CH₂), 7.20-7.87 (14H, ArH); \]
\[ \delta_{\text{C}} \text{ (100 MHz; CDCl}_3\) 69.52-70.32 (C-1" to C-26"), 77.48 (C-1'), 114.83 (C-2), 126.41, 126.56, 127.84, 128.43, 128.73, 128.98, 132.87, 141.02, 156.49 (C-1); \]
\[ m/z \text{ (FAB}^+) \ 672 \text{ (M}^+, 32\%), 581 \text{ (M}^+ - \text{Bz, 10) and 91 (Bz}^+, 17). \]
Bisphenyl-4,4'-yloxy-26-benzyloxy-3,6,9,12,15,18,21,24-octaoxahexacosyloxy (204)

The reaction was carried out under anhydrous conditions. To a solution of 4,4'-dihydroxybiphenol (186 mg, 1.00 mmol) in THF/DMF (10:1), potassium carbonate (828 mg, 6.00 mmol) was added in one portion. Following warming to 40 °C, 151 (5.712 g, 8.00 mmol) was added dropwise. The resulting solution was stirred at 60 °C for 6 d. The reaction mixture was quenched with iced water (2 x 5 ml) and washed with saturated lithium chloride solution (3 x 50 ml). The crude product was extracted into chloroform (3 x 40 ml) and dried (NaSO₄), filtered and evaporated in vacuo to yield the crude product as an orange oil. Flash chromatography (hexane/ethyl acetate, 3:1) afforded the title compound as a viscous yellow oil (211 mg, 18%).

νmax /cm⁻¹ (Film) 2884 s, 1458 s, 1124 s;

δH (300 MHz, CDCl₃) δ 3.61-3.80 (72H, m, 1 to 26-CH₂), 5.07 (4H, s, 1'-CH₂), 7.55 (18H, ArH);

δC (100 MHz; CDCl₃) 68.96-70.42 (C-1" to C-26"), 77.39 (C-1'), 114.71 (C-2"), 126.82 (C-2'), 127.43 (C-3''), 128.43 (C-3'), 128.73 (C-4''), 128.98 (C-5'), 133.44 (C-4''), 157.78 (C-1'');

m/z (FAB⁺) 688 (M⁺, 24%) and 91 (Bz⁺, 21%).

191
The reaction was carried out under anhydrous conditions. To a solution of 4,4'-dihydroxybiphenol (50.0 mg, 0.27 mmol) in acetonitrile (10 ml) at room temperature, potassium carbonate (149 mg, 1.10 mmol) was added and 146 (227 mg, 0.80 mmol) in acetonitrile (2 ml) was added dropwise. The reaction mixture was stirred at 80 °C for 4 d. The reaction mixture was quenched with iced water (2 ml), the crude product extracted into dichloromethane (20 ml), and washed with brine (10 ml). Following drying (phase separator), evaporation in vacuo afforded the crude product as a brown oil. Purification via solid phase extraction cartridge (ethyl acetate/methanol) afforded the title compound as an oil (122 mg, 51%).

$\nu_{\text{max}}$ /cm$^{-1}$ (Film) 2887 $s$, 1447 $s$, 1118 $s$;

$\delta_H$ (400 MHz, CDCl$_3$) 1.50–1.90 (12H, m, 2 x 3'-CH$_2$ to 5'-CH$_2$), 3.60–4.20 (52H, m, 2 x 1'-CH$_2$ to 17-CH$_2$ and 2 x 6'-CH$_2$), 4.64 (2H, t, $J_{3.7}$, 2 x 2'-CH), 6.90 (4H, d, $J_{8.0}$, ArH) and 7.45 (4H, d, $J_{8.0}$, ArH);

$\delta_C$ (75 MHz; CDCl$_3$) 19.29 (C-5"), 25.28(C-4"), 30.41(C-3"), 61.98(C-17), 67.41(C-6"), 69.60-70.68 (C-1 to C-16), 98.74 (C-2"), 114.77 (C-2'), 127.45 (C-3'), 133.43 (C-4'), 157.78 (C-1');

$m/z$ (LCMS) 901 (M$^+$ + NH$_4^+$, 80%), 817 (M$^+$ - THP + NH$_4^+$, 50), 732.52 (M$^+$ - 2THP + 2 NH$_4^+$, 100);

$\lambda_{\text{max}}$/nm (KOH/MeOH) 260.
Synthesis of Bisphenyl-4,4'-yloxy-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy) (209)

To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 199 (122 mg, 0.14 mmol) was added and the solution stirred at room temperature for 3 h. Following evaporation the residue was suspended in an aqueous solution of potassium hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml), dried (phase separator) and evaporated in vacuo to afford the title compound as a viscous oil (74 mg, 74%).

$\nu_{\text{max}}$/cm$^{-1}$ (Film) 2883 s, 1444 s, 1112 s;

$\delta_{\text{H}}$ (400 MHz; CDCl$_3$) 1.27 (2H, s, 2 x OH), 3.58–4.21 (48H, m, 2 x 1-CH$_2$ to 17-CH$_2$), 6.90 (4H, d, $J$ 8.1 2'-CH) and 7.51 (4H, d, $J$ 8.1, 3'-CH);

$\delta_{\text{C}}$ (75 MHz; CDCl$_3$) 61.59 (C-17), 67.43 (C-16), 69.64 – 72.64 (C-3 to C-14), 114.84 (C-2'), 127.54 (C-3'), 133.46 (C-4'), 157.73 (C-1');

$m/z$ (LCMS) 732.52 (M$^+$+NH$_4^+$, 100 %), 714.46 (M$^+$, 30);

$\lambda_{\text{max}}$/nm (KOH/MeOH) 260.
Synthesis of 2-\{17-[2-(3-[[17-(tetrahydro-2H-pyran-2-yloxy)-
3,6,9,12,15-pentaoxahexadecyl]oxy]benzyl]phenoxy]-3,6,9,12,15-
pentaoxahexadecyl]oxy)tetrahydro-2\/-/-pyran (200)

The reaction was carried out under anhydrous conditions. To a solution of bis(2-
hydroxyphenyl)methane (50.0 mg, 0.25 mmol) in acetonitrile (10 ml) at room
temperature, potassium carbonate (138 mg, 1.00 mmol) was added. 146 (213 mg, 0.75
mmol) in acetonitrile (2 ml) was added dropwise, and the reaction mixture stirred at 80
°C for 4 d. The reaction mixture was quenched with iced water (2 ml), washed with
brine (5 ml) and the crude product extracted into dichloromethane (20 ml). Dried
(phase separator) and evaporated in vacuo to afford the crude product as a brown oil.
Purification via a solid phase extraction cartridge (ethyl acetate/methanol) afforded the
title compound as a viscous oil (92 mg, 41%).

\( \nu_{\text{max}} / \text{cm}^{-1} \) (Film) 2870 s, 1450 s and 1103 s;
\( \delta_{\text{H}} \) (400 MHz; CDCl\(_3\)) 1.50–1.90 (12H, m, 2 x 3"-CH\(_2\) to 5"-CH\(_2\)), 3.60–4.18 (52H, m, 2
x 1-CH\(_2\) to 17-CH\(_2\) and 2 x 6"-CH\(_2\)), 3.96 (2H, s, 7'-CH\(_2\)), 4.65 (2H, t, \( J = 3.7 \), 2 x 2"-CH)
and 6.75 – 7.20 (8H, m, ArH);
\( \delta_{\text{C}} \) (100 MHz; CDCl\(_3\)) 19.29 (C-5"), 25.98 (C-4"), 30.50 (C-7"), 34.67 (C-3"), 67.05 (C-
6") 68.00–73.00 (C-1 to C-17), 98.27 (C-2"), 111.70 (C-2'), 120.90 (C-4'), 127.40 (C-
3'), 131.10 (C-5'), 133.20 (C-6') and 148.0 (C-1');
m/z (LCMS) 916.13 (M\(^{+}\)+NH\(_4\)^{+}, 50%), 831.82 (M\(^{+}\)-THP+NH\(_4\)^{+}, 45), 746.50 (M\(^{+}\-
2THP+NH\(_4\)^{+}, 100).
Synthesis of 2-{17-[2-(3-[17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl]oxy)benzyl]phenoxy]-3,6,9,12,15-pentaoxaheptadecyl}oxy)hydroxyl (210)

To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 200 (92.0 mg, 0.10 mmol) was added and the solution stirred at room temperature for 3 h. Following evaporation *in vacuo* the residue was suspended in an aqueous solution of potassium hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml) and dried (phase separator). Evaporation *in vacuo* afforded the title compound as a viscous oil (56 mg, 77%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (Film) 3110 br, 2871 s, 1453 s and 1102 s;

$\delta_{\text{H}}$ (400 MHz; CDCl$_3$) 1.25 (2H, s, 2 x OH), 3.63–4.20 (50H, m, 2 x 1-CH$_2$ to 17-CH$_2$ and 7'-CH$_2$) and 6.78–7.20 (8H, m, ArH);

$\delta_{\text{C}}$ (100 MHz; CDCl$_3$) 30.5 (C-7'), 62.0 (C-17), 70.2–72.6 (C-1 to C-16), 111.3 (C-2'), 120.5 (C-4'), 127.0 (C-3'), 129.6 (C-6') 130.7 (C-5') and 158.0 (C-1');

$m/z$ (LCMS) 746.51(M$^+$/NH$_4^+$,100%).
The reaction was carried out under anhydrous conditions. To a solution of bis(4-hydroxyphenyl)methane (50.0 mg, 0.25 mmol) in acetonitrile (10 ml), potassium carbonate (138 mg, 1.00 mmol) was added. 146 (213 mg, 0.75 mmol) in acetonitrile (2 ml) was added dropwise. The reaction mixture was stirred at 80 °C for 4 d, the reaction was quenched with iced water (2 ml), washed with brine (2 ml), and the crude product was extracted into dichloromethane (20 ml). Drying (MgSO₄), filtration and evaporation in vacuo afforded the crude product as a brown oil. Purification via solid phase extraction cartridge (ethyl acetate/methanol) afforded the title compound as a viscous oil (96 mg, 43%).

$\nu_{\text{max}}$ cm$^{-1}$ (Film) 2927 s, 1385 m and 979 m;

$\delta$ (400 MHz; CDCl₃) 1.40–1.90 (12H, m, 2 x 3‴-CH₂ to 5‴-CH₂), 3.55–4.10 (54H, m, 2 x 1-CH₂ to 17-CH₂ and 5′-CH₂ and 2 x 6‴-CH₂), 4.58 (2H, t, $J$ 3.7, 2 x 2‴-CH), 6.80 (4H, d, $J$ 7.9, ArH) and 7.05 (4H, d, $J$ 7.9, ArH);

$\delta$ (100 MHz; CDCl₃) 19.57 (C-5″), 25.65 (C-4″), 30.65 (C-3″) 32.2 (C-5′), 67.0 (C-6″), 70.2–73.0 (C-1 to C-17), 98.91 (C-2″), 116.9 (C-2′), 130.1 (C-3″), 135.0 (C-4″) and 157.0 (C-1′);

$m/z$ (LCMS) 830.68 (M$^+$-THP+NH$_4^+$, 100%), 746.52 (M$^+$-2THP+NH$_4^+$, 100).
Synthesis of 4-({17-[4-(4'-{[17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl]oxy}benzyl)phenoxy]-3,6,9,12,15-pentaoxaheptadecyl]oxy)hydroxyl (211)

To a solution of hydrochloric acid (18 M; 5 ml), and dichloromethane (5 ml), 201 (96.0 mg, 0.10 mmol) was added and the solution stirred at room temperature for 3 h. Following evaporation in vacuo the residue was suspended in an aqueous solution of potassium hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml) and dried (phase separator). Evaporation in vacuo afforded the title compound as a viscous oil (50 mg, 68%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (Film) 2925 s, 1386 m, 979 w;

$\delta_H$ (400 MHz; CDCl$_3$) 3.29–4.07 (50H, m, 2x 1-CH$_2$ to 17-CH$_2$ and 5'-CH$_2$), 6.81 (4H, d, J 8.1, ArH) and 7.03 (4H, d, J 8.1, ArH);

$\delta_C$ (100 MHz; CDCl$_3$) 31.05 (C-5'), 61.32 (C-17), 67.29–72.39 (C-1 to C-16), 114.45 (C-2'), 129.49 (C-3'), 133.71 (C-4') and 156.94 (C-1');

$m/z$ (LCMS) 728 (M$^+$, 100%).
Synthesis of 4-octyl-phenoxy-[17-(2H-tetrahydropyran-2-yloxy)-3,6,9,12,15-pentaoxaheptadecyloxy] (202)

The reaction was carried out under anhydrous conditions. To a suspension of n-octylphenol (100 mg, 0.48 mmol) in acetonitrile (30 ml), potassium carbonate (201 mg, 1.45 mmol) and 146 (409 mg, 0.95 mmol) in acetonitrile (5 ml) were added and the reaction mixture stirred at reflux temperature for 4 d. The reaction was washed with water (10 ml) and brine (10 ml), the crude product extracted into dichloromethane (30 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a viscous brown oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a viscous oil (78 mg, 29%).

$\nu_{\text{max}}/\text{cm}^{-1}$ (Film) 2925 s, 2857 s, 1123 s;

$\delta_H$ (300 MHz; CDCl$_3$) 0.86 (3H, t, $J$ 6.5, Me), 1.25-1.82 (18H, m, 3' to 5'-CH$_2$, 2 to 7-CH$_2$), 2.51 (2H, t, $J$ 7.7, 1-CH$_2$), 3.46-3.85 (24H, m, 1'' to 16''-CH$_2$, 6''-CH$_2$), 4.08 (2H, t, $J$ 4.9, 17''-CH$_2$), 5.06 (1H, t, $J$ 3.2, 2''-CH), 6.75 (2H, d, $J$ 8.4, ArH), 6.99 (2H, d, $J$ 8.4, ArH);

$\delta_C$ (75 MHz; CDCl$_3$) 14.06 (C-8), 19.49 (C-5'), 22.65(C-7), 25.45 (C-4'), 29.27 (C-6), 29.47 (C-5), 30.58 (C-3'), 31.69 (C-4), 31.88 (C-3, C-2), 35.05 (C-1), 62.21 (C-17''),
66.67 (C-6'), 69.84-70.82 (C-1" to C-16"), 98.96 (C-2'), 114.47 (C-2"'), 129.19 (C-3"'), 136.41 (C-4"'), 157.98 (C-1');
m/z (FAB') 493 (M^+ - Me(CH\_2)_7 + Na, 25%), 475 (M^+, 10), 469 (M^+ - Me(CH\_2)_7, 8).

Synthesis of 4-octyl-phenoxy-[17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy] (212)

\[ 202 \rightarrow c. \text{HCl/DCM} \rightarrow 212 \]

To a solution of hydrochloric acid (18 M; 2 ml), and dichloromethane (10 ml), \textit{202} (50 mg, 0.09 mmol) was added and the solution stirred at room temperature for 1 h. Following evaporation the residue was suspended in an aqueous solution of potassium hydroxide (10%; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml) and dried (phase separator). Evaporation \textit{in vacuo} afforded the \textit{title compound} as a viscous oil (36 mg, 86%).

\[ v_{\text{max}}/\text{cm}^{-1} \text{ (Film)} \]: 2884 s, 1440 s, 1093 s;
\[ \delta_H \text{ (300 MHz; CDCl}_3) \]: 0.86 (3H, t, J 6.75), 1.21-1.28 (12H, m, 2 to 7-CH\_2), 2.51 (2H, t, J 7.7, 1-CH\_2), 3.59-3.82 (24H, m, 1' to 17'-CH\_2), 6.82 (2H, d, J 7.8, ArH), 7.05 (2H, d, J 7.8, ArH);
The reaction was carried out under anhydrous conditions. To a solution of 4,4'-biphenol (50.0 mg, 0.27 mmol) and potassium carbonate (295 mg, 2.14 mmol) in acetonitrile (50 ml) at room temperature, 155 (791 mg, 1.06 mmol) in acetonitrile (10 ml) was added dropwise. The reaction mixture was stirred at 80 °C for 6 d. The reaction mixture was quenched by addition of water (30 ml), and the crude product extracted into dichloromethane (40 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a viscous oil (294 mg, 78%).

\[
\begin{align*}
\delta_C (100 \text{ MHz; CDCl}_3) & \ 14.10 (C-8), 22.66 (C-7), 29.26 (C-6), 29.47 (C-5), 31.73 (C-4), 31.87 (C-3, C-2), 35.04 (C-1), 61.29 (C-17'), 69.68-72.53 (C-16' to C-1'), 114.40 (C-2''), 129.19 (C-3''), 135.32 (C-4''), 156.74 (C-1''); \\
\text{m/z (ES') } & \ 509.2 (M^+ + \text{Na}^+, 100\%).
\end{align*}
\]

Synthesis of Bisphenyl-4,4'-yloxy-[35-(2H-tetrahydropyran-2-yloxy)-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentriacontyloxy] (205)
Bisphenyl-4,4'-yloxy-(35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy) (213)

To a solution of hydrochloric acid (18 M; 2 ml), and dichloromethane (10 ml), 205 (294 mg, 0.21 mmol) was added and the solution stirred at room temperature for 1 h. Following evaporation the residue was suspended in an aqueous solution of potassium hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml) and dried (phase separator). Evaporation in vacuo afforded the title compound as a viscous oil (259 mg, 99%).

$\nu_{\text{max}}$ (Film) 2873 s, 1438 s, 1149 s;

$\delta_{\text{H}}$ (300 MHz, CDCl$_3$) 3.15-4.09 (96H, m, C-1 to C-35-CH$_2$), 6.76-6.79 (2H, d, $J$ 8.4, 2-CH$_2$), 7.42-7.46 (2H, d, $J$ 8.4, 3-CH$_2$);

$\delta_{\text{C}}$ (100 MHz; CDCl$_3$) 62.22 (C-35"), 70.01-73.59 (C-1" to C-34"), 114.34 (C-2), 127.28 (C-3), 132.91 (C-4), 156.40 (C-1);

$m/z$ (ES$^+$) 1265 (M$^+$ + Na$^+$, 48%), 1260 (M$^+$ + H$_2$O, 39).
Synthesis of 2-((35-[2-(3-{35-(tetrahydro-2H-pyran-2-yloxy)-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy)}benzyl)phenoxy]-3,6,9,12,15,18,21,24,27,30-undecaoxapentatriacontyloxy)tetrahydro-2H-pyran (207)

The reaction was carried out under anhydrous conditions. To a suspension of bis2-phenolmethane (50.0 mg, 0.25 mmol) in acetonitrile (50 ml) at room temperature, potassium carbonate (275 mg, 1.99 mmol) and 155 (736 mg, 0.99 mmol) in acetonitrile (5 ml) were added. The reaction mixture was stirred at reflux for 10 d. The reaction was quenched with water (20 ml), the crude product extracted into dichloromethane (30 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as an orange oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as an oil (201 mg, 57%).

νmax/cm⁻¹ (film) 2870 s, 1450 s and 1103 s;

δH (400MHz; CDCl3) 1.38-1.46 (12H, m, 2 x 1' to 35'-CH2, 7-CH2, 2 x 6''-CH2), 3.45-3.98 (102H, m, 2 x 1' to 35'-CH2, 7-CH2, 2 x 6''-CH2), 4.37 (2H, t, J 3.5 2 x 2''-CH), 6.67-7.03 (8H, m, ArH);

δC (100MHz; CDCl3) 19.28 (C-5''), 25.03 (C-4''), 29.81 (C-7), 30.35 (C-3''), 61.78 (C-35'), 68.03 (C-6''), 69.40-72.24 (C-1' to C-34'), 98.51 (C-2''), 110.95 (C-2), 120.22 (C-4), 126.62 (C-3), 129.08 (C-5), 129.77 (C-6), 156.33 (C-1);

m/z (ES⁺) 1424 (M⁺, 10%), 1356 (M⁺ - THP + H2O, 50), 1272 (M⁺ - 2xTHP + H2O, 100);

λmax/nm (KOH/MeOH) 235.
Synthesis of 2-((35-[2-(3-{35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-
undecaoxapentatriacontyloxy})benzyl]phenoxy)-
3,6,9,12,15,18,21,24,27,30-undecaoxapentatriacontyloxy)hydroxyl

(215)

To a solution of hydrochloric acid (18 M; 2 ml), and dichloromethane (10 ml), 207 (150
mg, 0.11 mmol) was added and the solution stirred at room temperature for 1 h.
Following evaporation the residue was suspended in an aqueous solution of potassium
hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5
ml) and dried (phase separator). Evaporation *in vacuo* afforded the *title compound* as a
viscous oil (98 mg, 78%).

$\nu_{\text{max}}/\text{cm}^{-1}$ (Film) 2864 s, 1438 s, 1116 s;

$\delta_{\text{H}}$ (300 MHz; CDCl$_3$) 3.57-4.08 (98H, m, 2 x 1'-CH$_2$ to 35'-CH$_2$, 7-CH$_2$), 6.77-7.16
(8H, m, ArH);

$\delta_{\text{C}}$ (75 MHz; CDCl$_3$) 29.66 (C-7), 61.68 (C-35'), 70.23-72.55 (C-1' to C-34'), 111.45 (C-2),
121.98 (C-4), 126.99 (C-3), 129.89 (C-5), 132.68 (C-6), 157.34 (C-1);

$m/z$ (ES$^+$) 1279 (M$^+$ + Na$^+$, 46%), 1274 (M$^+$ + H$_2$O, 18)
Synthesis of 4-({35-[4-(4'-{35-(tetrahydro-2H-pyran-2-yloxy)-3,6,9,12,15,18,21,24,27,30,33-undeca oxapentatriacontyloxy})benzyl]phenoxy}-3,6,9,12,15,18,21,24,27,30-undeca oxapentatriacontyloxy)tetrahydro-2H-pyran (206)

The reaction was carried out under anhydrous conditions. To a suspension of bis4-phenylmethane (50.0 mg, 0.25 mmol) at room temperature in acetonitrile (50 ml), potassium carbonate (275 mg, 1.99 mmol) and 155 (736 mg, 0.99 mmol) in acetonitrile (5 ml) were added. The reaction was stirred at reflux temperature for 10 d. The reaction mixture was washed with water (20 ml), the crude product extracted into dichloromethane (30 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as an orange oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as an oil (78 mg, 22%).

$\nu_{max}$/cm$^{-1}$ (Film) 2894 s, 1436 s, 1128 s;

$\delta$H (400 MHz; CDCl$_3$) 1.44-1.66 (12H, m, 2 x 3" to 5"-CH$_2$), 3.53-3.83 (101H, m, 2 x 1'-CH$_2$ to 35'-CH$_2$, 6''-CH$_2$, 5-CH$_2$), 4.56 (3H, m, 2 x 2"-CH), 6.74-7.50 (8H, m, ArH);

$\delta$C (100 MHz; CDCl$_3$) 19.34 (C-5'"), 25.11 (C-4'"), 30.23 (C-3'"), 30.39 (C-5), 60.08 (C-35'), 67.09 (C-6"), 70.21-70.30 (C-1' to C-34'), 98.62 (C-2'"), 114.24 (C-2), 115.06 (C-3), 129.39 (C-4), 149.68 (C-1);

m/z (ES$^+$) 1447 (M$^+$ + Na$^+$, 74%), 1363 (M$^+$ + Na$^+$-THP, 24), 650 ([M$^+$-2 x THP + 2xNa$^+$]/2, 16).
Synthesis of 4-({35-[4-(4'-{35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy})benzyl]phenoxy}-3,6,9,12,15,18,21,24,27,30-undecaoxapentatriacontyloxy)hydroxyl (214)

![Chemical Structure]

to a solution of hydrochloric acid (18 M; 2 ml), and dichloromethane (10 ml), 206 (50 mg, 0.04 mmol) was added and the solution stirred at room temperature for 1 h. Following evaporation the residue was suspended in an aqueous solution of potassium hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml) and dried (phase separator). Evaporation in vacuo afforded the title compound as a viscous oil (36 mg, 82 %).

νmax/cm⁻¹ (Film) 2873 s, 1444 s, 1119 s;

δH (300 MHz; CDCl3) 3.30-4.07 (98H, m, 2 x 1'-CH₂ to 35'-CH₂, 5'-CH₂), 6.76-7.06 (8H, m, ArH);

δC (75 MHz; CDCl₃) 30.28 (C-5), 62.18 (C-35'), 69.41-72.36 (C-1' to C-34'), 114.38 (C-2), 116.19 (C-3), 130.75 (C-4), 151.86 (C-1);

m/z (ES⁺) 1256 (M⁺, 8%), 651 ([M⁺ + 2x Na⁺]/2, 28)
Synthesis of 4'-Phenyl-Phenyl-4-yloxy-[35-(2H-Tetrahydropyran-2-yloxy)-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy] (208)

The reaction was carried out under anhydrous conditions. To a suspension of phenylphenol (80.0 mg, 0.47 mmol) in acetonitrile (50 ml) potassium carbonate (259 mg, 1.88 mmol), and 155 (1.04 g, 1.41 mmol) were added and the reaction was stirred at 80 °C for 11 d. The reaction mixture was quenched with water (15 ml), the crude product extracted into dichloromethane (30 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as an orange oil. Purification via flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as an orange oil (265 mg, 72%).

$v_{max}/\text{cm}^{-1}$ (Film) 2855 s, 1467 s, 1103 s;

$\delta_{\text{H}}$ (300 MHz; CDCl$_3$) 1.44-1.74 (6H, m, 3" to 5"-CH$_2$), 3.46-4.14 (50H, m, 6"-CH$_2$, 1' to 35'-CH$_2$), 4.48-4.49 (1H, br, 2"-CH), 6.90-7.69 (9H, m, ArH);

$\delta_{\text{C}}$ (75 MHz; CDCl$_3$) 19.76 (C-5"), 25.10 (C-4"), 30.52 (C-3"), 63.74 (C-35"), 66.64 (C-6"), 68.85-71.15 (C-1' to C-34"), 100.07 (C-2"), 114.85 (C-2), 126.40 (C-6), 126.54 (C-8), 127.88 (C-3), 128.52 (C-7), 133.88 (C-4), 140.35 (C-5), 157.99 (C-1);
Synthesis of 4’-Phenyl-Phenyl-4-yloxy-[35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy] (217)

To a solution of hydrochloric acid (18 M; 2 ml), and dichloromethane (10 ml), phenylphenol-dodecaethyleneglycol-THP (200 mg, 0.26 mmol) was added and the solution stirred at room temperature for 1 h. Following evaporation the residue was suspended in an aqueous solution of potassium hydroxide (10 %; 3 ml) for 10 min. The product was extracted into dichloromethane (5 ml) and dried (phase separator). Evaporation in vacuo afforded the title compound as a viscous oil (170 mg, 95%).

ν<sub>max</sub> /cm<sup>-1</sup> (Film) 2858 s, 1423 s, 1127 s;
δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 3.57-4.15 (48H, m, 1' to 35'-CH<sub>2</sub>), 6.94-7.59 (9H, m, ArH);
δ<sub>C</sub> (75 MHz; CDCl<sub>3</sub>) 61.39 (C-35'), 69.53-72.53 (C-1' to C-34'), 114.74 (C-2), 126.41 (C-6), 126.46 (C-8), 127.85 (C-3), 128.45 (C-7), 133.73 (C-4), 140.73 (C-5), 158.16 (C-1);
m/z (ES<sup>+</sup>) 722 (M<sup>+</sup> + Na<sup>+</sup>, 59%).
Synthesis of Trimethylsilyl-propenyl-benzene (192)

The reaction was carried out under anhydrous conditions. To a solution of Grubbs’ Catalyst (82 mg, 0.10 mmol) in dichloromethane (3 ml) at room temperature, 189 (0.11 ml, 1.00 mmol) and 188 (0.32 ml, 2.00 mmol) were added via syringe. The reaction was stirred at reflux for 24 h. Evaporation in vacuo afforded a brown viscous oil which upon flash column chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a colourless oil (171 mg, 45%).

$\nu_{\text{max}} /\text{cm}^{-1}$ (Film) 2876 s, 1270 s 845 m, 725 m; 
$
\delta_H \text{ (300 MHz; CDCl}_3\text{) } 0.00 \text{ (TMS), 1.28 (2H, d, } J 3.4, 1\text{-CH}_2\text{), 6.07 (1H, q, } 2\text{-CH), 6.41 (1H, d, } J 15.9, 3\text{-CH), 7.14-7.30 (5H, m, } \text{ArH); }$

$
\delta_C \text{ (75 MHz; CDCl}_3\text{) } 0.00 \text{ (TMS), 18.85 (C-1), 121.71, 126.28, 127.86, 128.45, 134.91; }$

$m/z (ES^+) 117 (M^+ - \text{TMS, 18%).}$

Synthesis of 2-benzyloxy-phenoxy-bis-allyl (194)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (799 mg, 19.9 mmol) at room temperature in THF (40 ml), 193 (500 mg, 2.5 mmol) and allyl bromide (1.73 ml, 19.9 mmol) were added. The reaction was stirred at reflux for 3 d, quenched with iced water (4 ml), and washed with brine (20 ml). Evaporation in vacuo afforded the crude product as a yellow solid, which was
recrystallised from methanol to afford the title compound as a cream solid (487 mg, 71%).

\[ \nu_{\text{max}} / \text{cm}^{-1} (\text{KBr Disc}) \nu_{\text{max}} / \text{cm}^{-1} (\text{Film}) 2856 \text{ s, } 1445 \text{ s} ; \]

\[ \delta_{\text{H}} (300 \text{ MHz}; \text{CDCl}_3) 3.81 (2\text{H}, \text{s, 7'-CH}_2), 4.61 (4\text{H, J 4.3, 3'-CH}_2), 5.23-5.89 (6\text{H, m, 1'-CH}_2, 2'-CH), 6.69-7.31 (8\text{H, m, ArH}) ; \]

\[ \delta_{\text{C}} (75 \text{ MHz}; \text{CDCl}_3) 29.67 (7'-\text{CH}_2), 75.81 (\text{C-3'}), 114.65 (\text{C-2}), 115.75 (\text{C-1'}), 121.97 (\text{C-4}), 127.07 (\text{C-3}), 129.91 (\text{C-5}), 132.01 (\text{C-6}), 137.54 (\text{C-2'}), 159.92 (\text{C-1}); \]

\[ m/z (\text{FAB'}) 279 (M^+, 25\%), 238 (M^+ - \text{allyl, 14}). \]

\textbf{Synthesis of 2'-allyl-2-benzyloxy-phenoxy (195)}

The reaction was carried out under anhydrous conditions. To diethylaniline (16 ml) 194 (300 mg, 1.07 mmol) was added and the reaction stirred at reflux for 18 h. The reaction mixture was poured onto a solution of conc. HCl (5.00 ml) and ice (75.0 g). The resultant precipitate was recovered by filtration to afford the title compound as a brown solid (242 mg, 81%).

\[ \nu_{\text{max}} / \text{cm}^{-1} (\text{Film}) 3280 \text{ s, } 2848 \text{ s, } 1448 \text{ s} ; \]

\[ \delta_{\text{H}} (300 \text{ MHz}; \text{CDCl}_3) 3.22 (4\text{H, J 5.6, 3'-CH}_2), 3.78 (2\text{H, s, 7-CH}_2), 4.91-4.96 (4\text{H, m, 1'-CH}_2), 6.18-6.30 (2\text{H, m, 2'-CH}), 6.58-6.70 (6\text{H, m, ArH}) ; \]

\[ \delta_{\text{C}} (75 \text{ MHz}; \text{CDCl}_3) 29.01, 29.67, 115.91 (\text{C-1'}), 121.56 (\text{C-4}), 125.42 (\text{C-2}), 126.81 (\text{C-5}), 128.17 (\text{C-3}), 130.12 (\text{C-6}), 132.71 (\text{C-2'}), 157.91 (\text{C-1}); \]

\[ m/z (\text{FAB'}) 279 (M^+, 46\%). \]
The reaction was carried out under anhydrous conditions. To a solution of Grubbs' Catalyst (59.0 mg, 0.07 mmol) in dichloromethane (2 ml) at room temperature, 195 (200 mg, 0.71 mmol) and allyl-TMS (0.45 ml, 2.86 mmol) were added and the reaction stirred at reflux for 18 h. Evaporation in vacuo afforded a brown viscous oil which upon flash column chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a yellow oil (107 mg, 41%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (Film) 3265 s, 2828 s, 1484 s, 1264 s 848 m, 728 m;

$\delta_{\text{H}}$ (300 MHz; CDCl$_3$) 0.00 (TMS), 1.25-1.29 (4H, m, 1'-CH$_2$), 3.18-3.20 (4H, m, 4'-CH$_2$), 3.81 (2H, s, 7-CH$_2$), 5.40-6.04 (4H, m, 2'-CH, 3'-CH), 6.58-6.73 (6H, m, ArH);

$\delta_{\text{C}}$ (75 MHz; CDCl$_3$) 0.00 (TMS), 19.23 (C-1'), 28.61 (C-4'), 29.17 (C-7), 121.52 (C-4), 125.31, 125.76, 126.91, 128.20 (C-3), 130.25 (C-6), 157.75 (C-1);

$m/z$ (ES$^+$) 262 (M$^+$, 54%).
9.3 Synthesis of PEG-chains

Synthesis of 17-Benzylxoy-3,6,9,12,15-pentaoxaheptadecan-1-ol (125)$_{144}$

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (1.24 g, 31.0 mmol) in THF (300 ml) at 0 °C, 133 (9.90 g, 35.0 mmol), was added dropwise and the resulting solution was stirred for 1 h. Benzylbromide (4.10 ml, 35.0 mmol) was added dropwise and the solution heated at 60 °C for 5 h. The reaction mixture was quenched with iced water (2 x 25 ml), the crude product extracted into chloroform (3 x 50 ml) washed with brine (2 x 10 ml) and dried (Na$_2$SO$_4$), filtration and evaporation in vacuo yielded the crude compound which upon column chromatography (dichloromethane/ethyl acetate, 2:1) afforded the title compound as a yellow oil (7.98 g, 62%).

$\nu_{\text{max}}/\text{cm}^{-1}$ (film) 3445 br s, 3063 w, 2866 s, 1496 m, 1450 s, 1346 m, 1200 w, 1099 s;

$\delta_H$ (300 MHz; CDCl$_3$) 2.48 (1H, s, OH), 3.41–3.87 (24H, m, 1 to 17-CH$_2$), 4.55 (2H, s, 1'-CH$_2$) and 7.25–7.60 (5H, m, 3' to 7'-CH$_2$);

$\delta_C$ (75 MHz; CDCl$_3$) 62.10 (C-1), 69.20–70.39 (C-2 to C-17), 77.19 (C-1'), 126.29 (C-3'), 128.39 (C-4'), 128.50 (C-5'), 140.79 (C-2');

$m/z$ (FAB$^+$) 373 (M$^+$, 58%) and 91 (PhCH$_2^+$, 90);

Synthesis of 17-Benzylxoy-3,6,9,12,15-pentaoxaheptadecyl methanesulfonate (137)
The reaction was carried out under anhydrous conditions. To a solution of 125 (8.96 g, 0.02 mol) in dichloromethane at 0 °C, triethylamine (1.87 ml, 0.03 mol) was added. The resulting solution was stirred at 0 °C for 30 min. and mesyl chloride (2.76 g, 0.02 mol) was added. The mixture was stirred for a further 30 min. at 0 °C and then at r.t for 16 h. The reaction was quenched with iced water (2 x 15 ml), the product was extracted into chloroform (3 x 50 ml) then washed with brine (2 x 10 ml). After drying (NaSO₄), filtration and evaporation in vacuo yielded the title compound as an orange oil (9.16 g, 88%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (film): 2868 s, 1496 m, 1445 s, 1348 m, 1217 w, 1095 s;

$\delta_{\text{H}}$ (300 MHz, CDCl₃): 3.04 (3H, s, Me), 3.62 (24H, m, 1 to 17-CH₂), 4.56 (2H, s, 1'-CH₂), 7.32 (5H, m, ArH);

$\delta_{\text{C}}$ (75 MHz; CDCl₃): 38.20 (Me), 69.49-70.03 (C-2 to C-17), 77.70 (C-1'), 126.49 (C-3'), 128.07 (C-4'), 128.38 (C-5'), and 141.92 (C-2');

m/z (FAB⁺) 435 (M⁺, 46%);

Synthesis of 17-Benzylxoxy-3,6,9,12,15-pentaoxaheptadecan-chloro (139)

The reaction was carried out under anhydrous conditions. To a solution of 125 (40.0 g, 0.10 mol) in pyridine (9.00 ml, 0.110 mol) at 0 °C, sulfonyl chloride (10.0 ml, 0.140 mol) was added dropwise. The resulting solution was stirred at 0 °C for 30 min. and then at 80 °C for 5 h. The reaction mixture was quenched with iced water (2 x 10 ml) and the crude product extracted into chloroform (3 x 40 ml) then washed with brine (2 x 10 ml) and aqueous HCl (3 x 20 ml). Drying (NaSO₄) filtration and evaporation in vacuo yielded the title compound as an orange oil (30 g, 75%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (film): 2864 s, 1484 m, 1457 s, 1341 m, 1200 w, 815 w;

$\delta_{\text{H}}$ (300 MHz, CDCl₃) δ 3.74 (24H, m, 1 to 17-CH₂), 4.50 (2H, s, 1'-CH₂), 7.28 (5H, m, ArH);

212
Synthesis of 10-methanesulfonyl-3,6-dioxydecan-1-ol (135)

The reaction was carried out under anhydrous conditions. To a solution of triethylene glycol (20.0 g, 0.13 mol) in dichloromethane (300 ml) at 0 °C triethylamine (28.0 ml, 0.19 mol) and mesyl chloride (4.70 ml, 0.06 mol) were added. The reaction was warmed to 40 °C and stirred for 4 h. The reaction was quenched with iced water (2 x 15 ml), and the product extracted into chloroform (3 x 50 ml) then washed with brine (2 x 15 ml). Drying (NaSO₄), filtration and evaporation in vacuo yielded the title compound as an orange oil (10.8 g, 35 %).

υmax/cm⁻¹ (Film) 3404 br, 3022 w, 2876 s, 1455 m, 1348 s, 1249 w, 1070 s;
δH (300MHz, CDCl₃) 2.30 (1H, s, OH), 3.02 (3H, s, Me), 3.62 (10H, m, 1 to 7-CH₂), 4.30 (2H, t, J 3.0, 8-CH₂);
δC (75MHz, CDCl₃) 38.071 (Me), 62.12 (C-1), 69.39 to 72.96 (C-2 to C-8);
m/z (FAB⁺) 229 (M⁺, 43%);
m/z exact mass calculated for C₁₇H₇O₆S 229.0746; found 229.0754 (M+H).
Synthesis of 26-benzyloxy-3,6,9,12,15,18,21,24-octaoxyhexacosyl methanesulfonate (151)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (0.88 g, 0.02 mol) in THF (200 ml), 135 (4.56 g, 0.02 mol) was added dropwise and the resulting solution stirred for 1 h. 139 (7.82 g, 0.02 mol) was added and the reaction mixture was stirred vigorously at 70 °C for 4 d. The reaction was quenched with iced water (2 x 25 ml) and the product extracted into chloroform (3 x 50 ml) and washed with brine (2 x 10 ml). Drying (MgSO₄), filtration and evaporation in vacuo yielded the crude compound as an orange oil. Reverse phase chromatography (acetonitrile/water) afforded the title compound as a dark orange viscous oil (1.40 g, 12%)

νmax/cm⁻¹ (film) 2867 s, 1494 m, 1443 s, 1347 m, 1204 w, 1093 s;
δH (300 MHz, CDCl₃) 3.02 (3H, s, Me), 3.73 (36H, m, 1 to 26-CH₂), 4.50 (2H, s, 1'-CH₂), 7.28 (5H, m, ArH);
δC (75MHz; CDCl₃) 38.12 (Me), 69.55-70.12 (C-2 to C-26), 77.29 (C-1'), 126.73 (C-2'), 128.48 (C-3'), 128.73 (C-4'), and 128.98 (C-5');
m/z (FAB⁺) 491 (M⁺ - Bz, 5%) 91 (Bz, 90%).
Synthesis of 17-(4-Toluene-sulfonic-acid)-3,6,9,12,15-pentaoxaheptadecan-1-ol (37)

The reaction was carried out under anhydrous conditions. To a solution of 133 (14.2 g, 50.0 mmol) in dichloromethane (250 ml) at 0 °C, triethylamine (10.5 ml, 0.08 mol) was added in one portion. Tosyl chloride (4.76 g, 0.03 mol) was added in portions over 20 min. The resulting solution was stirred at room temperature for 3 h, and for a further 1 h at 38 °C. The reaction mixture was quenched with iced water (2 x 25 ml) and the product extracted into chloroform (3 x 50 ml) and then washed with brine (2 x 10 ml). Drying (Na₂SO₄), filtration and evaporation in vacuo afforded the title compound as a yellow oil (11.24 g, 52 %).

\[ v_{\text{max}} /\text{cm}^{-1}(\text{film}) \text{ 3362 br, 2874 s, 1597 m, 1356 s, 1177 s and 1097 s;} \]
\[ \delta_{\text{H}} (300 \text{ MHz; CDCl}_3) \text{ 2.13 (3H, s, Me), 2.50 (1H, s, OH), 3.47-3.70 (22H, m, 1 to 16-CH}_2\text{), 4.14 (2H, t, J 3.5, 17-CH}_2\text{), 7.32 (2H, d, J 8.0, 2 x 3'-H) and 7.78 (2H, d, J 8.0, 2 x 2'-H);} \]
\[ \delta_{\text{C}} (75 \text{ MHz; CDCl}_3) \text{ 21.36 (Me), 61.37 (C-1), 68.98 to 73.01 (C-2 to C-17), 125.61 (C-4'), 127.68 (C-3'), 129.54 (C-2') and 132.62 (C-1');} \]
\[ m/z (\text{FAB}^+) \text{ 437 (M}^+, 10%);} \]

Synthesis of 1-chloro-3,6,9,12,15-pentaoxaheptadecan-chloro (140)

The reaction was carried out under anhydrous conditions. To a solution of 133 (8.60 g, 0.03 mol) in pyridine (5.20 ml, 0.07 mol) at 0 °C, sulfonyl chloride (5.50 ml, 0.08 mol) was added dropwise. The resulting solution was stirred at 0 °C for 30 min, and for a
further 1 h at 80 °C. The reaction was quenched with iced water (2 x 25 ml) and the crude product extracted into chloroform (3 x 50 ml) then washed with brine (2 x 10 ml) and aqueous HCl (3 x 20 ml). Drying (Na$_2$SO$_4$), filtration and evaporation in vacuo afforded the title compound as a pale orange oil (9.04 g, 95%).

$v_{\text{max}}$/cm$^{-1}$ (Film) 2882 s, 1117 s, 750 s and 667 s;

$\delta_H$ (300MHz; CDCl$_3$) 3.32-3.97 (24H, m, 1 to I$_7$-CH$_2$);

$\delta_C$ (75MHz; CDCl$_3$) 42.46 (C-1, C-17) and 70.32-71.07 (C-2 to C-16);

$m/z$ (FAB$^+$) 319.1076 (M$^+$, 25%. C$_{12}$H$_{25}$O$_5$Cl$_2$ requires 319.1079).

**Synthesis of 8-benzyloxy-3,6-dioxyocta-1-ol (123)**

![Chemical structure of 123](image)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (4.80 g, 0.12 mol) in THF (300 ml), triethyleneglycol (20.0 g, 0.13 mol), was added dropwise and the resulting solution stirred for 1 h. Benzylbromide (15.8 ml, 0.13 mol) was added dropwise and the solution heated at 60 °C for 5 h. The reaction mixture was quenched with iced water (2 x 25 ml), the product extracted into chloroform (3 x 50 ml) and washed with brine (2 x 10 ml). Drying (MgSO$_4$), filtration and evaporation in vacuo afforded the title compound as a yellow oil (27 g, 85%).

$v_{\text{max}}$/cm$^{-1}$ (Film) 3445 br, 3062 w, 2866 s, 1494 m, 1454 s, 1350 w, 1205 s, 1099 s;

$\delta_H$ (300 MHz, CDCl$_3$) 2.67 (1H, s, OH), 3.65 (12H, m, 1 to 8-CH$_2$), 3.54 (2H, s, 1'-CH$_2$), 7.21 (5H, m, ArH);

$\delta_C$ (75 MHz, CDCl$_3$) 62.13 (C-1), 70.78 to 72.01 (C-2 to C-8), 72.93 (C-1'), 128.76 (C-5'), 128.79 (C-3'), 129.20 (C-4'), 141.44 (C-2');

$m/z$ (FAB$^+$) 241 (M$^+$, 9%) and 91 (PhCH$_2^+$, 94);

$m/z$ exact mass calculated for C$_{13}$H$_{29}$O$_4$Na 263.1259; found 263.1251 (M$^+$Na).
Synthesis of 26-benzyloxy-3,6,9,12,15,18,21,24-octaoxyhexacosan-1-chloro (149)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (1.24 g, 0.03 mol) in THF (200 ml), 123 (6.80 g, 0.03 mol) was added dropwise and the resulting solution stirred for 1 h. 140 (9.00 g, 0.03 mol) was added dropwise and the resulting solution stirred at 70 °C for 4 d. The reaction was quenched with iced water (2 x 25 ml) and the product extracted into chloroform (3 x 50 ml), washed with brine (2 x 10 ml) and dried (Na₂SO₄). Filtration and evaporation in vacuo afforded the crude compound as an orange oil. Reverse phase chromatography (acetonitrile/water) afforded the title compound as a dark orange viscous oil (7.81 g, 53%).

$\nu_{max}/\text{cm}^{-1}$ (Film) 2868 s, 1454 m, 1105 s, 750 s and 667 s;

$\delta_H$ (300MHz; CDCl₃) 3.55-3.71 (36H, m, 1 to 18-CH₂), 4.50 (2H, s, 1'-CH₂) and 7.26-7.29 (5H, m, ArH);

$\delta_C$ (75MHz; CDCl₃) 42.70 (C-1), 70.35-73.19 (C-2 to C-26), 77.09 (C-1'), 127.71 (C-3'), 128.32 (C-4'), 128.55 (C-5'), and 141.76 (C-2');

$m/z$ (FAB⁺) 545.2474 (M + Na⁺, 10%, C₂₅H₄₃O₇ClNa requires 545.2493).
Synthesis of 35-benzyloxy-3,6,9,12,15,18,21,24,27,30,33-
undeoxapentatriacontyl methanesulfonate (152)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (0.88 g, 0.02 mol) in THF (200 ml), 135 (4.56 g, 0.02 mol) was added dropwise and the resulting solution stirred for 1 h. Following addition of 149 dropwise (10.0 g, 0.02 mol) the reaction mixture was heated at 70 °C for 4 d. The reaction was quenched with iced water (2 x 25 ml) and the product extracted into chloroform (3 x 50 ml) and washed with brine (2 x 10 ml). Drying (MgSO₄), filtration and evaporation in vacuo yielded the crude compound as a brown oil. Reverse phase chromatography (acetonitrile/water) afforded the title compound as a dark orange viscous oil (683 mg, 5%) 

νₓᵧ/cm⁻¹ (film) 2866 s, 1484 m, 1438 s, 1344 m, 1212 w, 1099 s; 
δₓᵧ (300 MHz, CDCl₃) 3.01 (3H, s, Me), 3.63 (48H, m, 1 to 35-CH₂), 4.50 (2H, s, 1'-CH₂), 7.28 (5H, m, ArH); 
δₓᵧ (75 MHz, CDCl₃) 38.08 (Me), 69.41-72.89 (C-1 to C-35), 77.47 (C-1'), 128.041 (C-53'), 128.14 (C-4'), 128.19 (C-5'), 141.75 (C-2'); 
m/𝑧 (FAB⁺) 715 (M⁺, 2%).

Synthesis of 1-chloro-3,6-dioxadecan-chloro (147)

218
The reaction was carried out under anhydrous conditions. To a solution of triethyleneglycol (10.5 g, 0.07 mol) in pyridine (12 ml, 0.15 mol) at 0 °C, sulfonyl chloride (12.7 ml, 0.18 mol) was added dropwise. The resulting solution was stirred at 0 °C for 30 min, and for a further 4 h at 80 °C. The reaction was quenched with iced water (2 x 25 ml), the crude product extracted into chloroform (3 x 50 ml) and washed with brine (2 x 10 ml) and aqueous HCl (3 x 20 ml). Drying (Na₂SO₄), filtration and evaporation in vacuo yielded the title compound as a pale orange oil (13.0 g, 99%).

Vmax/cm⁻¹ (Film) 2866 s, 1450 m, 1100 s, 754 s and 669 s;

δH (300 MHz; CDCl₃) 3.30-3.72 (12H, m, 2x 1 to 3 CH₂);

δC (75 MHz; CDCl₃) 43.18 (C-1), 71.03 (C-2), 71.79 (C-3);

m/z (FAB⁺) 187.0294 (MH⁺, 15%. C₆H₁₅O₂Cl₂ requires 187.0293).

Synthesis of 11-benzyloxy-3,6,9-trioxaundecan-1-ol (124)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (3.60 g, 0.09 mol) in THF (300 ml), tetraethyleneglycol (20.0 g, 0.100 mol), was added dropwise, and the resulting solution stirred for 1 h. Benzyl bromide (11.9 ml, 0.1 mol) was added dropwise and the solution heated at 60 °C for 5 h. The reaction mixture was quenched with iced water (2 x 25 ml), and the product was extracted into chloroform (3 x 50 ml), washed with brine (2 x 10 ml) and dried (MgSO₄). Filtration and evaporation in vacuo yielded the title compound as a yellow oil (23.4 g, 82%)

νmax/cm⁻¹ (Film) 3440 br, 3068 w, 2866 s, 1493 m, 1456 s, 1352 m, 1213 w, 1097 s;

δH (300 MHz, CDCl₃) 2.21 (1H, s, OH), 3.10-3.59 (16H, m, 1 to 11-CH₂), 4.50 (2H, s, 1'-CH₂), 7.27-7.69 (5H, m, ArH);
\( \delta_C (75 \text{ MHz, CDCl}_3) 62.17 (\text{C-1}), 70.74 - 72.97 (\text{C-2 to C-11}), 77.01 (\text{C-1'}), 128.03 (\text{C-5'}), 128.20 (\text{C-3'}), 128.78 (\text{C-4'}), 129.21 (\text{C-2'}); \\
m/z (\text{FAB}^+) 285 (M^+, 15\%), \text{ exact mass calculated for } C_{15}H_{25}O_5 285.1702; \text{ found } 285.1706 (M+H). \\

**Synthesis of 11-benzyloxy-3,6,9-trioxaundecan-1-oxybenzyl (127)**

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (1.22 g, 0.05 mol) in THF (20 ml), tetraethyleneglycol (4.40 ml, 0.03 mol) and benzylbromide (6.10 ml, 0.05 mol) were added and the reaction stirred at 70 °C for 16 h. The reaction was quenched with iced water (10 ml) and the product extracted into chloroform and washed with brine (10 ml). Drying (phase separator) and evaporation in vacuo afforded the **title compound** as a yellow oil (9.65 g, 99%).

\( \nu_{\max} / \text{cm}^{-1} \) (Film) 2867 s, 1453 m, 1102 m, 698 m; 
\( \delta_H (300 \text{ MHz; CDCl}_3) 3.18-3.68 (16H, m, 1 to 11-\text{CH}_2), 4.58 (2H, s, 2x 1'-\text{CH}_2), 7.24-7.85 (10H, m, ArH); \\
\( \delta_C (75 \text{ MHz; CDCl}_3) 69.13 - 70.37 (\text{C-1 to C-11}), 77.01 (\text{C-1'}), 128.03 (\text{C-5'}), 128.20 (\text{C-3'}), 128.78 (\text{C-4'}), 129.21 (\text{C-2'}); \\
m/z (\text{ES}^+) 375 (M^+, 90%).

220
Synthesis of 11-hydroxy-3,6,9-trioxaundecan-1-ol (115)

Palladium hydroxide on carbon (100 mg) was presaturated with H₂ for 1 h. 127 (1.00 g, 2.67 mmol) in methanol (20 ml) was added and the reaction stirred at room temperature for 1 d. The crude product was extracted into dichloromethane (10 ml), and filtration of the catalyst and evaporation in vacuo afforded the crude compound as a pale yellow oil. Flash column chromatography (dichloromethane/ethyl acetate, 2:1) afforded the title compound as a colourless oil (501 mg, 98%).

ν_max /cm⁻¹ (Film) 3449 br, 3066 w, 2868 s;  
δ_H (300 MHz; CDCl₃) 2.56 (2H, s, OH), 3.19-3.78 (16H, m, 1 to 11-CH₂);  
δ_C (75 MHz; CDCl₃) 62.09 (C-1), 67.78-73.35 (C-2 to C-11);  
m/z (ES⁺) 216 (M⁺ + Na⁺, 64%).

Synthesis of 17-Benzyloxy-3,6,9,12,15-pentaoxaheptadecan-chloro (139)

From the chemical structures and reactions described, it is evident that the synthesis involves the formation of compounds with multiple oxyethylene units and chloro groups, which are likely targeted for specific applications in organic chemistry or material science.
The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (2.64 g, 0.07 mol) in THF (300 ml), 123 (14.4 g, 0.06 mol) was added dropwise and the resulting solution stirred for 1 h. Following the addition of 147 dropwise (11.2 g, 0.06 mol) the solution was stirred at 60 °C for 2 d. The reaction was quenched with iced water (2 x 25 ml) and washed with brine (2 x 10 ml). The product was extracted into chloroform (3 x 50 ml), dried (MgSO₄), filtered and evaporated in vacuo to yield the crude compound as an orange oil. Reverse phase chromatography (acetonitrile/water) afforded the title compound as an orange oil (14.5 g, 62%).

\[\nu_{\text{max}}/\text{cm}^{-1} (\text{Film}) \ 2869 \text{ s}, 1456 \text{ m}, 1108 \text{ s}, 755 \text{ s} \text{ and } 669 \text{ s};\]

\[\delta_H (300 \text{ MHz}; \text{CDCl}_3) 3.74 (24H, m, 1 \text{ to } 17-\text{CH}_2) 4.50 (2H, s, 1'-\text{CH}_2) 7.28 (5H, m, \text{ArH});\]

\[\delta_C (75 \text{ MHz}; \text{CDCl}_3) 41.84 (\text{C}-1), 68.30-69.82 (\text{C}-2 \text{ to } \text{C}-17), 77.74 (\text{C}-1'), 126.72 (\text{C}-3'), 128.48 (\text{C}-4'), 128.79 (\text{C}-5'), \text{ and } 141.90 (\text{C}-2');\]

\[m/z (\text{FAB}^-) 413.1720 (\text{M}^- + \text{Na}^+, 0.25\% , \text{C}_{19}\text{H}_{30}\text{O}_{6}\text{ClNa requires 413.1707}).\]

Synthesis of 11-(2H-Tetrahydropyran-2-yloxy)-3,6,9-trioxaundecan-1-ol (129)

The reaction was carried out under anhydrous conditions. To a solution of 115 (8.90 ml, 51.0 mmol) at 0 °C in dichloromethane, \(p\)-tolenesulfonic acid (970 mg, 5.10 mmol) was added. 3,4-Dihydro-2\(H\)-pyran (4.19 ml, 46.0 mmol) was added dropwise and the solution stirred at 0 °C for 1 h, then r.t for 16 h. The reaction mixture was quenched with iced water (2 x 50 ml), washed with brine (2 x 25 ml), and dried (phase separator). Evaporation in vacuo afforded the title compound as an orange oil (6.50 g, 46%).

\[\nu_{\text{max}}/\text{cm}^{-1} (\text{Film}) \ 3435 \text{ br}, 3064 \text{ w}, 2863 \text{ s}, 1492 \text{ m}, 1452 \text{ s}, 1353 \text{ m}, 1216 \text{ w}, 1099 \text{ s};\]

\[\delta_H (300 \text{ MHz, CDCl}_3) 1.59-1.83 (6H, m, 3' \text{ to } 5'-\text{CH}_2), 3.71-3.98 (18H, m,1 \text{ to } 11-\text{CH}_2, 6'-\text{CH}_2), 4.74 (1H, t, J 3.1, 2'-\text{CH});\]

\[\delta_C (75 \text{ MHz, CDCl}_3) 19.86 (\text{C}-5'), 25.84 (\text{C}-4'), 30.97 (\text{C}-3'), 62.59 (\text{C}-1), 67.06 (\text{C}-6'), 70.95-71.05 (\text{C}-2 \text{ to } \text{C}-11), 99.34 (\text{C}-2');\]

\[m/z (\text{ES}^-) 279 (\text{M}^+, 5\%), 195 (\text{M}^+ - \text{THP}, 20), 85 (\text{THP}, 96).\]
Synthesis of 17-chloro-3,6,9,12,15-pentaoxaheptadecan-1-ol (141)\(^{146}\)

\[
\begin{align*}
\text{HO} & \quad \text{SOCl}_2 \quad \text{Pyridine} \\
133 & \rightarrow 141
\end{align*}
\]

The reaction was carried out under anhydrous conditions. To a solution of 133 (2.00 g, 7.08 mmol) in pyridine (0.46 ml, 5.80 mmol) at 0 °C, thionyl chloride (0.42 ml, 5.80 mmol) was added dropwise. The reaction mixture was stirred at 80 °C for 5 h. The reaction was quenched by the addition of iced water (10 ml), the crude product extracted into chloroform (3 x 10 ml) and washed with brine (10 ml) and aqueous HCl (2 x 30 ml). Drying (Na\(_2\)SO\(_4\)), filtration of drying agent and evaporation in vacuo afforded the crude compound as a very dark orange oil (669 mg, 31%).

\(v_{\text{max}}/\text{cm}^{-1}\) (Film) 3449 br, 3057 w, 2858 s, 1493 s, 735 s, 648 s;

\(\delta_H\) (300 MHz; CDCl\(_3\)) 2.63 (1H, s, OH) and 3.33–3.80 (24H, m, 1 to 17-CH\(_2\));

\(\delta_C\) (75 MHz; CDCl\(_3\)) 42.55 (C-17), 61.55 (C-1) and 70.22–72.37 (C-2 to C-16);

\(m/z\) (ES\(^+\)) 324 (M\(^+\) + Na\(^+\), 39%).

Synthesis of 17-(2\(H\)-Tetrahydropyran-2-yloxy)-3,6,9,12,15-pentaoxaheptadecan-chloro (142)\(^{147}\)

The reaction was carried out under anhydrous conditions. To a solution of 141 (500 mg, 1.60 mmol) in dichloromethane (50 ml) at 0 °C, \(p\)-toluenesulfonic acid (30.0 mg, 0.16 mmol) and 3,4-dihydro-2\(H\)-pyran (0.15 ml, 1.60 mmol) were added and the reaction mixture stirred at room temperature for 16 h. The reaction mixture was quenched with iced water (2 x 20 ml), drying (Na\(_2\)SO\(_4\)), filtration and evaporation in vacuo afforded the crude compound as a dark yellow oil. Reverse phase chromatography (acetonitrile/water) afforded the title compound as a dark yellow oil (218 mg, 35%).
\( v_{\text{max}} / \text{cm}^{-1} \) (Film) 2939 s, 2867 s, 1119 s, 843 m;
\( \delta_H (300 \text{ MHz}; \text{CDCl}_3) 1.49-1.70 (6\text{H, m, 3'- to 5'-CH}_2), 3.58-3.85 (26\text{H, m, 1-CH}_2 \text{ to 17-CH}_2 \text{ and 6'-CH}_2), 4.92 (1\text{H, t, } J 4.7, 2'-\text{CH}); \)
\( \delta_C (75 \text{ MHz; CDCl}_3) 19.17 (\text{C-5'}), 25.14 (\text{C-4'}), 30.28 (\text{C-3'}), 42.37 (\text{C-17}), 61.41 (\text{C-1}), 66.35 (\text{C-6'}), 70.05-71.07 (\text{C-2 to C-16}), 98.62 (\text{C-2'}); \)
\( m/z \) (ES\(^+\)) 384 (M\(^+\), 39%).

**Synthesis of 10-methoxy-3,6-dioxadecan-bromo (144)**

To a solution of triethyleneglycolmonomethylether (9.70 ml, 0.06 mol) in toluene (80 ml) at room temperature, hydrobromic acid (48 % in water; 13.8 ml, 0.12 mol) was added dropwise. The reaction mixture was stirred vigorously at 110 °C for 3 d. The mixture was neutralised by addition of NaHCO\(_3\), washed with brine (30 ml) and the product extracted into ethyl acetate (40 ml). Drying (Na\(_2\)SO\(_4\)), filtration and evaporation \textit{in vacuo} afforded the title compound as a yellow oil (1.69 g, 12%).

\( v_{\text{max}} / \text{cm}^{-1} \) (Film) 2876 s, 1454 m and 880 w;
\( \delta_H (300 \text{ MHz, CDCl}_3) 3.32 (3\text{H, s, Me}) \text{ and } 3.39-3.99 (12\text{H, m, 1-CH}_2 \text{ to 8-CH}_2); \)
\( \delta_C (75 \text{ MHz, CDCl}_3) 30.18 (\text{C-1}), 58.89 (\text{Me}) \text{ and } 70.42 \text{ to } 71.82 (\text{C-2 to C-8}); \)
\( m/z \) (FAB\(^+\)) 250 (M\(^+\) + Na\(^+\), 44%), 227 (M\(^+\), 17).

**Synthesis of 17-bromo-3,6,9,12,15-pentaoxaheptadecan-1-ol (145)**

To a solution of 133 (8.00 g, 28.3 mmol) in toluene (50 ml) at room temperature, hydrobromic acid (48 % in water; 3.20 ml, 28.3 mmol) was added. The reaction mixture was stirred vigorously at 110 °C for 3 d. Following cooling to room temperature the mixture was neutralised by addition of NaHCO\(_3\) and the crude product extracted into ethyl acetate. Filtration and evaporation \textit{in vacuo} afforded the crude
compound which was washed with water (20 ml), extracted into dichloromethane (30 ml), dried (phase separator), filtered and evaporated in vacuo to afford the title compound as a pale yellow oil (5.50 g, 56%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (Film) 2889 s, 1445 m and 888 w;

$\delta_H$ (300 MHz; CDCl$_3$) 3.14 (1H, s, OH), 3.39 (2H, t, $J = 3.1$, 2-CH$_2$), 3.51-3.63 (20H, m, 3-CH$_2$ to 17-CH$_2$), 3.71 (2H, t, $J = 3.1$, 1-CH$_2$);

$\delta_C$ (100 MHz; CDCl$_3$) 30.27 (C-1), 62.50 (C-17), 71.10-73.49 (C-2 to C-16);

$m/z$ (ES$^+$) 347 (M$^+$, 30%), 367 (M$^+$ + Na$^+$, 20).

**Synthesis of 17-(2H-Tetrahydropyran-2-yloxy)-3,6,9,12,15-pentaoxaheptadecan-bromo (146)**

![Synthesis of 17-(2H-Tetrahydropyran-2-yloxy)-3,6,9,12,15-pentaoxaheptadecan-bromo (146)](image)

The reaction was carried out under anhydrous conditions. To a solution of 145 (2.00 g, 5.80 mmol) in dichloromethane at room temperature, $p$-toluenesulfonic acid (110 mg, 0.58 mmol), and 3,4-dihydro-2H-pyran (0.53 ml, 5.80 mmol) were added. The reaction mixture was stirred at room temperature for 16 h, quenched by the addition of water (10 ml) and washed with brine (10 ml). Drying (phase separator) and evaporation in vacuo afforded the crude compound as a yellow oil. Reverse phase chromatography (acetonitrile / water) afforded the title compound as a yellow oil (1.46 g, 59%).

$\nu_{\text{max}} / \text{cm}^{-1}$ (Film) 2924 s, 1456 m, 1124 s and 844 w;

$\delta_H$ (300 MHz; CDCl$_3$) 1.23-1.84 (6H, m, 3'-CH$_2$ to 5'-CH$_2$), 3.44 (2H, t, $J = 3.1$, 1-CH$_2$), 3.57-3.81 (20H, m, 3-CH$_2$ to 17-CH$_2$), 3.80 (2H, t, $J = 3.1$, 2-CH$_2$), 4.59 (1H, t, $J = 3.5$, 2'-CH);

$\delta_C$ (75 MHz; CDCl$_3$) 19.31 (C-5'), 25.29 (C-4'), 30.13, 30.41, 62.09 (C-17), 66.48 (C-6'), 70.11-71.05 (C-2 to C-16), 98.75 (C-2');

$m/z$ (LCMS) 446.27 (M$^+$ + NH$_4^+$, 100%).
Synthesis of 17-(2H-Tetrahydropyran-2-yloxy)-3,6,9,12,15-pentaoxaheptadecan-1-ol (130)

The reaction was carried out under anhydrous conditions. To a solution of 133 (8.90 ml, 35.0 mmol) at 0 °C in dichloromethane, p-toluenesulfonic acid (660 mg, 3.50 mmol) was added. 3,4-Dihydro-2H-pyran (2.80 ml, 32.0 mmol) was added dropwise and the solution stirred at 0 °C for 1 h, and at r.t for 16 h. The reaction mixture was quenched with iced water (2 x 50 ml), washed with brine (2 x 40 ml), and dried (phase separator). Evaporation in vacuo afforded the title compound as an orange oil (6.77 g, 53%).

\[ \nu_{\text{max}} / \text{cm}^{-1} (\text{Film}) \ 3422 \text{ br}, 3056 \text{ w}, 2865 \text{ s}; \]
\[ \delta_{\text{H}} (300 \text{ MHz}; \text{CDCl}_3) 1.69-1.83 (6\text{H, m, 3'} \text{ to 5'}-\text{CH}_2), 2.62 (1\text{H, s, OH}), 3.79-3.92 (26\text{H, m, 1 to 17-CH}_2, 6'-\text{CH}_2), 4.83 (1\text{H, t, J 3.5}, 2'-\text{H}); \]
\[ \delta_{\text{C}} (75 \text{ MHz}; \text{CDCl}_3) 19.86 (\text{C-5'}), 25.84 (\text{C-4'}), 30.97 (\text{C-3'}), 62.57 (\text{C-1}), 67.05 (\text{C-6'}), 70.94-73.00 (\text{C-2 to C-17}), 99.32 (\text{C-2}); \]
\[ m/z (\text{ES}^+) 389 (\text{M}^+ + \text{Na}^+, 17%), 283 (\text{M}^+ - \text{THP}, 11), 85 (\text{THP}, 96). \]

Synthesis of 17-methanesulfonate-3,6,9,12,15-pentaoxaheptadecan-methane-sulfonate (138)

The reaction was carried out under anhydrous conditions. To a solution of 133 (8.00 g, 28.3 mmol) in dichloromethane (40 ml) at 0 °C, triethylamine (11.8 ml, 85.0 mmol) was added. Methanesulfonylchloride (6.59 ml, 85.0 mmol) was added and the reaction mixture stirred at 0 °C for 2 h then at r.t for 16 h. The reaction was quenched by addition of water (30 ml), dried (phase separator) and evaporated in vacuo to afford the title compound as an orange oil (9.62 g, 78%).

\[ \nu_{\text{max}} / \text{cm}^{-1} (\text{Film}) 2878 \text{ s}, 1444 \text{ m}, 1354 \text{ s}, 1256 \text{ w}, 1078 \text{ s}; \]
Synthesis of 35-(2H-Tetrahydropyran-2-yloxy)-
3,6,9,12,15,18,21,23,24,27,30,33-undecaoxapentatriacontyl
methanesulfonate (153)

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (433 mg, 10.8 mmol) in THF (100 ml) at 0 °C, 130 (3.97 g, 10.8 mmol) dissolved in THF (10 ml) was added dropwise. 138 (9.50 g, 21.7 mmol) dissolved in THF (10 ml) was added dropwise and the reaction mixture stirred at 65 °C for 4 d. The reaction was quenched by addition of iced water (1 ml) and the crude product extracted into dichloromethane (20 ml). Separation and drying (phase separator), followed by filtration and evaporation in vacuo afforded the crude product as a yellow oil. Purification via reverse phase chromatography (acetonitrile/water) afforded the title compound as a colourless oil (3.82g, 50%).

$\nu_{max} / \text{cm}^{-1}$ (Film) 2928 s, 1480 s, 1380 s;

$\delta_H$ (300 MHz; CDCl$_3$) 1.26-1.80 (6H, m, 3' to 5'-CH$_2$), 3.01 (3H, s, Me), 3.36-3.69 (44H, m, 3 to 35-CH$_2$), 3.78 (2H, m, 2-CH$_2$), 4.32 (2H, m, 1-CH$_2$), 4.54 (1H, t, $J_{3.5}$, 2'-CH);

$\delta_C$ (75 MHz, CDCl$_3$) 19.39 (C-5'), 25.24 (C-4'), 30.40 (C-3'), 37.51 (Me), 62.08 (C-35), 66.57 (C-6'), 69.23-70.47 (C-1 to C-34), 98.99 (C-2');
Synthesis of 35-hydroxy-3,6,9,12,15,18,21,23,24,27,30,33-
undecaoxapentatriacontyl iodide (156)

To a solution of 153 (4.00g, 5.65 mmol) in acetone (40 ml) at room temperature,
sodium iodide (8.47 g, 56.5 mmol) was added. The reaction mixture was stirred at
reflux for 4 d. Filtration and evaporation in vacuo afforded a dark orange oil, which
was washed with dichloromethane (10 ml). Repeated filtration and evaporation in
vacuo afforded the title compound as a very viscous dark orange oil (3.69g, 99%).

\[
\begin{align*}
\text{C} & \quad \text{H} \quad \text{O} \quad \text{I} \\
\text{153} & \quad \text{156}
\end{align*}
\]

\( \nu_{\max} / \text{cm}^{-1} \) (Film) 3404 br, 3022 w, 2942 s, 1477 s, 1355 s;
\( \delta_H \) (300 MHz; CDCl\(_3\)) 2.59 (1H, s, OH), 3.54 (2H, t, 35-CH\(_2\), J 6.6), 3.85-4.08 (15H, m, 1 to 34-CH\(_2\));
\( \delta_C \) (75 MHz; CDCl\(_3\)) 3.98 (C35), 60.54 (C1), 68.42-72.26 (C2-C34);
\( m/z \) (ES\(^+\)) 679 (M\(^+\) + Na\(^+\), 76%), 697 (M\(^+\) + Na\(^+\) + H\(_2\)O, 28).

Synthesis of 35-(2H-Tetrahydropyran-2-yloxy)-
3,6,9,12,15,18,21,23,24,27,30,33-undecaoxapentatriacontyl iodide (155)

The reaction was carried out under anhydrous conditions. To a solution of 156 (3.79g,
5.65 mmol) in dichloromethane (100 ml) at room temperature, \( p \)-toluenesulfonic acid
(107 mg, 0.56 mmol) and 3,4-dihydro-2\( H \)-pyran (0.5ml, 5.65 mmol) were added. The
reaction mixture was stirred at room temperature for 16 h. The crude product was
washed with water (0.5 ml), dried and evaporated in vacuo to afford the title compound
as a brown oil (4.18g, 99%).

\( \nu_{\max} / \text{cm}^{-1} \) (Film) 2942 s, 1471 s, 1351 s;
$\delta_H$ (300 MHz; CDCl$_3$) 1.42-1.75 (6H, m, 3' to 5'-CH$_2$), 3.16-3.21 (2H, t, $J$ 6.9, 35-CH$_2$), 3.53-4.09 (48H, m, 1 to 34-CH$_2$, 5'-CH$_2$), 4.55-4.57 (1H, t, $J$ 3.4, 2'-CH);

$\delta_C$ (75 MHz; CDCl$_3$) 3.16 (C-35), 19.87 (C-5'), 25.85 (C-4'), 30.98 (C-3'), 62.58 (C-1), 67.06 (C-6'), 70.66-72.42 (C-2 to C-34), 99.34 (C-1');

$m/z$ (ES$^+$) 763.27 (M$^+$ + Na$^+$, 30%), 674.94 (M$^+$ - THP + H$_2$O, 10), 655.30 (M$^+$ - THP, 8), 649.08 (M$^+$ - 1 + 2 x H$_2$O, 12).
9.5 Synthesis of Fluorescently labelled compounds

Labelling of Bisphenyl-4,4'-yloxy-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy) with pyrene (219)

The reaction was carried out under anhydrous conditions. To a solution of 209 (50.0 mg, 0.07 mmol), in dichloromethane (10 ml), at room temperature, triethylamine (0.01 ml, 0.07 mmol) was added. Following stirring for 10 min, 218 (32.0 mg, 0.08 mmol) was added in one portion, and the reaction mixture stirred at room temperature for 6 d. The reaction was quenched with water (10 ml), and the crude product extracted into dichloromethane (20 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a viscous yellow oil. Flash chromatography (ethyl acetate/hexane, 2:1) afforded the title compound as a yellow oil (5 mg, 7%).

$\lambda_{\text{max}}$/nm (KOH/MeOH) 240, 260, 275, 325, 340;
$m/z$ (ES$^+$) 1002 ($M^+$ + H$_2$O, 15%);
$R_f$ 0.45 (Label), 0.12 (218) (ethyl acetate/hexane 2:1)
Labelling of Bisphenyl-4,4'-yloxy-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy) with Texas Red (220)

The reaction was carried out under anhydrous conditions. To a solution of 209 (7.00 mg, 0.01 mmol) in dichloromethane (10 ml) at room temperature, triethylamine (0.009 ml, 0.01 mmol) was added and the solution stirred for 5 mins. Texas Red–N-hydroxysuccinimide ester (5.00 mg, 0.01 mmol) in dichloromethane (2 ml) was added and the solution stirred at room temperature for 9 d. The reaction mixture was washed with water (2 ml), the organic phase separated, dried (phase separator) and evaporated in vacuo to afford the crude product as a blue solid. Flash chromatography (dichloromethane then dichloromethane/methanol) afforded the title compound as blue solid as a mixture with unlabelled compound 209.

$\lambda_{\text{max}}$/nm (KOH/MeOH) 250, 580;

m/z (ES$^+$) 1467 (M$^+$ + MeOH + H$_2$O, 68%), 1449 (M$^+$ + MeOH, 37%);

R$_f$ 0.89 (209 and 220), 0.00 (Texas Red) (Methanol)
Labelling of 2-((35-[2-(3-{35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy)}benzyl)phenoxy]-3,6,9,12,15,18,21,24,27,30-undecaoxapentatriacontyloxy)hydroxyl with Texas Red (221)

The reaction was carried out under anhydrous conditions. To a solution of 213 (15.0 mg, 0.01 mmol) at room temperature in dichloromethane (5 ml), triethylamine (0.009 ml, 0.01 mmol) was added and the solution stirred for five minutes. Texas Red-N-hydroxysuccinimide ester (5.00 mg, 0.01 mmol) in dichloromethane (2 ml) was added, and the reaction stirred at room temperature for 12 d. The mixture was washed with water (2 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a blue solid. Flash chromatography (dichloromethane then dichloromethane/methanol) afforded the title compound as a blue solid as a mixture with unlabelled compound 215.

\( \lambda_{\text{max}}/\text{nm} \) (KOH/MeOH) 240, 580;

\( R_f \) 0.91 (215 and 221), 0.00 (Texas Red) (Methanol)

The reaction was carried out under anhydrous conditions. To a solution of 180 (50.0 mg, 0.02 mmol) in dichloromethane (10 ml) at room temperature, triethylamine (0.003 ml, 0.02 mmol) was added and the solution stirred for 5 min. 218 (9.00 mg, 0.02 mmol) was added and the reaction mixture stirred at room temperature for 14 d. The reaction mixture was washed with water (5 ml), the organic phase separated, dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Flash chromatography (ethyl acetate/hexane, 2:1, the ethyl acetate/methanol, 10:1) afforded the title compound as a yellow viscous oil (4 mg, 7%).

$\lambda_{\text{max}}$/nm (KOH/MeOH) 240, 265, 275, 325, 345;

$R_f$ 0.45 (Label), 0.09 (222) (ethyl acetate/hexane 2:1)

The reaction was carried out under anhydrous conditions. To a solution of 180 (25.0 mg, 0.01 mmol) in dichloromethane (10 ml) at room temperature, triethylamine (0.009 ml, 0.01 mmol) was added and the solution stirred at room temperature for 5 min. Texas-Red-N-hydroxysuccinimide ester (5.00 mg, 0.01 mmol) in dichloromethane (2 ml) was added and the solution stirred at room temperature for 14 d. The reaction mixture was washed with water (2 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a blue solid. Flash chromatography (dichloromethane then dichloromethane/methanol) afforded the title compound as a blue solid as a mixture with the unlabelled compound 180.

$\lambda_{\text{max}}$/nm (KOH/MeOH) 225, 255, 580;

$R_f$ 0.88 (180 and 223), 0.00 (Texas Red) (Methanol)
Bibliography

7. A Wallgren, Tuberc., 1948, 245-251
10. A Q Wells, MRC Special Report no 259, London HMSO, 1946
17. S Capewell, Tubercule, 1986, 67, 125
23. BBC News Website (www.bbc.co.uk/news), 4/4/03
24. V Springett, Tubercule, 1965, 46, 76
34. A Cunningham, C Spreadbury, *J. Bact.*, 1998, 180, 801
37. Subcommittee of the Joint TB Committee, *Thorax*, 1990, 45, 403
43. W Fox, I Sutherland, *Quart. J. Med.*, 1959, 28, 77
50. F G Winder, The Biology of the Mycobacteria, Volume 1, 353, 1982

236
57. C S Stauffer, A Datta, Tet., 2002, 58, 9765
59. P T Davidson, H Q Le, Drugs, 1992, 43, 651
69. W McDermott, R Tompsett, Am. Rev. TB, 1954, 70, 748
71. E Brender, Tubercule, 1972, 53, 128

239
141. Hitachi Chemical Corporation, Patent 57214129, 1984, Japan
144. Butterworth JF; Moran JR; Whitesides GM; Strichartz GR; J. Med. Chem. 1987, 8, 30
146. Pederson CJ; Ger Offen DE 1963528 197000702, 1970
154. D U Hahn, personal communication, 2000
Appendices
Current Data Parameters
NAME  hahn
EXPNO  5
PROCNO  1

F2 - Acquisition Parameters
Date_  20000313
Time  15.53
INSIRUM  msl
PROBHD
PULPROG  10555_PC
T0  1024
SOLVENT  CDCl3
R0  4526
R5  574
R0  4526
SMH  29411.766 Hz
F1DES  287202427 Hz
AQ  0.0174580 sec
RG  455
DMW  17.000 usec
DE  625.00 usec
TE  297.0 Hz
D11  0.00000480 sec
H1L  0 dB
D5  0.00100000 sec
D25  0.00004101 sec
D27  0.00003907 sec
D0  2.00000000 sec
P1  0.00 usec
L1  1
D1  0.00000380 sec
SF01  75.4755000 MHz
NUCLEUS

F2 - Processing parameters
SI  4096
SF  75.4676780 MHz
NOW  EM
SSB  0
LB  2.00 Hz
GB  0
PC  0.70
SB  -302.00 Hz

1D NMR plot parameters
CX  30.00 cm
FP  270.000 ppm
F1  20376.27 Hz
FP  -30.000 ppm
F2  -2264.03 Hz
PPMCM  10.00000 ppm/cm
HZCM  754.57676 Hz/cm
The selective functionalisation and difunctionalisation of 
moieties

Gwénaëlle Hervé, Dirk Uwe Hahn, Anne-Cécile Hervé, Kerry J. Goodworth, Alison M. Hill and Helen C. Hailes

* Department of Chemistry, University College London, 20 Gordon Street, London, UK WC1H 0AJ E-mail: h.c.hailes@ucl.ac.uk

** School of Chemistry, University of Exeter, Stocker Road, Exeter, UK EX4 4QD

Received 4th September 2002, Accepted 14th November 2002
First published as an Advance Article on the web 19th December 2002

Methodologies to access water soluble large ringed calixarenes in good yield using efficient synthetic procedures have been investigated. Symmetrical partial functionalisations at the lower rim are described using activated [n]ethylene glycol chains and the addition behaviour contrasted with that of bromoalkanenitriles which proceeds with no observed regioselectivity. Full functionalisations of the calixarenes bearing hydrophilic groups are then investigated and a two-step procedure established which appears to be generally applicable for the addition of different [n]ethylene glycol chains. Furthermore, difunctionalisation under different reaction conditions are described. Throughout, strategies for the characterisation of these high mass compounds are outlined.

Introduction

In recent years, the chemistry of calixarenes has received much attention where they have been used as building blocks for host molecules with various applications in supramolecular chemistry. Research has focused on regio- and stereoselective functionalisation at the lower and upper rim and a range of procedures for this have been reported. Methods for the etherification of the calix[4]arenes at the lower rim have been well established and general procedures are available for the selective preparation of monoalkoxy-, 1,2- and 1,3-dialkoxy-calix[4]arenes, trialkoxy- and fully O-alkylated calix[4]arenes in high yields.

By comparison, the synthesis of derivatives of the larger ring calixarenes, calix[6]arenes and in particular the calix[8]arenes, have been explored to a lesser degree and their chemistry is therefore less established. However, with their larger ring sizes, they have significant potential for use as large molecular receptors for medium and large sized organic compounds or as enzyme mimics. Compared to the calix[4]arenes, the selective O-alkylation of calix[6]- and calix[8]arenes at the lower rim is more difficult, and can be unpredictable because of their conformational flexibilities and large number of reactive centres. However, synthetic procedures have been established for the selective monosubstitution, 1,4-disubstitution, 1,2,4,5-tetra-substitution, and hexa-substitution of calix[6]arenes in high yields. Tri-O-alkylated 1,2,3- or 1,3,5-calix[6]arenes have been obtained in lower yields. The factors governing the outcome of these reactions include the strength of the base used, the different solubilities of intermediates, the possibility of generating mono- or polyalanines which have different stabilities, and conformational and metal template effects. However, the selective functionalisations reported have involved, almost exclusively, the use of reactive or unfractionalised electrophiles such as benzy1 and methyl groups where further functionalisation of these groups is not possible.

Similarly, synthetic procedures using calix[8]arenes have been reported to generate 1,3,5,7-tetraalcohol- and O-substituted derivatives, where an alternate alkylation mechanism was proposed to explain the regioselectivity observed. However, as with the calix[6]arenes most reports involve the addition of reactive or unfractionalised electrophiles such as the benzy1 halides. Interesting exceptions include the preparation of octopus-type calix[6]arenes in low yield, monosubstituted calix[8]arene using Cl(CH2CH2O)3Tl, and the interbridging of calix[4]arenes and calix[8]arenes using mono- to twiceethylene glycol ditosylates. Full ether functionalisations at the lower rim of calix[8]arenes have been reported, generally involving the use of large excesses of strong bases together with a large excess of a reactive electrophile such as the methyl or benzy1 halides. The full O-alkylation of calix[8]arenes using activated [n]ethylene glycol moieties has also been described, but up to 80 equivalents of electrophile were required, and with longer chain ethylene glycols (>diethylene glycols) incomplete reactions were reported. There is therefore a significant need to establish simple efficient procedures for the partial or full derivatisation of calix[8]arenes using less reactive functionalised electrophiles, in particular, the attachment at the lower rim of electrophiles of lower reactivity than benzyl and methyl halides, with different properties such as solubilising, catalytic, complexation or fluorescent moieties.

Herein we report our study to establish a general and simple procedure for the partial or full O-alkylation of calix[6]arenes and calix[8]arenes using electrophiles including 4-bromobutyronitrile, 7-bromoheptanenitrile and activated tri-, hexa- and dodecaethylene glycols, due to our interest in the preparation of hydrophilic and functionalised calixarenes.

Results and discussion

In our studies we selected two types of chain for partial or full attachment, the bromoalkanenitriles, which have potential for further elaboration, and poly(ethylene glycol) chains, which have solubilising properties or could be further functionalised. 

Neri et al. have reported the selective alkylation of tert-butyl-calix[8]arene at the lower rim using benzyl bromides, which under strongly basic conditions led to the formation of octa-substituted products, whilst the use of weak bases (K2CO3 and
CaF) generated 1,3,5,7-tetrasubstituted compounds.44 However, when the mild bases were used with methyl iodide the 1,2,4-triethoxy- and 1,2,3,4-tetramethoxy derivatives predominated.7 Furthermore, Neri et al. rationalised that the size of the electrophile is important and reported that when using butyl iodide both the 1,2,4-triethoxy- and 1,3,5,7-tetraethoxy derivatives were generated. In our preliminary study, we investigated the reaction between bromobutyronitrile and p-tert-butylcalix[8]arene 1 with NaH in THF or DMF with the aim of generating the fully O-alkylated calix[8]arene. However, negligible amounts of alkylated products were generated. Interestingly, when carrying out the same reaction, but using KjCOj as a mild base in acetonitrile, alkylations were readily observed. When using 0.5 to 2 equivalents of bromobutyronitrile (per phenolic OH), the reaction evolved to give a complete mixture of polyalkylated compounds with no preference for the formation of particular partially alkylated derivatives. These were therefore not isolated, but were reacted directly in a second step this time using NaH as the base which promoted the formation of p-tert-butyl-octakis(cyanopropoxy)calix[8]arene 4, using in total 20–32 equivalents of the bromobutylcalixarene (i.e. 2.5 to 4 equivalents per phenolic OH) in 30–35% yield over the two steps. Although the use of NaH did not generate the fully O-alkylated product in one step, we interestingly observed that 4 could also be formed in 70% yield but in one step using a larger amount of mild base (KjCOj) and an extended reaction time. This simple one-step methodology was then used with the calixarenes 2 and 3 forming the butyronitrile derivatives, 5 and 6, in 72% and 71% yields respectively. The use of KjCOj with 1 and bromoheptanonomnirane was similarly effective, and 7 was formed in 80% yield. The solubilities of compounds 1–3 are approximately 0.3 mg ml⁻¹ in water whilst those of 4–7 were measured as 7–8 mg ml⁻¹. Using ¹³C NMR spectroscopy four aromatic signals were observed indicating conformational interconversion of the calixarenes. Indeed, a reduction in calixarene mobility was only noted for the calix[6]arene derivative 6 where in the ¹H NMR spectra at room temperature signal broadening was observed.

These results highlighted two strategies to generate the fully alkylated products: one involving two steps and a combination of a mild base (with no selective O-alkylation) and strong base; the second, a one step procedure using larger quantities of a mild base. The requirement for the use of mild bases with bromoalkanenitriles is contrary to previous reports using electrophiles of similar reactivity where for full alkylations, stronger bases have been used.6 The need for the use of a mild base for initial alkylation is unclear though likely to involve several parameters including greater monoanion stability in the presence of the electrophiles used together with template effects. Although full alkylations could be achieved using the bromoalkanenitriles, up to 32 equivalents of the electrophile were required. For the synthesis of compounds with greater aqueous solubilities, incorporating groups such as [p]ethylene glycol, a more efficient process would be required, particularly if more elaborate compounds were synthesised for attachment. Previous reports have highlighted the challenging nature of such O-alkylation reactions.45 Initial studies focussed on the use of p-tert-butylcalix[8]arene 1 and mesylate or tosylate activated methoxytriethylene glycol46 since mesylated or tosylated compounds have been used in previous calixarene additions.47,48 A range of different bases (NaH, KjCOj, PhLi, n-BuLi), reaction times, temperatures and solvent systems (DMF, THF, DMF-THF, benzene) were used together with a 20-fold excess of the electrophile. However, at best the mono-substituted calix[8]arene 8 was obtained in a 10% yield (Table 1, entry 1), together with traces of disubstituted derivatives detectable by ESMS. The use of further equivalents of electrophile or base had no effect on the reaction outcome. Bearing in mind the ease with which the bromoalkanenitriles had been added, further O-alkylations were performed but using 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane.49 When carrying out the reaction with 1 in the presence of NaH using 30 equivalents of base and 50 equivalents of 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane, 9 was formed in 55% yield after purification by alumina chromatography (Scheme 1, Table 1, entry 2). The use of a milder base was also explored and when using KjCOj (16 equivalents) and only 8 equivalents of 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane, interestingly 9 was also formed, in 40% yield. Remarkably, for the reaction to proceed with NaH a large excess of both base and [p]ethyleneglycol chain were required and despite this no fully alkylated calixarene was generated. When using p-tert-octylcalix[8]arene 2 the use of NaH led to the formation of no O-alkylation products (Table 1, entry 4), but with a mild base the 1,3,5,7-substituted product 10 (Scheme 1, Table 1, entry 5) was readily isolated.

The structural characterisation of compounds 9 and 10 was carried out using mass spectrometry and NMR spectroscopy. Broadening of the signals was observed in the ¹H NMR spectra and the phenolic protons were never observed in this series of compounds. However, ¹³C NMR proved to be particularly powerful indicating the symmetrical nature of the partially alkylated compounds with two pairs of signals for the tert-butyl carbons (C(CH₃)₃) and C(CH₃)₂ at approximately 31 ppm and 34 ppm for 9 and with a pair of signals corresponding to C(CH₃)₃ at 38 ppm for 10. Two sets of signals for the aromatic carbons were observed in some cases (although for several compounds the signals were superimposed). MS analysis proved to be useful for the tert-butyl series, however, with larger groups at the upper rim such as tert-octyl molecular ions could not always be observed. The ability of these hydrophilic compounds to complex to several metal ions also led to complications when attempting to observe the molecular ions and in such cases the use of ¹³C NMR spectroscopy proved to be particularly effective. Compounds 9 and 10 possessed solubilities of 9–10 mg ml⁻¹ in water.

Interestingly, 9 and 10 were the products predominantly generated regardless of whether mild or strong bases were used, the amount of base and electrophile present and the reaction conditions. In general, the use of KjCOj was preferable to NaH since fewer equivalents of electrophile (and base) were required and the procedure was more reliable with different groups at the upper rim. The formation of these partially alkylated...
protected and bromo activated hexaethylene glycol monobrominated to give 17-bromo-3,6,9,12,15-pentaoxapentadecy prepared (Scheme 2). Accordingly, hexaethylene glycol was used in additions to calix[4]arenes. The partial low yielding additions observed when using strong bases suggests poor polyanion stability in the presence of the activated triethylene glycol chains, 12 was reacted with p-tert-butylcalix[4]arene 1 and NaH, THF, electrophile; iii, 10% conc. HCl in MeOH-CH₂Cl₂; iv, NaH, Et₃N, CH₂COCl, electrophile; i, HBr (48%); ii, TsOH, THP.

Scheme 1 Reagents and conditions: i, K₂CO₃, CH₃CN, electrophile; ii, NaH, THF, electrophile; iii, 10% conc. HCl in MeOH-CH₂Cl₂; iv, NaH, Et₃N, CH₂COCl. 

Table 1 Selective O-alkylations of p-alkylcalix[n]arenes

<table>
<thead>
<tr>
<th>Entry</th>
<th>Calixarene</th>
<th>Electrofile/equivalents</th>
<th>Base/equivalents</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1, R' = tert-Butyl</td>
<td>MeO(CH₂CH₂O)₄Me/20 eq.</td>
<td>NaH/10 eq.</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>1, R' = tert-Butyl</td>
<td>Br(CH₂CH₂O)₄Me/60 eq.</td>
<td>NaH/30 eq.</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>1, R' = tert-Butyl</td>
<td>Br(CH₂CH₂O)₄Me/8 eq.</td>
<td>K₂CO₃/16 eq.</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>2, R' = tert-Octyl</td>
<td>Br(CH₂CH₂O)₄Me/60 eq.</td>
<td>NaH/16 eq.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>2, R' = tert-Octyl</td>
<td>Br(CH₂CH₂O)₄Me/8 eq.</td>
<td>K₂CO₃/16 eq.</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>1, R' = tert-Butyl</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>NaH/16 eq.</td>
<td>mono- and di-alkylation</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>1, R' = tert-Butyl</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>K₂CO₃/16 eq.</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>8</td>
<td>2, R' = tert-Octyl</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>NaH/16 eq.</td>
<td>14</td>
<td>62</td>
</tr>
<tr>
<td>9</td>
<td>2, R' = tert-Octyl</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>K₂CO₃/16 eq.</td>
<td>14</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>3, R' = tert-Butyl</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>K₂CO₃/12 eq.</td>
<td>15</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>Br(CH₂CH₂O)₄Me/8 eq.</td>
<td>NaH/16 eq.</td>
<td>19</td>
<td>76</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>Br(CH₂CH₂O)₄Me/8 eq.</td>
<td>NaH/16 eq.</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>NaH/16 eq.</td>
<td>21</td>
<td>55</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>NaH/16 eq.</td>
<td>22</td>
<td>45</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>12, Br(CH₂CH₂O)₄THP/8 eq.</td>
<td>NaH/16 eq.</td>
<td>23</td>
<td>45</td>
</tr>
<tr>
<td>16</td>
<td>1, R' = tert-Butyl</td>
<td>27, I(CH₂CH₂O)₄THP/8 eq.</td>
<td>K₂CO₃/16 eq.</td>
<td>29</td>
<td>'25</td>
</tr>
<tr>
<td>17</td>
<td>28</td>
<td>27, I(CH₂CH₂O)₄THP/8 eq.</td>
<td>NaH/16 eq.</td>
<td>30</td>
<td>'27</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>CH₂COCl/20 eq.</td>
<td>NaH/20 eq.</td>
<td>31</td>
<td>54</td>
</tr>
<tr>
<td>19</td>
<td>13</td>
<td>CH₂COCl/10 eq.</td>
<td>NaH/20 eq.</td>
<td>32</td>
<td>54</td>
</tr>
</tbody>
</table>

iv. 16 to 31, v. 13 to 32

Some calix[4]arene products were observed to complex to triphenylphosphine, as observed by MS (M⁺ - t - PPh₃). Interestingly, the triphenylphosphine could not be detected in the starting material 12 or products by ¹³C NMR spectroscopy, but could be using ³¹P NMR spectroscopy. The contamination by triphenylphosphine was clearly undesirable, and therefore the use of this synthetic route was avoided.

Following the results from the additions of short PEG chains, 12 was reacted with p-tert-butylicalix[4]arene 1 and NaH (Table 1, entry 6), however, an inseparable mixture of mono- and di-alkylated products were formed in low yield, as detected.

heptadecan-1-ol 11 in 56% yield using Chong’s methodology for the preparation of bromoalkanols and 11 was purified using reverse phase chromatography. This was subsequently THP protected to give 17-tetrahydropyranyloxy-3,6,9,12,15-pentaoxapentadecyl bromide, 12.

The deprotection of hexaethylene glycol was initially carried out via THP monoprotection and this compound was then brominated using carbon tetrabromide and triphenylphosphine with subsequent purification. However, when 12, prepared via this route, was used in additions to calixarenes, some calix[8]arene products were observed to complex to triphenylphosphine, as observed by MS (M⁺ + PPh₃). Interestingly, the triphenylphosphine could not be detected in the starting material 12 or products by ¹³C NMR spectroscopy, but could be using ³¹P NMR spectroscopy. The contamination by triphenylphosphine was clearly undesirable, and therefore the use of this synthetic route was avoided.

Following the results from the additions of short PEG chains, 12 was reacted with p-tert-butylicalix[8]arene 1 and NaH (Table 1, entry 6), however, an inseparable mixture of mono- and di-alkylated products were formed in low yield, as detected.
by ESMS. When using 8 equivalents of 12 and K₂CO₃ as base, the C₅ symmetrical product, 1,3,5,7-tetra-O-substituted tert-butylcalix[8]arene 13 was exclusively formed in 65% yield, after purification by alumina chromatography. Interestingly the alkylation of 9 under strongly basic conditions led to the formation of 14 in 62% and 63% yield respectively, possibly due to co-solvent or conformational effects in the presence of the substituted hexaethyleneglycol. However, for the addition of 12 to 3, the use of a weak base was required to give the 1,3,5-tri-O-substituted tert-butylcalix[6]arene 15 (Table 1, entry 10). The THP groups were readily removed from compounds 13–15 to give the corresponding alcohols 16, 17 and 18 using 10% HCl in dichloromethane–methanol which had solubilities of 15–20 mg ml⁻¹ in water.

The structural characterisation of compounds 13–18 was carried out using MS (ES and MALDI-TOF) and NMR spectroscopy as outlined above. Again, ¹³C NMR spectroscopy indicated the symmetrical nature of the compounds, as shown in Fig. 1 for compounds 13 and the tert-octyl-substituted calixarenes, displaying only one signal for the tert-butyl CH₂ and quaternary carbons as shown in Fig. 1 for compound 21. Furthermore the λmax of the compounds were recorded and observed at approximately 270 nm and 278 nm in agreement with Comforth et al.’s observations.[5]

The general applicability of this methodology was explored further with the attachment of THP protected dodecylethylene glycol PEG chains (PEG12-THP). The synthesis of activated PEG12-THP was initially explored using several strategies, however, the most successful, concise route is shown in Scheme 3. Dimethylethylene glycol and mono-THP protected hexaethyleneglycol were prepared as previously reported.[10,21] These were then coupled under basic conditions to give PEG12-THP mesylate 26, in 50% yield after purification by reverse phase chromatography. To improve the efficiency of coupling to the calixarene, activation to give the iodo-derivative was carried out. Accordingly, 26 was reacted with sodium iodide in acetone. However, partial deprotection also occurred under these reaction conditions. The material was therefore directly reprotected to give PEG12-THP iodoide, 27 in 90% yield.

Compound 27 was then reacted with calixarene 1 and K₂CO₃ to give the tetrasubstituted symmetrically PEGylated calix[8]arene 28 (Scheme 1, Table 1, entry 16). Compound 28 was directly deprotected to give 29. Analysis by MS revealed the formation of multiply charged species and product degradation (for example, a fragment corresponding to M⁺ + 7 - CH₂O was observed). However, further confirmation of the synthesis of the 1,3,5,7-tetrasubstituted compound was provided by ¹³C NMR analysis. The second coupling between 28 and 27 was as before carried out under strongly basic conditions and the fully alkylated product generated was then directly deprotected to give 30 (with a solubility of 35 mg ml⁻¹ in water) in 27% yield over the two steps. Finally, ¹³C NMR confirmed the formation of the fully alkylated product with a single peak for each of the tert-butyl carbons and UV analysis revealed peaks at λmax 270 nm and 280 nm.

Having established a facile procedure for the synthesis of partially substituted symmetrical calixarenes, the second addition of tetraethylene glycol chains was explored to access fully alkyalted materials.[13] These partially substituted calixarenes exhibited a λmax at approximately 305 nm and 280 nm.

Reagents and conditions: i, NaH; ii, NaI, acetone, then TsOH, THP.

Fig. 1  tert-Butyl and tert-octyl regions of the ¹³C NMR spectra for compounds 13, 17, and 21.

Calix[8]arene 17. Compound 13 has two non-identical sets of C(CH₃)₃ groups, at approximately 31.5 ppm and 31.7 ppm and non-equivalent quaternary carbons at 33.8 ppm and 34.0 ppm, whilst the non-equivalent quaternary carbons adjacent to the aromatic ring in 17 can be observed at 37.5 ppm and 37.9 ppm. However, with the increasing mass and poor relaxation of these high molecular mass compounds not all of the quaternary aromatic carbons could be observed.

Corknforth et al. has previously reported a shift in the UV maxima from 300 nm for unsubstituted calixarenes to 270 nm and 280 nm for the fully alkylated materials.[15] These partially substituted calixarenes exhibited a λmax at approximately 305 nm and 280 nm.

Scheme 3  Reagents and conditions: i, NaH; ii, NaI, acetone, then TsOH, THP.

The selectivity observed in these addition reactions will enable symmetrical multifunctionalised calixarenes to be readily prepared possessing combined properties. To investigate the selectivity for the addition of activated carboxylic acid groups, tert-butyl-tetra(hexaethyleneglycol)tetrahydroxycalix[8]arene 16 was reacted with an excess of acetyl chloride and THP ether. The hydroxyl groups of 16 were acylated with acetyl chloride and triethylamine tert-butyl-tetra(hexaethyleneglycol) TEP ether triacetoxycalix[8]arene 32 was generated as the major product (33% yield). ¹³C NMR spectroscopy again confirmed the highly symmetrical nature of the calixarenes. This differential addition of further functionalities opens up numerous possibilities. ¹³C NMR spectroscopy again confirmed the highly symmetrical nature of the calixarenes. This differential addition of further functionalities opens up numerous possibilities.
opportunities for the synthesis of highly functionalised large ringed calixarenes.

In summary, we have identified versatile high yielding procedures for the mono-functional full and partial substitution of calix[6]arenes and also for the symmetrical bifunctionalisation of calix[6]arenes. For the addition of ethylene glycol chains the 2-step procedure developed utilised only 16 equivalents of the electrophile to generate the fully alkylated product. This methodology has the potential to significantly extend applications of calix[6]arenes with the introduction of different properties into one molecule, including solubilising groups, complexation or fluorescent moieties, to numerous applications including the design of sensors and the synthesis of biological probes.

Experimental

All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Unless otherwise indicated, reagents were obtained from commercial suppliers and were used without further purification. All solvents were dried over standard drying agents and freshly distilled prior to use. Reactions were monitored by TLC on Kieselgel 60 F254 plates with detection by UV, or permanganate and phosphomolybdic acid stains. Flash column chromatography was carried out using silica gel (particle size 40-63 μm) and a Bruker Reflex III MALDI-TOF mass spectrometer. The elemental analyses of calixarenes are very often uncorrected.

The procedure outlined above was used to give 4 as a white solid (0.642 g, 70%) (Found: C, 73.3; H, 7.33; N, 5.29; Br 4.4; C15H12N2O4KBr.H2O requires: C, 73.3; H, 7.33; N, 5.29; Br 4.1%). mp 225-230 °C; νmax(Nujol)/cm-1: 2960, 2247 (CN), 1583; δmax(300 MHz; CDC13) 1.07 (12H, s, C(CH3)3), 1.35 (16H, t, J 6.7 and 5.8 Hz, CH2CH2CN), 2.37 (16H, t, J 6.7 Hz, CH2CN), 3.61 (16H, t, J 5.8 Hz, OCH2), 3.98 (16H, s, CH2 bridge), 6.94 (16H, s, ArH); δmax(75 MHz; CDCl3) 13.9 (CH2CH2CN), 25.9 (CH2CN), 29.8 (CH2 bridge), 31.3 (C(CH3)3), 34.1 (C(CH3)3), 70.6 (OCH2), 119.5 (CN), 126.0 (CH), 132.1, 146.3, 152.5; m/z (HRFAB) 1856.170 (M + Na)+, C26H21N2O6Na requires 1856.163.

The procedure outlined above was used to give 5 as a cream solid (0.830 g, 72%) (Found: C, 80.0; H, 9.32; N, 5.13; C12H10N2O4 requires: C, 79.9; H, 9.54; N, 4.91%) mp 195-197 °C; νmax(Nujol)/cm-1: 2977, 2249 (CN), 1598, 1582; δmax(300 MHz; CDCl3) 0.71 (72H, s, C(CH3)3), 1.17 (48H, s, CH2-C(CH3)3), 1.52 (16H, s, CH2CH2CN), 1.84 (16H, m, CH2CH2-CN), 2.30 (16H, t, J 6.3 Hz, CH2CN), 3.60 (16H, t, J 6.6 Hz, OCH2), 3.95 (16H, s, CH2 bridge), 6.96 (16H, s, ArH); δmax(75 MHz; CDCl3) 13.9 (CH2CH2CN), 25.9 (CH2CN), 30.2, 31.7, 31.9 (C(CH3)3), 32.3, 38.2 (C(CH2)2), 56.7 (OCH2), 70.9 (OCH2), 119.7 (CN), 126.9 (CH), 132.6, 145.6, 152.5; m/z (HRFAB) 2304.660 (M + Na)+, C37H24N4O8Na requires 2304.664.

The elemental analyses of calixarenes are very often uncorrected and particularly 13C NMR analysis. The p-tert-butyl- and p-tert-octyl-substituted calix[8]arenes and p-tert-butylcalix[8]arene were prepared following literature procedures. X-ray crystallography was carried out using a Siemens SMART APEX diffractometer equipped with a CCD detector. The crystals were analysed using the TEXSAN and TEXSAN/SAINT softwares. The structures were solved by direct methods and refined using full-matrix least-squares techniques.

The elemental analyses of calixarenes are very often uncorrected and particularly 13C NMR analysis. The p-tert-butyl- and p-tert-octyl-substituted calix[8]arenes and p-tert-butylcalix[8]arene were prepared following literature procedures. X-ray crystallography was carried out using a Siemens SMART APEX diffractometer equipped with a CCD detector. The crystals were analysed using the TEXSAN and TEXSAN/SAINT softwares. The structures were solved by direct methods and refined using full-matrix least-squares techniques.
Monoalkylation of p-tert-butylcalix[8]arene with 3,6,9-trioxadecyl methanesulfonate

To p-tert-butylcalix[8]arene 1 (1.30 g, 1 mmol) was added THF-DMF (120 ml, 5:1) and the mixture was stirred at 50 °C until a clear solution was obtained. Sodium hydride (60% dispersion in mineral oil; 0.80 g, 20.0 mmol) was added and stirring was continued for 1 h. 3,6,9-Trioxadecyl methanesulfonate (4.85 g, 20.0 mmol) in THF-DMF (30 ml, 5:1) was then added dropwise at rt and the reaction mixture was heated for 2 d at 80 °C. The reaction was quenched with the addition of ice-cold water and extracted with chloroform (3 × 30 ml). The combined organic extracts were washed with saturated LiCl solution (30 ml), brine (30 ml) and water (30 ml), and the solvent evaporated affording a crude product. This was recrystallised using dichloromethane-diethyl ether to yield 5,11,17,23,29,35,41,47-octa-tert-butyl-49,51,53,55-tetrakis-(3,6,9-trioxadecyloxy)-50,52,54,56-tetrahydroxycalix[8]arene (5.11.17.23.29.35.41.47-Octa-tert-butyl-49,51,53,55-tetrakis-(3,6,9-trioxadecyloxy)-50,52,54,56-tetrahydroxycalix[8]arene) as an oil (477 mg, 41%).

The reaction was carried out as described above and purified using a neutral alumina column (gradient: ethyl acetate-methanol) to give 10 as an oil (747 mg, 31%).

Partial alkylation of p-tert-butylcalix[8]arene 1, p-tert-octylcalix[8]arene 2 and p-tert-butylcalix[8]arene 3 with bromohexaethylene glycol THF ether (12) (1 mmol per available OH) or 3,6,9-trioxa-decyl bromide to give 14 as a viscous oil (0.973 g, 62%).

To 17-bromo-3,6,9,12,15-pentaoxahapte-decyl bromide 12

To 17-bromo-3,6,9,12,15-pentaoxahapte-decan-1-ol (11) (0.42 g, 2.21 mmol) in CHCl3 (60 ml) was added toluene-p-sulfonic acid monohydrate (0.42 g, 2.21 mmol) and 3,4-dihydro-2H-pyran (2.05 g, 24.4 mmol). The reaction was stirred at rt for 2 d, then washed with water (20 ml) and dried (sodium sulfate). The solvent was removed in vacuo to give the title compound as a clear oil (7.72 g, 77%); \( \lambda_{max}^{film/cm} \) 2924, 2869, 1456; \( \delta_h (300 MHz; CDCl_3) \) 1.38–1.78 (6H, m, CH_3), 3.39 (2H, t, \( \delta_c (75 MHz; CDCl_3) \) 6.79–7.19 (16H, m, ArH); \( \delta_c (75 MHz; CDCl_3) \) 9.33 (4H, s, CHO), 70.2, 70.3, 70.9, 71.0, 71.6, 74.8, 125.3–128.0 (several signals), 133.5, 143.1–150.5 (several signals), \( \text{m/z} \ (ES) \ 1443.1 \) (M^+, \text{C}_{28}H_{24}O_{10}.O requires 1442.9).

The reaction was carried out as described above and purified using a neutral alumina column (gradient: ethyl acetate-methanol) to give 13 as a viscous oil (0.874 g, 65%) (Found: C, 66.2; H, 9.26. \text{C}_{36}H_{34}O_{10}.H_2O requires C, 66.1; H, 9.03%); \( \lambda_{max}^{film/cm} \) 2950, 2840, 2700; \( \delta_h (300 MHz; CDCl_3) \) 0.86–1.36 (72H, m, C(CH_3)_2), 3.16–4.03 (76H, m, OCH_2, OCH, CH_2 bridge), 6.79–7.20 (16H, m, ArH); \( \delta_c (75 MHz; CDCl_3) \) 30.0 (CH_2 bridge), 31.2 and 31.3 (2 × C(CH_3)_2), 33.8 and 34.0 (2 × C(CH_3)_2), 58.7 (OCH), 70.3 (signals superimposed), 71.7, 125.7 and 126.5 (2 × CH), 132.9, 146.1, 152.4 (m/z (ES) 1881.3). \( \text{M}^+ \) (C_{15}H_{24}O_{10}.HCl requires 1881.2).

To 17-bromo-3,6,9,12,15-pentaoxahapte-decan-1-ol (11) (1.76 g, 22.1 mmol) in CHCl_3 (60 ml) was added toluene-p-sulfonic acid monohydrate (0.42 g, 2.21 mmol) and 3,4-dihydro-2H-pyran (2.05 g, 24.4 mmol). The reaction was stirred at rt for 2 d, then washed with water (20 ml) and dried (sodium sulfate). The solvent was removed in vacuo to give the title compound as a clear oil (7.72 g, 77%); \( \lambda_{max}^{film/cm} \) 2924, 2869, 1456; \( \delta_h (300 MHz; CDCl_3) \) 1.38–1.78 (6H, m, CH_3), 3.39 (2H, t, \( \delta_c (75 MHz; CDCl_3) \) 6.79–7.19 (16H, m, ArH); \( \delta_c (75 MHz; CDCl_3) \) 9.33 (4H, s, CHO), 70.2, 70.3, 70.9, 71.0, 71.6, 74.8, 125.3–128.0 (several signals), 133.5, 143.1–150.5 (several signals), \( \text{m/z} \ (ES) \ 1443.1 \) (M^+, \text{C}_{28}H_{24}O_{10}.O requires 1442.9).

The reaction was carried out as described above and purified using a neutral alumina column (gradient: ethyl acetate-methanol) to give 13 as a viscous oil (0.874 g, 65%) (Found: C, 66.2; H, 9.26. \text{C}_{36}H_{34}O_{10}.H_2O requires C, 66.1; H, 9.03%); \( \lambda_{max}^{film/cm} \) 2950, 2840, 2700; \( \delta_h (300 MHz; CDCl_3) \) 0.86–1.36 (72H, m, C(CH_3)_2), 3.16–4.03 (76H, m, OCH_2, OCH, CH_2 bridge), 6.79–7.20 (16H, m, ArH); \( \delta_c (75 MHz; CDCl_3) \) 30.0 (CH_2 bridge), 31.2 and 31.3 (2 × C(CH_3)_2), 33.8 and 34.0 (2 × C(CH_3)_2), 58.7 (OCH), 70.3 (signals superimposed), 71.7, 125.7 and 126.5 (2 × CH), 132.9, 146.1, 152.4 (m/z (ES) 1881.3). \( \text{M}^+ \) (C_{15}H_{24}O_{10}.HCl requires 1881.2).
The reaction was carried out as described above and the product purified using a neutral alumina column (gradient: ethyl acetate–methanol) to give 15 as a viscous oil (0.676 g, 67%) \(\delta_{13}^{(300 \text{ MHz; CDCl}_3)} 1.13-1.35 \text{ (54H, m, CH}_2\text{OH), 1.44-1.89 \text{ (18H, m), 3.45-4.03 \text{ (90H, m, CH}_2\text{O and CH}_2\text{ bridge), 4.61} \text{ (3H, m, CHO), 6.70-7.24 \text{ (12H, m, ArH),}}\)

\(\delta_{13}^{(75 \text{ MHz; CDCl}_3)} 19.2, 25.3, 30.3-31.5 \text{ (signals superimposed), 33.6 and 34.0} \text{ (2 \times C(CH}_3)_2\text{), 62.0, 66.4, 69.8-70.4} \text{ (signals superimposed), 72.3, 98.7 \text{ (CHO), 125.3 and 126.3 (2 \times CH), 132.9, 142.3 and 146.6, 149.2 and 151.2; m/z (ES) 2017.1 (M^+ \text{, C}_{111}\text{H}_{220}\text{O}_{27}) requires 2017.3).}\


The calixarenes 13, 14 or 15 were stirred in dichloromethane–methanol (50 : 10 ml) containing 10% conc. HCl for 3 h at rt. Sodium hydrogencarbonate was added to neutralise the solution and the solvent removed in vacuo. The product was suspended in ethyl acetate and the inorganic salts removed by filtration. The ethyl acetate was then removed in vacuo to reveal the deprotected calixarenes.

5.11,17,23,29,35,41,47-Octa-tert-octyl-calix[49,50,51,52,53,54,55,56-octakis(3,6,9-trioxadecyloxy)calix[8]]arene 20

The reaction was carried out as described above using 10 (160 mg, 0.085 mmol) to give 20 as an oil (30 mmg, 47%): \(m/z_{\text{ES}} (\text{MALDI-TOF}) 3935.1 ([\text{MNa}^{+} - 3\text{THP}]^+ \text{, C}_{118}\text{H}_{236}\text{O}_{27}\text{Na} requires 3935.4).\)


The reaction was carried out as described above using 13 (187 mg, 0.070 mmol) to give 21 as an oil (150 mg, 55%): \(m/z_{\text{ES}} (\text{MALDI-TOF}) 4362.0 ([\text{MNa}^{+} - 2\text{THP}]^+ \text{, C}_{117}\text{H}_{235}\text{O}_{27}\text{Na} requires 4361.9).\)
To 26 (4.00 g, 5.65 mmol) in acetone (40 ml) was added sodium methoxide (4.74 g, 76.8 mmol) in 30 ml of methanol and the reaction was heated at reflux for 4 d. After filtration the filtrate was concentrated in vacuo, redissolved in dichloromethane and any remaining salt was removed by filtration. The removal of solvent in vacuo and NMR analysis revealed some loss of the THP protecting group. This material was therefore directly reprotected.

To the intermediate (3.80 g, approx. 5.1 mmol) in dichloromethane (50 ml) was added 3,4-dihydro-2H-pyran (0.428 g, 5.1 mmol) and toluenesulfonic acid (104 mg, 0.51 mmol) and the reaction was stirred for 18 h. The mixture was washed with water (2 ml), the organic phase dried (sodium sulfate) and evaporated in vacuo to give 27 as a viscous oil (3.70 g, 90%) which was used immediately.

*ν*<sub>max</sub>(film)/cm<sup>-1</sup> 2928s, 1480s, 1351s; <sup>1</sup>δ<sub>1H</sub> (300 MHz; CDCl<sub>3</sub>) 1.41–1.83 (1H, m, CH<sub>2</sub>-THP), 2.60 (4H, t, J 3.9 Hz, CH<sub>2</sub>-THP), 2.87 (6H, s, OH), 4.61 (4H, dd, J 3.9 and 2.9 Hz, CHO), 6.74–7.10 (16H, m, ArH), <sup>13</sup>δ<sub>1H</sub> (75 MHz; CDCl<sub>3</sub>) 29.7 (CH<sub>2</sub> bridge), 31.3 and 31.6 (2 × CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>), 33.9 and 34.1 (2 × C(CH<sub>3</sub>)), 61.8, 70.3–70.7 (signals superimposed), 72.6, 125.5 (CH), 132.9, 145.7; *m/z* (+ES) 3368.158 ([M - C<sub>3</sub>H<sub>4</sub>]+, C<sub>41</sub>H<sub>28</sub>O<sub>2</sub>Na requires 3568.083).


Compound 28 (325 mg, 0.09 mmol) was deprotected as described above and purified using alumina chromatography (gradient: ethyl acetate–methanol). After initial NMR analysis (see below) to confirm the selectivity, the material was directly deprotected: <sup>1</sup>δ<sub>1H</sub> (300 MHz; CDCl<sub>3</sub>) 0.95–1.28 (12H, m, C(CH<sub>3</sub>)), 1.47–1.79 (24H, m, CH<sub>2</sub>-THP), 3.33–4.06 (216H, m, CH<sub>2</sub>O and CH<sub>2</sub>-bridge), 4.61 (4H, dd, J 3.9 and 2.9 Hz, CHO), 6.74–7.10 (16H, m, ArH), <sup>13</sup>δ<sub>1H</sub> (75 MHz; CDCl<sub>3</sub>) 29.7 (CH<sub>2</sub> bridge), 31.3 and 31.6 (2 × CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>), 33.9 and 34.1 (2 × C(CH<sub>3</sub>)), 61.8, 70.3–70.7 (signals superimposed), 72.6, 125.5 (CH), 132.9, 145.7; *m/z* (+ES) 3368.158 ([M - C<sub>3</sub>H<sub>4</sub>]+, C<sub>41</sub>H<sub>28</sub>O<sub>2</sub>Na requires 3568.083).

The full alkylation reaction was carried out as described above using 28, and the material was directly deprotected by alumina chromatography (gradient: ethyl acetate–methanol) to give 29 as an oil (165 mg, 55%). <sup>1</sup>δ<sub>1H</sub> (300 MHz; CDCl<sub>3</sub>) 0.95–1.28 (12H, m, C(CH<sub>3</sub>)), 1.47–1.79 (24H, m, CH<sub>2</sub>-THP), 3.33–4.06 (216H, m, CH<sub>2</sub>O and CH<sub>2</sub>-bridge), 4.61 (4H, dd, J 3.9 and 2.9 Hz, CHO), 6.74–7.10 (16H, m, ArH), <sup>13</sup>δ<sub>1H</sub> (75 MHz; CDCl<sub>3</sub>) 29.7 (CH<sub>2</sub> bridge), 31.3 and 31.6 (2 × CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>), 33.9 and 34.1 (2 × C(CH<sub>3</sub>)), 61.8, 70.3–70.7 (signals superimposed), 72.6, 125.5 (CH), 132.9, 145.7; *m/z* (+ES) 3368.158 ([M - C<sub>3</sub>H<sub>4</sub>]+, C<sub>41</sub>H<sub>28</sub>O<sub>2</sub>Na requires 3568.083).
using a neutral alumina column (gradient: ethyl acetate-

References

13 D. Kraft, R. Amecke, V. Bohmer and W. Vogt,

J. Org. Chem., 1992, 57, 2611; K. Iwamoto, K. Araki and


C. Geraci, A. Bottino, M. Piattelli, E. Gavuzzo and P. Neri,

J. Chem. Soc, Perkin Trans. 2, 2000, 185; J. Li, Y. Chen and X. Lu,

Chem., 1993, 380; A. Casnati, P. Minari, A. Pochini and R. Ungaro,


P. Neri, E. Baticcolo, F. Cusolo, C. Geraci and M. Piattelli,

J. Org. Chem., 1994, 59, 3880; P. Neri, C. Geraci and M. Piattelli, 


P. Neri, G. M. L. Consoli, F. Cusolo, C. Rocco and M. Piattelli,


D. Krafit, R. Arce, V. Böhmer and W. Vogt, Tetrahedron, 1993, 49, 6019; C. Geraci, A. Bottino, M. Piattelli, E. Gavuzzo and P. Neri,

J. Chem. Soc, Perkin Trans 2, 2000, 185; J. Li, Y. Chen and X. Lu,


V. Bocchi, D. Fona, A. Pochini, R. Ungaro and G. D. Andreotti, 


J. W. Cornforth, E. D. Morgan, K. T. Potts and R. J. W. Rees,


