

**Synthesis and Transport Studies of Artificial Pore-Formers**

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**DOCTOR OF PHILOSOPHY**

in the Department of Chemistry

We accept this thesis as conforming  
to the required standard

*v* / *V* / *24*

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## ABSTRACT

The synthesis and characterization of simple mimics of pore forming antibiotics such as amphotericin B were explored. A sub-unit approach to the synthesis was employed which allowed for construction of a set of candidate structures. The targets are assembled by joining two "wall" units via a "linkage" unit with subsequent addition of polar head groups to either end of the structure. The wall units are macrocyclic diene tetraesters derived from maleic anhydride prepared by either acid catalyzed ester formation from diols or carboxylate substitution of dihalides (compounds 14, 15, 22, 23, 24, 30, 31, 34). Either set of reaction conditions limit the range of functionality possible in the starting diol or dihalide. Macrocycles 22, 23, and 24 were linked with m-xylylene dithiol via a 2:1 Michael addition reaction to give bis-macrocyclic alkene precursors. Alternatively, macrocycles 22 and 23 reacted with 3-thio-1-propanol and the mono-alcohol products were converted to iodides which were linked with 2R,3R-(+)-tartaric acid. Three types of polar head groups - neutral (1-thio- $\beta$ -D-glucose and 3-thio-1-propanol), cationic (2-aminoethanethiol), and anionic (2-thioacetic acid) - were added to the bis-macrocyclic alkene precursors via Michael addition reactions. A total of fourteen candidate structures were prepared for transport evaluation.

The activity of the fourteen mimics synthesized were determined by the pH-stat technique in which the transport of alkali metal cations across large

unilamellar lipid bilayer vesicles were monitored by the collapse of a proton gradient. All the active compounds showed a zero order decay in proton gradient. Of the fourteen mimics surveyed, three had activities comparable to amphotericin B (compounds 51, 52, and 59). The other eleven compounds were not sufficiently active for further characterization. The "add back" experiments, the kinetic orders, and the alkali metal ion selectivity studies are consistent with the proposal that the mimics behave as pore formers.

Examiners:

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DEDICATION

*To my parents*

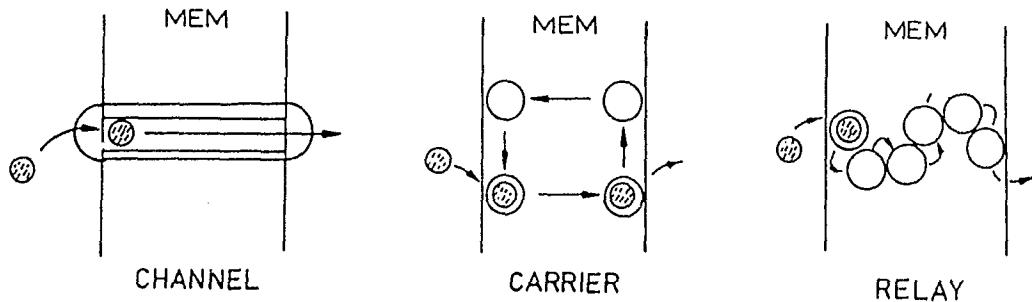
*with love*

## INTRODUCTION

### Background

Biological membranes<sup>1</sup> are highly organized, dynamic structures which surround cells and their various compartments, separating the contents from the outside environment. Natural membranes are noncovalent, fluid assemblies made up of mainly lipids and proteins. The basic structure of membranes consists of a layer of hydrocarbon (hydrophobic) topped with polar heads (hydrophilic) held together by noncovalent interactions. Membranes serve as a barrier and regulate the ionic and molecular composition of cells. In nature, membrane transport is regulated by membrane proteins which provide a path for transport of essential solutes.

There are three basic mechanisms for transport<sup>2</sup> (scheme 1). Channels or pores are tubes with ionophilic lumen which span bilayer membranes and are sufficiently hydrophilic to allow the diffusion of polar substrates across the cell membrane. Carriers form a complex with a substrate, shuttle across the membrane by diffusion, and release the substrate. In a relay mechanism, the substrate is transported by a series of hops between closely spaced substrate binding sites within the membrane. In general, natural protein transporters utilize channels or pores. The details of the mode of action



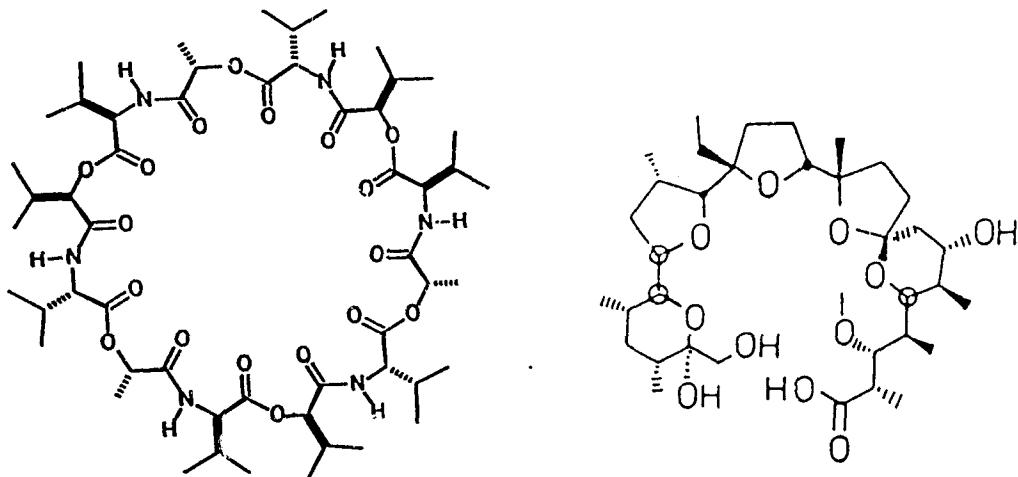
**Scheme 1.** Schematic mechanisms for transport.

of channels or pores are poorly understood at the molecular level, mainly due to lack of structural information from natural transporters.

Biomimetic studies of ion transport attempt to mimic the structure and function of natural transport systems in order to understand the fundamental principles of their behavior. Generally, the mimics are either from modified natural sources, or completely synthetic ones. In designing a biomimetic model, it is not possible to mimic the ion-transport proteins since the structures are too large (and in many cases not fully known) to be accessible by conventional synthesis. However, relatively simple antibiotics<sup>3-6</sup> are known to induce ion transport in biological and model membranes and it is possible to design synthetic model systems based on their structures. In consequence, most research in this area has focussed on these relatively simple antibiotics. These

antibiotics are of two kinds: Carrier ionophores or channels and pores.

Ionophores<sup>2</sup> or ion carriers may be either neutral carriers, such as valinomycin, or weak acid carriers, such as monensin A (scheme 2). Valinomycin is a cyclic dodecadepsipeptide in which the hydrophobic backbone points outward and the hydrophilic peptide oxygens point inward allowing it to interact with a cation in the centre of the ring. Monensin A forms a closed structure by head-to-tail hydrogen bonding and complexes cations using the oxygens which point inward to the ring. Synthetic analogues<sup>7-15</sup> of ionophores, crown ethers and cryptands, have been studied in detail and their fundamental properties have been established.



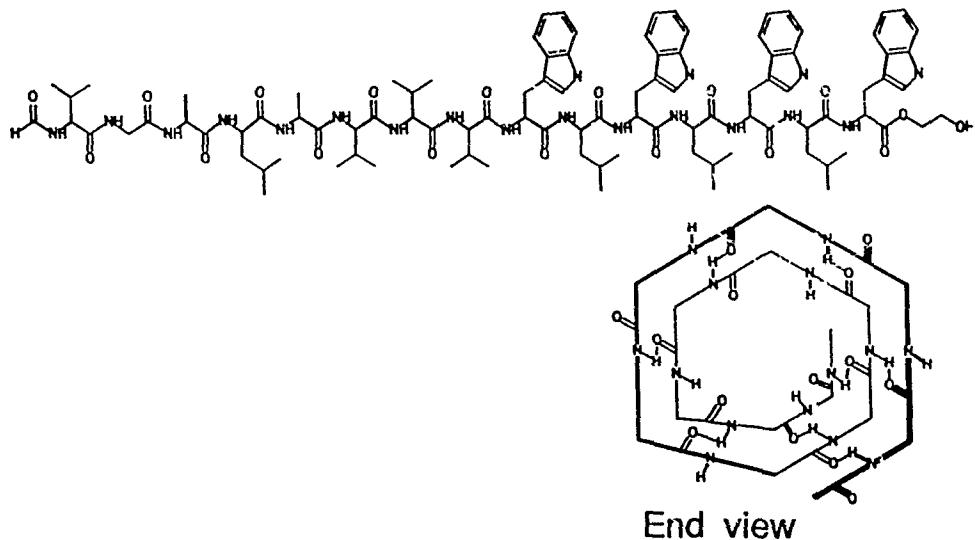
*Valinomycin*

*Monensin A*

Scheme 2. Structures of valinomycin and monensin.

The second kind of ion transporting antibiotics are channels or pore formers. The terminologies "channels" and "pores" are used interchangably in the literature, but for the purposes of this thesis it is important to distinguish the two. It will be useful to define a "channel" as a single molecule with a defined ion selective structure. In contrast, a "pore" is defined as an assembly of many molecules that forms defects and other nonspecific structures. By these definitions, the dimer of gramicidin A is a channel and amphotericin B a pore former.

Probably one of the most studied channel forming antibiotic is gramicidin A<sup>16-18</sup> (scheme 3). It contains fifteen alternative D- and L-amino acids arranged in a left-handed  $\beta$ -helix in non-



**Scheme 3.** The structure and schematic organization of gramicidine.

polar solvents. It has a length of 25 ~ 30 Å and a diameter of 4 Å. It forms an end-to-end dimer, joined by six intramolecular hydrogen bonds, which can span a bilayer membrane, where the hydrophobic amino acid residues face outward to the lipid of the membrane and the peptide carbonyl oxygens line the centre of the helix. Ion transport is believed to occur down the axis of the  $\beta$ -helix. Single channel unit conductance measurements in Black Lipid Membranes (BLM's) have shown that the transport occurs by a channel mechanism.

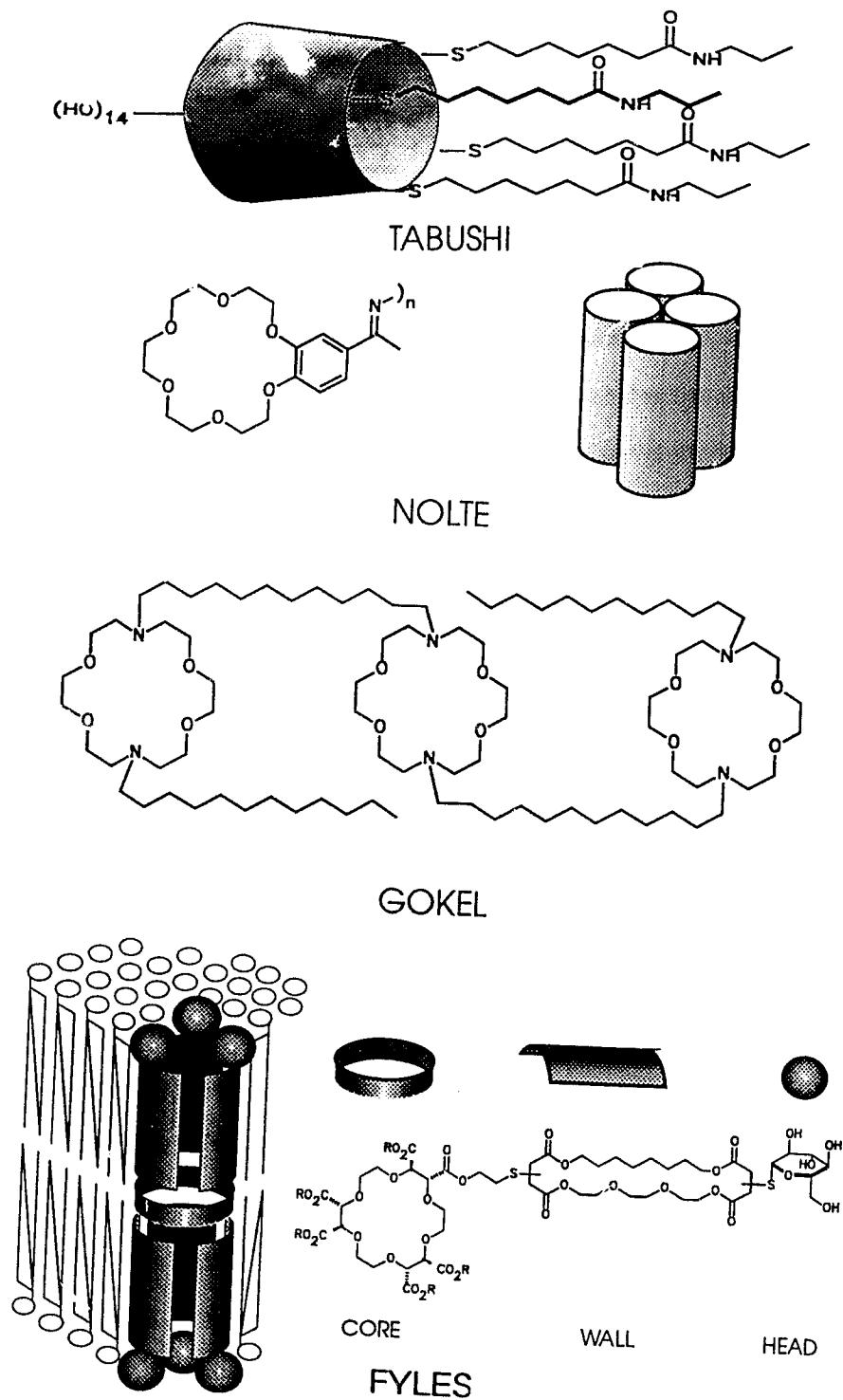
Peptide derived gramicidin mimics have been reported<sup>19-20</sup>. However, they generally tend to form large aggregates and have reduced selectivity compared to the gramicidin channel<sup>16-18,21</sup> which has a smaller and better defined channel structure. Rationally designed functional synthetic channels have been reported (Scheme 4).

Tabushi<sup>22</sup> reported an artificial channel forming compound based on a cyclodextrin framework with four hydrophobic tails and three potential metal ion binding sites. This "half channel" is proposed to form an end-to-end dimer, similar to gramicidin A, which spans a bilayer membrane made up of egg lecithin, incorporating Tiron (a UV active metal complexing dye) in its interior. The channel binds copper II and cobalt II ions in organic solvents. It also mediates cobalt II ion transport across vesicle bilayers with second order kinetics with respect to "the concentration" of the channel, and copper

II ion at a slower rate with first order kinetics. The transport of metal ions is proposed to occur by rapid metal ion "jumping" between the binding sites.

Nolte<sup>23-26</sup> has reported an artificial ion channel based on a polymer of isocyanide which contains benzo-18-crown-6 side chains. These polymers are rigid and form a tight helix with four repeating units per turn. As a consequence, the crown ether rings are stacked on top of one another and form four channels parallel to the polymer axis. This mimic exhibits both a hydrophilic interior with a large number of ion binding sites and a hydrophobic exterior, and is long enough (approximately 40 Å) to span a bilayer membrane. The channel was shown to enhance the cobalt II ion permeability of membranes of dihexadecyl phosphate (DHP) vesicles (monitored by internal UV absorptions of the dye 4-(2-pyridylazo)resorcinol monosodium salt) and was shown to have an Arrhenius activation energy of 24 kJ mole<sup>-1</sup> compared to 20.5 to 22.5 kJ mole<sup>-1</sup> for gramicidin. Nolte concluded that the mimic was a channel since a carrier transport mechanism has higher activation energy of 90 to 120 kJ mole<sup>-1</sup>. The transport in this system is independent of the fluid state of the bilayer and is consistent with a channel constructed of several relay type binding sites.

Gokel<sup>27</sup> reported a simple mimic believed to have a "channel-like" structure. It is a flexible tris-(macrocyclic) system, held together by two spacers, and with two side arms.



**Scheme 4.** Synthetic ion channels.

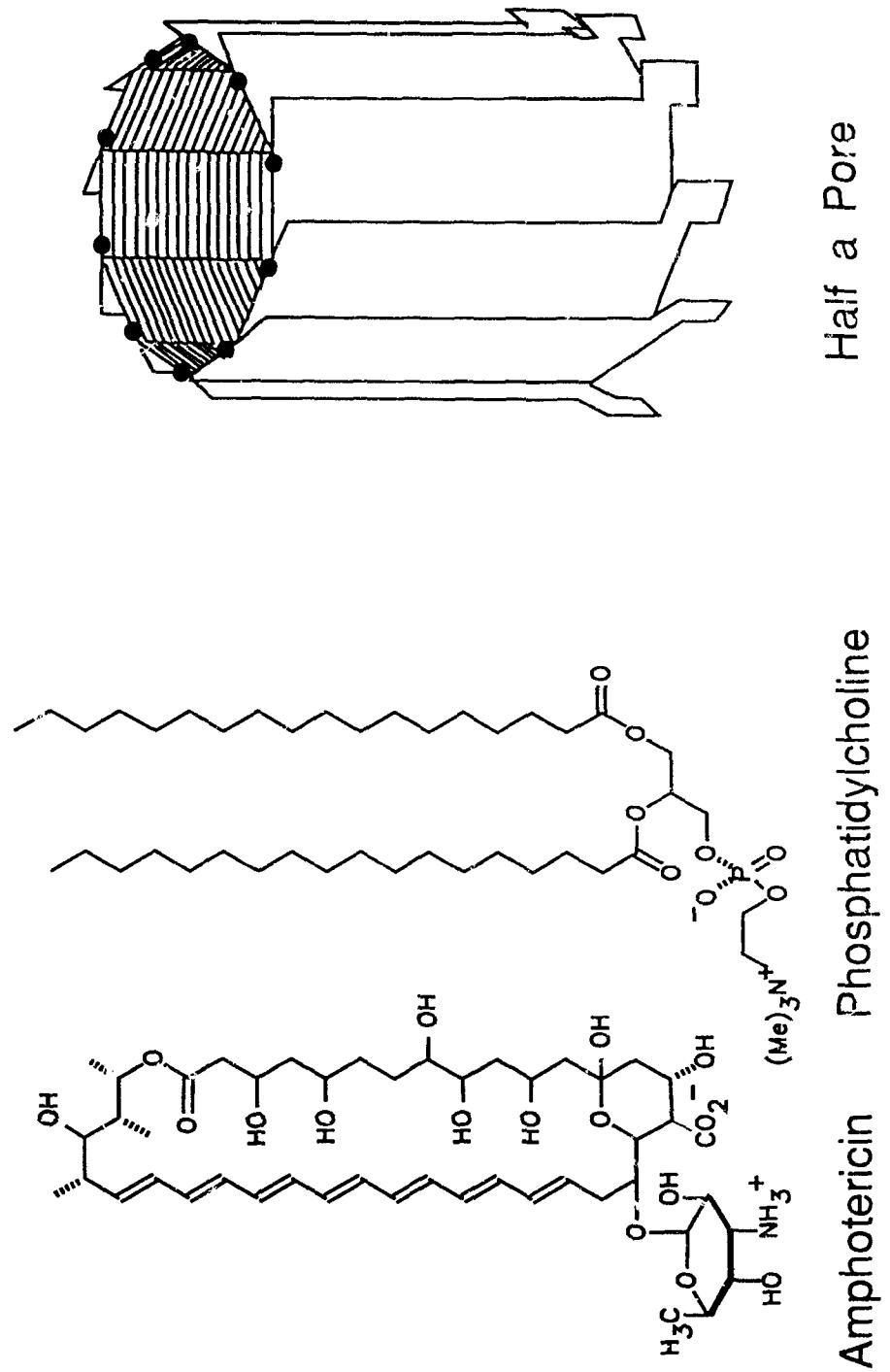
It was proposed that two of the macro rings are positioned at each end of the membrane surfaces providing donor relays and the third macro ring an internal relay point. The mimic was shown to enhance the sodium ion transport in phosphatidylcholine vesicles which was monitored by dynamic  $^{13}\text{Na}^+$  NMR spectroscopy. The sodium ion transport rate for the channel was compared to a diaza-crown ether carrier (which was simply the central unit with alkyl arms) and gramicidin. The channel transports sodium ions 40 times faster than the carrier, but 100 times slower than gramicidin. Gokel suggested that the mimic does not transport sodium ion via a carrier mechanism, since the mimic had first-order kinetics and the carrier second order kinetics.

Fyles<sup>28-29</sup> reported an innovative approach to unimolecular ion channels based on a crown ether hexaacid. The crown ether is derived from tartaric acid and is oriented such that the carboxylate derived groups are in axial positions. As a consequence the macrocyclic tetraester arms are projected above and below the crown ether. This mimic is long enough (~40 Å) to span a bilayer vesicle with the polar heads pointing at each end of the bilayer and the crown ether positioned well into the bilayer midplane. A series of similar ion channel analogues have been synthesized by James<sup>30</sup>. One of these mimics was thoroughly studied by Kaye<sup>31</sup> as part of her Master's thesis project. This mimic has six attached side-discriminated macrocyclic tetraesters with one of the

macrocyclic arms having a polyether function, and six glucose polar head groups. The James/Kaye mimic was shown to transport alkali metals (monitored by pH-stat technique) across lipid bilayer vesicles. Its transport behaviour was compared to gramicidin D and valinomycin in the same experimental system. Experimental observations (transporter and metal ion concentration dependence, activation energy, etc.) showed that the mimic was quite dissimilar to valinomycin but behaved similarly to gramicidin D. It was concluded that the mimic was an ion channel and not a carrier.

The mimics above are "channels". An example of a natural pore is the polyene antibiotic amphotericin B<sup>32-34</sup> which forms aqueous pores in bilayer membranes by aggregation of 10 to 15 molecules in each half of the bilayer (scheme 5). In this aggregate pore the polyene edge is hydrophobic and interacts with lipid and other polyene edges, and the hydroxyl edge interacts with water and other polyhydroxyl edges. Cholesterol or other sterols are also required for stable pore formation and are believed to interact with the hydrophobic edge of the pore. The pores formed are structurally much "looser" than the gramicidin channel and have a distribution of sizes and activities. Due to their "loose" structure, the pores are inherently less selective transporters than gramicidin which has a rigid, defined structure.

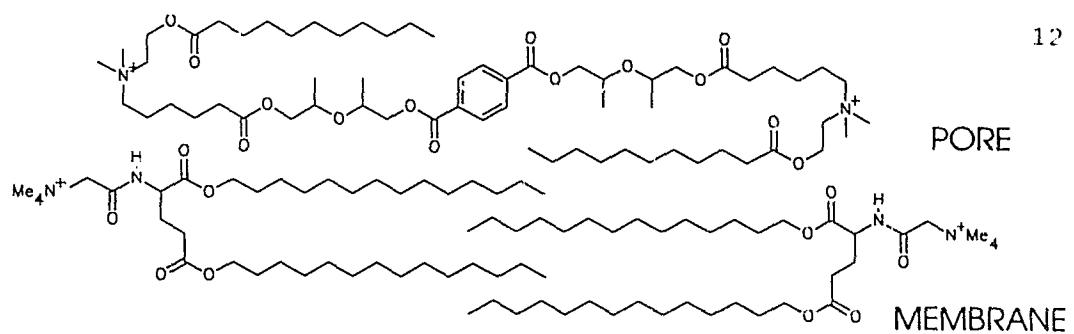
Synthetic pore formers have also been reported (scheme 6). Kunitaki<sup>35</sup> reported two amphotericin mimics. These are



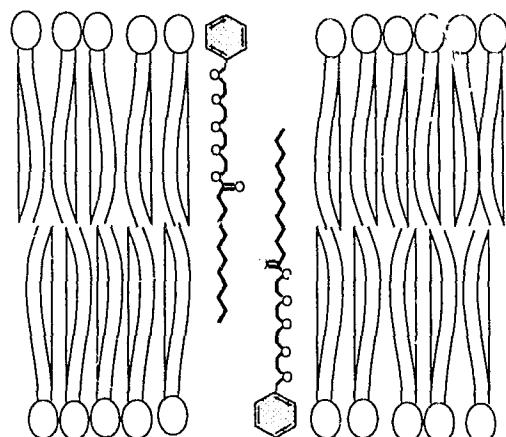
**Scheme 5.** The structure and schematic organization of amphotericin.

double-chain ammonium salts with a hydrophobic (hydrocarbon or fluorocarbon) chain and a hydrophilic chain made up of ether/ester linkages. These mimics were shown to transport hydroxide ions through a synthetic bilayer vesicle of similar dimensions, formed from a glutamate diester with entrapped riboflavin (a H dependent fluorescent dye); the transport was monitored by fluorescence quenching of the trapped riboflavin. The mimic with the fluorocarbon side chain was shown to be less effective than the one with the hydrocarbon side chain. In this system, the transport ability is due to cluster formation or phase separation; the mimics form aqueous defects or other nonspecific structures in the bulk hydrophobic membrane which allow the transport of ions across the bilayer membranes. In this system, the transport ability is clearly a function of membrane fluidity.

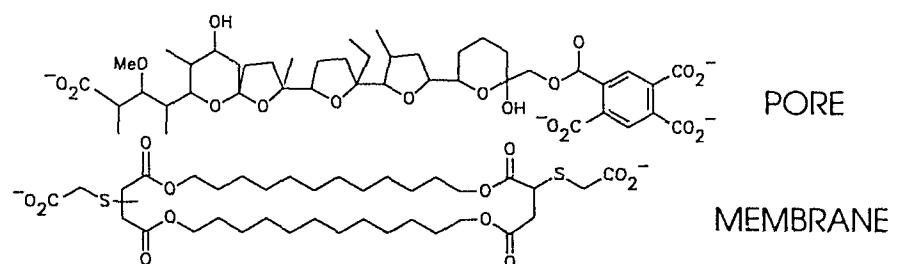
Menger<sup>36-37</sup> reported a series of simple compounds with a general structure RO(CH<sub>2</sub>CH<sub>2</sub>O)<sub>n</sub>R', that increase ion movement across a distearoylphosphatidylcholine (DSPC) bilayer membranes. These compounds were precursors to a target molecule which was found to be inactive. The most active of the series was found to be: R = dodecanoyl, n = 5, and R' = benzyl. Since the polyether part of the molecule is long enough to span only half the bilayer, it was suggested that a minimum of two molecules was required to span a bilayer membrane and to allow the passage of ions. In this arrangement, the benzyl group is associated with the DSPC



KUNITAKE



MENGER



FUHRHOP

Scheme 6. Synthetic pore formers.

quaternary nitrogen by an ion-dipole attraction and the hydrocarbon tail is embedded in the lipophilic region of the membrane. This molecule was shown to increase the potassium ion permeability across the DSPC membrane faster than 18-crown-6 and gramicidin D. Interestingly, in a U-tube experiment, the 18-crown-6 transported potassium ions through chloroform but the mimic was totally inactive.

Fuhrhop<sup>38-41</sup> reported an amphotericin mimic from a modified natural molecule. This mimic, the pyromellitate ester of monensin, has negative charges at both ends which when stretched is approximately 20 Å in length. The membrane used in this system is a fluid monolayer vesicle of approximately 20Å thickness. The small thickness of the membrane thus reduces the size requirements of the mimic. The membranes are readily prepared from macrocyclic tetraester derivatives with two polar head groups. These double headed molecules are known as bolaamphiphiles. The mimic readily forms pores inside the monolayer membrane and facilitates lithium ion transport, assessed by a gel-permeation chromatography experiment. The transport can be partly blocked by bis-quaternary ammonium salts of approximately the same length, leading towards the development of a switching mechanism for artificial pore formers.

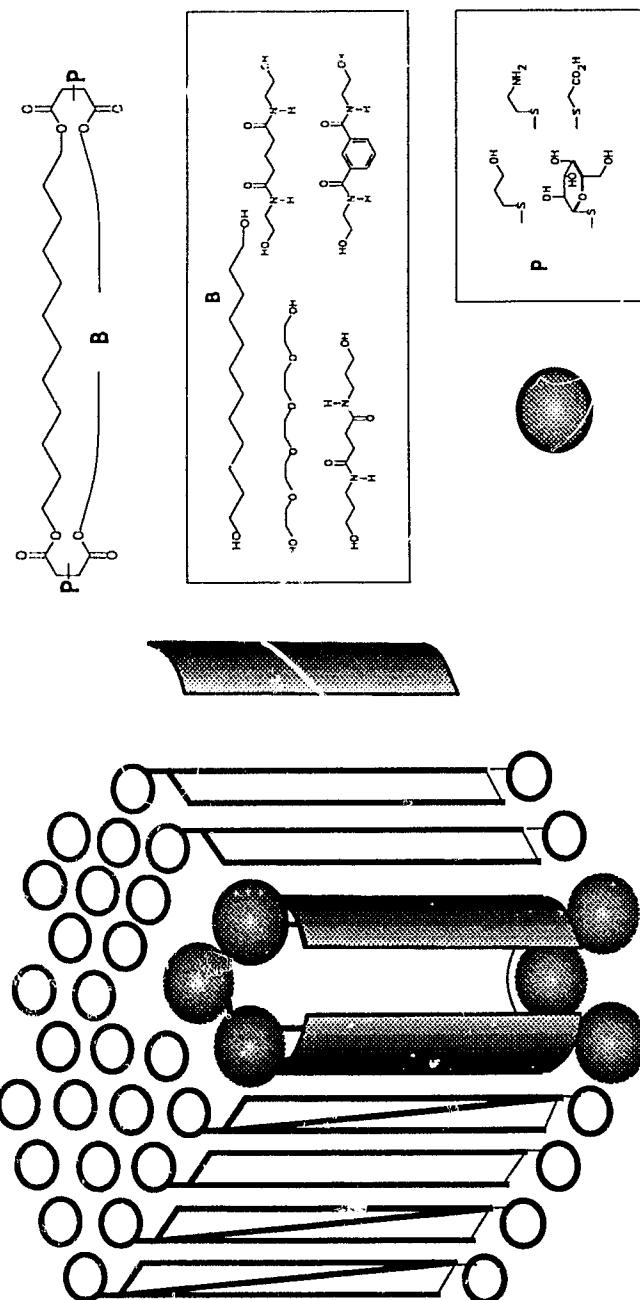
#### Design

The most notable difficulty facing the construction of

artificial pores or channels is synthesis. Large molecules with molecular weights of a few thousand are required to span a bilayer membrane of 35 to 40 Å thickness. This is possible: synthetic ion channels of 3000 to 5000 in molecular weight have been made in our labs<sup>28-30</sup>. The synthesis is quite ambitious and the preparation of structural variations needed for structure-activity studies is laborious and time consuming.

An alternative to this problem is the construction of structures of modest molecular weights which could self-aggregate and form pores in membranes similar to amphotericin B. Extrapolation from the structure of amphotericin suggests that the mimic should possess the following structural features: 1) The overall shape and size of the mimic should be compatible with the membrane forming amphiphiles utilized. This is to permit mixing and diffusion with the membrane. 2) The mimic should possess both hydrophobic and hydrophilic edges or faces. 3) The polar edge or face should permit self-hydrogen bonding to permit an aggregate to maintain itself. 4) The mimic should be sufficiently rigid to compel the hydrophilic edge or face to be held within the non-polar membrane core, roughly normal to the plane of the membrane.

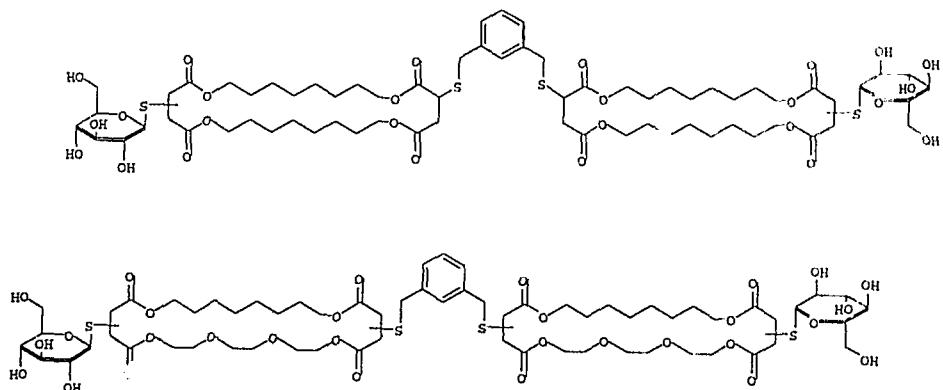
A simple approach to this design criteria focuses on Fuhrhop's bolaamphiphiles. Substitution of one of the polymethylene arms with a more polar arm will begin to satisfy the criteria outlined above (scheme 7). The polar arm might



**Scheme 7.** The pore forming bolaamphiphile design for Fuhrhop's monolayer membranes (12-series).

be as simple as a polyether or might involve hydrogen bonding amide groups.

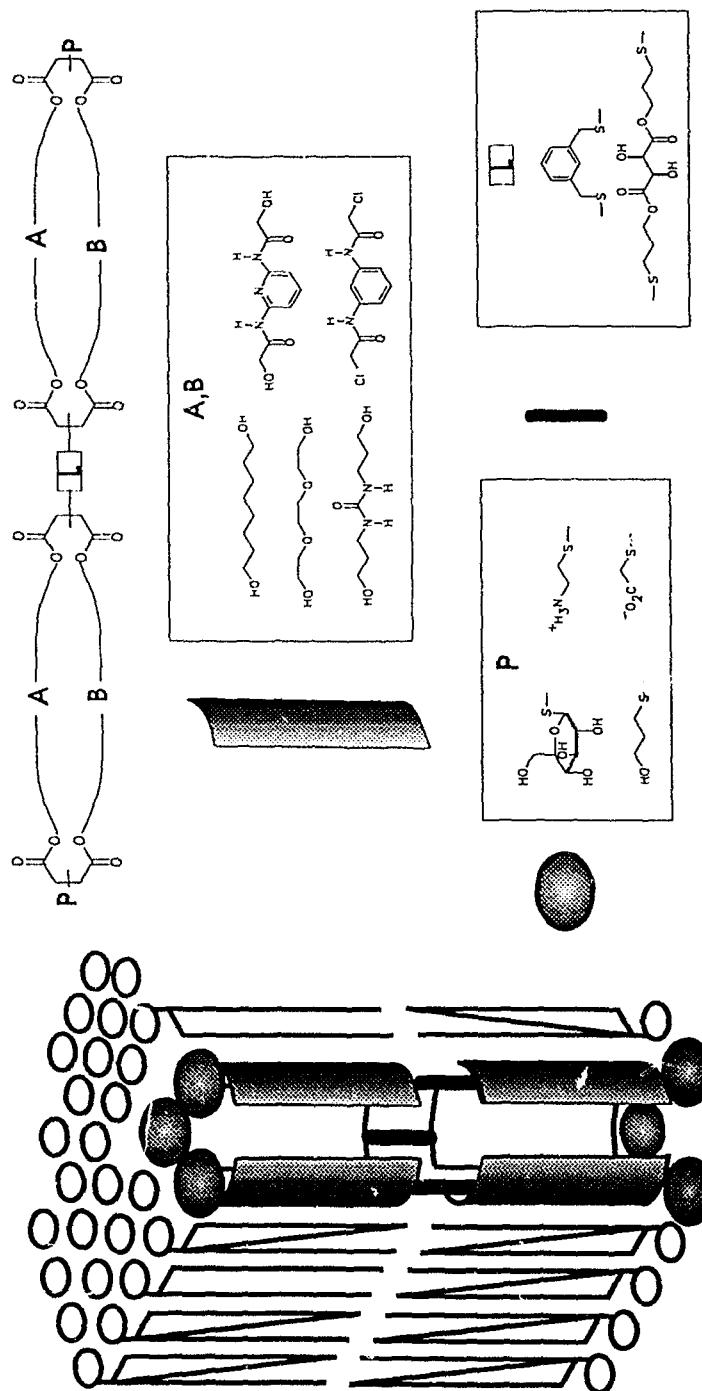
Extension of this system to bilayer membranes of about 40 Å length has been reported by Fyles<sup>42</sup> (scheme 8). These structures are about 40 Å in length and were shown to facilitate the transport of alkali metal ion across bilayer membranes.



**Scheme 8.** Fyles' synthetic pore formers.

The design of the bolaamphiphile pores and their longer analogues require functional groups that aid aggregation and pore formation. These can be achieved by synthesis of side-discriminated macrocyclic tetraesters capable of hydrogen bonding. The hydrogen bonding function can be incorporated into the wall, the linker, or the polar head group. Candidate structures for each of these subunits are proposed in Scheme 9.

Since our initial goal was to survey the general strategy



**Scheme 9.** The pore forming bolaamphiphile design for bilayer membranes (8-series).

of pore formation, the targets were chosen to simplify the synthetic task. The linkers and polar heads are all commercially available and inexpensive. The polar arms incorporating amide linkages may be synthesized quite easily from readily available, inexpensive starting materials. The entire synthesis thus is reduced to two kinds of reactions. Esterification and Michael addition. Ample literature precedence for these two types of reactions is available<sup>43-49</sup> and the synthesis was expected to proceed rapidly.

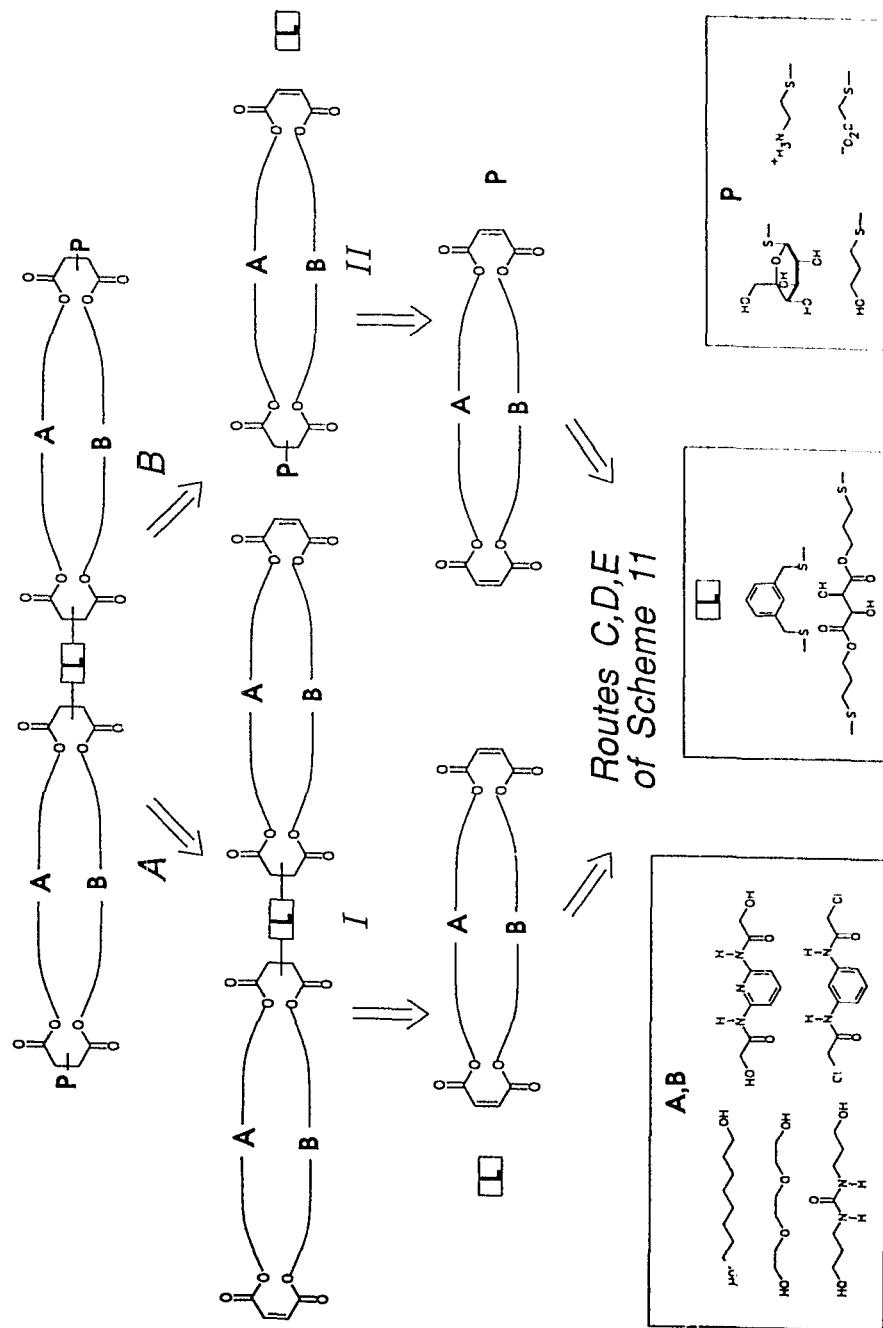
The aim of this project was: 1) to explore the synthesis of side-discriminated macrocycles with the goal of making self-aggregating pores, and 2) to examine the activity of these pores to see if the strategy for the pore formers is productive.

## SYNTHESIS

### Retrosynthesis

The retrosynthesis of the proposed pore formers is shown in scheme 10 (the 8-series). In principle, the target compounds may be made by two different routes: Route A was chosen in this project. The advantage of route A is the common intermediate I, from which a variety of pore formers varying in the head group can be formed. The disadvantage of this route is the possibility of competing polymerization during the linkage reaction with *m*-xylylene dithiol. The most notable disadvantage of route B is the intermediate II. The preparation and purification of this intermediate would need to be solved individually for all the possible variations of the polar head groups and the macrocycles. The purification of this type of mono-reacted macrocycle has proven extremely tedious in our labs; hence, route A was chosen over route B in this project. A variation of route B, similar to the reaction used by James<sup>30</sup> for the synthesis of artificial ion channels was also attempted using tartaric acid as the linking unit.

The elegance of this approach to the synthesis of these relatively large molecules is its simplicity. The entire synthesis is based on two basic reactions, ester formation and Michael addition. Although, in sum, the synthesis appears straightforward, in reality many problems were encountered,



**Scheme 10.** Retrosynthesis of the pore forming bolaamphiphiles for bilayer membranes (8-series).

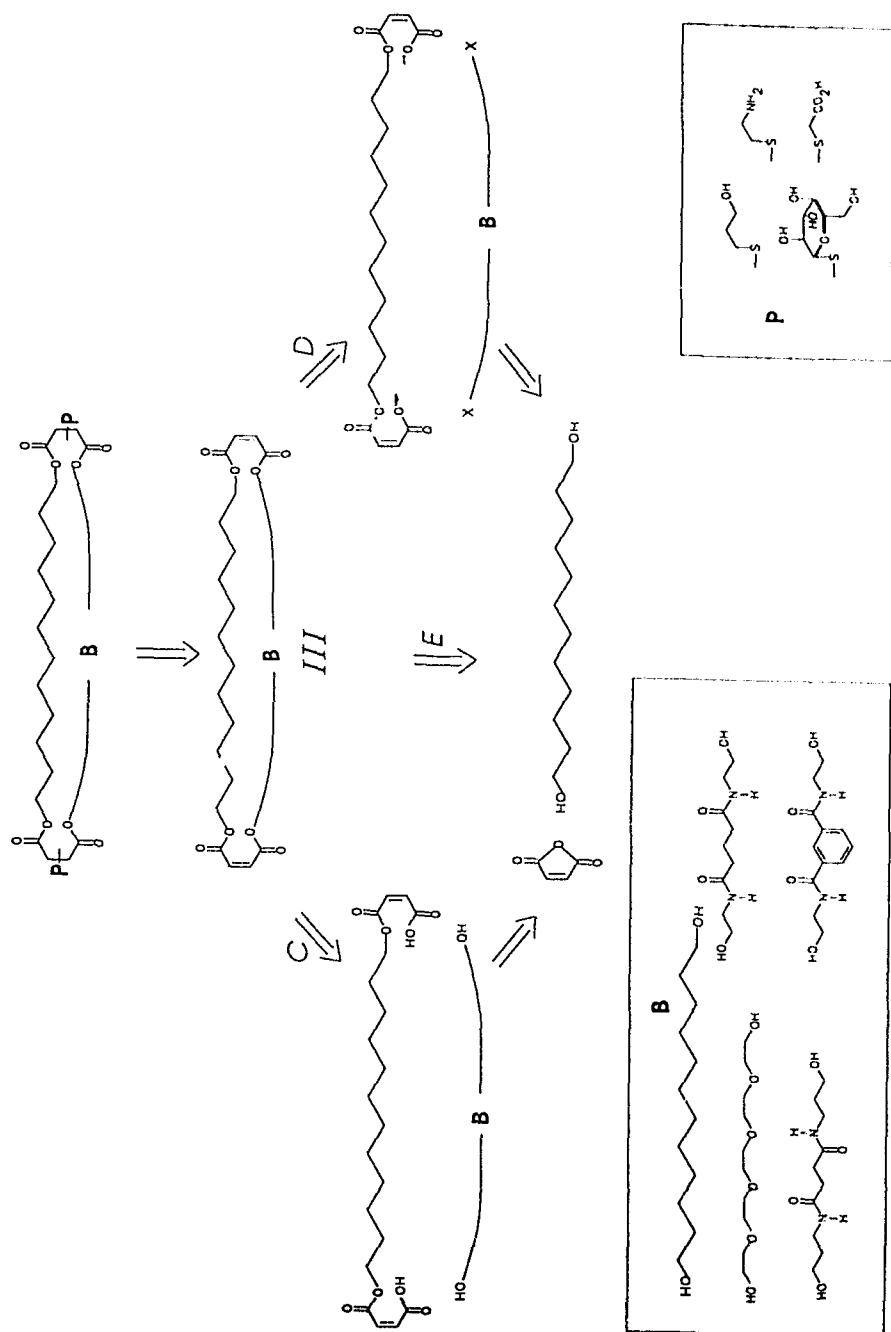
the details of which will be discussed in this chapter.

The retrosynthesis of target bolaphiles is drawn in Scheme 11 (the 12-series). The macrocyclic diene intermediate III can be made either in two steps, (routes C and D), or one step (route E). All the three approaches were explored in this project and the results are discussed below.

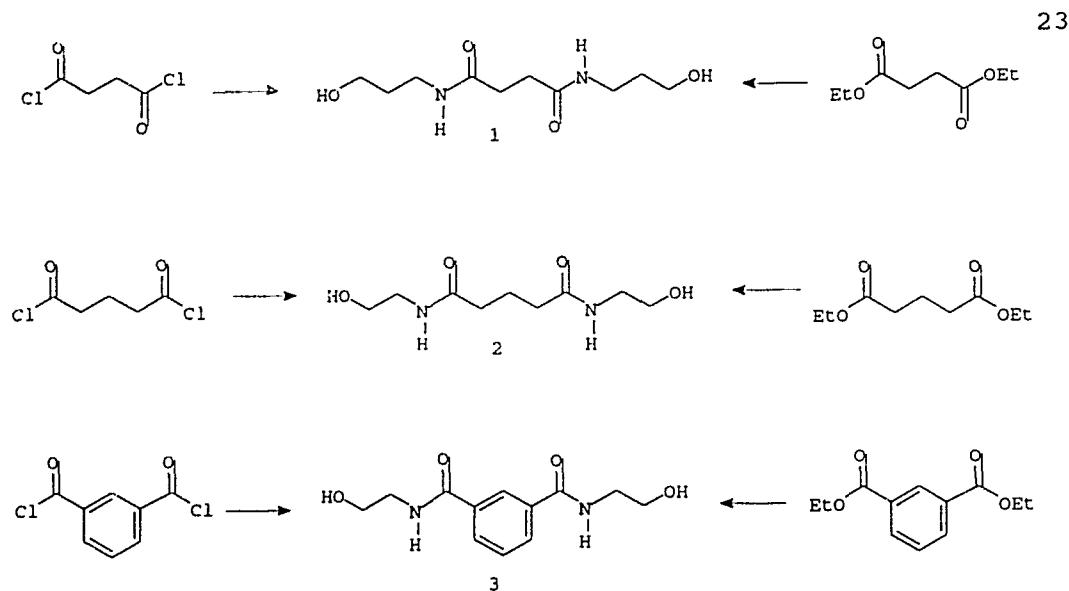
#### Polar Arms

The starting materials used for the synthesis of the polar arms are inexpensive and readily available. The polar arms 1, 2, and 3 were synthesized by reaction of succinoyl chloride, glutaroyl chloride, and isophthaloyl chloride with 3-amino-1-propanol or ethanolamine in the presence of base in very poor yields (10-20 %) (Scheme 12).

An alternative method was explored which proved fruitful both in terms of yield and ease of purification. Thus, the diethyl esters of succinic acid, glutaric acid, and isophthalic acid were reacted with the appropriate amine giving 1, 2, and 3 in reasonable yields (49 - 70%). The products were all solid and were easily purified by recrystallization from THF or acetonitrile. The reaction and purification were so facile that large quantities of the diol were obtained in the first attempt. The products were characterized by <sup>1</sup>H and <sup>13</sup>C NMR and mass spectroscopy. The NMR spectra were straightforward and could be solved by



**Scheme 11.** Retrosynthesis of the pore forming bolaamphiphiles for Fuhrhop's monolayer membranes (12-series).



**Scheme 12.** Synthesis of polar arms (12-series).

inspection. The purity of each product was confirmed by elemental analysis.

The synthesis of the shorter polar arms for the 8-series 4, 7, and 8 are shown in Scheme 13. All compounds were characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR and mass spectroscopy, and their purity was confirmed by elemental analysis.

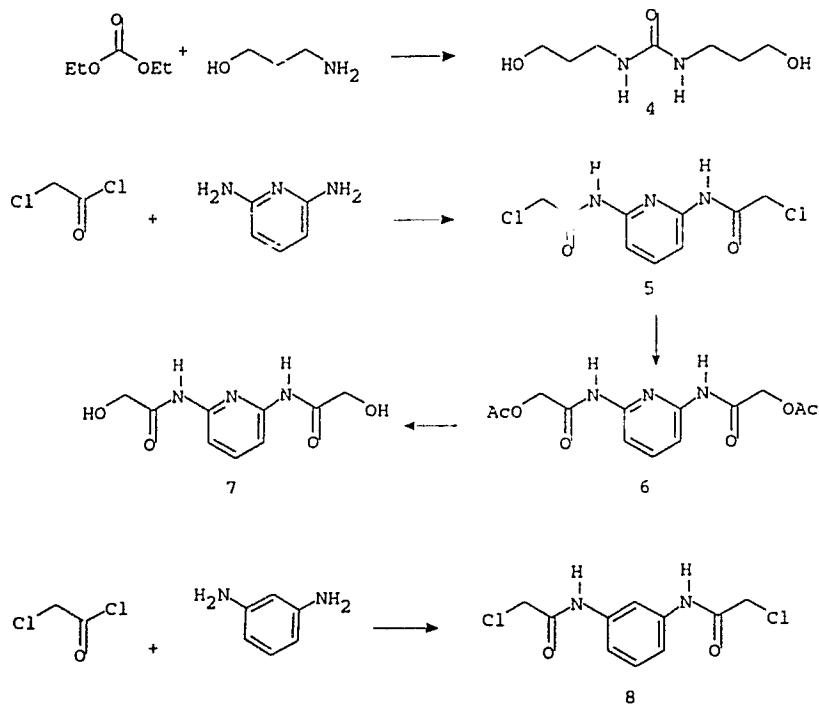
Both the  $^1\text{H}$  and  $^{13}\text{C}$  spectra were straightforward and could be solved by inspection. For example, the proton chemical shifts for  $\text{CH}_2\text{Cl}$ ,  $\text{CH}_2\text{OAc}$ , and  $\text{CH}_2\text{OH}$  for compounds 5, 6, and 7, were 4.2, 4.7, 4.1 ppm and the  $^{13}\text{C}$  chemical shift for C-X, C-OAc, and C-OH were 42.7, 63.1, and 62.9 ppm respectively.

Variations in reaction conditions for compounds in scheme 13 were attempted and the results merit discussion here. Compound 4 was synthesized in reasonable yield (71%) by simply heating the components for not more than 48 hours.

Purification was achieved by recrystallization from acetonitrile.

Compounds 5, 6, and 7 were obtained in reasonable yields (71%, 89%, and 76% respectively) and large quantities were synthesized to continue to the macrocyclic tetraesters. It should be noted that reaction temperature was very crucial in obtaining high yields of 5. When the reaction was carried out at 0°C or at room temperature, the yields were dramatically reduced to less than 1%. The reaction was also carried out using the Schotten-Baumann procedure at different temperatures, which failed to give the desired product. The substitution reaction using NaOH to convert 5 to 7, resulted in the hydrolysis of the amide bond. Hence, the dichloride 5 was converted to its diacetate derivative 6, which was further hydrolysed under mild conditions to the diol 7. Conversion of 6 to 7 also resulted in complete hydrolysis of the amide bond when the reaction was carried out above room temperature. The reaction of 5 with sodium iodide did not give the diiodo derivative; instead, the diol 7 was isolated as the major product resulting from the substitution at the CH<sub>2</sub>-X carbon during the work up.

Compound 8 was synthesized in 18% yield. A complex product mixture was obtained in this reaction and the major products were characterized by their <sup>13</sup>C chemical shift for the carbonyl carbon at 176 ppm, indicating direct acylation of the aromatic ring. The mass spectrum also showed masses higher



**Scheme 13.** Synthesis of polar arms (8-series).

than 600, indicating oligomerization. The purification of the desired product was tedious and required repeated extractions and chromatography. When the reaction was scaled up, the yield fell to less than 3%. Attempts at increasing the yield by lowering the reaction temperature (down to  $-78^{\circ}\text{C}$ ) failed. Attempts at carrying out the reaction using the Schotten-Baumann procedure at various temperatures failed to give the desired product. In light of the low yield and tedious purification, further conversion of compound 8 to its diol derivative was not pursued.

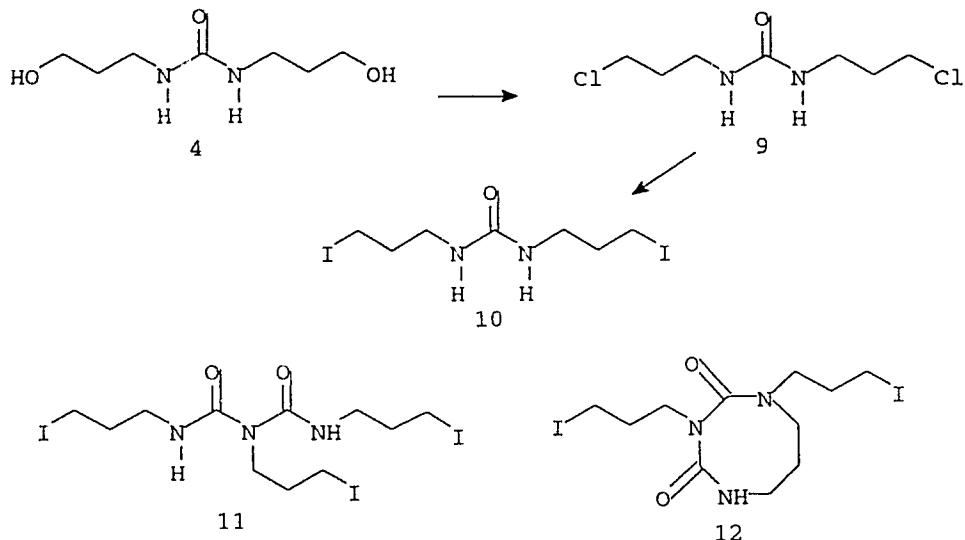
Conversion of 4 into its dihalo derivatives is shown in

Scheme 14. The diol 4 was reacted with thionyl chloride, giving 9 in 12% yield. The reaction of 9 with a large excess of sodium iodide gave the desired product 10 in less than 10% yield plus compounds 11 and 12 as the major products which result from intramolecular and/or intermolecular N-alkylation of 10. A small amount of unreacted dichloride 9 (8%) was also present in the product mixture.

To summarize, several polar arms incorporating amide bonds were synthesized (1, 2, 3, 4, and 7). The diol derivative of 8 was not prepared due to the low yield of the reaction, resulting from the competing acylation of the aromatic ring as well as oligomerization reactions. The diiodo derivative of 5 was also not isolated due to substitution of hydroxide at the work up stage. Synthesis of the diiodo derivative of 4 gave mostly 11 and 12 as a result of intramolecular and/or intermolecular N-alkylation reactions. The use of these polar arms in the synthesis of side-discriminated macrocycles is discussed below.

### Macrocycles

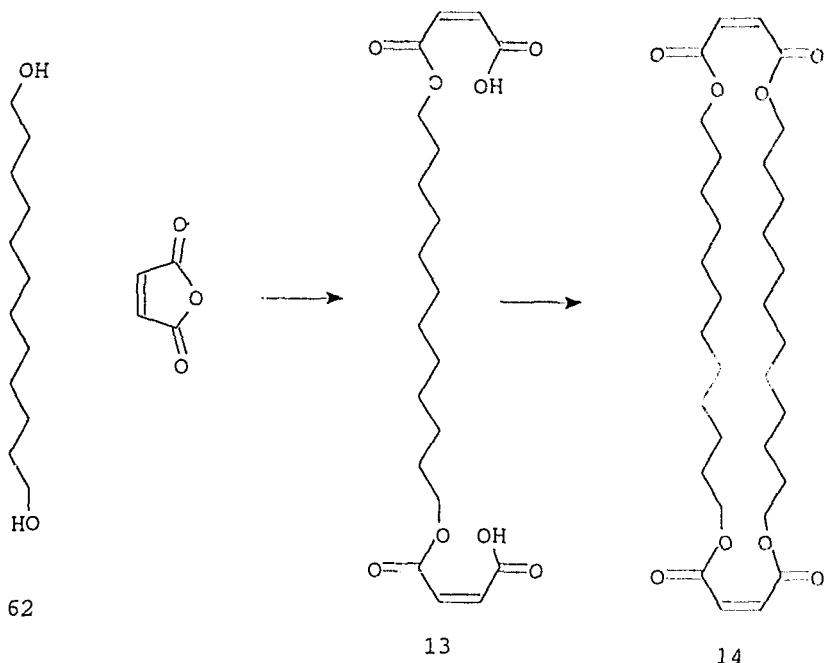
Preparations of esters have been studied in great depth, as indicated by the large number of reviews and monographs in the literature<sup>43-46</sup>. Esters are formed from the direct reaction of alcohols with carboxylic acids, activated acyl derivatives, such as acid chlorides and anhydrides, or other reactive



**Scheme 14.** Synthesis of dihalo derivatives of compound 4. intermediates generated in situ using coupling reagents like DCC.

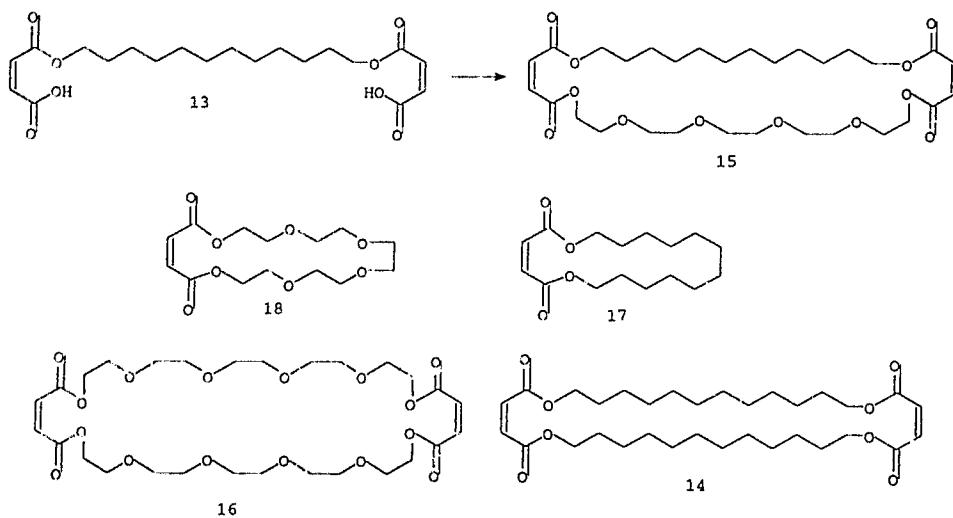
12-Series - The assembly follows the procedure described by Fuhrhop<sup>38</sup>. Treatment of two equivalents of maleic anhydride with one equivalent of 1,12-dodecanediol (62) gave the diacid 13 quantitatively. Cyclization of 13 gave 14 in 16 - 28% yield (Scheme 15). The <sup>1</sup>H and <sup>13</sup>C NMR and mass spectral data matched those of Fuhrhop. A one-pot synthesis (without isolating the diacid 13) of the macrocycle 14 was attempted and gave a 17% yield, similar to that of the two step reaction.

A side-discriminated macrocycle was prepared from the reaction of 13 with pentaethylene glycol, giving the



**Scheme 15.** Synthesis of Fuhrhop's symmetrical macrocycle.

macrocycle 15 in 9% yield plus four other cyclic products (Scheme 16). A one-pot synthesis of 15 was attempted and gave a 9% yield, identical to the two step reaction. The reaction gave a statistical mixture of all the possible cyclic products. In this case, the desired macrocycle 15 could be readily purified by column chromatography. The product was identified by its  $^1\text{H}$  and  $^{13}\text{C}$  NMR and mass spectra, and its purity was confirmed by elemental analysis. The  $^1\text{H}$  NMR spectrum showed the  $\text{CO}_2\text{CH}_2$  at 4.2 ppm. The  $^{13}\text{C}$  NMR spectrum showed two  $\text{C}=\text{O}$  at 165.1 and 165.0 ppm, two  $\text{C}=\text{C}$  at 130.1 and 129.2 ppm, and two  $\text{CO}_2\text{CH}_2$  at 65.3 and 64.2 ppm. It should be noted that the  $^1\text{H}$  and  $^{13}\text{C}$  NMR of 14 and 17 were very similar



**Scheme 16.** Synthesis of a side-discriminated macrocycle by acid catalyzed dehydration method.

and gave identical elemental analyte. They were distinguished by their mass spectra: for 17 ( $M + 1$ ) = 283 and for 14 ( $M + 1$ ) = 565. This was also true for compounds 16 and 18.

Synthesis of other side-discriminated macrocycles in the 12-series were attempted, using diols 1, 2, and 3 (Table 1). In a one-pot reaction of 1, 2, and 3 with maleic anhydride and 1,12-dodecanediol in benzene or toluene - similar to the conditions used for the preparation of 15 - starting materials were recovered (Table 1, entries 1-3). A small amount of the diacid 13 could be identified among the reaction products. Stepwise reactions of 1 and 2 also failed to give the desired

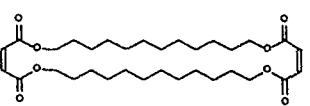
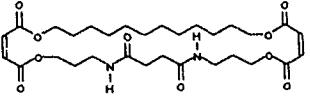
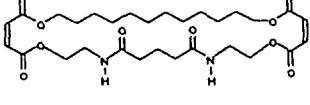
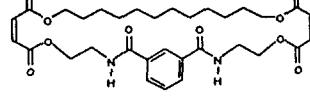
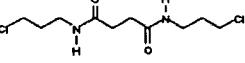
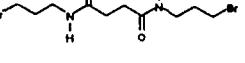
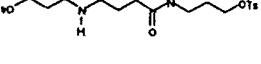
macrocycle (Table 1, entries 4-6). The essential problem was that the three diols were insoluble in the reaction mixture and therefore could not react with maleic anhydride or diacid 13 to give the desired product. Apparently the acid catalyst was associated with the solid, for no ester products were formed even though 1,12-dodecanediol and maleic anhydride were simultaneously present in the reaction mixture.

The solubility of the three diols was examined in various solvents. The only solvent that combined diol solubility with the formation of a water azeotrope<sup>50</sup> for the acid catalyzed dehydration reaction was dimethoxyethane (DME). Unfortunately a control reaction in DME with 13 and 1,12-dodecanediol failed to give the expected macrocycle 14 and starting materials were recovered (Table 1, entry 7). Alternative sequences of steps were attempted; when the diols 1, 2, and 3 were reacted with maleic anhydride as a homogeneous solution in DME or THF, starting materials were recovered.

To avoid the solubility problem, direct fusion of the starting materials without any solvent was examined. The goal here was to work at a temperature where the diols 1, 2, and 3 were molten and intermolecular H-bonding less important. Preparation of macrocycle 14 by fusion of maleic anhydride and 1,12-dodecanediol was attempted and resulted in the formation of polymers (Table 1, entry 8). When the same reaction was carried out in the high boiling "solvent" biphenyl, the

Table 1. Attempted Reactions

En <sup>1</sup>	Re <sup>2</sup>	Conditions	Expected Product	Fn <sup>3</sup>
1	<u>1</u>	MA <sup>4</sup> , <u>62</u> H <sup>+</sup> /-H <sub>2</sub> O		SM <sup>5</sup>
2	<u>2</u>	MA, <u>62</u> H <sup>+</sup> /-H <sub>2</sub> O		SM
3	<u>3</u>	MA, <u>62</u> H <sup>+</sup> /-H <sub>2</sub> O		SM
4	<u>1</u>	<u>13</u> H <sup>+</sup> /-H <sub>2</sub> O		SM
5	<u>1</u>	1) MA 2) <u>62</u> , H <sup>+</sup> /-H <sub>2</sub> O		SM
6	<u>2</u>	1) MA 2) <u>62</u> , H <sup>+</sup> /-H <sub>2</sub> O		SM
7	<u>13</u>	<u>62</u> /DME <sup>6</sup> H <sup>+</sup> /-H <sub>2</sub> O		SM

8	<u>62</u>	MA $H^+$ /fuse		P1 <sup>7</sup>
9	<u>1</u>	MA, <u>62</u> $H^+$ /fuse		SM
10	<u>2</u>	MA/ <u>62</u> $H^+$ /fuse		SM
11	<u>3</u>	MA/ <u>62</u> $H^+$ /fuse		SM
12	<u>1</u>	$PCl_3/DME$		CM <sup>8</sup>
13	<u>1</u>	$PBr_3/DME$		CM
14	<u>1</u>	$TsCl^9/Et_3N$ DME		SM

15	<u>4</u>	<u>21</u> $\text{H}^+ / -\text{H}_2\text{O}$		SM
16	<u>4</u>	MA/THF		SM
17	<u>4</u>	MA/DMAP <sup>10</sup> $\text{Et}_3\text{N}/\text{THF}$		SM
18	<u>22</u>	$\text{RSH}^{11}$ $\text{Pip}^{12}/\text{IPA}^{13}$		CM
19	<u>23</u>	$\text{RSH}$ $\text{Pip/IPA}$		CM
20	<u>24</u>	$\text{RSH}$ $\text{Pip/IPA}$		CM
21	<u>22</u>	$\text{RSH/TMP}^{14}$ $\text{THF/IPA}$		O1 <sup>15</sup>

22	<u>23</u>	RSH/TMP THF/IPA		ol
23	<u>24</u>	RSH/TMP THF/IPA		ol
24	<u>22</u>	RSH/TMP slow add.		ol
25	<u>23</u>	RSH/TMP slow add.		ol

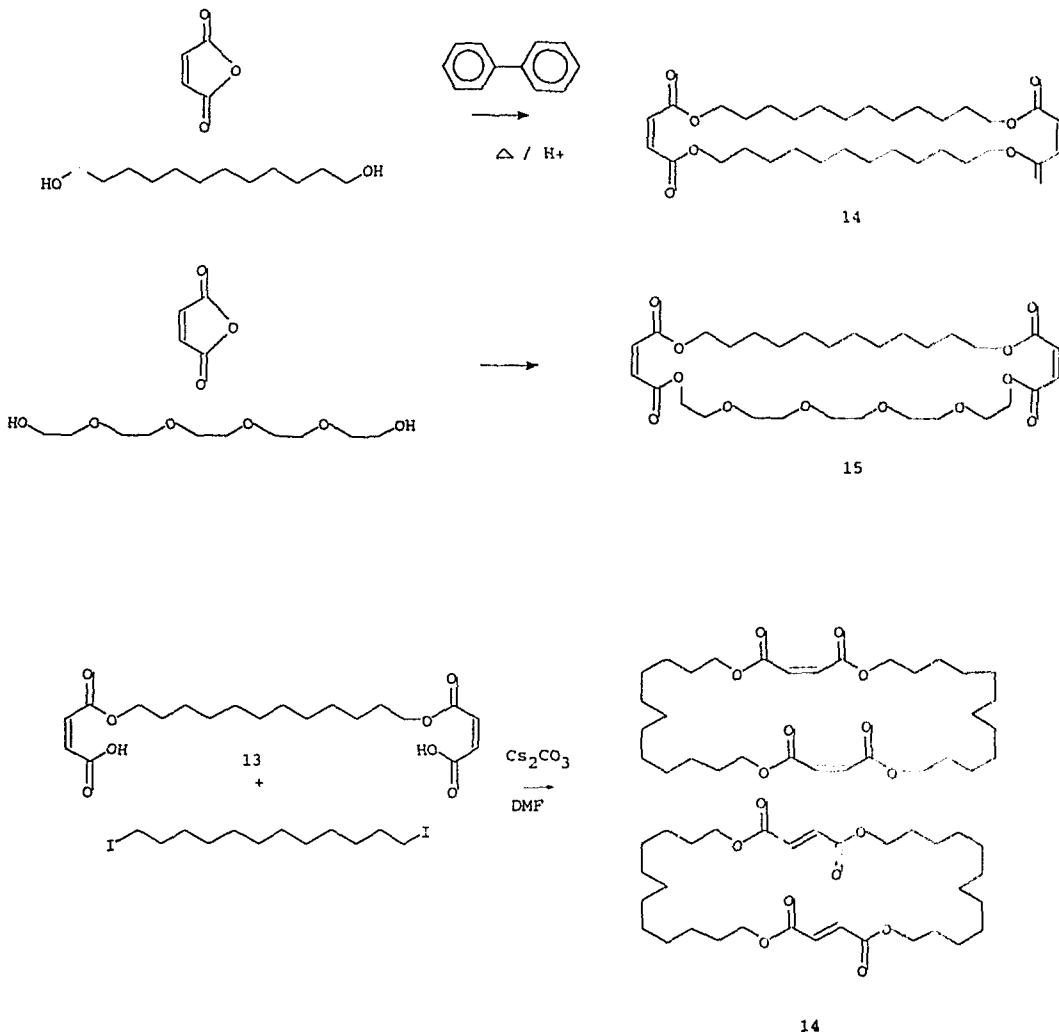
<sup>1</sup>En = Entry<sup>2</sup>Re = Reactant<sup>3</sup>Fn = Found<sup>4</sup>MA = Maleic Anhydride<sup>5</sup>SM = Starting Material<sup>6</sup>DME = Dimethoxyethane<sup>7</sup>P1 = Polymer<sup>8</sup>CM = Complex Mixture<sup>9</sup>TsCl = p-Toluenesulfonyl chloride<sup>10</sup>DMAP = 4-Dimethylaminopyridine<sup>11</sup>RSH = m-xylylene dithiol<sup>12</sup>Pip = Piperidine<sup>13</sup>IPA = Isopropyl Alcohol<sup>14</sup>TMP = 2,2,6,6-Tetramethylpiperidine<sup>15</sup>Ol = Oligomer

desired macrocycle was obtained in lower yield (5%) than the acid catalyzed dehydration reaction in benzene solution. Similarly, the reaction of maleic anhydride, 1,12-dodecanediol, and pentaethylene glycol in biphenyl gave 15 in a lower yield (3%) than the acid catalyzed dehydration reaction in benzene (scheme 17).

Starting materials were recovered from the fusion reaction of 1, 2, and 3 with maleic anhydride and 1,12-dodecanediol in biphenyl (Table 1, entries 9-11). The diols were not truly soluble in the reaction mixture; two liquid phases were apparent in the reaction flask. Moreover, the diols were not fully stable under the reaction conditions as indicated by the progressive discoloration of the molten diols.

Alternative reaction condition for the preparation of 14 using the diacid chloride derivative of 13 has been reported by Fuhrhop to give very poor yields<sup>38</sup>. Activation of the carbonyl group by DCC had been previously explored by Swan<sup>51</sup> and proved unsuccessful. The reaction of the diols 1, 2, and 3 with maleic anhydride using 4-dimethylaminopyridine<sup>52-53</sup> as an activating agent was also unsuccessful; in all three reactions unreacted starting materials were recovered. Therefore, macrocyclization by the activation of the carbonyl carbon was not pursued further.

An alternative approach to the synthesis of 14 was explored using cesium carbonate in DMF<sup>54-59</sup>. The diacid 13 was



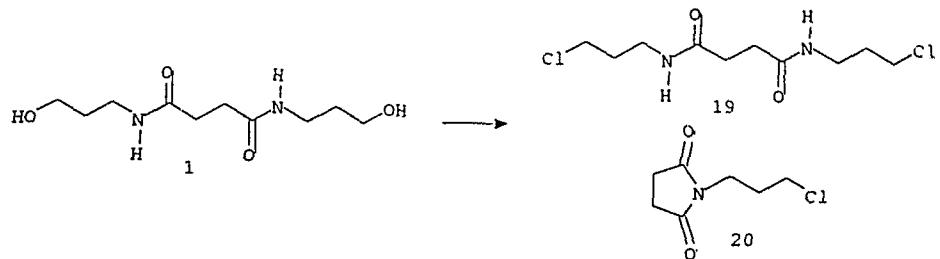
**Scheme 17.** Alternative approaches to the synthesis of macrocyclic tetraesters.

reacted with 1,12-diiodododecane in DMF, using cesium carbonate as the base and gave the desired macrocycle 14 in 4% yield (scheme 17). One notable feature of this reaction is the presence of the trans isomer of 14 in the isolated product. This is believed to result from isomerization by

small amounts of iodine present in 1,12-diiodododecane. The extent of isomerization was never uniform (5-95%) and depended on the shelf life of the diiodide compound; the older the sample, the higher the isomerization to the trans isomer. This isomerization was not a problem because in the subsequent step (the Michael addition reaction) the alkene function will be lost. The <sup>1</sup>H NMR spectra of the cis and trans isomers were quite distinct. The proton chemical shift for the cis CH=CH was at 6.2 ppm and for the trans isomer at 6.8 ppm.

Attempts at utilizing this new approach using the dihalo or ditosylate derivatives of 1 failed. Reaction of 1 with thionyl chloride gave the dichloro compound 19 in 9% yield, and lower yields (1-2%) were obtained when the reaction was scaled up. Only very small quantities of compound 19 could be isolated, thereby its conversion to the diiodo derivative and reaction with the diacid 13 using the cesium carbonate method was not worthwhile (scheme 18). Reaction of 1 with phosphorus trichloride or phosphorus tribromide gave complex product mixtures. When the diol 1 was reacted with P-toluenesulphonyl chloride, starting materials were recovered (Table 1, entries 12-14).

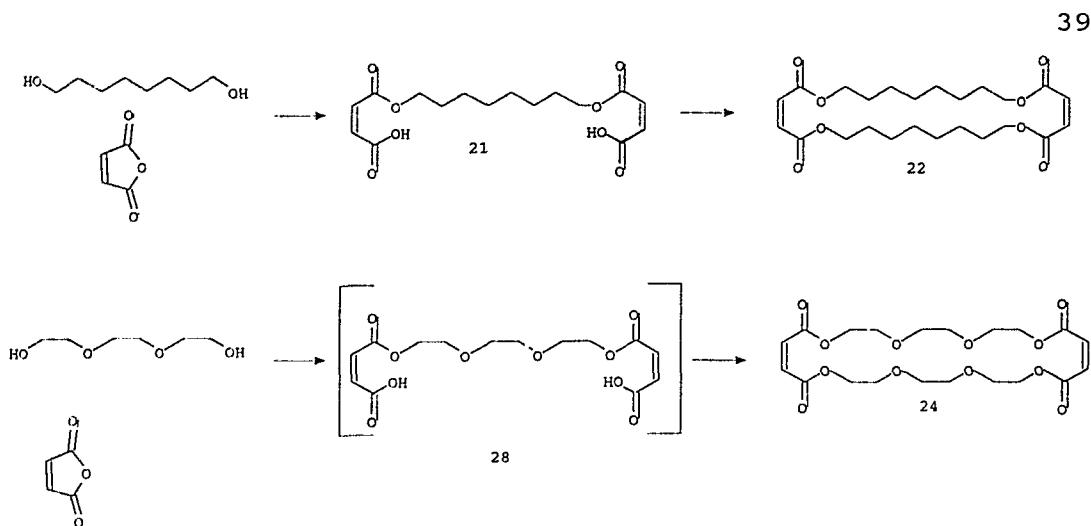
While the preparation of other side-discriminated macrocycles using the derivatives of the polar arms 2 and 3 were being investigated, severe problems with the transport experiment (work of James<sup>60</sup>), were encountered which conspired to terminate this approach to pore formers for monomolecular



**Scheme 18.** Synthesis of the dihalo derivative of compound 1.

membranes similar to Fuhrhop<sup>38</sup>. The key problem in the transport was vesicle leakage but subsequent work has sought to avoid significant background leakage whenever possible. Our solution to the vesicle leakage problem was to change to bilayer vesicle membranes prepared from egg phosphatidyl choline. Mimics need to be 40 Å long to span this bilayer membrane, twice the length required in the Fuhrhop system. The general conclusion from the synthetic work in this series is that acid catalyzed esterification is essential for macrocyclization, but this places severe limitations on the types of functional groups that can be incorporated in the polar side of the macrocycle.

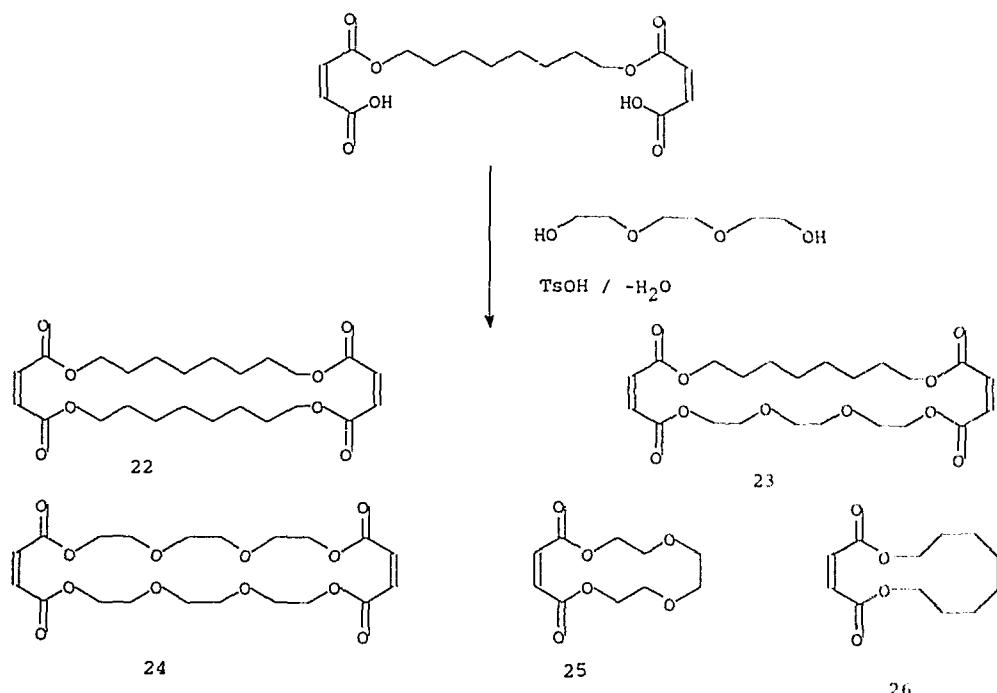
**8-Series** - The preparation of two symmetrical macrocycles, 22 and 24, was attempted (scheme 19). 1,8-Octanediol was reacted with two equivalents of maleic anhydride and gave the diacid 21 quantitatively. The acid catalyzed dehydration reaction of the diacid 21 with 1,8-Octanediol gave 22 in 11% yield. The



**Scheme 19.** Synthesis of symmetrical macrocycles by acid catalyzed dehydration method.

macrocycle 24 was synthesized in 11% yield in two steps without the isolation of the diacid intermediate 28. Another side-discriminated macrocycle was synthesized: the acid catalyzed dehydration of the diacid 21 with triethyleneglycol gave the macrocycle 23 in 6% yield (scheme 20). As with the preparation of 15, this reaction also gave a mixture of all the possible cyclic products. One-pot reactions of 22, 23, and 24 were attempted and gave the desired macrocycles in very poor yields (1-2%). However, all three macrocycles 22, 23, and 24 could readily be made in multigram quantities by the acid catalyzed dehydration method.

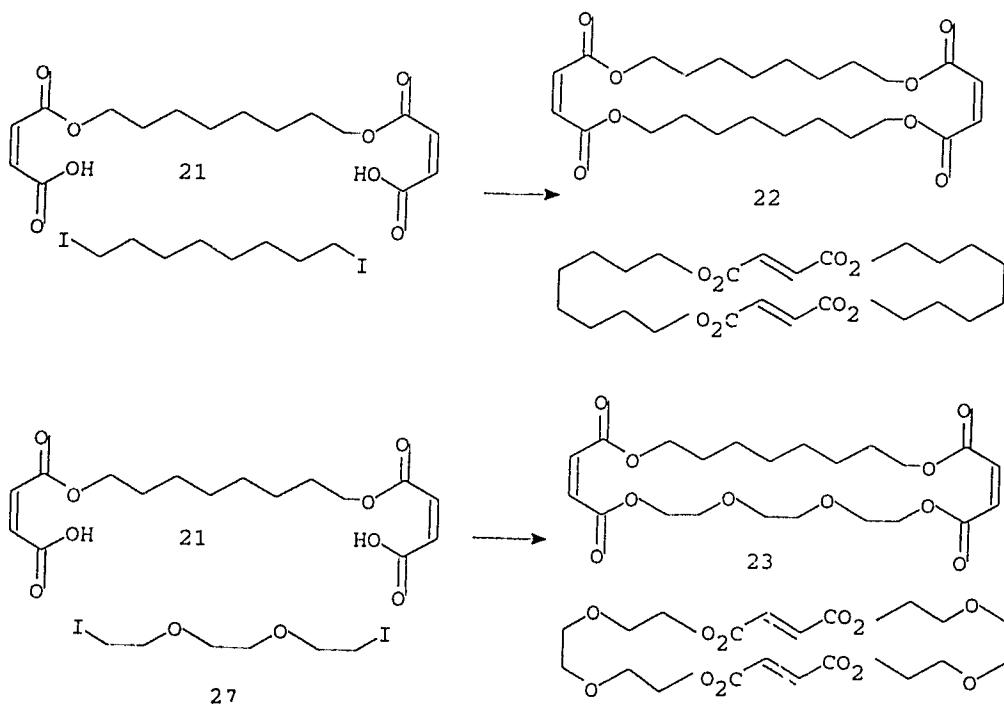
The synthesis of 22 and 23 by the cesium carbonate method was investigated. The reaction of the diacid 21 with 1,8-diiodooctane in DMF using cesium carbonate as the base, gave the desired product in 34% yield (scheme 21). Again a mixture



**Scheme 20.** Synthesis of side-discriminated macrocycle by acid catalyzed dehydration method.

of cis and trans isomers was obtained. The side-discriminated macrocycle 23 was synthesized: the reaction of the diacid 21 with the diiodide 27 in a solution of cesium carbonate in DMF gave a mixture of cis and trans isomers in 7% yield. A variation in the base used was explored: changing the base from cesium carbonate to tetrabutyl ammonium hydroxide did not improve the yield.

An alternative sequence of steps for the preparation of 23 was also explored. Triethyleneglycol was reacted with two equivalents of maleic anhydride to give the diacid 28. When



**Scheme 21.** Synthesis of macrocycles by cesium carbonate method.

the reaction was carried out in benzene, only partial reaction was observed. Longer reaction times did not improve the yield, and higher temperatures (toluene) gave mostly polymer. The diacid 28 was also very tedious to purify and required extensive chromatography. It was clear this route could not offer any advantage over the other two approaches noted above, consequently, this route was abandoned.

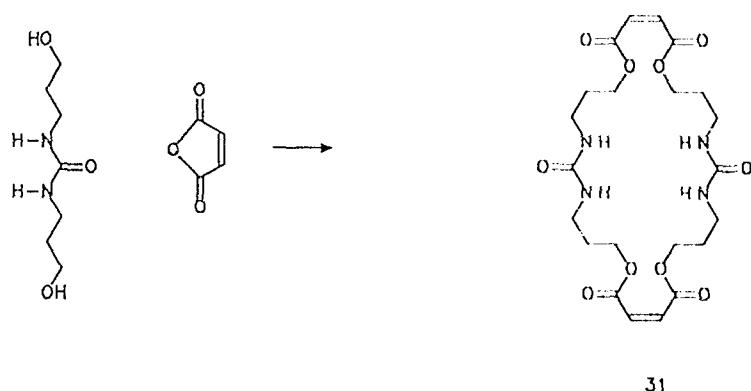
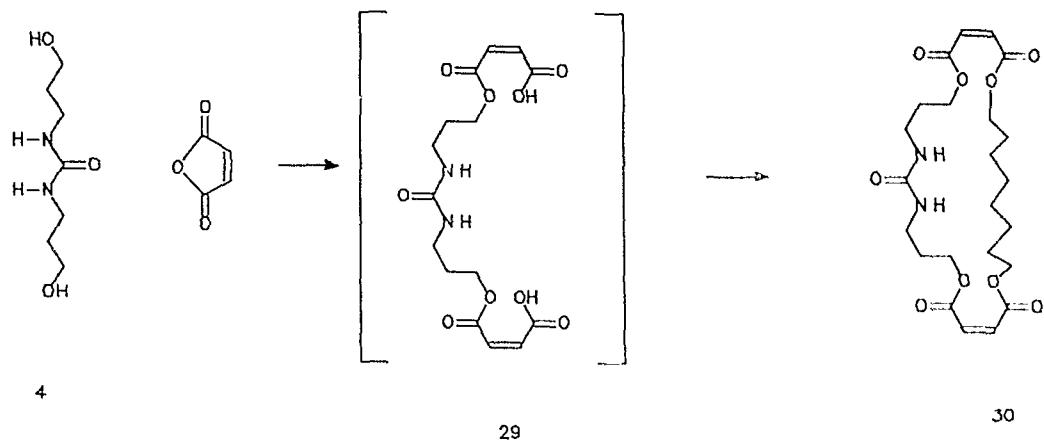
Thus far, two approaches to the synthesis of macrocyclic tetraesters have been examined. The acid catalyzed

dehydration method gives a mixture of cyclic products. The reaction and purification procedures are relatively straightforward and can be routinely used to obtain gram quantities of the desired macrocycles (22, 23, and 24). The cesium carbonate method gives a mixture of cis and trans isomers. This isomerization is not a problem since in the subsequent Michael addition reaction the alkene function is lost. This method proved to be better only for the preparation of 22: although the macrocyclization yield of this reaction is higher than in the acid catalyzed approach, the latter approach was more convenient to use for obtaining large quantities of 22.

Synthesis of another side-discriminated macrocycle using the diol 4 as the polar arm was attempted. Although the diol 4 was not soluble in a useful solvent such as benzene or toluene, its reaction with 21 under acid catalysis was attempted. When the diol 4 was reacted with the diacid 21 starting materials were recovered (Table 1, entry 15). Alternative sequences of steps - the reaction of 4 with maleic anhydride -under different reaction conditions were attempted. When the diol 4 was reacted with two equivalents of maleic anhydride in THF, unreacted starting materials were recovered. When the same reaction was carried out with one equivalents of 4-dimethylaminopyridine, again starting materials were recovered (Table 1, entries 16-17). The reaction of 4 with maleic anhydride in DMF and a catalytic amount of sulphuric

acid was successful in the preparation of the diacid 29. This was reacted without purification with 1,8-diiodooctane in cesium carbonate and DMF and gave the desired side-discriminated macrocycle 30 in less than 1% yield (scheme 22). The low yield is attributed to the first step of the reaction. Large amounts of the unreacted diol 4 plus some mono-reacted diol and polymeric products were isolated. Attempts at preparation of large quantities of 29 were not fully successful. Both the cis and the trans isomers of 30 were present in the reaction mixture. The cis isomer could be isolated and purified from the reaction mixture by ether precipitation from some chromatographic fractions. The cis isomer was characterized by <sup>1</sup>H and <sup>13</sup>C NMR and mass spectroscopy.

The acid catalyzed preparation of the symmetrical macrocycle 31 was attempted and proved successful (scheme 22). The macrocycle 31 was prepared in one step from the reaction of 4 with maleic anhydride in benzene in 2% yield. Although the diol 4 was insoluble in the reaction mixture, the product 31 still formed. This compound was very hygroscopic and was difficult to isolate and purify; satisfactory elemental analysis for compound 31 could not be obtained. The low yield is primarily due to losses during purification by column chromatography. Attempts at scaling up the reaction and isolating large quantities of 31 were not fully successful. Since the yield for the preparation of the diiodo derivative



**Scheme 22.** Synthesis of macrocycles incorporating urea function.

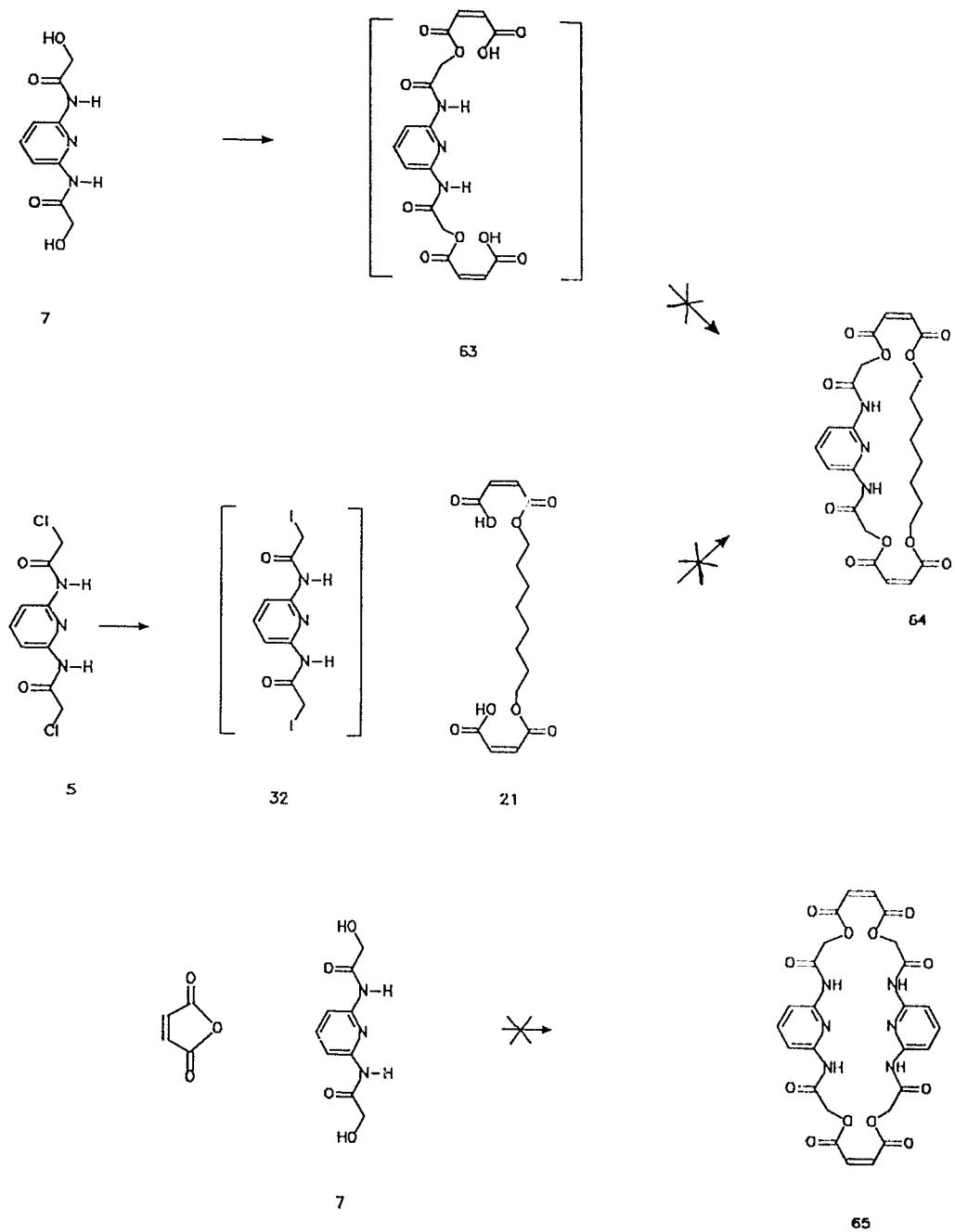
of 4 (10) was very low (1%), its reaction with the diacid 21 using the cesium carbonate method was not attempted.

To avoid the problems encountered with the aliphatic amides of the 12-series and 8-series, the polar arms 7 and 8 were used to synthesize other side-discriminated macrocycles. Although the diol 7 was insoluble in benzene, its reaction

under acid catalyzed dehydration condition was attempted. The reaction of diol 7 with maleic anhydride and 1,8-octanediol resulted in the formation of symmetrical macrocycle 22 and the smaller cyclic product 26. Alternative sequences of steps were also attempted. Unfortunately, the diol 7 did not react with maleic anhydride, and its reaction with maleic anhydride under acid catalyzed reaction conditions resulted in the hydrolysis to 2,6-diaminopyridine. A one-pot synthesis of the symmetrical macrocycle 65 under acid catalyzed dehydration conditions using the diol 7 was attempted, which again resulted in the degradation of the amide bond (scheme 23).

In another attempt, compound 7 was converted to its diiodo derivative 32 which was reacted without purification with the diacid 21, using cesium carbonate as the base. Compound 32 was not stable under the reaction conditions and was hydrolysed to 2,6-diaminopyridine (scheme 23). The desired side-discriminated macrocycle incorporating this polar arm thus could not be synthesized due to instability of the diol 7 and its dihalo derivatives.

Macrocyclization using the dichloro derivative 8 was attempted (scheme 24). Reaction of 8 with sodium iodide gave the diiodo derivative 33 which, without further purification, was reacted with 21 and gave the macrocycle 34 as its trans isomer in 3% yield. The product was purified by column chromatography and was characterized by its <sup>1</sup>H and <sup>13</sup>C NMR and mass spectra.

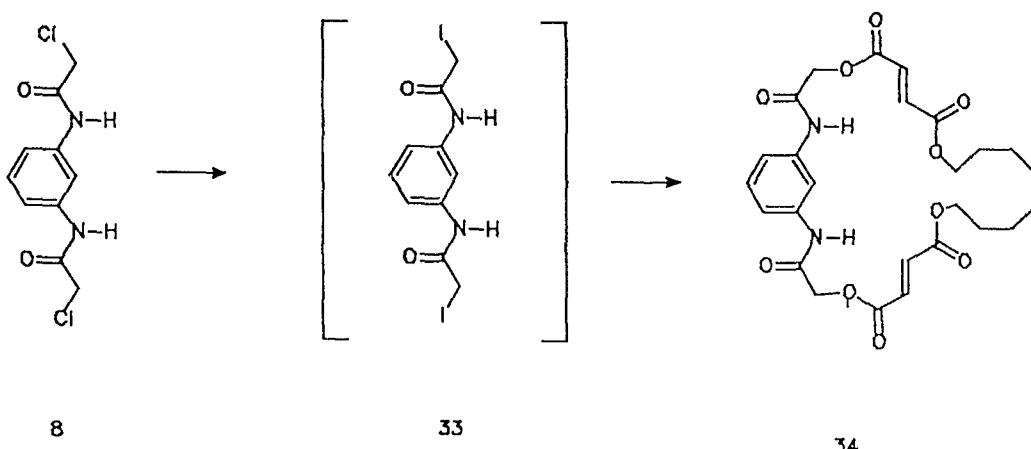


**Scheme 23.** Attempted macrocyclization reaction incorporating the polar arm **7** and its dihalo derivative.

To summarize, acid catalyzed esterification was an easy, convenient method for the preparation of 22 and 23 and 24. Macrocyclic 31 was conveniently prepared using this method, however, the yield was low and only small quantities could be isolated. The diols 1, 2, 3 and 4 were found to be unreactive, as no reactions with maleic anhydride or p-toluenesulfonylchloride were observed. The diol 7 was unstable and degraded under acid catalyzed macrocyclic dehydration reaction conditions.

The use of cesium carbonate in DMF provided a useful alternative to acid catalyzed esterification for the preparation of 30 and 34; however, this route was limited by the unavailability or instability of the diiodo derivatives of the polar arms. The yields were also much lower than the acid catalyzed esterification, except in the case of 22.

The problems encountered can be grouped into three cases:  
a) Although the final compounds were stable, some precursors were less stable and prone to self condensation. b) The diols were unreactive, probably due to self-hydrogen bonding and association with the acid catalyst. c) Restrictions on methods available for esterification imposed due to a) and b). To sum up, six macrocycles - 22, 23, 24, 30, 31, and 34 were synthesized. Unfortunately, very small quantities of the macrocycles 30, 31, and 34 were prepared such that their further conversions to the final products were not feasible; gram quantities of these macrocycles were required to continue



**Scheme 24.** Preparation of a side-discriminated macrocycle incorporating amide function.

the synthesis to the next two steps - the linkage reaction and addition of the polar head groups. Macrocycles 22, 23, and 24 were made in sufficient quantities to pursue their synthesis further.

#### Linkage

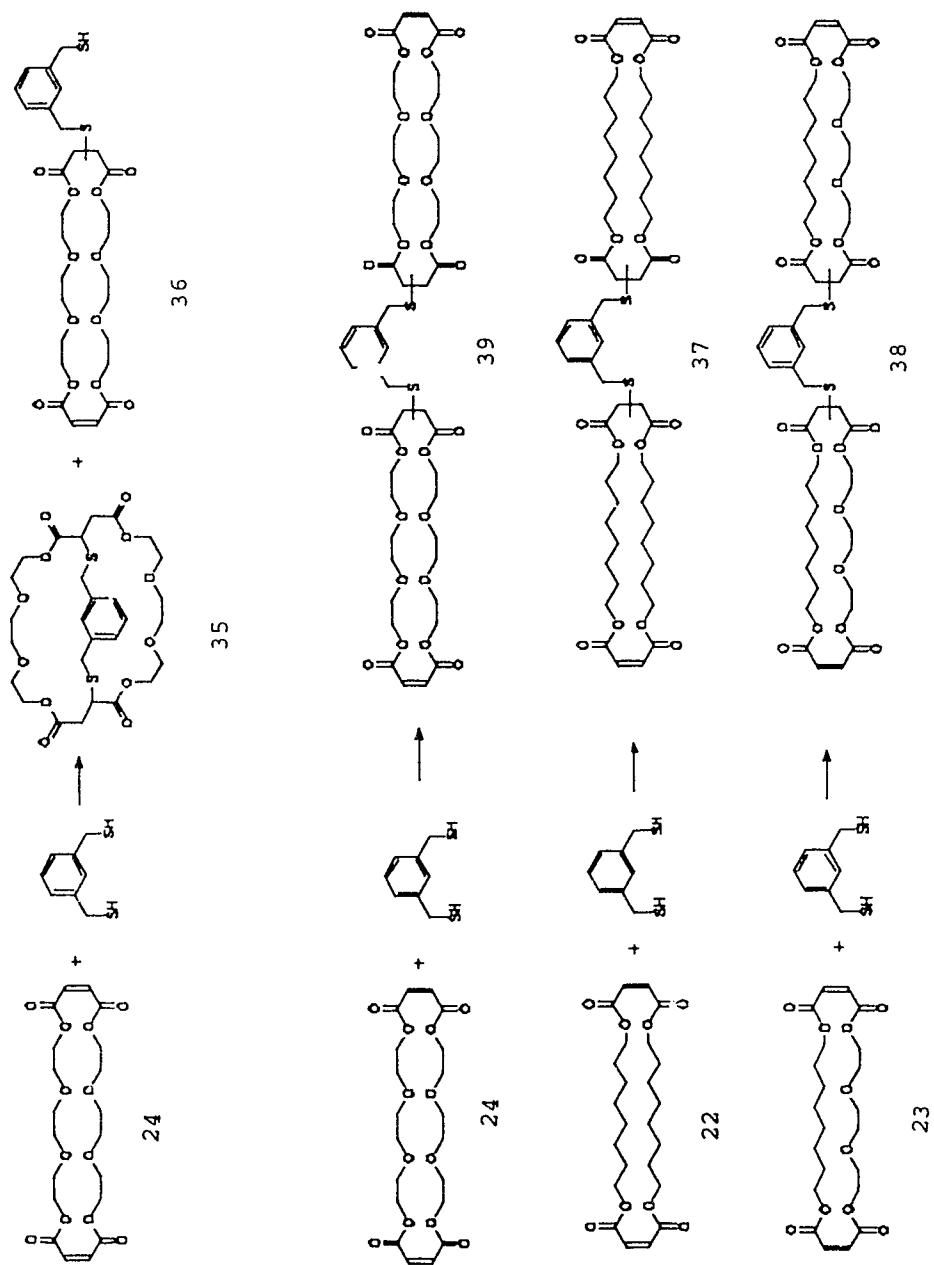
Amphotericin mimics using macrocyclic tetraesters have been reported<sup>42</sup>. The macrocycles were linked via Michael addition using meta-xylylene dithiol and piperidine in isopropanol. I used the same reaction conditions to link 22, 23, and 24 (Table 1, entries 18-20) and obtained a complex product mixture for all three reactions. Purification by column chromatography and close inspection of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of the fractions revealed: 1) the addition of the

piperidine to the double bond, and 2) the isomerization of the cis alkene to the trans isomer. This isomerization is believed to have resulted from the reversible addition of the piperidine to the conjugated alkene. The addition of the base to the double bond and isomerization has been overlooked and has to date not been reported; therefore, further work was performed to investigate these observations. A control reaction was carried out. Macrocycle 22 was refluxed in isopropyl alcohol (IPA) in the presence of piperidine for one hour.  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the crude product clearly showed the addition of the base and quantitative isomerization to the trans alkene. As far as the synthesis was concerned, isomerization of the alkene was not a problem since in the final step of the synthesis - the addition of polar head groups - the alkene function will be lost. However, analysis of the  $^{13}\text{C}$  NMR became very complicated and the purity of samples based on  $^{13}\text{C}$  NMR data was becoming questionable. As far as purification was concerned, addition of the base made the purification extremely tedious and undesirable.

In order to eliminate the possibility of addition of the base and isomerization of the double bond, a bulky base (2,2,6,6-tetramethylpiperidine) was used to link 22, 23, and 24. Also, a 1:1 mixture of THF:IPA was used to completely dissolve the macrocycle 22 (Table 1, entries 21-23). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the crude reaction mixture did not show the addition of the base and less than 5% isomerization of cis

alkene to the trans isomer. The crude products were purified by column chromatography. Close inspection of the  $^1\text{H}$  NMR of the fractions from silica gel chromatography showed that the ratio of aromatic and alkene protons was variable. Some fractions also showed the presence of a triplet at 1.9 ppm in the proton spectra, indicating the presence of the SH proton and a peak at 29 ppm in their  $^{13}\text{C}$  NMR spectra indicating an  $\text{ArCH}_2\text{SH}$ . Purification of the product mixtures with gel permeation chromatography gave fractions that had very similar  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, but the ratio of the aromatic to the alkene protons varied quite a bit, ranging from 2:1 to 1:2! It was clear that a mixture of oligomers was present in the product mixture, another notable feature of these linkage reactions which had to date not been reported.

One solution to the problem was slow addition of the dithiol to the reaction mixture, firstly to ensure the diene macrocycle remained in excess, and at the same time to conserve the stock of the macrocycles. This was unsuccessful: slow addition of the dithiol to 22 or 23 resulted in the formation of oligomers (Table 1, entries 24-25), and slow addition of thiol to 24 gave the capped macrocycle 35 in 62% yield formed by cross-linking the dithiol (scheme 25). Compound 35 was the fastest running compound in the gel column and could easily be isolated. The capped macrocycle 35 could easily be distinguished from compound 36 by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy, as it lacked the chemical shift due to alkene



**Scheme 25.** Linkage reaction using m-xylylene dithiol.

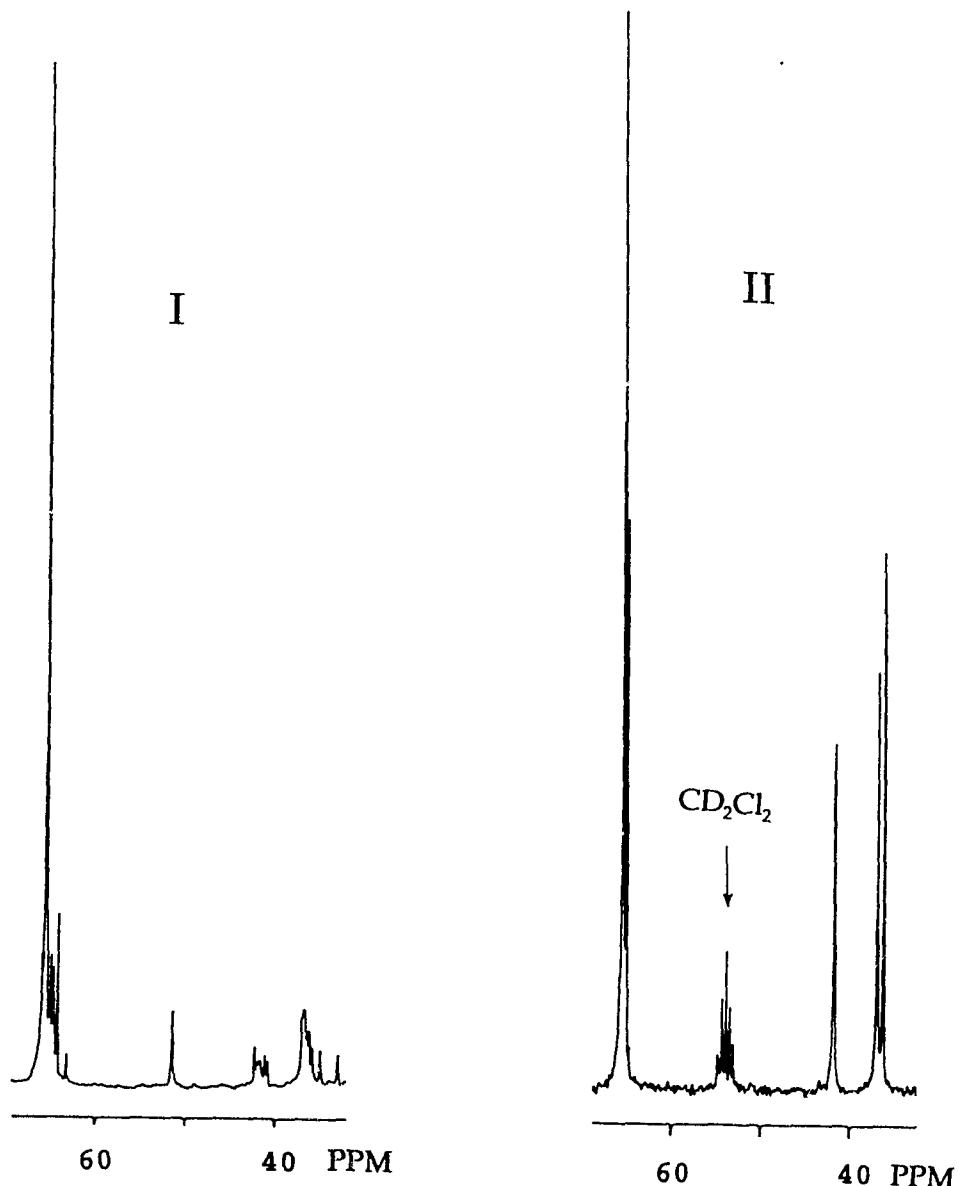
protons and carbons and  $\text{ArCH}_2\text{SH}$  protons and carbons. However, its regiochemistry could not be confirmed by NMR spectroscopy. Since both compounds 35 and 36 had identical masses, mass spectroscopy was not useful in distinguishing the two compounds. To avoid oligomerization, a large excess of macrocycles 22, 23, and 24 was used in the presence of a limiting amount of dithiol (scheme 25). At a molar excess of 10 equivalents of diene macrocycle to dithiol, the major products were the desired linked compounds 37, 38, and 39. In these cases the aromatic:alkene proton ratios were clearly 1:1. This reaction is quite efficient; nearly 85% of the excess unreacted macrocycles 22, 23, and 24 could be recovered by column chromatography. All the three compounds were characterized by their  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. They all showed the following characteristic chemical shifts. For  $^1\text{H}$  NMR: aromatic  $\text{C}=\text{CH}$  at 7.2-7.3 ppm, alkene  $\text{C}=\text{CH}$  at 6.2 ppm, ester  $\text{CO}_2\text{CH}_2$  at 4.2 ppm, and  $\text{ArCH}_2\text{SCH}$  at 3.5-3.9 ppm and  $\text{CH}_2\text{C}=\text{O}$  at 2.5-2.9 ppm. For  $^{13}\text{C}$  NMR:  $\text{C}=\text{O}$  at 165, 170, and 171 ppm, ester  $\text{CO}_2\text{CH}_2$  at 65 ppm,  $\text{CHS}$  at 42 ppm, and  $\text{CH}_2\text{C}=\text{O}$  and  $\text{ArCH}_2\text{S}$  at 37 and 36 ppm.

Given the complexity of the structures, the  $^{13}\text{C}$  NMR spectra are unusually simple. For compounds 37 and 39 with symmetrical wall units where the question of regioisomers does not arise, a maximum of eight  $\text{C}=\text{O}$  resonances might be expected (meso and racemic RR and SS; four unique  $\text{C}=\text{O}$  resonances for each diastereomer). The three regioisomers of 38 would be

even more complex, potentially giving 24 unique C=O resonances. However, the macrocycles are large and flexible, and the distance between the unique carbonyl carbons at the two ends of the macrocycles are large which would tend to minimize any differences. Accidental overlap of signals and the resultant spectral simplification is an unexpected benefit in these compounds. They are mixtures of diastereomers (and regioisomers) but behave as single compounds. Consequently, the "purity" of the mixture can be established from the simple spectra observed.

The proton decoupled  $^{13}\text{C}$  NMR spectrum obtained for compound 37 in this project is compared to the reported<sup>42</sup> proton decoupled  $^{13}\text{C}$  NMR spectrum in scheme 26. The multiple peaks in spectrum I, clearly indicate the presence of a mixture of products. The sharp peaks in spectrum II indicate the presence of a substantially purer product.

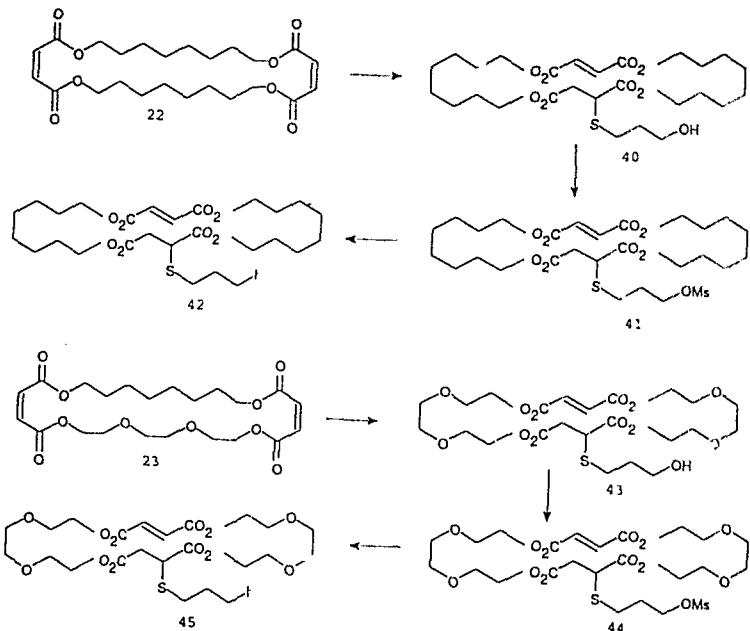
An alternative linkage reaction, similar to the reaction used by James<sup>30</sup> for the synthesis of artificial ion channels was used to link macrocycles 22 and 23 to tartaric acid. The symmetrical macrocycle 22 was reacted with one equivalent of 3-thio-1-propanol in the presence of piperidine and gave the mono-addition product 40 in 28% yield. Compound 40 was reacted with methanesulfonyl chloride at -10°C in the presence of triethylamine and gave the compound 41 in 76% yield. The reaction of 41 with sodium iodide in acetone gave the iodo compound 42 in 79% yield. In a similar manner, compounds 43,



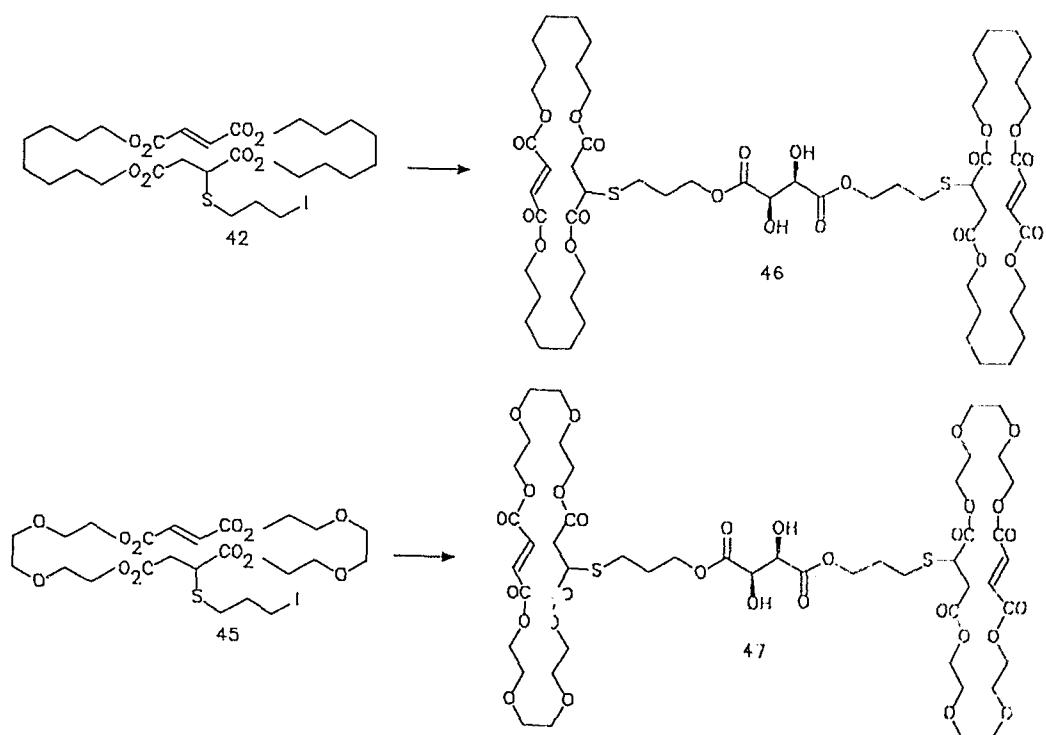
**Scheme 26.** Comparison of the proton decoupled  $^{13}\text{C}$  NMR spectrum for compound 37. The spectrum I is the reported<sup>42</sup> spectrum. Spectrum II is the  $^{13}\text{C}$  nmr spectrum obtained in this project.

44, and 45 were synthesized in 39%, 90% and 63% yield respectively (scheme 27). Note that all the macrocycles in Scheme 27 contain trans alkene bonds since in the first reaction step piperidine was used to make the mono-addition compounds 40 and 43. The reaction of compounds 42 and 45 with 2R,3R-(+)-tartaric acid in DMSO using tetramethyl ammonium hydroxide as the base gave the dimers 46 and 47 in 45% and 52% yield respectively (scheme 28). All the compounds were characterized by their <sup>1</sup>H and <sup>13</sup>C NMR spectra. The most characteristic <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts for compounds 46 and 47 were the CHOH at 4.5 ppm and CHOH at 72 ppm for tartaric acid.

To summarize, macrocycles 22, 23, and 24 were linked efficiently using an improved procedure. This is the best known procedure available to date. Thus, m-xylylene dithiol was reacted with a large excess of macrocycles 22, 23, and 24 in a 1:1 mixture of THF:IPA and catalytic amount 2,2,6,6-tetramethylpiperidine and the desired linked dimers 37, 38, and 39 were made in 24%, 13% and 24% yield. An alternative linkage reaction was attempted and proved superior to the linkage reaction with m-xylylene dithiol. Compounds 42 and 45 were reacted with 2R,3R-(+)-tartaric acid and tetramethyl ammonium hydroxide in DMF and gave the linked products 46 and 47 in 45% and 52% yield. The advantages of this linking reaction are: 1) Oligomerization is not possible and only the dimer product can be formed, and 2) only two equivalents of



**Scheme 27.** Synthesis of compounds 42 and 45.



**Scheme 28.** Linkage reaction using 2R,3R-tartaric acid.

the diiodo compounds 42 and 45 are required for the linkage reaction.

Thus far, five linked dimers (37, 38, 39, 46, and 47) were synthesized from the reaction of three different macrocycles with m-xylylene dithiol and tartaric acid. All the five linked dimers were made in sufficient quantity to carry their synthesis to the final step- the addition of the polar head groups.

#### Polar Head Groups

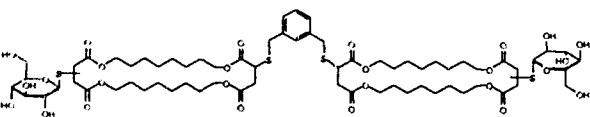
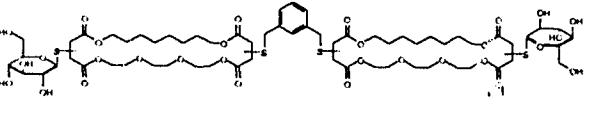
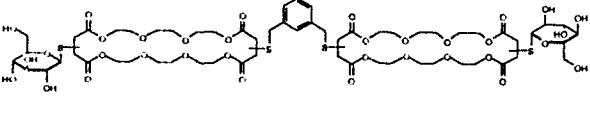
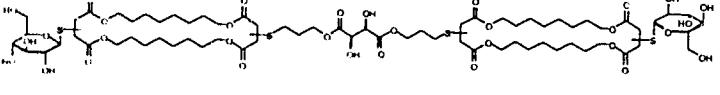
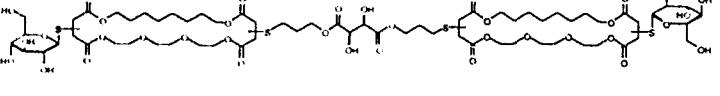
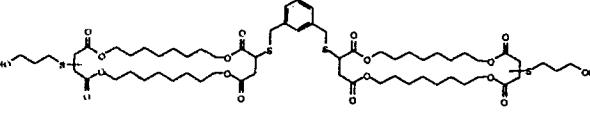
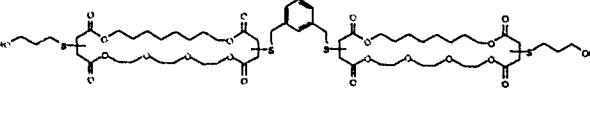
Three types of polar head groups are possible: neutral, cationic and anionic. The neutral polar head group may be represented by 1-thio- $\beta$ -D-glucose (large) and 3-thio-1-propanol (small), the cationic by 2-aminoethanethiol, and the anionic by 2-thioacetic acid. All the three different types of polar head groups were used for the synthesis of the mimics.

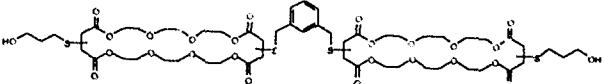
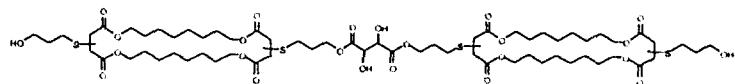
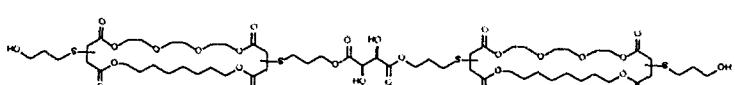
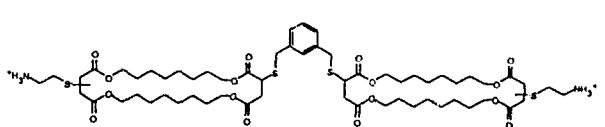
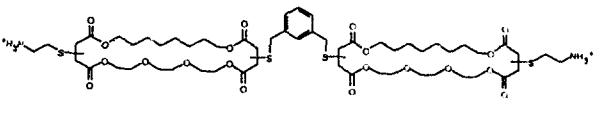
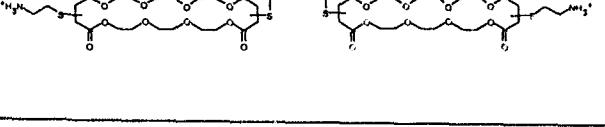
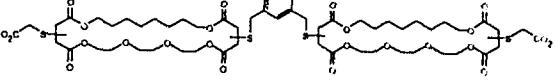
The reaction conditions used for the addition of the polar head groups were similar to the reported procedures by Fuhrhop<sup>38</sup>. Many control reactions were carried out on the macrocycle 22 to improve reaction conditions (solvent, temperature, acid and base used) for my system. The most notable difference in my synthesis was the use of 2,2,6,6-tetramethylpiperidine as the base instead of piperidine. The addition of 1-thio- $\beta$ -D-glucose was carried out in a 1:1

mixture of THF:IPA at 50°C for 10 hours, using methanesulfonic acid and 2,2,6,6-tetramethylpiperidine to adjust the pH to 9. The addition of the 3-thio-1-propanol was carried out in THF at 50°C for five hours using tetramethylpiperidine as the base catalyst. The addition of 2-aminoethanethiol was carried out in THF at 50°C for one hour using tetramethylpiperidine as the base catalyst. This reaction gave some ring opening products. The addition of 2-thioacetic acid was carried out in THF at 50°C for six hours using tetramethylpiperidine to adjust the pH to 9.

Addition of the polar head groups to compounds 37, 38, 39, 46, and 47 were successful in the first attempt (table 2). All the products were identified by their <sup>13</sup>C NMR spectra. The <sup>1</sup>H NMR spectra for these relatively large molecules were complex and were not informative, however, they showed the presence of ester CO<sub>2</sub>CH<sub>2</sub>, CH<sub>2</sub>C=O, CH<sub>2</sub>S, or tartrate CHO<sub>2</sub>H at 4.2, 2.5-2.9, 3.5-3.9, and 4.5 ppm respectively. Both the <sup>1</sup>H and <sup>13</sup>C NMR spectra showed the absence of alkene. The characteristic <sup>13</sup>C NMR spectra of the thioglucose head group showed the anomeric carbon at 87 and 88 ppm, for the thiopropanol CH<sub>2</sub>OH at 61 ppm, for the thioamine CH<sub>2</sub>N at 43 ppm, and for the thioacetic acid CH<sub>2</sub>CO<sub>2</sub>H at 34.0 and 33.9 ppm.

Table 2. The fourteen mimics synthesized

Product	CPd <sup>1</sup>	Yield <sup>2</sup> %
	<u>48</u>	90
	<u>49</u>	36
	<u>50</u>	29
	<u>51</u>	80
	<u>52</u>	78
	<u>53</u>	81
	<u>54</u>	89

	<u>55</u>	94
	<u>56</u>	94
	<u>57</u>	86
	<u>58</u>	88
	<u>59</u>	88
	<u>60</u>	82
	<u>61</u>	97

<sup>1</sup> compound number<sup>2</sup> percentage yield of the reaction

Summary

A combination of three different macrocycles, two different linkers, and four different polar head groups were used to synthesize fourteen potential pore forming mimics. The most notable feature of this approach is its simplicity. This is a short synthesis to relatively large molecules of varying structure. The macrocyclization, linkage reaction, and addition of the polar head group could be modified to allow structural variations in the target compounds. This enabled me to incorporate a number of different structural features into the final products in order to carry out a structure-activity study of these mimics. The synthesis proved very challenging yet quite satisfying once the final products were at hand.

**TRANSPORT**Introduction

The aim of this section is to survey the activities of the 14 potential pore forming mimics synthesized, and to correlate their activity to their structure.

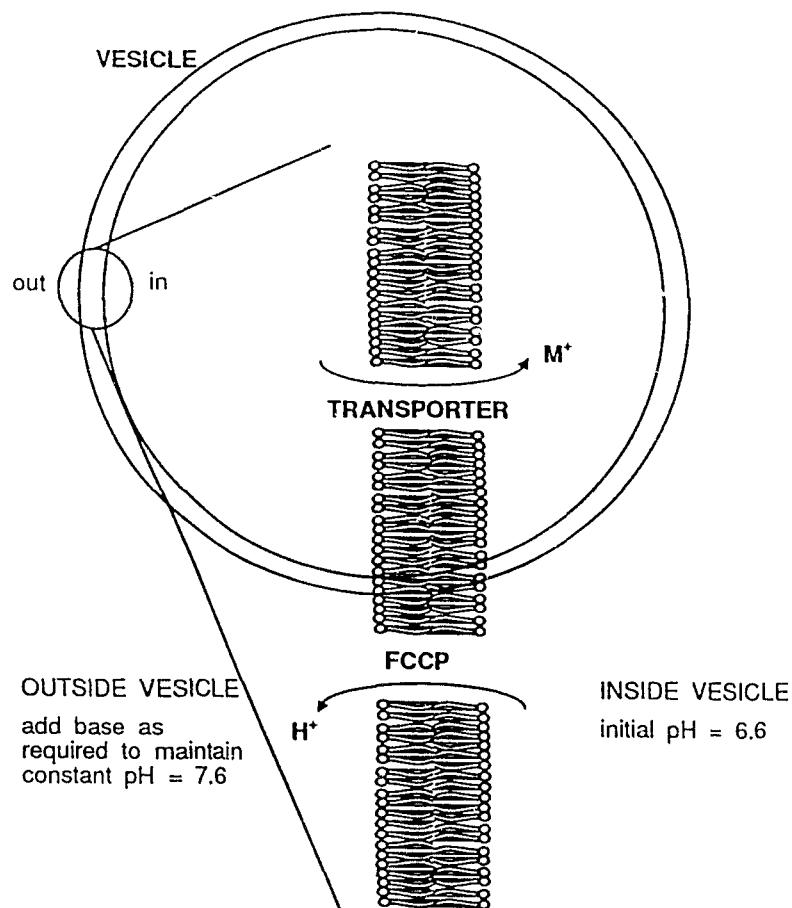
The activity of artificial pore formers could be analyzed in planar bilayers such as black lipid membranes (BLM's) or in bilayer lipid vesicles. Black lipid membranes<sup>20,61-63</sup> are formed across a thin hole separating two aqueous compartments by applying a small amount of lipid over the hole. The lipid film thins and a planar bilayer is formed, surrounded by a torus of excess lipid at the edge of the hole. Vesicles<sup>64-66</sup> are spherical, cell-like aqueous structures composed of one or more lipid bilayers. Their diameter can be small (100 Å) or large (5000 Å) and can be both uni- or multilamellar. Such vesicle systems are the model system closest to the bilayer component of cell structures and thus provide a useful model system in which to analyze transport by artificial pore formers.

In principle, vesicles are impermeable to ions and most polar molecules. This key feature has been used in vesicle studies where a set pH and ion gradient is maintained between inner and outer aqueous solutions. The addition of ionophores to the vesicle membrane collapses these gradients which can be

monitored by NMR<sup>66</sup>, fluorescence<sup>67-68</sup> or UV/VIS<sup>25</sup> absorption changes, and pH-stat titration<sup>19</sup>.

The pH-stat titration technique utilizes the electroneutral proton-cation antiport to study the kinetics of ion transport across vesicle membrane walls (Figure 1). In the presence of a transmembrane pH gradient, the protons are driven to the outside and the cations to the inside of the vesicles. Thus, the cation permeability induced by an ionophore can be measured indirectly by monitoring the proton movement across a membrane. With this technique, sufficient entrapped volume is required to detect the protons released; also, the proton release should not be rate limiting in the proton-cation exchange process.

The aim of the transport study is to survey the active compounds and their possible mechanisms. An active transporter could be a carrier, a unimolecular ion channel, or an aggregate pore. One guideline has been proposed for distinguishing between carriers and pores in vesicle membranes. Gary-Bobo et al<sup>66</sup> compared the cation permeability of valinomycin (a carrier), gramicidin D (an ion channel), and amphotericin B (an aggregate pore former) in bilayer lipid vesicles by the pH-stat titration method described above. Cation transport rate was found to be dependent on valinomycin concentration, but equilibrium (100% proton release) was always reached during the transport event. It was also observed that all vesicles appeared to be involved in the



**Figure 1.** Schematic of cation and proton antiport through a pore in a pH-stat experiment.

transport process. This progressive proton transport through the whole vesicle population suggests that valinomycin is mobile and exchanged between vesicles. In contrast, both amphotericin B and gramicidin D showed a very fast proton transport which reached a plateau value dependent on the ionophore concentration. The addition of Triton X-100

resulted in release of the remaining entrapped protons. This result coupled with a  $^{31}\text{P}$  NMR study of the transport events showed that upon addition of the ionophore gramicidin D, two vesicle populations were formed: one population which was rapidly permeabilized (through incorporation of gramicidin D), and the other not at all. These results are consistent with the idea that these ionophores are immobile between vesicles, and that they show an "all-or-none" mode of action.

#### RESULTS AND DISCUSSIONS

The egg L- $\alpha$ -lecithin vesicles used in this study were prepared by reverse evaporation<sup>69</sup>. Large unilamellar vesicles (LUV's) with high entrapment volumes and low permeation rate could be readily made and reproduced. Detailed procedures for vesicle preparation were established by Kaye<sup>31</sup>. An excerpt from her M.Sc. thesis is provided as Appendix I. My studies followed these procedures as closely as possible.

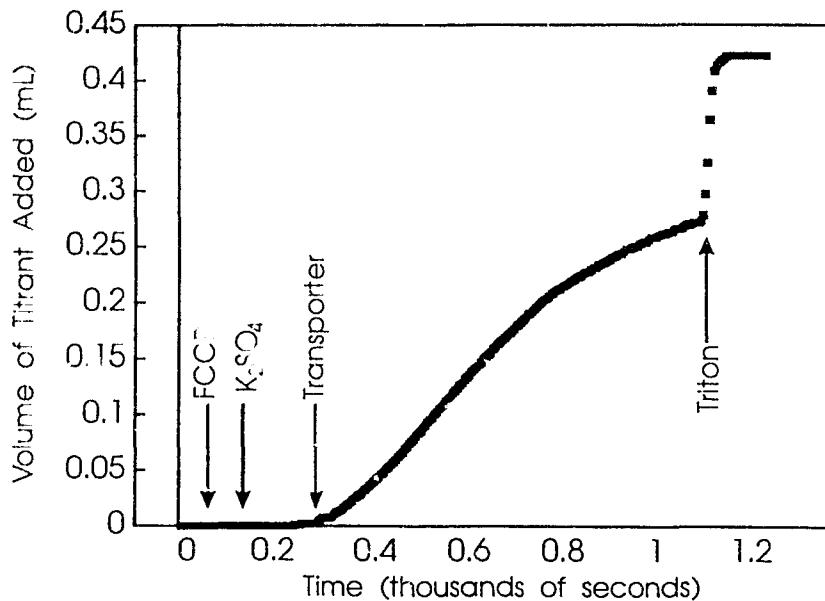
The quality of the vesicles produced by this method has been determined by Kaye<sup>31</sup>. Analysis of transmission electron micrographs has shown that vesicles possess an average diameter of 1200 Å; about 5% of the vesicles were between 2500 and 3500 Å, and 5% were less than 600 Å. The vesicle solution also contained some large lipid aggregates. The melittin assay<sup>70</sup> results indicated that in these vesicle preparations 95% of the entrapped buffer was present in unilamellar

vesicles. Therefore, the aggregates contain very little entrapped buffer.

In a typical experiment, 0.2 mL of vesicle solution was used which corresponded to 1.9 mg of phospholipid. The entrapped volume was sufficient for pH-stat detection, which required about 0.3 to 0.5 mL of choline hydroxide solution to neutralize. In all experiments more than 100 data points were collected. A typical plot of titrant volume versus time is shown in Figure 2.

The pH-stat experiment was carried out as follows: 1.8 mL of external solution (choline sulphate D-mannitol) was added to the titration cell containing 0.2 mL of the aliquot of vesicle solution. The pH of the solution was approximately 6.5 to 6.9. A pH gradient was established with the addition of choline hydroxide to the external solution; the pH was increased to 7.6, one unit higher than the internal solution (pH = 6.6). The data collection was started after this point. This is the starting point of Figure 2.

The proton carrier, carbonyl cyanide-p-trifluoromethoxy-phenyl hydrazone (FCCP), was added (1  $\mu$ L aliquot of 0.766 mM solution in methanol) to ensure that proton permeability was rapid and not the rate limiting factor in the proton-cation exchange process. The added FCCP induces a very slow base rate leakage (less than  $1 \times 10^{-10}$  mol H $^+$  s $^{-1}$ ) in the presence of metal ions and in the absence of a transporter.



**Figure 2.** Typical zero order plot of titrant volume added versus time elapsed for a pH-stat experiment (see Table 3, entry 5 for values).

Next the metal cation was added. Alkali metal sulphate salts at concentrations of 0.500 M with no pH adjustment were used. Upon addition of the metal ion solution, the pH of the solution dropped below 7.6. The lowering of pH was small for lithium and cesium, large for sodium and potassium, and largest for rubidium. This lowering of the pH was due neither to the antiport of the metal ion and proton across the membrane, nor to the lysis of vesicles; the lyse volume for the systems with and without metal ion were identical. The titrimeter was turned off before the addition of the metal

ion. After the addition, the pH was allowed to stabilize, then the titrimeter was turned on and the set pH of 7.6 was reestablished with the addition of choline hydroxide. Finally, the transporter was added and transport was observed as a function of added base.

The rate constants for all transport events were calculated according to a zero order analysis. This was done by plotting the volume of the base added versus time and calculating the slope of the best straight line. The rates obtained were reproducible to  $\pm 10\%$  within the same vesicle batch. The day to day reproducibility between different batches of vesicles was approximately  $\pm 40\%$ . For a semi-quantitative comparison of the absolute rates of all the mimics, this variability was compensated by normalization of the rates with respect to a particular compound and concentration. All other quantitative experimental comparisons were carried out within a single vesicle batch; therefore, no normalization was required.

The complete data set obtained in pH-stat experiments is tabulated in Appendix 2, and the results are discussed below. The relative activities of all the 14 mimics synthesized were compared to amphotericin, and the results are shown in Table 3. A similar table which includes the mimic structures is reproduced in Appendix 3 for easy perusal. The activity is defined here as the observed rate divided by the concentration of the mimic in the vesicle solution. Three of the mimics

(51, 52 , 59 ) had specific activities similar to or greater than that of amphotericin. By close examination of the relative activities of the 14 mimics, several general observations may be made with respect to their head groups, linking units, and walls incorporated.

There were two linking units examined: m-xylylene dithiol and tartaric acid. In general the mimics that possess tartaric acid as the linking units are active, whereas the mimics that possess m-xylylene dithiol are inactive (entries 5-9 and 11-15). These results are consistent with the notion that the tartaric acid linking unit would be able to aggregate due to the hydrogen bonding ability of the alcohol function.

Entries 3, 8, 10, and 14 may be compared to illustrate the effect of the polar head groups. Unfortunately, entries 6 and 12 cannot be directly compared because they have a different linking unit. Transport activity is found to decrease in the following order: positively charged > negatively charged > neutral. Both amine and acetate head groups are charged, a feature which is absent in the glucose and propanol head groups. The activity of the mimic with an amine head group is higher than that with acetate possibly because it has more favourable interaction with the phospholipid head groups. This observation is consistent with Gary-Bobo's observation that positively charged antibiotics are more active than negatively charged ones in anionic vesicles<sup>71</sup>.

The activity of mimics 58, 59, and 60 may be compared to illustrate the effect of the wall units (entries 2, 3, and 4). The mimic with the side-discriminated macrocycle, 59, is more active than the symmetrical mimics 58 and 60. This is consistent with the design criteria; if the mimic aggregates and forms a pore, the hydrophobic edges could point outward to the vesicle hydrocarbon and the hydrophilic edges point inward and provide a medium where metal ion transport would be facilitated. The same observation is true for the mimics 56/57 (entries 11, 12); the mimic with the side-discriminated macrocycle is the more active compound. However, this observation is not generally true. The mimics 51/52 (entries 5, 6) show the opposite behaviour. The head group in this case is much larger and this may play a dominant role.

The mimics 48 and 49 (entries 7 and 8) were found to be inactive in this study. However, an earlier report<sup>42</sup> has shown these mimics to be active. This inconsistency is believed to be due to two factors: 1) The vesicle systems used in the two studies were different. Thus, a difference in incorporation and transport of the metal ion across the bilayer vesicles would be expected. Previously these mimics were found to be 20 - 50 fold less active than amphotericin B. In my study the observed rates were small and fell short

En <sup>1</sup>	Cpd <sup>2</sup> (T)	M <sup>+</sup>	[M <sup>+</sup> ] ---- mM	[T] ---- μM	Rate ----- 1x10 <sup>-10</sup>	Activity Rate/[T] ----- 1x10 <sup>-4</sup>	RA <sup>4</sup>
1	Am <sup>3</sup>	K <sup>+</sup>	92	4.3	1.9	0.4	1.0
2	<u>58</u>	K <sup>+</sup>	97	27	3.0	0.11	0.25
3	<u>59</u>	K <sup>+</sup>	97	33	29	0.88	2.0
4	<u>60</u>	K <sup>+</sup>	97	20	1.6	0.08	0.18
5	<u>51</u>	K <sup>+</sup>	100	19	15	0.79	1.8
6	<u>52</u>	K <sup>+</sup>	100	22	10	0.46	1.0
7	<u>48</u>	K <sup>+</sup>	100	23	Inactive		
8	<u>49</u>	K <sup>+</sup>	100	17	Inactive		
9	<u>50</u>	K <sup>+</sup>	100	19	Inactive		
10	<u>61</u>	K <sup>+</sup>	100	29	3.2	0.11	0.25
11	<u>56</u>	K <sup>+</sup>	93	14	1.9	0.14	0.32
12	<u>57</u>	K <sup>+</sup>	93	21	5.6	0.27	0.61
13	<u>53</u>	K <sup>+</sup>	100	24	Inactive		
14	<u>54</u>	K <sup>+</sup>	100	18	Inactive		
15	<u>55</u>	K <sup>+</sup>	100	20	Inactive		

TABLE 3. Activity of amphotericin B and mimics. Rates are normalized for entries 1, 11, 12 only. The rates are in moles H<sup>+</sup>s<sup>-1</sup>. The activities are in liter s<sup>-1</sup>. The inactive compounds had rates 20 - 50 times smaller than amphotericin B. The relative activity is defined as the activity of the transporter divided by the activity of Amphotericin B (Amph.).

- 1) Entry number
- 2) Compound number
- 3) Amphotericin
- 4) Relative Activity

of the dynamic range of the experiment: The activity of any compound which is 20-50 times less active than amphotericin could not be quantified in my study. 2) Close examination of the <sup>13</sup>C NMR spectra of the two mimics in the previous work showed the presence of mixtures in the samples. Gel permeation chromatography had not been used, therefore, the samples could have contained mixtures of oligomers with varying length. It is not known which of the products or combination of products was active, but it points to the possibility that the length of the mimic is important in its transport ability. This could provide a clue for the further structural modification needed to improve the transport ability of these groups of mimics.

Further experiments were carried out to gain insight into the transport behaviour of the "active" mimics ( 51, 52, and 59 ). In this study, any mimic that has a relative activity of 1.0 or higher is defined as "active". The first experiment was to observe the activity of these transporters in the absence of FCCP and metal ion. The results are shown in Table 4.

All three "active" mimics were capable of proton transport in the absence of the proton carrier FCCP. This suggests that the active structures are large enough to allow proton-sulphate symport or proton-choline antiport. Amphotericin under similar conditions, is capable of proton transport. The rate of proton flux in the absence of FCCP

Compound (T)	M <sup>+</sup> ----	[M <sup>+</sup> ] ----	[T] ----	Rate before addition ----- 1x10 <sup>-10</sup>	Rate after addition ----- 1x10 <sup>-10</sup>
<u>52</u>	K <sup>+</sup>	93	21	3 ± 0.3	8 ± 0.8
<u>51</u>	K <sup>+</sup>	93	17	6 ± 0.6	15 ± 1.5
<u>59</u>	K <sup>+</sup>	94	32	30 ± 3	"lysis"

↓  
Increasing  
Pore  
Size

TABLE 4. Transport rates in presence and absence of FCCP. The rates are in moles H<sup>+</sup> s<sup>-1</sup>. Addition of 59 caused lysis; addition of Triton X-100 to the vesicle solution did not result in any further proton release.

and metal ion increases going down the table; this increase in rate could be attributed to an increase in the size of the active structures. Addition of potassium ion (in absence of FCCP) to the vesicle solution containing the mimics 51, 52, and 59 did not alter the rates. However, once FCCP was added, the rates increased for both 51 and 52. This implies that the proton transport is rate limiting and addition of FCCP in all transport experiments is required to ensure that the observed rates are due to metal ion transport and not the protons. One interesting observation should be noted: addition of Triton X-100 to the vesicle solution containing the mimic 59 did not result in any further proton release. This suggests that the mimic can act as a "detergent" and release all the entrapped

protons from the vesicles, both uni- and multilamellar.

To establish whether the mimics move between vesicles, two "add back" experiments were carried out (Table 5, Figure 3). In this experiment, 2.0 mL of the vesicle solution with the imposed pH gradient is removed from the pH-stat cell prior to the addition of the transporter. The transporter is then added to the solution and the transport is monitored. Once the transport is finished, the vesicle solution that was removed earlier is added back to the titrimeter cell and the transport is monitored. If the mimic is mobile between vesicles, some further transport would be observed. If the mimic remains bound to the first set of vesicles, no further transport would be observed (zero in the limit). The maximum possible rate can only be about half that of the rate before adding back the vesicle solution, since the concentration in the transporter is reduced by a factor of two. The results in Table 3 clearly indicate that the mimic 56 remains with the vesicles and is not mobile. Also, the transport rate after addback was sufficiently low to imply that very little mimic remained in the bulk solution. The mimic 51 shows a similar trend; however, the reduction in rate is not as large as for the mimic 58. For this reason, the full immobility of 51 in vesicles cannot be definitively concluded. This behaviour is also characteristic of amphotericin<sup>66</sup>. In contrast, the carrier ionophore valinomycin<sup>66</sup>, is mobile and permeabilizes all the vesicles. In an "add back" experiment, the transport

Compound (T)	$M^+$	$[M^+]$ mM	[T] $\mu M$	Rate before addition $1 \times 10^{-10}$	Rate after addition $1 \times 10^{-10}$
51	$K^+$	92	17	$19 \pm 2$	$5 \pm 0.5$
58	$K^+$	92	86	$29 \pm 3$	$2 \pm 0.2$

Table 5. Transport rates in "add back" experiments. The rates are in moles  $H^+ s^{-1}$ .

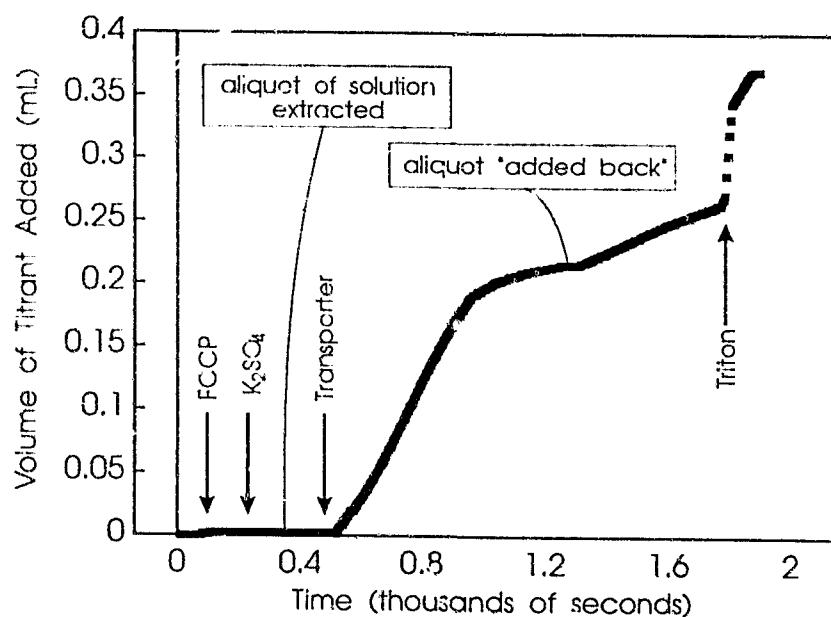


Figure 3. Typical plot of titrant volume added versus time elapsed for a pH-stat "add back" experiment (see Table 5, entry 1 for values).

rate would be the maximum possible, which is about half the initial rate.

A transport concentration dependence study of the mimics 52 and 58 was also carried out (Table 6, Figures 5-8). The apparent kinetic order of the mimics was determined by the method of initial rates.

$$\text{Rate} = k_{\text{obs}}[A]^a \cdots [N]^n$$

$$\log(\text{Rate}) = \log k_{\text{obs}} + a \log[A] + \cdots n \log[N]$$

The slope of the plot of log (initial rate) versus the log (concentration) gives the apparent kinetic order. The apparent kinetic orders for 52 and 58 are  $1.5 \pm 0.1$  and  $1.6 \pm 0.1$  respectively. The apparent kinetic orders for gramicidin D and the synthetic ion channel studied by Kaye<sup>31</sup> both showed an apparent kinetic order of 0.5. The higher order for 52 and 58 is consistent with the formation of small aggregates which increase ionic permeability. Although the transport rates for the mimic 51 had been measured at different concentrations, later analysis of the data had revealed that the rates have not been measured at low enough transport concentrations. The data obtained for 51 was mostly near the plateau region and therefore could not be used to determine the apparent kinetic order.

The alkali metal cation selectivity study for the mimics 52 and 59 was carried out (Figures 9-10). Clearly, neither of

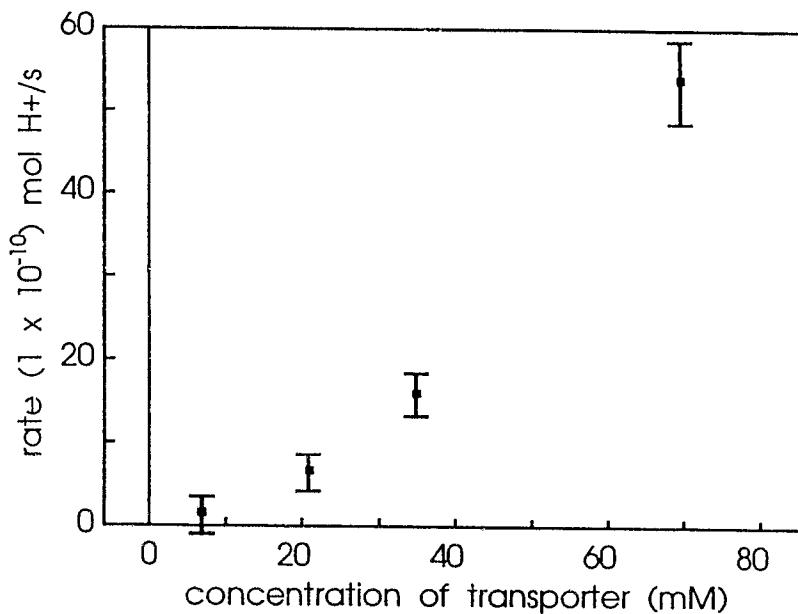
Compound (T)	$M^+$ ----	$[M^+]$ ----	[T] ----	Rate ----- $1 \times 10^{-10}$	Apparent order	$r^2$
<u>52</u>	K <sup>+</sup>	94	7	1.6	1.5	0.99
	K <sup>+</sup>	94	21	6.7		
	K <sup>+</sup>	94	35	16		
	K <sup>+</sup>	94	70	54		
<u>58</u>	K <sup>+</sup>	92	26	2.3	1.6	0.99
	K <sup>+</sup>	92	87	18		
	K <sup>+</sup>	92	130	30		
	K <sup>+</sup>	92	173	4.		

TABLE 6. Transport concentration dependence experiments. The rates are in moles H<sub>2</sub>O<sup>-1</sup>.

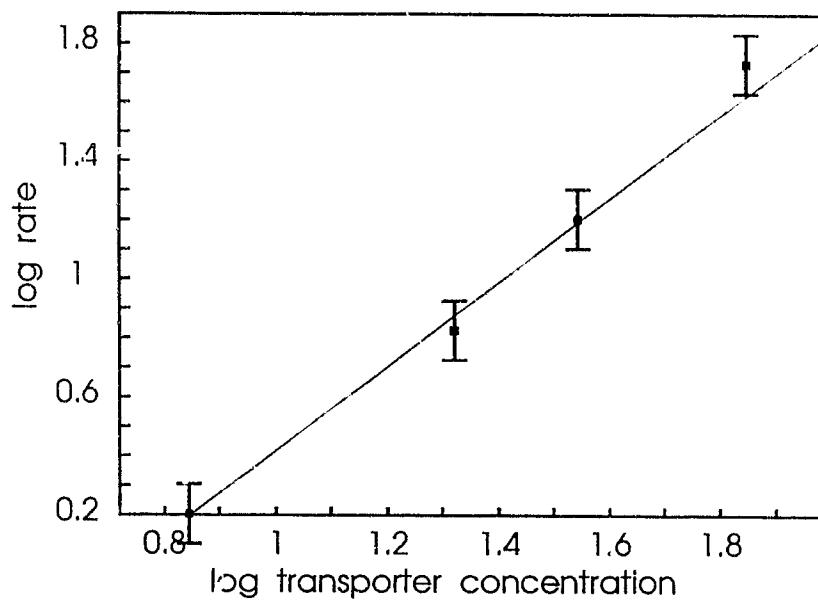
the mimics show any selectivity for a particular cation. Amphotericin<sup>34,72</sup> also shows no selectivity for alkali metal cations under the same conditions. In contrast, valinomycin<sup>73</sup> is distinctively selective towards potassium cation. This is consistent with the notion that these mimics are not carriers and behave more like the pore forming antibiotic amphotericin.

#### Summary and Conclusion

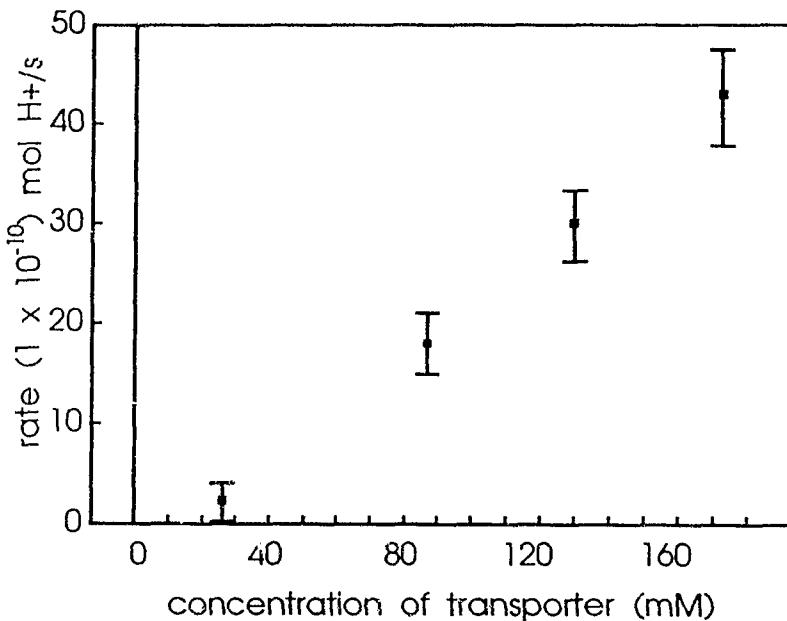
The activities of the 14 mimics were surveyed and compared to amphotericin activity. Of all the mimics synthesized, only three (51, 52, and 59) had activity comparable to or greater than amphotericin. The activity of



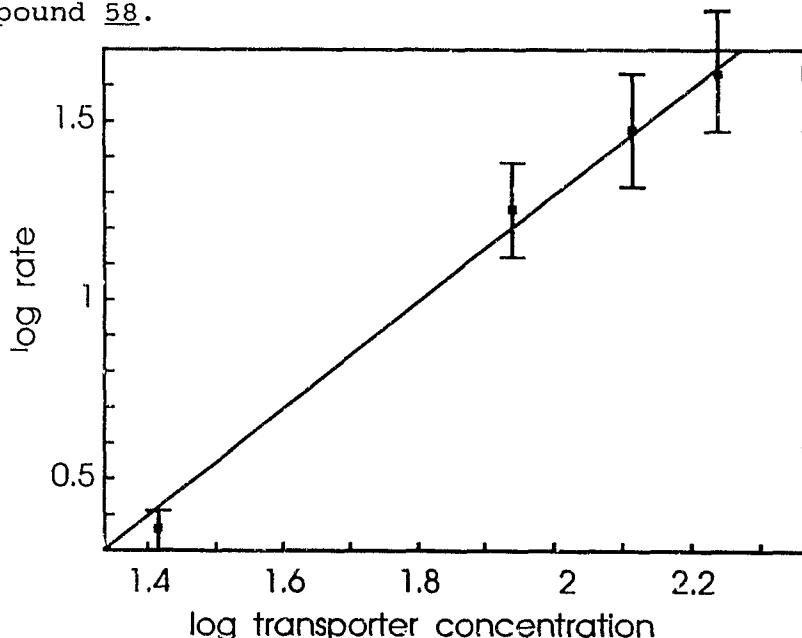
**Figure 4.** Dependence of rate on transport concentration for compound 52.



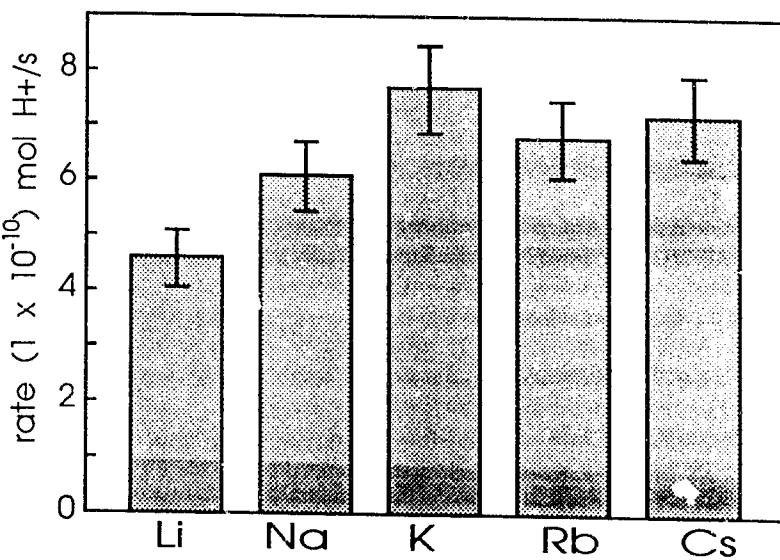
**Figure 5.** Graph of log (rate) versus log (transport concentration) for compound 52. The apparent order for the transporter is  $1.5 \pm 0.1$ . The  $r^2$  is 0.99.



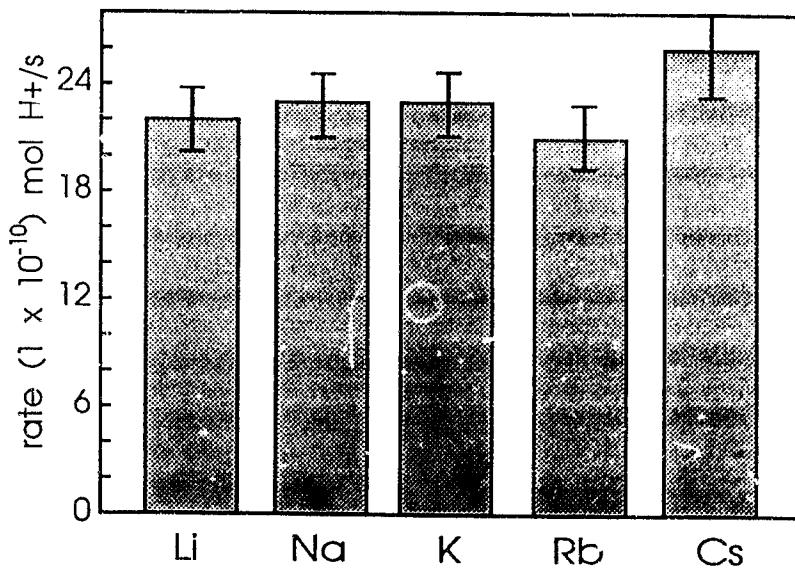
**Figure 6.** Dependence of rate on transport concentration for compound 58.



**figure 7.** Graph of log (rate) versus log (transport concentration) for compound 53. The apparent order for the transporter is  $1.6 \pm 0.1$ . The  $r^2$  value is 0.99.



**Figure 8.** Bar graph of alkali metal cation selectivities for compound 52 (see Table 8 for values).



**Figure 9.** Bar graph of alkali metal cation selectivities for compound 59 (see Table 8 for values).

the mimics with tartaric acid linking units was greater than that of the ones with m-xylylene dithiol, in a series where the polar head groups were the same. The mimics with amine and acetate (charged) polar head groups were active, whereas the mimics with glucose and propanol head groups were inactive, in a series where the linking group was m-xylylene dithiol. The transport rates in the absence of FCCP decreased in the order of 59 > 51 > 52. The "add back" experiments, the apparent kinetic orders, and the alkali metal selectivity studies are all consistent with the proposal that the mimics are not carriers but behave similarly to the pore forming antibiotic amphotericin B.

The results reveal the minimum requirements for an active pore forming mimic. The aim of this project was to rationally design molecules capable of aggregation, pore formation, and transport of metal cations. It is my belief that this goal has been met and has provided us with hope and new directions to pursue.

**EXPERIMENTAL**

Melting points were taken on a Riechardt hot stage microscope and are uncorrected. All solvents used were reagent grade. Proton NMR spectra were recorded with a Perkin Elmer R32 (90 MHz, CW), Bruker WM 250 (250.13 MHz, FT), or Bruker AMX 360 (360.14 MHz, FT) spectrometer. The spectra recorded with the R32 (90 MHz) were referenced to Me<sub>4</sub>Si as internal standard. All spectra recorded on the WM 260 or AMX 360 were referenced to the central solvent line as standard (7.24 ppm for CDCl<sub>3</sub>, 5.32 ppm for CD<sub>2</sub>Cl<sub>2</sub>, and 3.30 ppm for CD<sub>3</sub>OD, all relative to Me<sub>4</sub>Si. Some spectra were recorded in D<sub>2</sub>O with a frequency reference for this solvent previously calibrated against external Me<sub>4</sub>Si in CDCl<sub>3</sub>. Carbon NMR spectra were recorded with a Bruker WM 250 (62.89 MHz) or Bruker AMX 360 (90.57 MHz) with the central solvent line as standard (77.0 ppm for CDCl<sub>3</sub>, 53.8 ppm for CD<sub>2</sub>Cl<sub>2</sub>, 29.8 for CD<sub>3</sub>COCD<sub>3</sub>, and 49.0 for CD<sub>3</sub>OD, all relative to Me<sub>4</sub>Si). Some spectra were recorded in D<sub>2</sub>O with a frequency reference for this solvent previously calibrated against external Me<sub>4</sub>Si in CDCl<sub>3</sub>. The mass spectra were recorded with a Finnigan 3300 GC-MS instrument with methane chemical ionization. Elemental analysis were performed by Canadian Microanalytical Services, New Westminster, B.C., and are quoted as percentages.

Preparation of Polar Arms

**N,N'-bis(3-Hydroxypropyl)succinamide (1):**

A mixture of diethyl succinate (17.42 g, 100 mmol) and 3-amino-1-propanol (15.0 g, 200 mmol) was heated at 70°C for 12 hrs. The crude product was recrystallized from THF to give 1 as white crystals (16.2 g, 70%): mp = 145 - 146°C; <sup>1</sup>H NMR 250 MHz ( $\delta$ , D<sub>2</sub>O): 3.4 (t, J = 7 Hz, 4H, CH<sub>2</sub>O), 3.1 (t, J = 7 Hz, 4H, CH<sub>2</sub>N), 2.3 (s, 4H, CH<sub>2</sub>C=O), 1.5 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , D<sub>2</sub>O): 177.2 (C=O), 61.6 (CH<sub>2</sub>O), 38.7 (CH<sub>2</sub>N), 33.8 (CH<sub>2</sub>C=O), 33.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (CI, m/e): 233 (M + 1); Analysis calculated for C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>: C 51.71%, H 8.69%, N 12.06%; Found: C 51.60%, H 8.45%, N 11.86%.

**N,N'-bis(2-Hydroxyethyl)glutaramide (2):**

A mixture of diethyl glutarate (18.8 g, 100 mmol) and ethanolamine (12.2 g, 200 mmol) was heated at 80°C for 13 hrs. The crude product was recrystallized from THF: MeOH (4:1) to give 2 as white solid (10.7 g, 49%): mp = 120 - 121°C; <sup>1</sup>H NMR 250 MHz ( $\delta$ , D<sub>2</sub>O): 3.5 (t, J = 6 Hz, 4H, CH<sub>2</sub>O), 3.1 (t, J = 6 Hz, 4H, CH<sub>2</sub>N), 2.1 (t, J = 7 Hz, 4H, CH<sub>2</sub>C=O), 1.7 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , D<sub>2</sub>O): 178.6 (C=O), 62.4 (CH<sub>2</sub>O), 43.9 (CH<sub>2</sub>N), 37.3 (CH<sub>2</sub>C=O), 24.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (CI, m/e): 219 (M + 1); Analysis calculated for C<sub>9</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>: C 49.53%, H 8.31%, N 12.84%; Found: C 49.57%, H 8.07%, N 12.63%.

**N,N'-bis(2-Hydroxyethyl)isophthalamide (3):**

Diethyl isophthalate (8.9 g, 40 mmol) and ethanolamine (4.9 g, 80 mmol) were heated at 98 - 105°C for 20 hrs. The crude product was recrystallized from THF to give 3 as white solid (6.5 g, 65%): mp = 146 - 147°C; <sup>1</sup>H NMR 250 MHz ( $\delta$ , D<sub>2</sub>O): 7.1 - 7.6 (m, 4H, aromatic CH), 3.5 (t, J = 7 Hz, 4H, CH<sub>2</sub>O), 3.2 (t, J = 7 Hz, 4H, CH<sub>2</sub>N); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , D<sub>2</sub>O): 172.1 (C=O), 136.3, 132.7, 131.6, 128.2 (aromatic CH), 62.4 (CH<sub>2</sub>O), 44.5 (CH<sub>2</sub>N); MS (CI, m/e): 253 (M + 1); Analysis calculated for C<sub>12</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>: C 57.13%, H 6.39%, N 11.1%; Found C 57.64%, H 6.41%, N 11.04%.

**N,N'-bis(3-Hydroxypropyl)urea (4):**

A mixture of diethyl carbonate (20.0 g, 169 mmol) and 3-amino-1-propanol (25.4 g, 338 mmol) was heated at 148 - 150°C for 41 hrs. The crude product was recrystallized from acetonitrile to give 4 as a white powder (21.2 g, 71%): mp 93 - 94°C; <sup>1</sup>H NMR 90 MHz ( $\delta$ , D<sub>2</sub>O): 3.5 (t, J = 7 Hz, 4H, CH<sub>2</sub>O), 3.1 (t, J = 7 Hz, 4H, CH<sub>2</sub>N), 1.6 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , D<sub>2</sub>O): 160.7 (C=O), 59.2 (CH<sub>2</sub>O), 46.7 (CH<sub>2</sub>N), 31.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (CI, m/e): 177 (M + 1); Analysis calculated for C<sub>7</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>: C 47.71%, H 9.15%, N 15.9%; Found: C 47.66%, H 9.24%, N 15.75%.

**2,6-bis(Chloroacetamido)pyridine (5):**

Chloroacetyl chloride (82.8 g, 733 mmol, 4 eq) was added

dropwise to a solution of 2,6-diaminopyridine (20.0 g, 183 mmol) and triethylamine (200 mL, excess) in 600 mL of dry THF at -78°C under N<sub>2</sub> atmosphere. The reaction was allowed to come to room temperature and was stirred overnight. The solvent was evaporated under reduced pressure. To the black colored solid residue was added 800 mL of H<sub>2</sub>O and the product was extracted with chloroform (400 mL x 3). The combined organic extract was dried over MgSO<sub>4</sub> and was filtered through 30 grams of silica-gel. The yellow colored eluent was evaporated under reduced pressure and the yellow solid residue was triturated with diethyl ether (200 mL). Filtration gave 5 as a white powder (34.1 g, 71%): mp = 167 - 168°C; <sup>1</sup>H NMR 90 MHz ( $\delta$ , CDCl<sub>3</sub>): 8.6 (br, 2H, NH), 7.6 - 8.0 (m, 3H, aromatic CH<sub>2</sub>) 4.2 (s, 4H, CH<sub>2</sub>Cl); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CDCl<sub>3</sub>): 164.3 (C=O), 148.7, 141.1, 110.2 (aromatic CH), 42.7 (CH<sub>2</sub>Cl); MS (CI, m/e): 263 (M + 1); Analysis calculated for C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>Cl<sub>2</sub>: C 41.24%, H 3.46%, N 16.03%, Cl 27.05%; Found: C 40.95%, H 3.44%, N 15.80%, Cl 28.75%.

#### **2,6-bis(Acetoxyacetamido)pyridine (6):**

A mixture of 5 (34.0 g, 130 mmol) and sodium acetate (23.4 g, 285 mmol, 2.2 eq) in 500 mL of dry DMF was heated at 38°C for 14 hrs under a N<sub>2</sub> atmosphere. The temperature was increased to 50°C and the mixture was stirred for another 8 hr. The solvent was evaporated under reduced pressure. To the black colored solid residue was added 400 mL of H<sub>2</sub>O and

the product was extracted with dichloromethane (400 mL x 3). The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$  and were passed through 30 grams of silica-gel. Evaporation of the eluent gave 6 as a white solid (35.9 g, 89%): mp = 150 - 151°C;  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 8.2 (br, 2H, NH), 7.6 - 8.0 (m, 3H, aromatic CH), 4.7 (s, 4H,  $\text{CH}_2\text{O}$ ), 2.2 (s, 6H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR 62.89 (MHz ( $\delta$ ,  $\text{CDCl}_3$ )): 169.5 (ester C=O), 165.5 (amide C=O), 148.6, 141.0, 110.0 (aromatic CH), 63.1 ( $\text{CH}_2\text{O}$ ), 20.7 ( $\text{CH}_3$ ); MS (CI, m/e): 310 (M + 1); Analysis calculated for  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_6$ : C 50.49%, H 4.89%, N 13.59%; Found: C 50.63%, H 4.80%, N 13.70%.

**2,6-bis(Hydroxyacetamido)pyridine (7):**

A mixture of the diacetate 6 (32.09 g, 104 mmol) and potassium hydroxide (11.6 g, 208 mmol, 2 eq) were dissolved in a mixture of methanol (500 mL) and water (200 mL) at room temperature. The reaction mixture was stirred at room temperature for 40 hrs. The solid product was filtered and was placed into a flask containing 200 mL of  $\text{H}_2\text{O}$ . The mixture was allowed to stir for two hours, and the solid product was filtered and washed with water (100 mL) and acetone (50 mL). Residual water was removed with a vacuum pump to give 7 as a white powder (17.7 g, 76%): mp = 221 - 222°C;  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CD}_3\text{OD}$ ): 8.3 (br, 2H, NH), 7.6 - 8.0 (m, 3H, aromatic CH), 4.1 (s, 4H,  $\text{CH}_2\text{O}$ );  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CD}_3\text{OD}$ ): 173.4 (C=O), 150.8, 141.6, 110.8 (aromatic CH), 62.9 ( $\text{CH}_2\text{O}$ ); MS (CI, m/e):

226 (M + 1); Analysis calculated for C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub>:C 48.00%, H 4.92%, N 18.66%; Found C 47.84%, H 4.94%, N 18.52%.

**1,3-bis(Chloroacetamido)benzene (8):**

Chloroacetyl chloride (8.4 g, 74 mmol, 4 eq, in 50 mL of dry THF was added dropwise into a solution of 1,3-phenylenediamine (2.0 g, 18 mmol) and triethylamine (12 mL, excess) in 50 mL of dry THF at room temperature under a N<sub>2</sub> atmosphere. The reaction was stirred for 48 hr. The solvent was removed under reduced pressure and the black colored residue was pre-adsorbed on a silica-gel column. The product was eluted with dichloromethane:methanol (98:2). The solvent was evaporated under reduced pressure. The pale brown residue was taken up in 500 mL of dichloromethane which was washed with H<sub>2</sub>O (400 mL) and saturated sodium bicarbonate solution (200 mL). It was dried over MgSO<sub>4</sub>, filtered and evaporated to dryness. The solid residue was recrystallized from acetone and activated carbon to give 8 as a white solid (0.9 g, 18%): mp = 220 - 221°C; <sup>1</sup>H NMR 90 MHz ( $\delta$ , CD<sub>3</sub>COCD<sub>3</sub>): 8.0 (br, 2H, NH), 7.2 - 7.5 (m, 4H, aromatic CH), 4.2 (s, 4H, CH<sub>2</sub>Cl); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CD<sub>3</sub>COCD<sub>3</sub>): 165.4 (C=O), 139.9, 130.0, 116.2, 111.7 (aromatic CH), 44.1 (CH<sub>2</sub>Cl); MS (CI, m/e): 261 (M + 1); Analysis calculated for C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>:C 46.0%, H 3.86%, N 10.73%, Cl 27.16%; Found: C 46.02%, H 3.88%, N 10.74%, Cl 29.48%.

Preparation of macrocycles (12-series)**5,18-Dioxa-4,19-dioxodocosa-2,20-diene-1,22-dicarboxylic acid (13):**

A mixture of maleic anhydride (9.8 g, 100 mmol, 2 eq) and 1,12-dodecanediol (10.2 g, 50 mmol) were refluxed in dry benzene for 8 hr. On standing at room temperature 13 crystallized from the solution and was recrystallized from methyl ethyl ketone to give 13 as a white solid (18.9 g, 95%); mp = 117 - 118°C. It's <sup>1</sup>H and <sup>13</sup>C NMR spectra were identical to reported spectra by Fuhrhop.<sup>38</sup>

**1,6,19,24-Tetraoxa-2,5,20,23-tetraoxocyclohexatricosa-3,21-diene (14):**

A mixture of the dicarboxylic acid 13 (3.9 g, 10 mmol), 1,12-dodecanediol (2.0 g, 10 mmol), and p-toluenesulfonic acid (1 g) were refluxed in 700 mL of benzene for 12 hr with azeotropic removal of water (Dean-Stark). The solvent was removed under reduced pressure and the residue was recrystallized twice from ethyl acetate to give 14 as a white solid (1.6 g, 28%); mp = 104 - 105°C. It's <sup>1</sup>H and <sup>13</sup>C NMR spectra were identical to reported spectra by Fuhrhop.<sup>38</sup>

A one-pot synthesis of 14 was attempted and gave 14 in 17% yield.

**Preparation of 14 by fusion:**

Maleic anhydride (0.98 g, 10 mmol), 1,12-dodecanediol

(2.0 g, 10 mmol), p-toluenesulfonic acid (0.2 g) and biphenyl (100 g) were ground to a fine powder. The mixture was heated at 130°C for 3 hrs. On cooling to room temperature, the mixture solidified. The product was purified on a silica-gel column (300 g), and was eluted with hexane:ethyl acetate (7:3). Yield: (0.14 g, 5%).

**Preparation of 14 by cesium carbonate method:**

1,12-diiodooctane (2.2 g, 5 mmol) in 100 mL of dry DMF was added dropwise over a 2 hour period into a solution of the diacid 13 (2.1 g, 5 mmol) and cesium carbonate (3.4 g, 10 mmol, 2 eq) in 200 mL of dry DMF at 80°C under N<sub>2</sub> atmosphere. The reaction was heated for 15 hrs and solvent was evaporated under reduced pressure. The product was purified on a silica-gel column (100 g). and was eluted with dichloromethane:methanol (99.5: 0.5) Yield: (0.12 g, 4%). The spectra matched the earlier spectra obtained in other preparations. The only notable difference in the <sup>1</sup>H NMR spectra was the presence of a trans CH=CH at 6.8 ppm.

**1,6,9,12,15,18,21,26-octaoxa-2,5,22,25-tetraoxocyclooctatricosa-3,23-diene (15):**

A mixture of the diacid 13 (5.0 g, 13 mmol), pentaethylene glycol (3.0 g, 13 mmol), and p-toluenesulfonic acid (1 g) were refluxed in toluene (1.5 L) for 18 hr with azeotropic removal of water (Dean-Stark). The solvent was evaporated under reduced pressure. The pale yellow syrupy

residue was taken up in ethyl acetate (100 mL) and was washed with water (30 mL x 3). The organic solution was dried over magnesium sulphate and was evaporated under reduced pressure. The residue was purified on a silica-gel column (300 g) and the product was eluted with hexane:ethyl acetate (2:8) to give 15 as a colorless oil (0.70 g, 9%);  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 6.2 (s, 4H,  $\text{CH}=\text{CH}$ ), 4.2 (m, 8H,  $\text{CO}_2\text{CH}_2$ ), 3.7 (m, 4H,  $\text{CO}_2\text{CH}_2\text{CH}_2\text{O}$ ), 3.6 (s, 12H,  $\text{OCH}_2\text{CH}_2\text{O}$ ), 1.6 (m, 4H,  $\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ), 1.3 (br, s, 16H,  $\text{CH}_2\text{CH}_2\text{CH}_2$ );  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 165.1, 165.0 ( $\text{C}=\text{O}$ ), 130.1, 129.2 ( $\text{CH}=\text{CH}$ ), 70.5 ( $\text{OCH}_2\text{CH}_2\text{O}$ ), 68.7 ( $\text{CO}_2\text{CH}_2\text{CH}_2\text{O}$ ), 65.3 ( $\text{CO}_2\text{CH}_2\text{CH}_2\text{O}$ ), 64.2 ( $\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ), 29.4, 29.1, 28.9, 28.3, 28.2, 25.7, 25.6 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ); MS (CI, m/e): 601 (M + 1); Analysis calculated for  $\text{C}_{30}\text{H}_{48}\text{O}_{12}$ : C 59.98%, H 8.05%; Found: C 59.88%, H 7.97%.

A one-pot synthesis of 15 was attempted and gave 15 in 9% yield.

#### Preparation of 15 by fusion:

Maleic anhydride (1.9 g, 20 mmol), 1,12-dodecanediol (2.02 g, 10 mmol), p-toluenesulfonic acid (0.2 g) and biphenyl (100 g) were ground to a fine powder which was added to a flask containing pentaethylene glycol (2.4 g, 10 mmol). The mixture was heated at 130°C for 3 hr. On cooling to room temperature the mixture solidified. The product was purified on a silica-gel column (300 g), and was eluted with hexane:ethyl acetate (2:8). Yield: (0.09 g, 3%).

Preparation of macrocycles (8-series)

**5,4-dioxa-4,15-dioxooctadecan-2,16-diene-1,18-dicarboxylic acid (21):**

A mixture of 1,8-octanediol (20.0 g, 137 mmol) and maleic anhydride (33.5 g, 342 mmol, 2.5 eq) were refluxed in 100 mL of benzene for 10 hrs. The solvent was evaporated under reduced pressure and the solid residue was triturated with diethyl ether (20 mL) to give the diacid 21 as a white powder (45.4 g, 97%); <sup>1</sup>H NMR 90 MHz ( $\delta$ , CDCl<sub>3</sub>): 9.8 (br, s, 2H, CO<sub>2</sub>H), 6.2 (s, 4H, CH=CH), 4.1 (t, J = 6 Hz, 4H, CH<sub>2</sub>O), 1.5 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.2 (br, s, 8H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CDCl<sub>3</sub>): 167.1, 166.3 (C=O), 133.5, 130.5 (CH=CH), 66.5 (CH<sub>2</sub>O), 28.7, 28.1, 25.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (CI, m/e): 343 (M + 1).

**1,6,15,20-tetraoxo-2,5,16,19-tetraoxocyclooctacosa-3,17-diene (22):**

A mixture of the diacid 21 (15.3 g, 45 mmol), 1,8-octanediol (6.6 g, 45 mmol) and methanesulfonic acid (5 drops) were refluxed in benzene (1.0 L) for 12 hr with azeotropic removal of water (Dean-Stark). The solvent was removed under reduced pressure and the solid residue was triturated with diethyl ether (100 mL) followed by recrystallization from ethyl acetate to give 22 as a colorless crystalline solid (2.3 g, 11%): mp = 104 - 105°C; <sup>1</sup>H NMR 90 MHz ( $\delta$ , CDCl<sub>3</sub>): 6.2 (s, 4H, CH=CH), 4.2 (t, J = 6 Hz, 8H, CH<sub>2</sub>O), 1.6 (m, 8H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.4 (br, s, 16H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>; <sup>13</sup>C NMR 62.89 (MHz ( $\delta$ ,

$\text{CDCl}_3$ ): 165.2 (C=O), 129.6 (CH=CH), 65.3 ( $\text{CH}_2\text{O}$ ), 29.1, 28.4, 25.8 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ); MS (CI, m/e): 453 (M + 1); Analysis calculated for  $\text{C}_{24}\text{H}_{36}\text{O}_8$ : C 63.69%, H, 8.01%; Found: C 63.6%, H 7.96%.

A one-pot synthesis of 22 was attempted and gave 22 in 4% yield. The spectra were identical to the spectra obtained in the two step reaction.

**Preparation of 22 by cesium carbonate:**

1,8-diiodooctane (10.7 g, 29 mmol) in 100 mL of dry DMF was added dropwise over a 2 hr. period into a solution of the diacid 21 (10.0 g, 29 mmol) and cesium carbonate (19.0 g, 58 mmol, 2 eq) in 400 mL of dry DMF at 80°C under a  $\text{N}_2$  atmosphere. The reaction was heated for 12 hr and the solvent was evaporated under reduced pressure. The product was purified on a silica-gel column (200g) and was eluted with hexane:ethyl acetate (8:2). Yield: (4.4 g, 34%). The spectra matched the earlier spectra obtained in the acid catalyzed dehydration reaction. The only notable difference in the  $^1\text{H}$  NMR spectra was the presence of trans CH=CH at 6.8 ppm.

**1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-3,17-diene (23):**

A mixture of the diacid 13 (20.5 g, 59 mmol), triethylene glycol (9.00 g, 59 mmol), and methanesulfonic acid (10 drops) in benzene (1.5 L) was refluxed for 16 hrs with azeotropic

removal of water (Dean-Stark). The solvent was removed under reduced pressure and the oily residue was preadsorbed on neutral alumina (50 g) which was added to a silica-gel column (200 g). The crude product (3.2 g) was eluted with hexane: ethyl acetate (1:1) which was purified by bulb-to-bulb distillation (220°C, 10<sup>-3</sup> mm Hg) to give 23 as a colorless oil (1.6 g, 6%); <sup>1</sup>H NMR ( $\delta$ , CDCl<sub>3</sub>): 6.2 (s, 4H, CH=CH), 4.2 (m, 8H, CO<sub>2</sub>CH<sub>2</sub>; 3.7 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.6, (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>O), 1.6 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.4 (br s, 8H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR ( $\delta$ , CDCl<sub>3</sub>): 165.2, 165.0 (C=O), 130.2, 129.1 (CH=CH), 70.5 (OCH<sub>2</sub>CH<sub>2</sub>O), 68.8 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.3 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 64.2 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 28.8, 28.3, 25.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (Cl, m/e): 457 (M + 1); Analysis calculated for C<sub>22</sub>H<sub>32</sub>O<sub>10</sub>: C 57.89%, H 7.07%; Found: C 58.14%, H 7.10%.

**Preparation of 23 by cesium carbonate method:**

A mixture of the diacid 13 (6.8 g, 20 mmol) and the diiodide 25 (7.4 g, 20 mmol) in 200 mL of dry DMF was added dropwise over a 54 hr period into a solution of cesium carbonate (13.0 g, 40 mmol, 2 eq) in 200 mL of dry DMF at 82°C under a N<sub>2</sub> atmosphere. The reaction was heated for 67 hr and the solvent was evaporated under reduced pressure. The solid residue was preadsorbed on neutral alumina (70 g) which was added to a silica-gel column (70 g). The product was eluted with hexane: ethyl acetate to give 23 as a colorless oil (0.62 g, 7%).

The same reaction was carried out with tetrabutyl ammonium hydroxide and gave 23 in 4% yield.

**1,6,9,12,15,20,23,26-octaoxa-2,5,16,19-tetraoxocyclooctacosa-3,17-diene (24):**

Three separate reactions were carried out simultaneously as follows: A mixture of triethylene glycol (10.0 g, 67 mmol) and maleic anhydride (13.1 g, 134 mmol) were refluxed in 1.0 L of benzene overnight. Triethylene glycol (10.0 g, 67 mmol), methanesulfonic acid (15 drops), and benzene (500 mL) were added to the cold reaction, and the mixture was refluxed with azeotropic removal of water (Dern-Stark) for 24 hr. The solvent was removed under reduced pressure. The combined oily residue from all the three reactions were preadsorbed on neutral alumina (200 g) and were added to a silica-gel column (2090 g). The product was eluted with ethyl acetate. Solvent was evaporated under reduced pressure and the oily residue was seeded and triturated with diethyl ether (100 mL) or ethyl acetate (50 mL) to yield 24 as colorless crystals (5.1 g, 11%); <sup>1</sup>H NMR ( $\delta$ , CDCl<sub>3</sub>): 6.2 (s, 4H, CH=CH), 4.2 (t, J = 3 Hz, 8H, CO<sub>2</sub>CH<sub>2</sub>), 3.6 (m, 8H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.5 (s, 8H, OCH<sub>2</sub>CH<sub>2</sub>O); <sup>13</sup>C NMR ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 165.4 (C=O), 130.0 (CH=CH), 70.9 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.0 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 64.7 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O); MS (CI, m/e): 461 (M + 1); Analysis calculated for C<sub>20</sub>H<sub>28</sub>O<sub>12</sub>: C 52.17%, H 6.13%; Found: C 51.9%, H 6.03%.

**10,12-diamido-1,6,16,21-tetraoxa-2,5,11,17,20-pentaoxacyclononacosa-3,18-diene (30):**

A solution of the diol 4 (2.0 g, 11 mmol), maleic anhydride (2.23 g, 22 mmol, 2 eq) and one drop of conc. sulfuric acid in 100 mL of dry DMF was heated at 65°C for 29 hr. This solution was added to a dropping funnel containing 1,8-diiodooctane (4.15 g, 11 mmol) and dry DMF (150 mL). This was added dropwise over a 24 hr period into a solution of cesium carbonate (7.50 g, 23 mmol, 2.1 eq) in dry DMF (1.0 L) at 80°C. The reaction was heated for a total of 39 hr. The solvent was evaporated under reduced pressure. The residue was taken up in dichloromethane (600 mL) and was filtered and evaporated under reduced pressure. The product was purified on a silica-gel column (120 g) and was eluted with dichloromethane:methanol (95:5). The solvent was evaporated to dryness and the pale yellow oily residue was triturated with 50 mL of diethyl ether, to give 30 as white crystalline needles (11 mg, 0.2%): mp = 133 - 134°C; <sup>1</sup>H NMR ( $\delta$ , CDCl<sub>3</sub>): 6.2 (s, 4H, CH=CH), 4.9 (br, 2H, NH), 4.2 (m, 8H, CO<sub>2</sub>CH<sub>2</sub>), 3.2 (t, J = 7 Hz, 4H, CH<sub>2</sub>N), 1.9 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.6 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.4 (br s, 8H, CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>); <sup>13</sup>C NMR ( $\delta$ , CDCl<sub>3</sub>): 165.7, 165.4 (ester C=O), 158.2 (urea C=O), 130.3, 129.2 (CH=CH), 65.4 (CO<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>6</sub>CH<sub>2</sub>O), 63.0 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 36.9 (CH<sub>2</sub>N), 28.8, 28.6, 28.2, 25.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (CI, m/e): 483 (M + 1); Analysis calculated for C<sub>23</sub>H<sub>34</sub>N<sub>2</sub>O<sub>9</sub>: C 57.25%, H 7.10%, N 5.81%; Found: C 56.63%, H 6.80%, N 5.67%.

**10,12,25,27-tetraamido-1,6,16,21-tetraoxa-2,5,11,17,20,26-hexaoxocyclotricosa-3,18-diene (31):**

A mixture of the diol 4 (2.0 g, 113 mmol), maleic anhydride (1.1 g, 113 mmol) and methanesulfonic acid (10 drops) was refluxed in 500 mL of benzene with azeotropic removal of water (Dean-Stark) for 48 hr. The diol 4 was insoluble in the reaction mixture, but slowly dissolved as the reaction proceeded. The solvent was evaporated under reduced pressure and the oily residue was purified on a silica-gel column (100 g). The product was eluted with dichloromethane: methanol (1:1) and the solvent was evaporated to dryness. The residue was dissolved in methanol (50 mL). Upon addition of diethyl ether (12 mL) to this solution, the product 31 came out of the solution as a sticky oil (53 mg, 2%); <sup>1</sup>H NMR ( $\delta$ , CD<sub>3</sub>OD): 6.2 (s, 4H, CH=CH), 4.2 (t, J = 7 Hz, 8H, CO<sub>2</sub>CH<sub>2</sub>), 3.2 (t, J = 7 Hz, 8H, CH<sub>2</sub>N), 1.7 (m, 8H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N); <sup>13</sup>C NMR ( $\delta$ , CD<sub>3</sub>OD): 167.2 (ester C=O), 161.1 (urea C=O), 131.1 (CH=CH), 64.1 (CO<sub>2</sub>CH<sub>2</sub>), 37.8 (CH<sub>2</sub>N), 30.2 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N); MS (CI, m/e): 513 (M + 1); Analysis calculated for C<sub>22</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>: C 51.56%, H 6.29%, N 10.93%; Found: C 48.32%, H 6.22%, N 10.18%.

**bicyclo[26,3,1]-2,27-diamido-5,10,19,24-tetraoxa-6,9,20,23-tetraoxo-1,7,21,28,30-pentaene (34):**

A mixture of the dichloride 5 (2.0 g, 7.6 mmol) and sodium iodide (11.5 g, 76 mmol, 10 eq) in 200 mL of acetone was refluxed under a N<sub>2</sub> atmosphere for 20 hr. The solvent was evaporated under reduced pressure. The crude diiodo compound

33 was mixed with the diacid 21 (2.6 g, 7.6 mmol) in 300 mL of dry DMF and the mixture was added dropwise over a 12 hr period to a solution of cesium carbonate (5.0 g, 15 mmol, 2 eq) in 200 mL of dry DMF at 80°C under a N<sub>2</sub> atmosphere. The reaction was heated for a total of 24 hrs. The solvent was removed under reduced pressure. The orange solid residue was taken up in chloroform (800 mL) and was washed with water (200 mL x 3). The organic layer was separated, dried over magnesium sulphate, filtered, and evaporated under reduced pressure. The residue was purified on a silica-gel column (60 g) and the product was eluted with dichloromethane:methanol (98:2). Solvent was evaporated under reduced pressure and the residue was triturated with diethyl ether (300 mL) to give 34 as a white powder (0.13 g, 3%): mp = 219 - 220°C; <sup>1</sup>H NMR ( $\delta$ , CDCl<sub>3</sub>): 7.2 - 7.5 (m, 4H, aromatic CH), 6.8 (s, 4H, CH=CH), 4.8 (s, 4H, CO<sub>2</sub>CH<sub>2</sub>C=O), 4.2 (t, J = 6 Hz, 4H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.6 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.4 (br, s, 8H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR ( $\delta$ , CDCl<sub>3</sub>): 164.6, 164.5, 163.8 (C=O); 137.3, 130.1, 116.8, 111.4 (aromatic CH=CH), 135.8, 131.5 (alkene CH=CH), 65.5 (CO<sub>2</sub>CH<sub>2</sub>C=O), 63.4 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 28.9, 28.6, 25.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (CI, m/e): 531 (M + 1); Analysis calculated for C<sub>26</sub>H<sub>30</sub>N<sub>2</sub>O<sub>10</sub>: C 58.86%, H 5.70%, N 5.28%; Found: C 58.84%, H 5.50%, N 5.27%.

Linkage Reactions

**Bis(3- and/or 4-,  $\alpha,\alpha'$ -dithia)-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-m-xylene (37):**

Meta-xylene dithiol (113 mg, 663  $\mu$  mol, from a stock solution in THF:isopropanol, 1:1) was added dropwise over a 1.5 hr period into a solution of 22 (3.0 g, 663 mmol, 10 eq) and 2,2,6,6-tetramethylpiperidine (5 drops) in 15 mL of THF at 79 - 80°C under a N<sub>2</sub> atmosphere. The reaction was heated for a total of 14 hr. The solvent was evaporated under reduced pressure and the residue was purified on a silica-gel column (50 g) to remove excess unreacted 22. The product was eluted with dichloromethane:methanol (95:5); the solvent was evaporated under reduced pressure and the oily residue was further purified on a gel permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform: methanol (4:3) and was collected in fraction 9 - 10 (2 mL). Evaporation of the solvent gave 35 as a colorless oil (172 mg, 24%); <sup>1</sup>H NMR ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 7.3 - 7.2 (br m, 4H, aromatic CH), 6.8, 6.2 (S, 4H, trans and cis CH=CH), 4.2 (m, 16H, CO<sub>2</sub>CH<sub>2</sub>), 3.9 - 3.5 (m, 6H, ArCH<sub>2</sub>SCH), 2.9 - 2.5 (m, 4H, CH<sub>2</sub>C=O), 1.6 (m, 16H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.3 (br s, 32H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.3, 170.3, 165.5 (C=O), 138.1, 133.7, 130.0, 129.8, 128.9, 128.3 (CH=CH), 65.6, 65.5, 65.2 (CO<sub>2</sub>CH<sub>2</sub>), 41.7, 41.6 (CHS), 36.8, 36.0 (CH<sub>2</sub>C=O, ArCH<sub>2</sub>), 29.5, 28.9, 28.8, 26.1, 25.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); Analysis calculated for C<sub>56</sub>H<sub>82</sub>O<sub>16</sub>S<sub>2</sub>: C 62.55%, H 7.69%, S 5.96%; Found: C 61.80%, H 7.42%, S 6.80%.

Bis(3-*and/or* 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-m-xylene (38):

meta-xylene dithiol (112 mg, 657  $\mu$  mol, from a stock solution in THF:isopropanol, 1:1) was added dropwise over a 1.5 hr period into a solution of 23 (3.0 g, 657 mmol, 10 eq) and 2,2,6,6-tetramethylpiperidine (5 drops) in 15 mL of THF at 79 - 71°C under a N<sub>2</sub> atmosphere. The reaction was heated for a total of 14 hr. The solvent was evaporated under reduced pressure and the residue was purified on a neutral alumina column (50 g) to remove excess unreacted 23. The product was eluted with ethyl acetate:methanol (95:5); the solvent was evaporated under reduced pressure and the oily residue was further purified on a gel permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fraction 12 - 14 (3 mL). Evaporation of the solvent gave 38 as a colorless oil (92 mg, 13%); <sup>1</sup>H NMR 250 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 7.3 - 7.2 (br m, 4H, aromatic CH), 6.8, 6.2 (s, 4H, trans and cis CH=CH), 4.2 (m, 16H, CO<sub>2</sub>CH<sub>2</sub>), 4.0 - 3.5 (m, 22H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>O, ArCH<sub>2</sub>SCH), 2.9 - 2.5 (m, 4H, CH<sub>2</sub>C=O), 1.6 (m, 8H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.3 (br, s, 16H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR 90.57 MHz: 171.5, 171.3, 170.7, 170.6, 170.3, 165.5, 165.4, 165.2, 165.1 (C=O), 138.0, 134.3, 133.8, 133.2, 130.5, 130.4, 130.3, 130.1, 129.8, 129.5, 128.9, 128.4 (CH=CH), 72.8, 71.0, 70.8, 70.6 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.2, 69.1 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.6, 65.5, 65.1, 65.0, 64.8, 64.6, 64.3 (CO<sub>2</sub>CH<sub>2</sub>), 41.8, 41.7, 41.6, 41.5 (CHS), 36.7, 36.5, 36.1, 35.9 (CH<sub>2</sub>C=O, ArCH<sub>2</sub>), 29.4, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 26.2, 26.1, 25.9, 25.8

(two peaks), 25.7, 25.6 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ); Analysis calculated for  $\text{C}_{52}\text{H}_{74}\text{O}_{20}\text{S}_2$ : C 57.66%, H 6.89%, S 5.92%; Found: C 57.57%, H 6.92%, S 5.84%.

**Bis(3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20,23,26-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-m-xylene (39):**

meta-xylene dithiol (100 mg, 587  $\mu$  mol, from a stock solution in THF:isopropanol, 1:1) was added dropwise over a 1.5 hr period to a solution of 24 (2.7 g, 587 mmol, 10 eq) and 2,2,6,6-tetramethylpiperidine (5 drops) in 15 mL of THF at 78 - 80°C under  $\text{N}_2$  atmosphere. The reaction was heated for a total of 20 hr. The solvent was evaporated under reduced pressure and the residue was purified on a neutral alumina column (50 g) to remove excess unreacted 24. The product was eluted with ethyl acetate:methanol (98:2); the solvent was evaporated under reduced pressure and the oily residue was further purified on a gel permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 9 - 11 (3 mL). Evaporation of the solvent gave 39 as a colorless oil (151 mg, 24%);  $^1\text{H}$  NMR 250 MHz ( $\delta$ ,  $\text{CD}_2\text{Cl}_2$ ): 7.3 - 7.2 (br m, 4H, aromatic CH), 6.8, 6.2 (s, 4H, trans and cis ( $\text{CH}=\text{CH}$ )), 4.2 (m, 16H  $\text{CO}_2\text{CH}_2$ ), 3.9 - 3.5 (m, 54H,  $\text{CO}_2\text{CH}_2\text{CH}-\text{OCH}_2\text{CH}_2\text{O}$ ,  $\text{ArCH}_2\text{SCH}$ ), 3.0 - 2.6 (m, 4H,  $\text{CH}_2\text{C=O}$ );  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CD}_2\text{Cl}_2$ ): 171.0, 170.9, 170.0, 169.9, 164.9, 164.5 ( $\text{C=O}$ ), 137.3, 133.4, 129.6, 129.5, 128.5, 128.0 ( $\text{CH}=\text{CH}$ ), 70.6, 70.4 ( $\text{OCH}_2\text{CH}_2\text{O}$ ), 68.8, 68.7, 68.6 ( $\text{CO}_2\text{CH}_2\text{CH}_2\text{O}$ ), 64.3, 64.2, 63.8 ( $\text{CO}_2\text{CH}_2$ ), 41.2, 41.0 ( $\text{CHS}$ ), 36.0,

35.5 ( $\text{CH}_2\text{C=O}$ ,  $\text{ArCH}_2$ ); Analysis calculated for  $\text{C}_{48}\text{H}_{66}\text{O}_{24}\text{S}_2$ : C 52.84%, H 6.10%, S 5.88%; Found: C 52.61%, H 6.10%, S 5.10%.

**Preparation of the capped compound (35):**

Meta-xylylene dithiol (70 mg, 412  $\mu$  mol, from a stock solution in THF:isopropanol, 1:1) was added dropwise over a 26 hr period to a solution of 24 (380 mg, 826  $\mu$  mol, 2 eq) and 2,2,6,6-tetramethylpiperidine (5 drops) in a 1:1 mixture of THF: isopropanol (10 mL) at 79 - 81°C under a  $\text{N}_2$  atmosphere. Once the addition was complete the reaction mixture was heated for a further 10 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 14 - 19 (6 mL). Evaporation of the solvent gave 35 as a colorless oil (160 mg, 62%);  $^1\text{H}$  NMR 360 MHz ( $\delta$ ,  $\text{CD}_2\text{Cl}_2$ ): 7.3 - 7.2 (br m, 4H, aromatic  $\text{CH}$ ), 4.2 (m, 8H,  $\text{CO}_2\text{CH}_2$ ), 3.9 - 3.5 (m, 22H,  $\text{CO}_2\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{O}$ ,  $\text{ArCH}_2\text{SCH}$ ), 2.9 (dd,  $J = 17$  and  $J = 10$ , 2H,  $\text{CH}_A\text{H}_B\text{C=O}$ ), 2.6 (dd,  $J = 17$  and  $J = 10$ , 2H,  $\text{CH}_A\text{H}_B\text{C=O}$ ;  $^{13}\text{C}$  NMR 90.57 MHz ( $\delta$ ,  $\text{CD}_2\text{Cl}_2$ ): 171.1, 170.2 ( $\text{C=O}$ ), 137.5, 129.8, 128.7, 128.2 ( $\text{C=C}$ ), 70.6, 70.5 ( $\text{OCH}_2\text{CH}_2\text{O}$ ), 69.0 ( $\text{CO}_2\text{CH}_2\text{CH}_2\text{O}$ ), 64.5, 64.1 ( $\text{CO}_2\text{CH}_2$ ), 41.3 ( $\text{CHS}$ ), 36.1, 35.7 ( $\text{CH}_2\text{C=O}$ ,  $\text{ArCH}_2$ ); MS (CI, m/e) 631 ( $M + 1$ ).

**3-(4-hydroxy-1-thiabutyl)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (40):**

Macrocyclic 22 (12.52 g, 27 mmol) and 3-mercaptop-1-

propanol (2.55 g, 27 mmol) were added to isopropanol (200 mL). Piperidine (10 drops) was added and the mixture was heated at reflux for 1 hr 45 min. The solvent was removed under reduced pressure and the product was chromatographed on 8% deactivated alumina (250 g) with a dichloromethane: hexane gradient (1:1 to 100%  $\text{CH}_2\text{Cl}_2$ ). Evaporation of the solvent gave 40 as a clear oil (4.15 g, 28%):  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 6.8 (s, 2H, trans  $\text{CH}=\text{CH}$ ), 4.2 (m, 8H,  $\text{CH}_2\text{O}$ ), 3.7 (m, 3H,  $\text{CH}_2\text{OH}$ , CHS), 3.2 - 2.7 (m, 4H,  $\text{CH}_2\text{CHS}$ ,  $\text{CH}_2\text{S}$ ), 2.4 (s, 1H, OH), 2.0 - 1.2 (m, 26H,  $\text{CH}_2$ ;  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 171.7, 170.6, 165.0 (C=O), 133.5 (trans C=C), 55.3, 64.9 ( $\text{CO}_2\text{CH}_2$ ), 41.7 (CHS), 36.6 ( $\text{CH}_2\text{C=O}$ ); MS (CI, m/e): 545 (M + 1); Analysis calculated for  $\text{C}_{27}\text{H}_{44}\text{O}_9\text{S}$ : C 59.54%, H 8.14%, S 5.89%; Found C 60.04%, H 7.91%, S 5.52%.

**3-(4-methanesulphonyl-1-thiabutyl)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (41):**

Compound 40 (4.15 g, 7.6 mmol) and triethylamine (4.04 g, 40 mmol, 5 eq) were dissolved in dichloromethane (150 mL) and the mixture was cooled to -10°C. Methanesulfonyl chloride (1.72 g, 15 mmol, 2 eq) in dichloromethane (5 mL) was added dropwise over 30 minutes. The reaction was allowed to warm to room temperature and was stirred for a further 2 hr. The reaction mixture was washed with saturated sodium chloride (100 mL x 2), 10% hydrochloric acid (100 mL x 2), 10% sodium bicarbonate (100 mL x 2) and with saturated sodium chloride (100 mL x 2), dried over sodium sulfate, and the solvent was

removed to give 41 as a yellow oil (3.62 g, 76%); <sup>1</sup>H NMR 90 MHz ( $\delta$ , CDCl<sub>3</sub>), 6.8 (s, 2H, trans CH=CH), 4.2 (m, 10H, CH<sub>2</sub>O, CH<sub>2</sub>OMeS), 3.6 (dd, J = 3 Hz and 6 Hz, 1H, CHS), 2.9 (s, 3H, SO<sub>2</sub>CH<sub>3</sub>), 3.1 - 2.6 (br m, 4H, CH<sub>2</sub>C=O, CH<sub>2</sub>S), 2.0 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>S), 1.8 - 1.2 (br m, 24H, CH<sub>2</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CDCl<sub>3</sub>): 171.2, 170.3, 164.8 (C=O), 133.4 (trans C=C), 65.2, 64.8 (CO<sub>2</sub>CH<sub>2</sub>), 41.5 (CHS), 36.4 (CH<sub>2</sub>C=O); MS (CI, m/e): 623 (M + 1).

**3-(4-iodo-1-thiabutyl)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (42):**

Compound 41 (3.62 g, 5.8 mmol) and sodium iodide (7 g, 47 mmol, 8 eq) were refluxed in acetone (100 mL) overnight. The solvent was removed under reduced pressure and the residue was taken up in dichloromethane (200 mL), washed with water (100 mL x 2), and dried over sodium sulfate. Evaporation of the solvent under reduced pressure gave 42 as a yellow oil (~0 g, 79%); <sup>1</sup>H NMR 90 MHz ( $\delta$ , CDCl<sub>3</sub>): 6.8 (s, 2H, trans CH=CH), 4.2 (m, 8H, CH<sub>2</sub>O), 3.65 (dd, J = 3 Hz and 6 Hz, 1H, CHS), 3.2 (t, J = 6 Hz, 2H, CH<sub>2</sub>I), 3.0 - 2.7 (m, 4H, CH<sub>2</sub>C=O, CH<sub>2</sub>S), 2.1 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>I), 1.8 - 1.3 (br m, 24H, CH<sub>2</sub>); <sup>13</sup>C NMR 62.89 (CDCl<sub>3</sub>): 171.5, 170.4, 164.9 (C=O), 133.5 (trans C=C), 65.4, 64.9 (CO<sub>2</sub>CH<sub>2</sub>), 41.5 (CHS), 36.6 (CH<sub>2</sub>C=O); MS (CI, m/e): 655 (M + 1); Analysis calculated for C<sub>27</sub>H<sub>43</sub>O<sub>8</sub>SI: C 49.54%, H 6.62%, S 4.90%, I 19.39%; Found: C 50.21%, H 6.56%, S 5.10%, I 19.64%.

**2R,3R-Bis(3-and/or 4-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxo(cyclooctacosa-17-3nyl)-4-thiabutyl-tartarate (46):**

Compound 42 (500 mg, 764  $\mu$  mol), 4 eq) in DMSO (10 mL) was added to a solution of 2R,3R-(+)-tartaric acid (29 mg, 191  $\mu$  mol) and tetramethyl ammonium hydroxide pentahydrate (69 mg, 382  $\mu$  mol, 2 eq) in DMSO (10 mL) at 59 - 60°C under a N<sub>2</sub> atmosphere. The reaction mixture was heated for 3 hr and the solvent was removed under reduced pressure. The product was purified on a gel permeation column (LH-20, 3 x 20 cm), eluted with chloroform:methanol (4:3), and collected in fractions 8 - 10 (3 mL). Evaporation of the solvent gave 46 as a clear oil (103 mg, 45%); <sup>1</sup>H NMR 250 MHz ( $\delta$ , CDCl<sub>3</sub>): 6.8 (s, 4H, trans CH=CH), 4.5 (s, 2H, CHO), 4.4 - 4.0 (m, 20H, CO<sub>2</sub>CH<sub>2</sub>), 3.6 (m, 2H, CHS), 2.9 - 2.6 (m, 8H, CH<sub>2</sub>C=O, CH<sub>2</sub>S), 1.9 (m, 2H, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 1.6 (m, 16H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>), 1.3 (br s, 32H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CDCl<sub>3</sub>): 171.5, 171.2, 170.5, 164.9 (C=O), 133.5 (trans C=C), 72.2 (CHO), 65.3, 64.9, 64.4 (CO<sub>2</sub>CH<sub>2</sub>), 41.6 (CHS), 36.5 (CH<sub>2</sub>C=O), 28.8, 28.3, 27.6, 25.9, 25.3 (CH<sub>2</sub>CH<sub>2</sub>S, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); Analysis calculated for C<sub>58</sub>H<sub>86</sub>O<sub>22</sub>S<sub>2</sub>: C 58.08%, H 7.23%; Found: C 57.66%, H 7.50%.

**3-and 4-(4-hydroxy-1-thiabutyl)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (43):**

Compound 23 (50 g, 11 mmol) and 3-mercaptopropanol (1.0 g, 11 mmol, 1 eq) were added to isopropanol (150 mL). Piperidine (10 drops) was added and the mixture was heated at reflux for 1 hr 30 min. The solvent was removed under reduced

pressure, and the product was chromatographed on 8% deactivated alumina (120 g) with a dichloromethane: hexane gradient (1:1 to 100%  $\text{CH}_2\text{Cl}_2$ ). Evaporation of the solvent gave 43 as a clear oil (2.38 g, 39%):  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 6.8 (s, 2H, trans  $\text{CH}=\text{CH}'$ ), 4.2 (m, 8H,  $\text{CO}_2\text{CH}_2$ ), 3.6 (m, 11H,  $\text{CH}_2\text{O}$ ,  $\text{CH}_2\text{OH}$ , CHS), 3.0 - 2.6 (m, 4H,  $\text{CH}_2\text{C=O}$ ,  $\text{CH}_2\text{S}$ ), 1.8 - 1.1 (m, 15 H,  $\text{CH}_2$ , OH);  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 171.6, 170.5, 164.9 (C=O), 134.0, 133.1 (trans C=C), 70.7, 70.6, 70.4, 69.2, 69.0, 68.9, 65.3, 65.2 (ether  $\text{CH}_2\text{O}$ ), 64.7, 64.5, 64.4, 64.2, 63.9 ( $\text{CO}_2\text{CH}_2$ ), 41.7, 41.5 (CHS), 36.5, 36.4 ( $\text{CH}_2\text{C=O}$ ); MS (CI, m/e): 549 (M + 1).

**3-and 4-(4-methanesulphonyl-1-thiabutyl)-1,6,9,12,15,20-hexaoxa-2,5,16,17-tetraoxocyclooctacosa-17-ene (44):**

Compound 43 (720 mg, 1.3 mmol) and triethylamine (2.02 g, 20 mmol, 15 eq) were dissolved in dichloromethane (50 mL) and the mixture cooled to -10°C. Methanesulfonyl chloride (860 mg, 7.5 mmol, 6 eq) in dichloromethane (5 mL) was added dropwise over a 30 minute period. The reaction was allowed to warm to room temperature and was stirred for a further 2 hr. The reaction mixture was washed with saturated sodium chloride (100 mL x 2), 10% hydrochloric acid (100 mL x 2), 10% sodium bicarbonate (100 mL x 2), saturated sodium chloride (100 mL x 2), and dried over sodium sulfate. The solvent was removed to give 44 as a yellow oil (740 mg, 90%);  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 6.8 (s, 2H, trans  $\text{CH}=\text{CH}'$ ), 4.2 (m, 10H,  $\text{CO}_2\text{CH}_2$ ,  $\text{CH}_2\text{OMeS}$ ), 3.6 (m, 9H,  $\text{CH}_2\text{O}$ , CHS), 3.0 (s, 3H,  $\text{SO}_2\text{CH}_3$ ), 2.9 -

2.6 (m, 4H,  $\text{CH}_2\text{C=O}$ ,  $\text{CH}_2\text{S}$ ), 2.0 (m, 2H,  $\text{CH}_2\text{CH}_2\text{OMeS}$ ), 1.8 - 1.3 (m, 12H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 171.3, 170.3, 164.9 (C=O), 134.0, 133.1 (trans C=C), 70.7, 70.6, 70.4, 69.2, 69.0, 65.3 (ether  $\text{CH}_2\text{O}$ ), 64.8, 64.5, 64.3, 63.9 ( $\text{CO}_2\text{CH}_2$ ), 41.6, 41.5 (CHS), 36.4, 36.3 ( $\text{CH}_2\text{C=O}$ ).

**3- and 4-(4-iodo-1-thiabutyl)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (45):**

Compound 44 (740 mg, 1.2 mmol) and sodium iodide (3 g, 20 mmol, 17 eq) were refluxed in acetone (100 mL) overnight. The solvent was removed under reduced pressure and the residue was taken up in dichloromethane (200 mL), washed with water (100 mL x 2), and dried over sodium sulfate. Evaporation of the solvent under reduced pressure gave 45 as a yellow oil (500 mg, 63%):  $^1\text{H}$  NMR 90 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 6.8 (s, 2H, trans  $\text{CH}=\text{CH}$ ), 4.2 (m, 8H,  $\text{CO}_2\text{CH}_2$ ), 3.6 (m, 9H,  $\text{CH}_2\text{O}$ , CHS), 3.2 (t,  $J = 6$  Hz,  $\text{CH}_2\text{I}$ ), 2.9 - 2.6 (m, 4H,  $\text{CH}_2\text{C=O}$ ,  $\text{CH}_2\text{S}$ ), 2.0 (m, 2H,  $\text{CH}_2\text{CH}_2\text{I}$ ), 1.7 - 1.2 (m, 12H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ ,  $\text{CDCl}_3$ ): 171.3, 170.3, 164.8 (C=O), 134.0, 133.0 (trans C=C), 70.7, 70.6, 70.4, 69.1, 69.0, 65.3 (ether  $\text{CH}_2\text{O}$ ), 64.8, 64.5, 64.4, 64.3, 63.9 ( $\text{CO}_2\text{CH}_2$ ), 41.5, 41.4, (CHS), 36.4, 36.3 ( $\text{CH}_2\text{C=O}$ ); MS (CI, m/e): 659 (M + 1).

**2R,3R-Bis(3- and/or 4-(1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-tartarate (47):**

Compound 45 (500 mg, 760 ( $\mu$  mol, 4 eq) in DMSO (10 mL) was added to a solution of 2R,3R-(+)-tartaric acid (28 mg, 190

$\mu$  mol) and tetramethyl ammonium hydroxide pentahydrate (69 mg, 380  $\mu$  mol, 2 eq) in DMSO (10 mL) at 54 - 56°C under a N<sub>2</sub> atmosphere. The reaction mixture was heated for 3 hr and the solvent was removed under reduced pressure. The product was purified on a gel permeation column (LH-20, 3 x 20 cm), eluted with chloroform: methanol (4:3), and collected in fractions 9 - 10 (2 mL). Evaporation of the solvent gave 47 as a clear oil (121 mg, 52%); <sup>1</sup>H NMR 250 MHz ( $\delta$ , CDCl<sub>3</sub>): 6.8 (s, 4H, trans CH=CH), 4.5 (s, 2H, CHOH), 4.4 - 4.0 (m, 2OH, CO<sub>2</sub>CH<sub>2</sub>), 3.7 - 3.6 (m, 18H, CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>O, CHS), 3.0 - 2.6 (m, 8H, CH<sub>2</sub>C=O, CH<sub>2</sub>S), 2.0 (m, 2H, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 1.6 (m, 16H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>), 1.3 (br s, 16H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CDCl<sub>3</sub>): 171.4, 171.3, 170.4, 164.9 (C=O), 134.0, 133.1 (trans C=C), 72.2 (CHOH), 70.8, 70.4 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.0 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.3 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 64.9, 64.4, 64.0 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 41.7 (CHS), 36.3 (CH<sub>2</sub>C=O), 28.7, 28.6, 28.3, 27.7, 25.7, 25.3 (CH<sub>2</sub>CH<sub>2</sub>S); Analysis calculated for C<sub>54</sub>H<sub>78</sub>O<sub>26</sub>S<sub>2</sub>: C 53.72%, H 6.51%; Found: C 52.56%, H 6.74%.

#### Addition of Polar Head Groups

**Bis[17-and/or 18-( $\beta$ -D-glucopyranosylthio)-3-and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (**48**):**

To a stirred solution of 37 (50 mg, 46.5  $\mu$  mol) in a 1:1 mixture of THF:isopropanol (20 mL) at 50°C under a N<sub>2</sub> atmosphere, was added methanesulfonic acid (18.9 mg, 197  $\mu$

mol, 1 eq) from a stock solution in isopropanol. 1-Thio- $\beta$ -D-glucose sodium salt dihydrate (50 mg, 197  $\mu$  mol, 4 eq) was added (pH 5). 2,2,6,6-Tetramethylpiperidine (5 drops) was added (pH 9) and the cloudy mixture was stirred for 10 hr. The solvent was evaporated under reduced pressure. The residue was washed with saturated sodium chloride solution (20 mL) and chloroform (10 mL). The solvent was decanted and the residue was dried under vacuum to give 48 as a pale yellow oil (61.3 mg, 90%);  $^{13}\text{C}$  NMR 62.89 MHz ( $\delta$ , CD<sub>3</sub>OD): 173.4, 173.1, 172.4, 172.1, 171.9 (C=O), 139.2, 130.9, 129.8, 129.2 (C=C), 66.9, 66.7, 66.5, 66.1 (CO<sub>2</sub>CH<sub>2</sub>), 42.3 (two peaks, CHS), 37.6, 37.2, 36.7 (CH<sub>2</sub>C=O, ArCH<sub>2</sub>), 30.3, 29.7, 27.0, 26.9 -(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); For assignment of glucose carbons see Table 7.

**Bis[17- and/or 18-( $\beta$ -D-glucopyranosylthio)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (49):**

To a stirred solution of 38 (62 mg, 57.2  $\mu$  mol) in a 1:1 mixture of THF:isopropanol (20 mL) at 50°C under a N<sub>2</sub> atmosphere, was added methanesulfonic acid (18.9 mg, 197  $\mu$  mol, 3 eq) from a stock solution in isopropanol. 1-Thio- $\beta$ -D-glucose sodium salt dihydrate (50 mg, 197  $\mu$  mol, 3 eq) was added (pH 5). 2,2,6,6-Tetramethylpiperidine (5 drops) was added (pH 9) and the cloudy mixture was stirred for 10 hr. The solvent was evaporated under reduced pressure. The residue was washed with saturated sodium chloride solution (20 mL) and chloroform (10 mL). The solvent was decanted and the

residue was dried under vacuum to give 49 as a pale-yellow oil (84.4 mg, 36%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>3</sub>OD): 173.4, 173.1, 172.3, 172.1, 172.0, 171.9 (C=O), 139.2, 131.0, 129.9, 129.3 (C=C), 69.2, 69.1 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.2, 65.1, 64.8, 64.4, 64.3, 62.9 (CO<sub>2</sub>CH<sub>2</sub>), 41.8, 41.7, 41.2 (CHS), 36.7, 36.5, 36.1, 35.9, 35.5 (CH<sub>2</sub>C=O, ArCH<sub>2</sub>), 30.1, 29.9, 29.6, 28.9, 28.7, 26.7, 26.2, 25.9, 25.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); For assignment of glucose carbons see Table 7.

**Bis[17- and/or 18-( $\beta$ -D-glucopyranosylthio)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20,23,26-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]- $\alpha$ -xylene (50):**

To a stirred solution of 39 (69 mg, 63.2  $\mu$  mol) in a 1:1 mixture of THF: isopropanol (20 mL) at 50°C under a N<sub>2</sub> atmosphere, was added methanesulfonic acid (18.9 mg, 197  $\mu$  mol, 3 eq) from a stock solution in isopropanol. 1-Thio- $\beta$ -D-glucose sodium salt dihydrate (50 mg, 197  $\mu$  mol, 3 eq) was added (pH 5). 2,2,6,6-Tetramethylpiperidine (5 drops) was added (pH 9) and the cloudy mixture was stirred for 10 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 10 (7 mL). Evaporation of the solvent gave 50 as a pale yellow oil (27.3 mg, 29%); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CD<sub>3</sub>OD): 173.5, 173.4, 173.2, 172.2, 172.0, 171.9 (C=O), 139.2, 131.1, 129.9, 129.3 (C=C), 65.9, 65.8, 65.3 (CO<sub>2</sub>CH<sub>2</sub>), 42.6, 41.2 (CHS), 38.6, 37.3, 36.7 (CH<sub>2</sub>C=O,

$\text{ArCH}_2$ ); For assignment of glucose carbons see Table 7.

**2R,3R-Bis[17- and/or 18-( $\beta$ -D-glucopyranosylthio)-3- and/or 4-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl]tartarate (51):**

To a stirred solution of 46 (43 mg, 35.9  $\mu$  mol) in a 1:1 mixture of THF:isopropanol (20 mL) at 50°C under a N<sub>2</sub> atmosphere, was added methanesulfonic acid (18.9 mg, 197  $\mu$  mol, 5 eq) from a stock solution in isopropanol. 1-Thio- $\beta$ -D-glucose sodium salt dihydrate (50 mg, 197  $\mu$  mol, 5 eq) was added (pH 5). 2,2,6,6-Tetramethylpiperidine (5 drops) was added (pH 9) and the cloudy mixture was stirred for 10 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform: methanol (4:3) and was collected in fractions 3 - 10 (7 mL). Evaporation of the solvent gave 51 as a pale yellow oil (57 mg, 80%); <sup>13</sup>C NMR 62.89 MHz ( $\delta$ , CD<sub>3</sub>OD): 173.5, 173.2, 172.8, 172.1, 172.0 (C=O), 73.9 (CHOH), 66.8, 66.6, 66.1, 64.9 (CO<sub>2</sub>CH<sub>2</sub>), 43.1, 42.8, 41.3 (CHS), 38.8, 38.1, 37.8, 36.0 (CH<sub>2</sub>C=O), 30.3, 29.7, 29.0, 27.7, 27.0 (CH<sub>2</sub>CH<sub>2</sub>S, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>; For assignment of glucose carbons see Table 7.

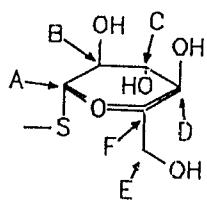
**2R,3R-Bis[17- and/or 18-( $\beta$ -D-glucopyranosylthio)-3-and/or 4-(1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-3nyl)-4-thiabutyl]-tartarate (52):**

To a stirred solution of 47 (59 mg, 48.9  $\mu$  mol) in a 1:1 mixture of THF:isopropanol (20 mL) at 50°C under N<sub>2</sub>

atmosphere, was added methanesulfonic acid (18.9 mg, 197  $\mu$  mol, 4 eq) from a stock solution in isopropanol. 1-Thio- $\beta$ -D-glucose sodium salt dihydrate (50 mg, 197  $\mu$  mol, 4 eq) was added (pH 5). 2,2,6,6-Tetramethylpiperidine (5 drops) was added (pH 9) and the cloudy mixture was stirred for 10 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 52 as a pale yellow oil (60.6 mg. 78%).  $^{13}\text{C}$  NMR 90.56 MHz ( $\delta$ , CD<sub>3</sub>OD): 173.5, 173.3, 173.2, 172.8, 172.2, 172.1 172.0 (C=O), 73.9 (CHOH), 66.7, 66.6, 66.4, 66.0, 65.9, 65.8, 65.2, 64.9 (CO<sub>2</sub>CH<sub>2</sub>), 43.0, 42.8, 42.4, 41.2, 41.0 (CHS), 39.5, 38.7, 38.6, 37.8, 37.5, 36.0 (CH<sub>2</sub>C=O), 30.2, 29.9, 29.5, 29.4, 29.0, 27.6, 26.9, 26.7 (CH<sub>2</sub>CH<sub>2</sub>S, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); For assignment of glucose carbons see Table 7.

Table 7.  $^{13}\text{C}$  NMR Data

	COMPOUND				
	48	49	50	51	52
Glucose	86.5(A) 85.6(A) 82.0(F) 81.7(F) 79.6(B) 79.5(B) 74.3(D) 63.0(E)	86.5(A) 85.8(A) 82.0(F) 81.9(F) 79.6(B) 79.5(B) 74.3(D) 62.9(E)	86.4(A) 85.7(A) 82.0(F) 81.9(F) 79.7(B) 79.5(B) 74.3(D) 62.9(E)	86.5(A) 85.8(A) 82.0(F) 81.9(F) 79.6(B) 79.5(B) 74.3(D) 62.9(E)	86.4(A) 86.8(A) 82.0(F) 81.9(F) 79.6(B) 79.5(B) 74.3(D) 62.9(E)
Glucose(C), $\text{OCH}_2\text{CH}_2\text{O}$	71.4(C) 71.3(C)	71.6 71.3 71.1 70.0	71.7 71.3 70.0	71.4(C) 71.3(C)	71.6 71.5 71.3 71.2 70.0



**Bis[17- and/or 18-(4-hydroxy-1-thiabutyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (53):**

To a solution of 37 (69.4 mg, 64.5  $\mu$  mol) in THF (20 mL) at 50°C under a N<sub>2</sub> atmosphere was added 3-mercaptopropanol (23.8 mg, 258  $\mu$  mol, 4 eq) from a stock solution in THF. 2,2,6,6-Tetramethylpiperidine (5 drops) was added and the mixture was stirred for 5 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 53 as a colorless oil (81.2 mg, 81%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.8, 171.5, 170.6, 170.5 (C=O), 138.1, 130.1, 129.0, 128.4 (C=C), 65.8, 65.6, 65.3 (CO<sub>2</sub>CH<sub>2</sub>), 61.3 (CH<sub>2</sub>OH), 42.2, 41.7, 41.6 (CHS), 37.1, 36.8, 36.0 (CH<sub>2</sub>C=O), ArCH<sub>2</sub>, 32.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>S), 29.6, 29.5, 29.3, 28.9, 28.4, 26.2, 26.1, 26.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

**Bis[17- and/or 18-(4-hydroxy-1-thiabutyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (54):**

To a solution of 38 (38.7 mg, 35.7  $\mu$  mol) in THF (20 mL) at 50°C under a N<sub>2</sub> atmosphere was added 3-mercaptopropanol (27.9 mg, 346  $\mu$  mol, 10 eq) from a stock solution in THF. 2,2,6,6-Tetramethylpiperidine (10 drops) was added and the mixture was stirred for 5 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-

permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 54 as a colorless oil (45.3 mg, 89%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.9, 171.7, 171.6, 171.5, 170.7, 170.6, 170.5 (C=O), 138.1, 130.1, 129.0, 128.4 (C=C), 70.9 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.3 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.7, 65.2, 64.8, 64.4 (CO<sub>2</sub>CH<sub>2</sub>), 61.3 (CH<sub>2</sub>OH) 42.3, 42.0, 41.8, 41.6 (CHS), 37.0, 36.9, 36.7, 36.5, 36.1, 36.0 (CH<sub>2</sub>C=O, ArCH<sub>2</sub>), 32.4, 32.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>S), 29.2, 29.1, 28.8, 28.5, 28.3, 26.1, 25.9, 25.8, 25.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

**Bis[17- and/or 18-(4-hydroxy-1-thiabutyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20,23,26-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (55):**

To a solution of 39 (50.0 mg, 45.8  $\mu$  mol) in THF (20 mL) at 50°C under a N<sub>2</sub> atmosphere was added 3-mercaptopropanol (31.9 mg, 346  $\mu$  mol, 8 eq) from a stock solution in THF. 2,2,6,6-Tetramethylpiperidine (10 drops) was added and the mixture was stirred for 5 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 55 as a colorless oil (58.4 mg, 94%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.9, 171.5, 170.6, 170.5 (C=O), 138.0, 130.1, 128.9, 128.5 (C=C), 70.9 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.3 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 64.9, 64.4 (CO<sub>2</sub>CH<sub>2</sub>), 61.1 (CH<sub>2</sub>OH), 42.1, 41.8 (CHS), 36.8, 36.5, 36.0 (CH<sub>2</sub>C=O,

$\text{ArCH}_2$ ), 32.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{S}$ ), 28.4 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{S}$ ).

**2R,3R-Bis[17- and/or 18-(4-hydroxy-1-thiabutyl)-3- and/or 4-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl]-tartarate (56):**

To a solution of 46 (34.5 mg, 28.8  $\mu$  mol) in THF (20 mL) at 50°C under a  $\text{N}_2$  atmosphere was added 3-mercaptopropanol (31.9 mg, 346  $\mu$  mol, 12 eq) from a stock solution in THF. 2,2,6,6-Tetramethylpiperidine (10 drops) was added and the mixture was stirred for 7 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 56 as a colorless oil (37.5 mg, 94%).  $^{13}\text{C}$  NMR 90.56 ( $\delta$ ,  $\text{CDCl}_3$ ): 171.4, 171.3, 170.3 ( $\text{C=O}$ ), 72.2 ( $\text{CHOH}$ ), 65.5, 65.4, 65.1, 65.0, 64.4 ( $\text{CO}_2\text{CH}_2$ ), 61.1 ( $\text{CH}_2\text{OH}$ ), 41.8, 41.6 ( $\text{CHS}$ ), 36.7, 36.5 ( $\text{CH}_2\text{C=O}$ ), 31.7 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{S}$ ), 29.6, 29.1, 29.0, 28.4, 28.2, 28.0, 27.6, 25.7 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ).

**2R,3R-Bis[17- and/or 18-(4-hydroxy-1-thiabutyl)-3- and/or 4-(1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-3nyl)-4-thiabutyl]-tartarate (57):**

To a solution of 47 (50.5 mg, 41.4  $\mu$  mol) in THF (20 mL) at 50°C under a  $\text{N}_2$  atmosphere was added 3-mercaptopropanol (31.9 mg, 346  $\mu$  mol, 8 eq) from a stock solution in THF. 2,2,6,6-Tetramethylpiperidine (10 drops) was added and the mixture was stirred for 7 hr. The solvent was evaporated

under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 57 as a colorless oil (57.6 mg, 86%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CDCl<sub>3</sub>): 171.6, 171.5, 171.3 (two peaks), 171.2, 170.4, 170.3 (C=O), 72.2 (CHOH), 70.5 (OCH<sub>2</sub>CH<sub>2</sub>O), 68.9, 68.8 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 65.4, 65.3, 64.9, 64.8, 64.4, 64.3, 64.2, 63.9 (CO<sub>2</sub>CH<sub>2</sub>), 60.9 (CH<sub>2</sub>OH), 41.8, 41.6, 41.5 (CHS), 36.5, 36.4, 36.3 (CH<sub>2</sub>C=O), 31.8, 31.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>S), 29.6, 28.7, 28.6, 28.3, 28.2, 28.0, 27.9, 27.7, 25.4, 25.3 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

**Bis[17- and/or 18-(3-amino-1-thiapropropyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (58):**

A mixture of compound 37 (50.0 mg, 46.5  $\mu$  mol), 2-aminoethanethiol (50.0 mg, 440  $\mu$  mol, 10 eq), and 2,2,6,6-tetramethylpiperidine (10 drops) in an 8:2 mixture of THF:isopropanol (20 mL) was heated at 50°C under a N<sub>2</sub> atmosphere for 1 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 58 as a colorless oil (50.3 mg, 88%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.8, 171.7, 171.4, 170.6, 170.5, 170.4 (C=O), 138.1, 130.0, 129.0, 128.4 (C=C), 66.0, 65.7, 65.4, 65.3 (CO<sub>2</sub>CH<sub>2</sub>), 43.5 (CH<sub>2</sub>N),

42.1, 42.0, 41.7, 41.6 (**CHS**), 37.2, 37.0, 36.8, 36.0, 35.6 (**CH<sub>2</sub>C=O**, **CH<sub>2</sub>S**), 29.6, 29.5, 28.9, 26.2, 26.1 (**CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>**).

**Bis[17- and/or 18-(3-amino-1-thiapropropyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacos-17-enyl]-m-xylene (59):**

A mixture of compound 38 (56.2 mg, 51.9  $\mu$  mol), 2-aminoethanethiol (50.0 mg, 440  $\mu$  mol, 9 eq), and 2,2,6,6-tetramethylpiperidine (10 drops) in an 8:2 mixture of THF:isopropanol (20 mL) was heated at 50°C under a N<sub>2</sub> atmosphere for 1 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20), 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 59 as a colorless oil (64.2 mg, 88%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>3</sub>OD): 171.9, 171.8, 171.7, 171.4, 171.2, 170.8, 170.7, 170.5 (**C=O**), 138.0, 130.1, 129.0, 128.4 (**C=C**), 70.9 (**OCH<sub>2</sub>CH<sub>2</sub>O**), 69.3 (**CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O**), 65.9, 65.7, 65.4, 65.2, 64.9, 64.7, 64.5, 64.4 (**CO<sub>2</sub>CH<sub>2</sub>**), 43.5 (**CH<sub>2</sub>N**), 41.8, 41.6, 41.3, 40.5, 40.4 (**CHS**), 36.9, 36.8, 36.7, 36.5, 36.4, 36.1, 36.0, 35.6 (**CH<sub>2</sub>C=O**, **CH<sub>2</sub>S**), 33.1, 29.6, 29.4, 29.2, 28.9, 28.8 (two peaks), 27.7, 27.2, 26.1, 26.0, 25.9, 25.8).

**Bis[17- and/or 18-(3-amino-1-thiapropropyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,9,12,15,20,23,26-octaoxa-2,5,16,19-tetraoxocyclooctacos-17-enyl]-m-xylene (60):**

A mixture of compound 39 (34.2 mg, 31.3  $\mu$  mol), 2-

aminoethanethiol (50.0 mg, 440  $\mu$  mol, 14 eq), and 2,2,6,6-tetramethylpiperidine (10 drops) in an 8:2 mixture of THF:isopropanol (20 mL) was heated at 50°C under a N<sub>2</sub> atmosphere for 1 hr. The solvent was evaporated under reduced pressure and the residue was purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 60 as a colorless oil (39.0 mg, 82%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.8, 171.1, 170.8, 170.5 (C=O), 138.0, 130.2, 129.0, 128.5 (C=C), 70.8 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.2 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 64.9, 64.8, 64.5, 64.3 (CO<sub>2</sub>CH<sub>2</sub>), 43.4 (CH<sub>2</sub>N), 41.2 (two peaks, CHS), 36.8, 36.3, 35.9, 35.8 (CH<sub>2</sub>C=O, CH<sub>2</sub>S).

**Bis[17- and/or 18-(1-thio-2-carboxylethyl)-3- and/or 4-( $\alpha,\alpha'$ -dithia)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl]-m-xylene (61):**

To a solution of 38 (59.9 mg, 55.3  $\mu$  mol) and mercaptoacetic acid (12.6 mg, 111  $\mu$  mol, 2 eq, from a stock solution in THF) in THF (20 mL) at 50°C under a N<sub>2</sub> atmosphere was added 2,2,6,6-tetramethylpiperidine (10 drops, pH 9). The reaction mixture was stirred for 6 hr and the solvent was removed under reduced pressure. The residue was dissolved in a 4:3 mixture of chloroform:methanol (5 mL) and was added to a cation exchange resin (Dowex 50 x 8 - 100, 1 x 5 cm) which had been activated with 2M sulfuric acid, washed with water, methanol, and a 4:3 mixture of chloroform:methanol. The

acidic fractions were combined, concentrated (~ 2 mL), and purified on a gel-permeation column (LH-20, 3 x 20 cm). The product was eluted with chloroform:methanol (4:3) and was collected in fractions 3 - 14 (12 mL). Evaporation of the solvent gave 61 as a pale-yellow oil (67.6 mg, 97%); <sup>13</sup>C NMR 90.56 MHz ( $\delta$ , CD<sub>2</sub>Cl<sub>2</sub>): 171.6, 171.4, 171.1, 170.8, 170.7, 170.5, 170.3, 170.0, 169.4 (C=O), 138.1, 130.1, 129.0, 128.4 (C=C), 70.9 (OCH<sub>2</sub>CH<sub>2</sub>O), 69.2 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 66.0, 65.8, 65.7, 65.5, 65.3, 65.1, 65.0, 64.9, 64.4, 64.3, 64.1 (CO<sub>2</sub>CH<sub>2</sub>), 42.7, 42.3, 42.1, 41.8, 41.7 (CHS), 36.8, 36.5, 36.4, 36.1, 36.0 (CH<sub>2</sub>C=O, CH<sub>2</sub>S), 34.0, 33.9 (CH<sub>2</sub>CO<sub>2</sub>H), 29.5, 29.2, 29.1, 29.0, 28.8, 25.9, 25.8, 25.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

**Appendix I****EXPERIMENTAL****General Instruments**

The titration system was a Metrohm 655 Dosimat buret and titration cell, 614 Impulsomat automatic titrator, and 632 pH-meter. The buret was linked to an HP-85 microcomputer for data acquisition. Vesicle preparation required use of a Heat Systems W385 Ultrasonic sonicator, located in the University of Victoria's Biochemistry and Microbiology Departments. Size distribution analysis of vesicles was performed using a Philips EM360 Transmission Electron Microscope, located in the Biology Department, and the Perkin-Elmer Model Lambda 4B Ultraviolet/Visible Spectrophotometer in the Chemistry Department.

**Vesicle Preparation**

Egg phosphatidylcholine and egg phosphatidic acid (egg PC and PA) were purchased from Avanti Polar Lipids, Inc., Pelham, Alabama. Cholesterol was purchased from Sigma/Aldrich. Anhydrous diethyl ether and HPLC grade chloroform were purchased from BDH; methanol, choline hydroxide (20% in water), D-mannitol, and Bis-Tris (2,2,-bis(hydroxymethyl)-

2,2',2"-nitrilotriethanol) from Sigma/Aldrich; sulphuric acid (ultrapure) from Fluka; and PD-10 Sephadex G-25M columns from Pharmacia. Only D<sup>3</sup> (deionized, double distilled) water was used.

#### *Stock Solutions*

Choline Sulphate - Choline hydroxide (250 mL of 20% solution) was titrated to pH 6.5 with concentrated sulphuric acid. Activated charcoal was added, and the solution stirred for 10 minutes; the charcoal was removed by filtration through Celite, and the solution concentrated by rotary evaporation. One half liter of 100% ethanol was added and the tarry residue was dissolved with heating. Anhydrous ether (200 mL) was added and the solution cooled to -10°C for 16 hours; the crystalline precipitate was removed by filtration. The mother liquor was returned to the freezer overnight to yield a final crop of precipitate. The combined batches of choline sulphate precipitate were dried for 24 hours by vacuum.

Internal Buffer Solution - 0.20 M bis-tris, and 0.054 M D-mannitol; the pH was adjusted to 6.60 using 0.45 M H<sub>2</sub>SO<sub>4</sub>. Internal buffer was made in 500 mL batches.

External solution - 0.110 M choline sulphate, and 0.093 M D-mannitol was made in one liter batches, portioned into 200 mL glass bottles and kept refrigerated as much as possible. Filtration through Millipore GS 0.22 µm filters extended the

life of the external solution.

Choline Hydroxide Titrant - one liter of 4.75 mM choline hydroxide, and 0.35 M D-mannitol, was dissolved in D<sup>3</sup> water that was boiled for 30 minutes to release carbon dioxide. This solution was made in a nitrogen filled glove bag to avoid carbon dioxide contamination. The base titer was established by titration versus standard potassium hydrogen phthalate.

#### *Egg Lecithin Vesicle Preparation*

The egg lecithin vesicles were made by reverse evaporation<sup>69</sup>, followed by sizing filtration and size exclusion chromatography. Each batch generated vesicles for approximately 16 pH-stat experiments. The following prescriptive procedure yields the most reproducible and reliable batches of vesicles for pH-stat work; that is, most of the entrapped buffer is within large unilamellar vesicles.

The vesicle bilayer composition is an 8:1:1 molar ratio of the egg PC and PA and cholesterol (or a 16:2:1 weight ratio). Make a chloroform stock solution of the 8:1:1 mixture, at a concentration of 50 mg PC per 6 mL; this limits the lipids, exposure to air, and simplifies the vesicle making procedure. Store under nitrogen in the refrigerator (0-5°C). Do not leave the solution at room temperature more than 5 minutes at a time.

Transfer 6 mL (50 mg PC) of the lipid\cholesterol

solution to a 50 mL round-bottom flask, and immediately evaporate to dryness on a rotary evaporator. Remove all traces of chloroform from the lipid film by drying under vacuum overnight at ambient temperature, or for four hours with the flask in a 50°C water bath. After the film is completely dry, add 6 mL of anhydrous diethyl ether to the flask, and quickly dissolve the dried lipid film. Add 2 mL of internal buffer. Stopper the flask with a nitrogen filled balloon to reduce the lipids' exposure to air.

Sonication of the lipid/ether/buffer solution mixes this "water in oil" mixture to homogeneity. Once the sonicator is tuned (13 mm tip), apply 2 second pulses (at 50% duty cycle and 5 power output) until the mixture is translucent gray. Stopper the flask with the nitrogen balloon. Reverse evaporation of the solution yields mostly large unilamellar vesicles with a high percentage of buffer entrapment. Remove the ether from the mixture by slow rotary evaporation, warming the flask with a 25°C water bath. As the ether begins to evaporate, the solution coats the sides of the flask; further evaporation promotes spontaneous bubbling. This bubbling continues for about 5 minutes, until almost all the ether has evaporated; the system goes through a gelatinous phase, then becomes a liquid. Add 3 mL of the external buffer, and continue rotary evaporation for half an hour at a slightly reduced vacuum.

The filtration unit sizes the vesicles by forcing the

vesicle solution first through a 1  $\mu\text{m}$  Nucleopore filter, then a 0.4  $\mu\text{m}$  filter. Using a 16 gauge 10 mL disposable syringe collect the vesicle solution, draw a further 2 mL of air, and inject the vesicle solution into the first cell. Use 10 psig nitrogen gas to force the vesicle solution through the first filter. Higher pressure may be required to force the solution through the second filter. The slower the filtration, the better the sizing of the vesicles; have the solution filter at 1 or 2 drops per second.

Equilibrate the Sephadex G-25M disposable columns with 10 mL of the external solution. Load all the vesicle solution. There will be three fractions: 1) multi-lamellar vesicles (MLVs); 2) large unilamellar vesicles (LUVs) (the desired fraction); and 3) small lipid aggregates and excess internal solution. There is a dead volume of 2.5 mL, and the first 15 drops of the vesicle band must also be discarded (contains mostly MLVs). Watch for a cloudy "doughnut" to form in the clear eluent in the receiving vial - this is the front of the vesicle band. Collect the next 4.5 mL of vesicles.

The prepared vesicles can be made and used on the same day, or used for the next two days. Batch-to-batch reproducibility is a problem, and within one batch there are differences noted for use over the 72 hour usable lifetime of the vesicles. It appears best to use the entire batch within 36 hours of its preparation.

### *Phospholipid Concentration Analysis*

Complexation of the phosphate headgroups of the phospholipid with a visible light absorbing species, phosphomolybdate, allows quantitative analysis of its concentration.

#### *Solutions*

Solution 1 - 16.00 g ammonium molybdate  $4\text{H}_2\text{O}$ , 120.0 mL  $\text{D}^3\text{H}_2\text{O}$ .

Solution 2 - 80.0 mL Solution 1, 40.0 mL concentrated hydrochloric acid, 10.0 mL mercury. After stirring for 30 minutes, three layers will separate upon standing for 5 minutes.

Solution 3 - all the filtrate of solution 2, remaining portion of solution 1, 200.0 mL concentrated sulphuric acid.

Chromogenic acid solution - 25 mL solution 3, 45 mL methanol, 5 mL chloroform, 20 mL water. These solutions have approximately a six month shelf life if kept in glass bottles and refrigerated.

#### *Procedure*

Transfer 50  $\mu\text{L}$  of vesicle solution into a large 1.5 cm diameter test tube. The vesicle solution has a typical concentration of about 10  $\text{mgmL}^{-1}$  phospholipid (thus,

approximately 0.5 mg phospholipid is being analyzed). Transfer appropriate quantities of the vesicle stock solution in chloroform into three test tubes to cover the range 0 - 1 mg phospholipid (at 50 mgmL<sup>-1</sup> use 0 - 20 µL). Add 0.4 mL chloroform and 0.1 mL of the chromogenic solution; boil for 1 minute for all test tubes. Cool to room temperature. Add 4 mL chloroform, shake gently, and wait 30 minutes. Carefully pipette out the chloroform layer into a 1 cm quartz cell, and record the UV absorption at 710 nm. The blank is 50 µL of chloroform as the solution aliquot in the procedure. The concentration of the vesicle solution can be extrapolated from a Beer-Lambert plot.

#### *Microscopic Analyses*

The following materials were purchased from the University of Victoria electron microscopy laboratory: Formvar, phosphotungstic acid, 150 mesh copper grids, microscope slides, and slide dipping tank.

#### *Solutions*

Formvar solution - 0.04 g Formvar, 10.0 mL chloroform; filter into dipping tank.

Staining solution - 2% (w/w) phosphotungstic acid (PTA) in water; adjust pH to 7.2 using 5 M potassium hydroxide; make

in 50 mL batches.

#### *Preparation of Grids*

Dip a microscope slide half of its length into the Formvar solution in the dipping tank. Remove after 20 seconds and let dry for 30 minutes. With a razor blade, cut the dried Formvar film close to the leading edge, across the entire width of the slide, one side only. Lift the leading edge of the film off the slide by blowing moist air parallel to the plane of the slide. Fill a 20 cm diameter Petri dish with water. Dip the slide at a shallow angle, cut film side up, into the water. The hydrophobicity of the film will force it to float on the surface of the water once it detaches from the slide. Make sure the film is floating and completely detached from the slide before continuing.

Using tweezers, place the copper grids dull side down on the floating Formvar film no closer than 2mm apart. Carefully place a fresh piece of Parafilm (large enough to easily cover the Formvar film) on top of the film/grids; the film will stick to the Parafilm. Remove and dry, grid side up, and protect from dust.

#### *Loading and Staining*

Place a drop of vesicle solution on the Formvar covered

side of a prepared grid. After 20 seconds lift the grid with tweezers, and remove the excess solution by placing it to the edge of a filter paper.

To stain the vesicle grid, put a drop of phosphotungstic acid solution on a piece of Parafilm and place the grid, vesicle layer down, on the drop for 30 seconds. Remove the grid from the stain and remove the excess staining solution by placing the grid to the edge of a filter paper. Dry the grid under a hot lamp for 2-3 minutes.

#### *Transmission Electron Microscope Analysis*

Under the microscope the vesicles appear as round, dark objects on a pale background. Their size distribution and number of lamellae can be determined by examination of photomicrographs taken using the microscope.

#### *The pH-stat Experiment*

Carbonylcyanide-trifluoromethoxyphenylhydrazone (FCCP), amphotericin, Triton X-100, and the sulphate salts of potassium, sodium, lithium, rubidium, and cesium, were purchased from Sigma/Aldrich.

FCCP was dissolved in methanol to concentration of  $7.7 \times 10^{-4}$  M. Amphotericin was dissolved to  $2.3 \times 10^{-4}$  M, in dimethylsulphoxide (Aldrich). Triton X-100 was diluted by a

factor of 20 with D<sup>3</sup> water. The mimics were dissolved in methanol or a 1:1 mixture of methanol:THF.

The following is a brief summary of the protocol used in this study. The vesicles were prepared with an entrapped buffer at pH 6.6. The buffer was composed of Bis-Tris, which was observed not to permeate nor be transported through the membrane. A one pH unit gradient was imposed by addition of the choline hydroxide titrant solution. A cation concentration gradient was created by adding a cation sulphate salt solution to the system. The transporter was then added, and the pH and cation gradients began to collapse. If necessary, a proton gradient decoupler (FCCP) is added if the transporter cannot achieve antiport translocation of the protons and cations. As the gradients collapse, the protons are released into the bulk soluiton and the pH drops. The titration instrumentation continuously adds sufficient choline hydroxide titrant to increase the pH to its initial point. The volume of base added versus time data was accumulated until the transport event was complete. The remaining protons were then released into the solution through lysis of the vesicles with Triton X-100, and were subsequently titrated. The data provided a rate constant for the release of protons from the vesicles which correlated with the translocation of the cations across the membrane.

*The pH-stat Data Accumulation and Manipulation Program*

The pH-stat program written for this project is divided into two parts. The first part of the program deals with the accumulation of time versus volume data, allows for changing the time delay, and records time events as required by the operator. The second part of the program is for data manipulation; re-calling, re-plotting, linear and first order regression.

The first order rate equation for the time versus volume curve generated by the pH-stat experiment is determined with minimal operator intervention. The operator defines the initial and final times of the experiment, then chooses a secondary initial time to create a new time range for the data manipulation (this is to overcome the lag-time experienced by the titrimeter when dealing with a fast titration). The operator then enters a low/high delta volume value for the titration, and enters a positive/negative increment volume value to guide the program's search for the best fit to the data. The algorithm is based on comparing the regression coefficients generated by changing the delta volume of the titration; there will be a maximum value of the regression coefficient corresponding to the best fit equation for the data. The algorithm is:

```
enter record number of time and volume initial → V0
enter record number of secondary time initial
enter record number of time final
```

enter estimate of delta volume for titration ( $\Delta V$ )

enter increment volume value

LOOP 1: determines record number of maximum volume allowable for the titration since logarithm of negative numbers not allowed.

LOOP 2: determines coefficients (slope, y-intercept, and regression) of the rate equation for the graph:

time elapsed versus  $\ln(1 - V_{t.e.} - V_0) / (\Delta V)$

for the time range specified by the secondary time initial and the time at which the maximum volume allowable was reached.

IF present  $r^2$  value lower than previous  $r^2$  value

THEN best fit has been found (i.e. previous value of delta volume)

ELSE apply increment to delta volume and GO TO LOOP 1

A graph of the manipulated data and first order line is printed out, along with the constants and variables involved.

**APPENDIX II**

Table 8. Results from the transport studies carried out on the fourteen mimics synthesized<sup>5</sup>.

Mimic <sup>3</sup> (T)	Batch <sup>2</sup>	M <sup>+</sup>	[M <sup>+</sup> ] --- mM	[T] --- μM	Rate <sup>1</sup> --- 1x10 <sup>-10</sup>
48	11	K	100	23	Inactive
49	1	K	100	17	Inactive
50	1	K	100	19	Inactive
51	1	K	100	19	15
51	2	K	92	29	28
51	2	K	92	58	30
51	2	K	92	86	31
51	2	K	92	115	34
52	1	K	100	22	10
52	4	K	94	7	1.6
52	4	K	94	21	6.7
52	4	K	94	35	16
52	4	K	94	35	17
52	3	K	94	70	54
52	4	K	4.1	38	9.6

52	4	K	20	38	11
52	4	K	30	38	12
52	3	Li	93	21	4.6
52	3	Na	93	21	6.1
52	3	K	93	21	7.7
52	3	Rb	93	21	6.8
52	3	Cs	93	21	7.2
53	1	K	100	24	Inactive
54	1	K	100	18	Inactive
55	1	K	100	20	Inactive
56	2	K	93	14	1.9
57	2	K	93	21	5.6
58	1	K	97	27	3.0
58	2	K	92	26	2.3
58	2	K	92	87	18
58	2	K	92	130	30
58	2	K	92	173	43
59	1	K	97	33	29
59	3	K	20	34	18

59	3	K	10	35	17
59	3	Li	93	32	22
59	3	Na	93	32	23
59	3	K	93	32	23
9	3	Rb	93	32	21
59	3	Cs	93	32	26
60	1	K	97	20	1.6
61	1	K	100	29	3.2
Amph <sup>4</sup>	2	K	92	4.3	1.9

1) Rates calculated using zero order analysis.

The units are in moles H<sup>+</sup> s<sup>-1</sup>

- 2) Indicates batch of vesicles that the measurements were carried out in.
- 3) FCCP concentration was 0.15 - 0.18 mM.
- 4) Amphotericin B.
- 5) The data in tables 4 and 5 are not shown.

## APPENDIX III

Table 9. Relative activity of the fourteen mimics synthesized.

En <sup>1</sup>	CPD <sup>2</sup>	STRUCTURE	RA <sup>3</sup>
1	Am <sup>4</sup>		1.0
2	<u>58</u>		0.25
3	<u>59</u>		2.0
4	<u>60</u>		0.18
5	<u>51</u>		1.8
6	<u>52</u>		1.0
7	<u>48</u>		IA <sup>5</sup>

8	<u>49</u>		IA
9	<u>50</u>		IA
10	<u>61</u>		0.25
11	<u>56</u>		0.32
12	<u>57</u>		0.61
13	<u>53</u>		IA
14	<u>54</u>		IA
15	<u>55</u>		IA

<sup>1</sup> En = Entry<sup>2</sup> CPd = compound number<sup>3</sup> RA = Relative Activity<sup>4</sup> Am = Amphotericin<sup>5</sup> IA = Inactive

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