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THE DESIGN, MODIFICATION OF CYCLODEXTRINS AS A POTENTIAL ORGANIC ELECTRONIC MATERIAL AND SYNTHESIS OF TETHERED LIPIDS FOR BIOELECTRONIC APPLICATIONS

presented by

KUN LI

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of the requirements for the

M.S. degree in Chemistry

Major Professor's Signature

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Date

THE DESIGN, MODIFICATION OF CYCLODEXTRINS AS A POTENTIAL ORGANIC ELECTRONIC MATERIAL AND SYNTHESIS OF TETHERED LIPIDS FOR BIOELECTRONIC APPLICATIONS

By

Kun Li

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ABSTRACT

THE DESIGN, MODIFICATION OF CYCLODEXTRINS AS A POTENTIAL ORGANIC ELECTRONIC MATERIAL AND SYNTHESIS OF TETHERED LIPIDS FOR BIOELECTRONIC APPLICATIONS

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In recent years, some organic materials which can exhibit many interesting optical, electrical, photoelectric, and magnetic properties have become very promising because of advantages compared with traditional inorganic materials. The major objective of this project is to design and make a supramolecular system that is a potential organic electronic material. We used cyclodextrins (CD) as molecular template for supramolecule formation because CDs not only have well-defined microenvironments for molecular recognition but also have many hydroxyl groups suited for functionalization. After combining the modified cyclodextrins with some photo sensitive compounds, a supramolecular system is formed which can be a potential electronic material or used to make biosensor. In the second part of this thesis, the synthesis of two different tethered bilayer lipids was described. By binding a membrane protein to the tethered lipid bilayers on a gold surface, the activities of protein can be coupled to an electrical signal and can be expressed and measured. This system is potentially used for bioelectronic applications.

ACKNOWLEDGEMENT

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TABLE OF CONTENTS

Appendices

LIST OF SCHEMES

LIST OF FIGURES

Chapter 1

Chapter 1. Background

1.1 Organic materials for electronic and optoelectronic devices

Some organic materials can exhibit many interesting optical, electrical, photoelectric, and magnetic properties in the solid state. One unique character of organic materials is organic π-systems which have played an important role in the construction of potential photo-and electro-active materials.[1]

Photo and electroactive organic materials have been used as organic semiconductors, organic metals (including superconductors, organic photoconductors, organic photoactive materials for solar cells), resist materials and in many other applications. Among them, organic photoconductors, liquid crystals and resist materials have been practically used for photo-receptors in electrophotography and display devices. In addition, organic materials have shown some potential applications for use in electronic and optoelectronic devices such as sensors, plastic batteries, solar cells, optical data storage, switching devices, and so on.

Compared with traditional inorganic materials, organic materials have many advantages. Organic materials made from the cheap and renewable resources can be used to replace natural, rare metal. This can significantly make them more available and lower the cost of materials. When these organic materials are used in electronic and optoelectronic devices, they are much easier than inorganic materials to be processed into thin films by

various techniques, e.g., solvent casting from solution, vacuum vapor deposition and monolayer self-assembly techniques. For example, to develop low-cost disposable plastic/paper electronic devices, conventional inorganic conductors, such as metals, and semiconductors, such as silicon, require multiple etching and lithographic steps in fabricating them for use in electronic devices. These processing and etching steps increase the price. On the other hand, conducting polymers have many advantages of plastics, such as flexibility and processing from solution.

1.1.1. Organic charge-transfer metals

Almost 95 years ago it was suggested that organic solids might have electrical conductivities comparable with metals.[3] The field remained speculative until synthesis of a bromine salt of perylene by Akamatu,[4] which showed significant conductivity. It was the first time that metallic properties were seen in materials which did not contain metal atoms. In 1962, Melby synthesized the first stable, highly conducting organic molecule-TCNQ (7,7,8,8-tetracyano-p-quinodimethane),[5] whose structure is shown in Fig 1.1 .

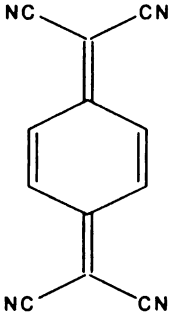


Figure 1.1 The structure of TCNQ

Since then, interest in conducting organic solids developed quickly when it was discovered that many salts of TCNQ were electrically conductive.[6] The TCNQ anions in the metallic salts are packed in pancake-like molecular stacks with the extended π-electronic systems above and below the molecular planes; see figure 1.2. Electrons are delocalized in these systems and move from plane to plane along the TCNQ stacks. The conductivities are very anisotropic, as much as 500 greater in the direction parallel to the stacks than in the perpendicular direction.[3]

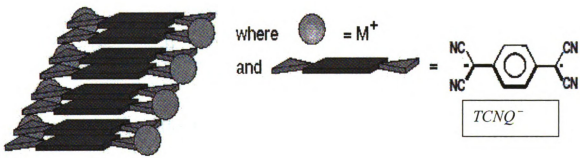


Figure 1.2 Complexed metal TCNQ salts (Figure adapted from http://www.soton.ac.uk/~moldev/research/solstate.htm)

More than 400 salts of TCNQ have been prepared.[7] These materials showed a wide range of novel magnetic, electrical, and structural properties. The reaction between donor (D) and acceptor (A) molecules in these salts can be illustrated in Fig 1.3.

$$DA \quad [D^{+}\cdot][A^{-}\cdot]$$

Figure 1.3 Charge-transfer compounds

In 1970, Wudl synthesized the organic electron-donor molecule TTF (tetrathiafulvalene) and found that highly conductive materials could be made when it reacted with halogens and pseudo-halogens.[8] TTF has four sulfur atoms and is a good electron donor. It is able to give up an electron to form a stable, positively charged cation. In combination with TCNQ a 1:1 salt crystallizes, between the temperatures of 298 and 54 K, TCNQ-TTF possesses the characteristics of a metal. Figure 1.4 shows the structure of TTF.

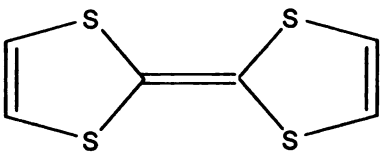


Figure 1.4 The structure of TTF

1.1.2. Conductive organic conjugated polymer

It has been known for more than 40 years that the electric conductivity of conjugated polymer chains is much higher than that of other polymeric materials.[8] Conjugated polymers have a structure of alternating single and double carbon-carbon (sometimes carbon-nitrogen) bonds. Single bonds are σ bonds, and double bonds have a σ bond and a π bond. Conjugated polymers have a σ bond backbone of overlapping sp^2 hybrid orbital. The p_z orbitals of the carbon atoms overlap with p_z orbitals of neighboring carbons to form the π bond. These π bonds can lead to electron delocalization along

the backbone of the polymer. This provides the possibility of charge mobility in the polymer chain.

Some conjugated polymers, such as polyaniline (PAn) and poly (N-vinylcarbazole) (PVCZ), do not have strict alternating single and double bonds structure, but nitrogen p orbitals help the delocalization of π electrons, so these are also considered to be conjugated polymers. The Fig 1.5 shows some examples of conjugated polymers.

In 1961, Hatano found that polyacetylene conducted electrical current. In 1977, it was found that the conductivity of polyacetylene could be increased by 13 orders of magnitude when it was doped with various donor or acceptor species to give p-type or n-type semiconductors and conductors.[10, 11] This finding attracted a lot of interest in research in the field of conductive polymers.

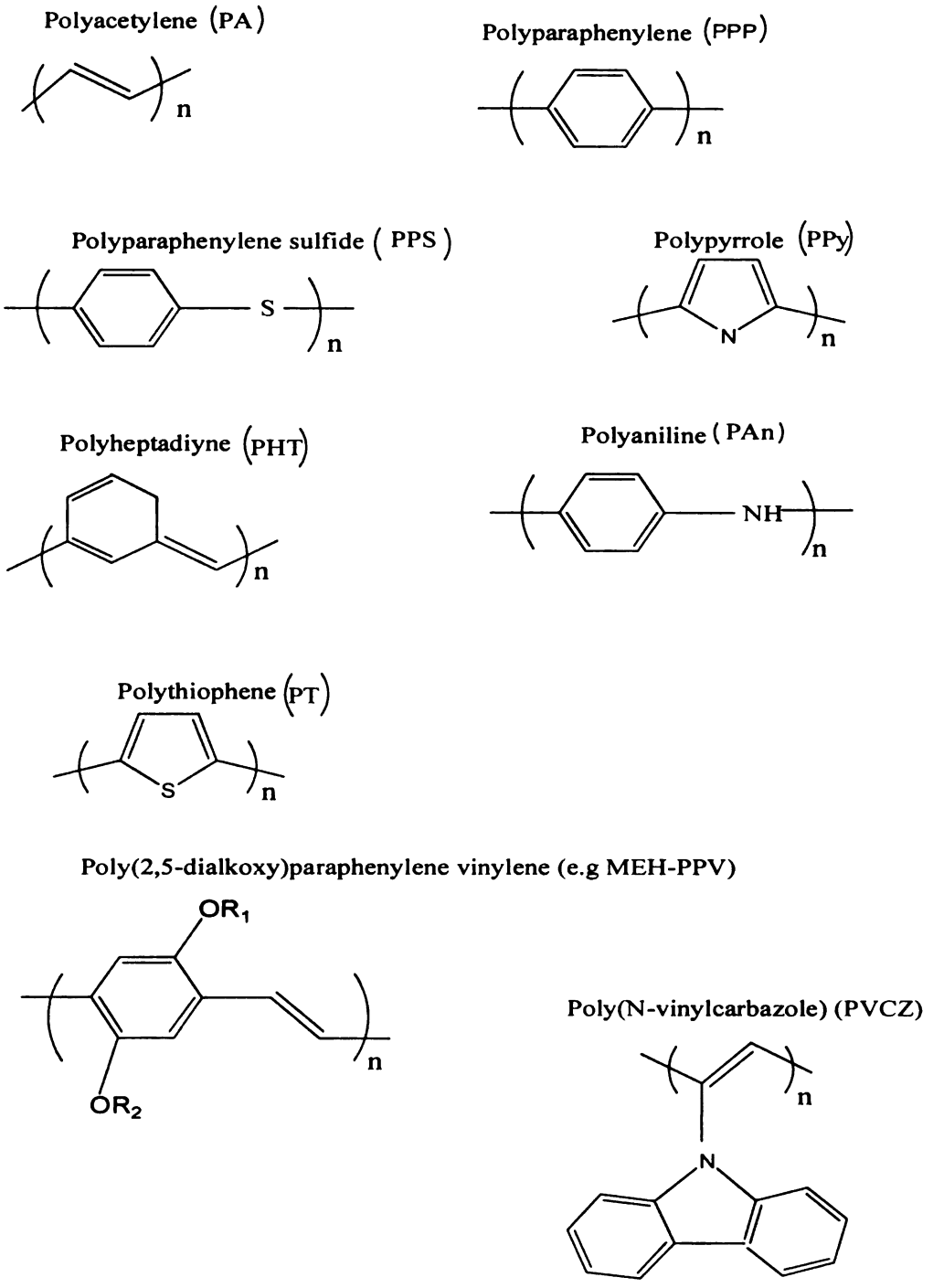


Figure 1.5 Molecular structures of examples of conjugated polymers

The term "intrinsically conducting polymer" (ICP) is used to describe those organic polymers that not only have the electrical, electronic, magnetic and optical properties of

a metal, but retain the mechanical properties and processibility commonly associated with a traditional polymer. Their properties are intrinsic to a "doped" form of the polymer. Figure 1.6 illustrates the conductivity of some doped electronic polymers.

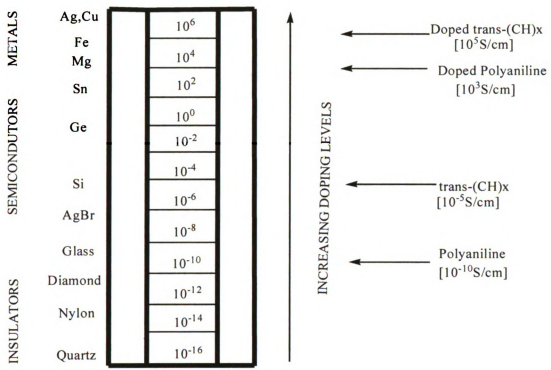


Figure 1.6 Conductivity of electronic polymers (Figure adapted from Alan G. MacDiarmid. Angew. Chem. Int. Ed. **2001**, 40, 2581-2590)

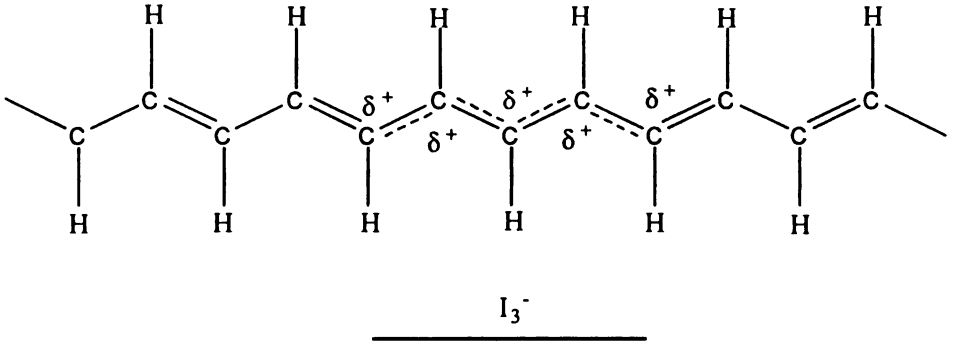
The reason why conjugated polymers dramatically increase the conductivity is doping. The followings are several important doping types.

P-Doping is a treatment that partially oxidizes the π backbone of an organic polymer. The most extensively investigated p-doped polyacetylenes (PA) are the halogen

derivatives.[12] Analysis of structural data suggests that the following chemical reaction takes place between PA and iodine:

$2(CH)_x + 3I_2 \rightarrow 2\,(CH)_x^{+}I_3^{-}$

This process increased conductivity of PA from 10^{-5} S cm^{-1} to 10^{3} S cm^{-1}. Scheme 1.1 shows a positive soliton of p-doped polyacetylenes.



Scheme 1.1 A positive soliton of p-doping. (Figure adapted from Alan G. MacDiarmid. Angew. Chem. Int. Ed. **2001**, 40, 2581-2590.)

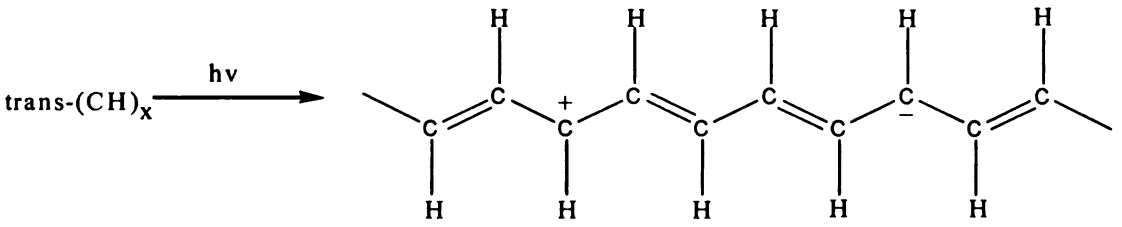
N-Doping is a partial reduction of an organic polymer. The n doping of PA is carried out by strong reducing agents, such as alkali metal. The most common method of alkali metal doping is the reaction with the naphthalenide salts of alkali metals in THF solution:

$(CH)_x + Na^{+}C_{10}H_8^{-} \rightarrow Na^{+}(CH)_x^{-} + C_{10}H_8$

This process, which partially populates the antibonding π system, can increase conductivity by about 10^{3} S cm^{-1}.

Photo-Doping is achieved by exposing trans-$(CH)_x$ to radiation of energy greater than or equal to its band gap. During this process, electrons are promoted across the gap.

Scheme 1.2 shows a representative of positively charged and negatively charged solitons in trans-$(CH)_x$.[13]



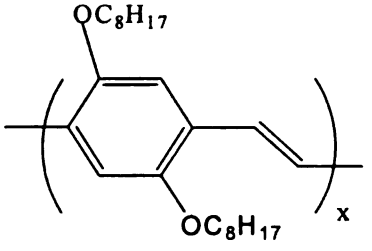
Scheme 1.2 A soliton of photo-doping. (Figure adapted from Alan G. MacDiarmid. Angew. Chem. Int. Ed. **2001**, 40, 2581-2590)

1.1.3 Conjugated polymers used as new materials for photovoltaic

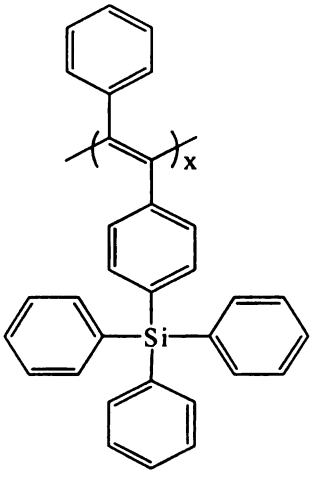
Photovoltaics (PV), sometimes called solar cells, are semiconductor devices that convert sunlight to direct current electricity. The first conventional photovoltaic cells were made in the late 1950s. Then they were mainly used to provide power for earth-orbiting satellites. In the 1970s, with improvements in performance and quality of PV, they were widely used to reduce the cost and give opportunities for powering remote terrestrial applications, such as battery charging navigational aids, signals and telecommunication equipment. In 1980s, PV became a powerful source for consumer electronic devices, including calculators, watches and small battery-charging applications. After the energy crises of the 1970s, more efforts began to develop PV power system for residential and commercial uses, both for remote power and for

utility-connected applications. Today, the industry's PV production is increasing at 25 percent annually.[14]

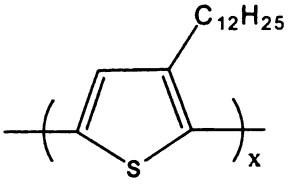
Though common materials for photovoltaics are inorganic, there has been a lot of effort to develop organic solar cells in the last several decades.[15] First, the small organic molecules (pigments) were used on PV.[15] Later, since conjugated polymers were found to have the characteristics of conductors and semiconductors, these materials were applied in PV, resulting in big improvements within the past years.[16, 17, 18] Fig 1.7 shows some examples of conjugated polymers used in PV.



Poly (2,5-dioctyloxy-p-phenylenvinylene
OOPPV



Poly (1-phenyl-2-p-triphenylsilyl phenyl acetylene)
PDPA-TPSi



Poly (3-dodecylthiophene)
PAT-12

Figure 1.7 The molecular structures of examples of conjugated polymers used in PV

How do conjugated polymers work as photovoltaics?

For inorganic semiconductors, the mechanism of charge generation from incident photons is well known. Because these materials are crystalline solids, their electronic structure can be shown in terms of energy bands. Their electronic structure has a conduction band and a valence band which are separated by an energy gap. The size of the gap depends on the materials. For most semiconducting materials, the band gaps are between 0.1eV and 2.2 eV. If these materials are irradiated with light, the electrons from the valence band can be excited to the conducting band. As a result, two charge carriers are produced-an electron in the conduction band and a hole in the valence band. For conjugated polymers, the characteristics of the π bonds are the source of the semiconducting properties of these polymers. The low energy π orbital is like the valence band, and the higher energy π^*-orbital is like the conduction band. The difference in energy between the two orbitals is the band gap which decides the optical properties of material. Most semiconducting conjugated polymers have a band gap between 1.5-3 eV. This makes them very suitable as optoelectronic devices working in the optical light range.

The first generation of organic photovoltaic solar cells were made by sandwiching single organic layers between two metal electrodes.[15] Their power conversion efficiencies were poor (in the range of 10^{-3} to 10^{-2} %). In 1986, two-layer organic photovoltaic cell (a phthalocyanine derivative as p-type semiconductor and a perylene

derivative as n-type semiconductor sandwiched between a transparent conducting oxide and a semitransparent metal electrode) was made.[21] It had about 1% power conversion efficiency.

In 1992, Sariciftci found a photoinduced electron transfer from optically excited conjugated polymers to the C_{60} molecule.[22] Then, highly increased photoconductivities were found after adding C_{60} to the conjugated polymers.[23, 24] The above results led to the development of polymer-fullerene bilayer heterojunction devices incorporating C_{60} and C_{60}–derivatives. These devices' efficiencies can be improved to 1.5%-4%.[25, 26] Why does adding fullerene improve the efficiency so much? The C_{60} molecule is an acceptor which can take on as many as six electrons.[27] After photoexcitation of the conducting polymer with light, electrons transfer to the C_{60} molecule. This results in an effective quenching of the excitonic photoluminescence of polymer. The photoinduced charge transfer is shown in Fig 1.8.

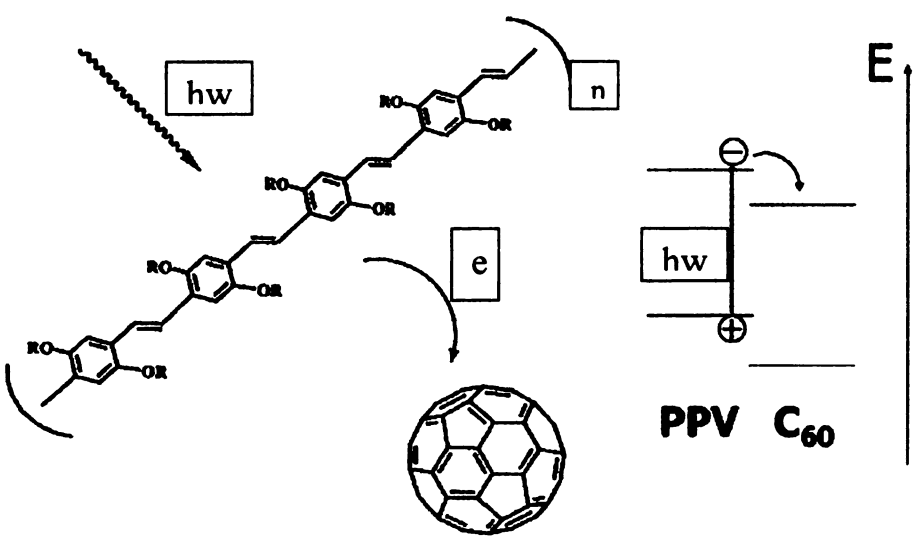


Figure 1.8 Photoinduced charge transfer (left) and a sketch of the energy level scheme (right). (Figure adapted from Harald Hoppe, Niyazi Sedar Sariciftci; Organic solar cells: An overview; J. Mater. Res., **2004**, 19, 1924-1945)

1.2 Supramolecular Chemistry: Host–Guest chemistry

1.2.1. Perspective of supramolecular chemistry

Supramolecular chemistry, which involves chemistry, biochemistry physical and materials science emerged only a few decades ago. According to the definition proposed by Jean-Marie Lehn, supramolecular chemistry is "the chemistry beyond the molecule".[28] Supramolecules are different from large molecules. They are complexes formed from two or more molecules by intermolecular forces (electrostatic forces, hydrogen bonding, van der waals forces, etc.). The molecular components are subunits that exist independently and have their own properties which may be kept or changed within the supramolecule. Figure 1.9 shows a process in which molecular chemistry leads to supramolecular chemistry.

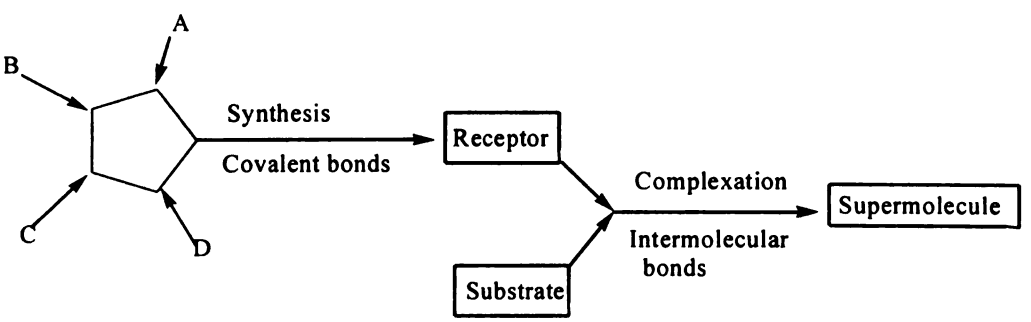


Figure 1.9 A molecular level diagram showing a process of molecular chemistry to supramolecular chemistry. (A, B, C and D represent small molecules)

1.2.2. Host-Guest chemistry

Host-Guest chemistry is a branch of supramolecular chemistry. It describes complexes that consist of two or more molecules held together in unique structural relationships by

hydrogen bonding or by ion pairing or by Van der waals force other than those of full covalent bonds.

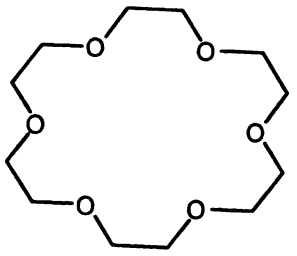
In 1967, Pedersen synthesized polyether macrocycles which he called crown ethers.[30] Figure 1.10 a shows examples of crown ethers. He showed that these compounds can bind the alkali metal ions of lithium, sodium, potassium into complexes. This began the research in host-guest chemistry. Crown ethers are macrocycles consisting of only ethyleneoxy units. Different numbers of ethyleneoxy units in the crown ethers define different-sized cavities.

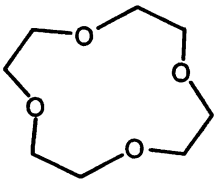
In 1969, based on Pedersen's discovery, Jean-Marie Lehn made bicyclic compounds of the crown ether type called cryptands, which are shown in Figure 1.10 b. These compounds display higher selectivity than crown ethers when forming complexes.[31]

In 1986, Cram designed host molecules called spherands that can form strong complexes with much higher selectivity than cryptands.[32] Figure 1.10c shows examples of spherands.

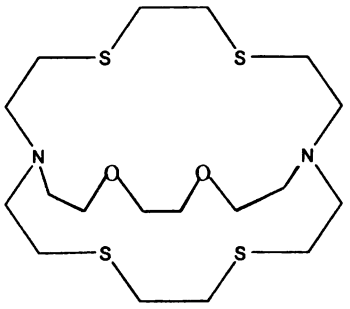
In 1987, the Nobel prize in chemistry was awarded to Donald J. Cram, Jean-Marie Lehn, and Charles J. Pederson.

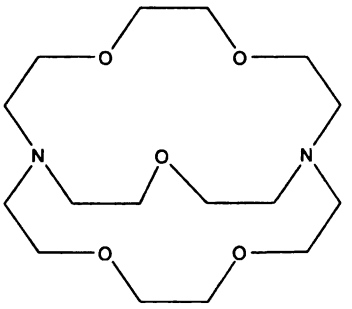
a



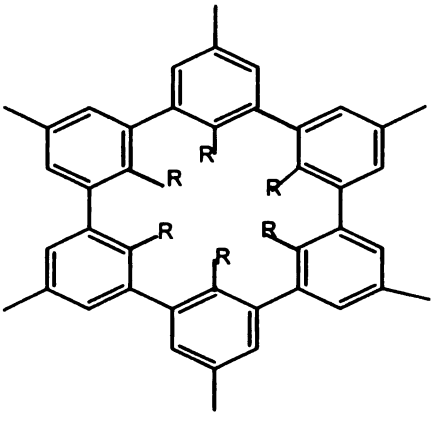


b

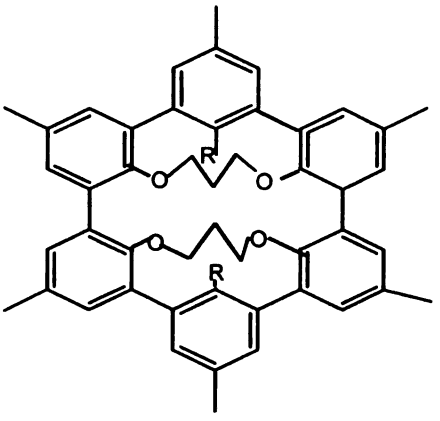




c



R= OCH3



R= OCH3

Figure 1.10 a Examples of crown ethers; **b**. Examples of cryptands; **c**. Examples of spherands

1.2.3. Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharide molecules consisting of 6, 7 or 8 α-I-4 linked D-glucose units (α-, β-, and γ-CD, respectively). As a result of the $^4C_1$ conformation of the glucopyranose units, all primary hydroxyl groups are positioned on one of the two edges of the ring, whereas all the secondary hydroxyl groups are on the other edge. The shape of the CDs is like a truncated cone. Figure 1.11 shows the structure and shape of CDs.

The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges, so the inside cavity has hydrophobic properties. Because of the many hydroxyl groups of CDs, the exterior of CDs is hydrophilic, making them soluble in water.

Cyclodextrins provide an ordered medium capable of molecular organization since they can form inclusion complexes with a variety of aliphatic and aromatic molecules by inserting the appropriate size guest into their cavity.[33-35] The binding forces responsible for the inclusion of guests are due to hydrophobic interactions, van der Waals interactions, hydrogen bonding and stabilization due to the displacement of water from the cavity. The size of cavity and the complexation properties depend on the number of gluopyranose units forming the ring.

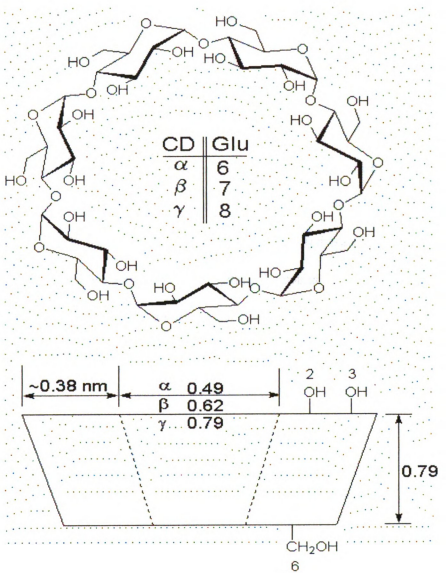


Figure 1.11 Structure and shape of cyclodextrins (Figure adapted from http://jindrich.org/CD/sbcdcon2.gif)

Hydroxyl groups offer good opportunities to modify the cyclodextrins. Reasons range from getting solubility in a desired solvent to investigating the mechanisms of enzyme-catalyzed reactions. Modifying cyclodextrins makes them more useful in industry and research. There are challenges to the modification of cyclodextrins. Challenges come

from the hydrophobic cavity and the large number of different hydroxyl groups.[36] Hydroxyl groups at the 2-, 3-, and 6- positions compete for reactants and make selective modification very difficult. The hydrophobic cavity can also complex with the reagent to direct its activity to an unexpected place.[37]

The strategy for modification depends on the purpose of the targeted product. There are two strategies for modification. The first one is the easy one: nonselective modification. For example, if a highly water soluble cyclodextrin is needed for application in a drug formulation, then a random conversion of hydroxyl groups to sulfate groups can be easily made and the product mixture will have a good solubility in water.[38] Similarly, if a cyclodextrin is needed with a high solubility in organic solvents, it is possible to convert the hydroxyl groups to silyl ethers in a random way.[39] This product will be very soluble in organic solvents and can be used to disperse indicator dyes which would otherwise tend to stay together. The other strategy is a much longer method: selective modification. It involves a series of protection and deprotection steps in order to selectively modify specific targeted positions. The final product is then homogeneous with a structure that is well characterized.

1.3 Photoinduced electron transfer.

1.3.1 Introduction

Photoinduced electron transfer is a branch of photochemistry. It concerns the properties of certain photoexited molecules to act as strong oxidizing or reducing species. Photoinduced electron transfer is involved in many areas of science. For example, biological photosynthesis is a process by which sunlight is harnessed for the growth and nourishment of plants. The early events in photosynthesis start with light absorption by an antenna system followed by a series of electron transfers. Photoinduced electron transfer also attracts organic chemists who seek to synthesize novel organic compounds that may be difficult to make by other routes.

Figure 1.12 shows a classification of photochemical pathways.

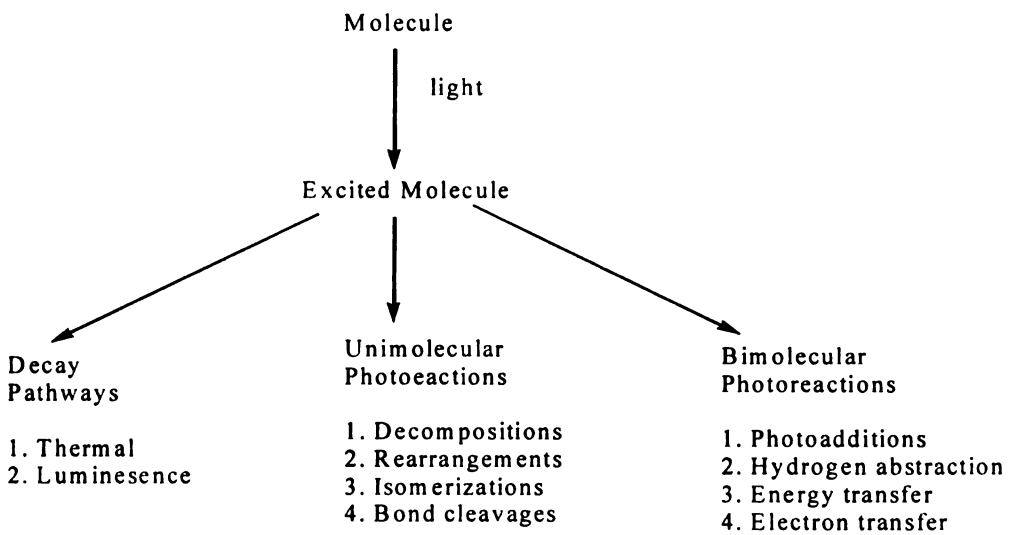


Figure 1.12 A classification of photochemical pathways

When ground-state molecules absorb visible or ultraviolet light, electrons in the highest occupied molecular orbitals (HOMO) are exited to the lowest unoccupied molecular orbitals (LUMO) which are at higher energy level. See Figure 1.13.

By absorbing a photon of light, the ground state is converted into a higher energy state, or electronically excited state.

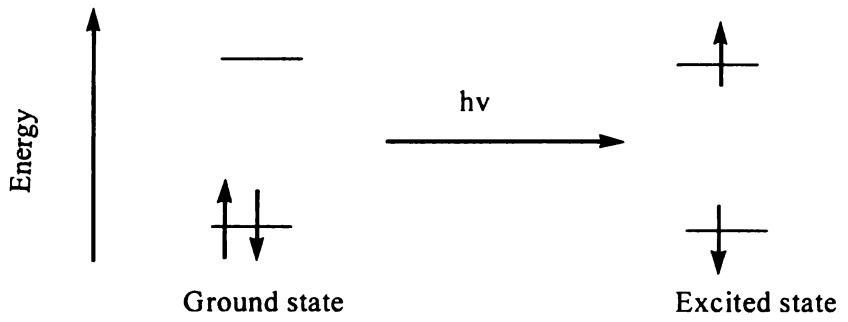


Figure 1.13 Photoexcitation results in an electronic transition

1.3.2 Types of electronic transitions

Why can some molecules easily get to their excited state by absorbing light, while others can not? It depends on the structure of the molecules. Different bonds that they have decide different electronic transitions. Figure 1.14 shows the most common types of electronic transitions in organic molecules.

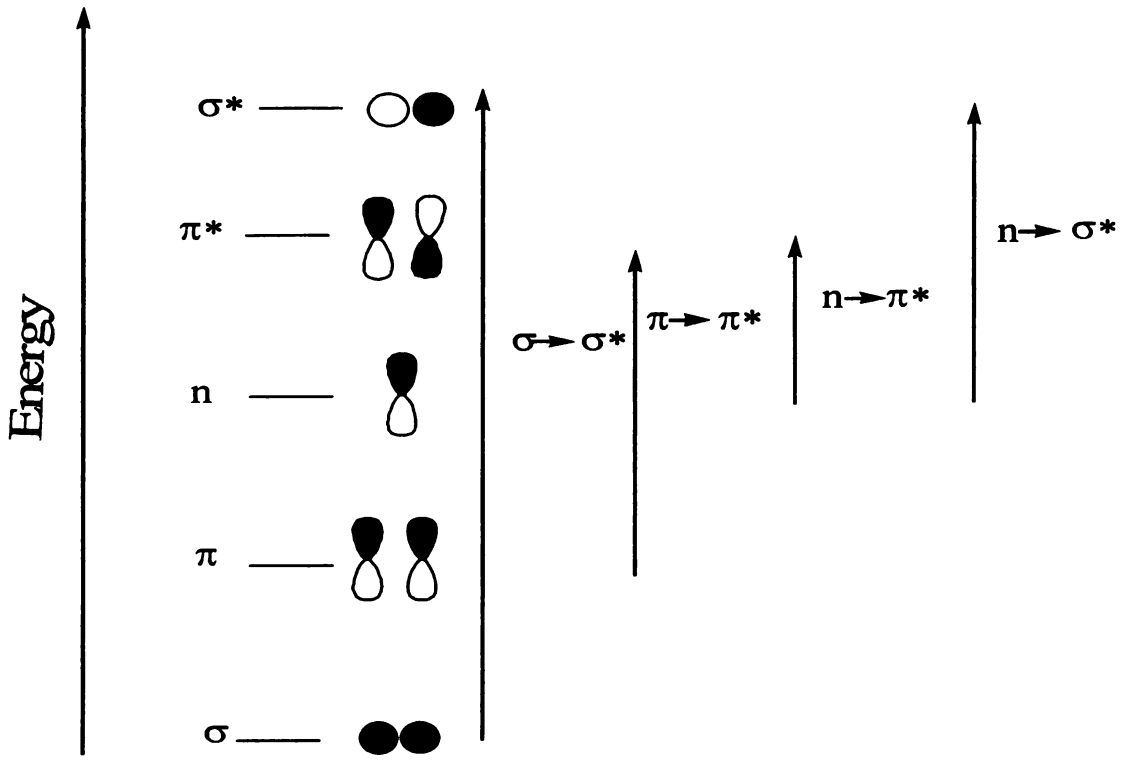


Figure 1.14 The most common types of electronic transitions in molecules

There are four main types of electron transitions: $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, and $n \rightarrow \sigma^*$.

The $\sigma \rightarrow \sigma^*$ transitions occur between the lowest energy orbital to the highest energy orbitals, so they require large amounts of energy, usually about 200 kcal mol^{-1}.

In a $\pi \rightarrow \pi^*$ transition, an electron from a bonding π-orbital is promoted to an antibonding π^*-orbital, as found in unsaturated organic molecules. The gap between π and π^*-orbitals is smaller than that between σ and σ^* since the energy of the bonding π-orbital is higher than bonding σ-orbital, and the energy of the π^*-orbital is lower than σ^*-orbital. As a result, photon excitation of an electron in the bonding π-orbital to the antibonding π^*-orbital requires less energy than a $\sigma \rightarrow \sigma^*$ transition.

An $n \rightarrow \pi^*$ transition is between non-bonding orbital and π^*-orbital. Since the n-orbital's energy is higher than the σ- and π-orbital energy, the $n \rightarrow \pi^*$ transition to create an n,π^* excited state usually needs less energy than $\pi \rightarrow \pi^*$ transition. It is most commonly found in organic molecules containing the carbonyl group such as ketones, aldehydes, esters and so on.

The $n \rightarrow \sigma^*$ transitions involve the promotion of an n-electron to an antibonding σ^* orbital. This transition is found in compounds containing heteroatom such as aliphatic amines and halogens.

1.4 Supramolecular Photochemistry

1.4.1 Mechanisms of Photophysical processes in Supramolecules

The field of supramolecular photochemistry has been very attractive for many studies. On one hand, it can model systems like those that perform photochemical processes in living organisms. This can help understand detailed mechanisms of some biological electron transfers which usually occur between donor and acceptor partners held together by noncovalent interactions. On the other hand, the design of artificial systems capable of performing useful light functions leads to the development of photoactive devices. The organizational architecture of a supramolecular system gives arrangements of photoactive subunits in space so that photochemical processes such as charge separation by electron transfer and selective photochemical reactions can be studied. In

most cases, the event that triggers the processes is molecular recognition of a species to form the supramolecular structure, as shown in Figure 1.15.

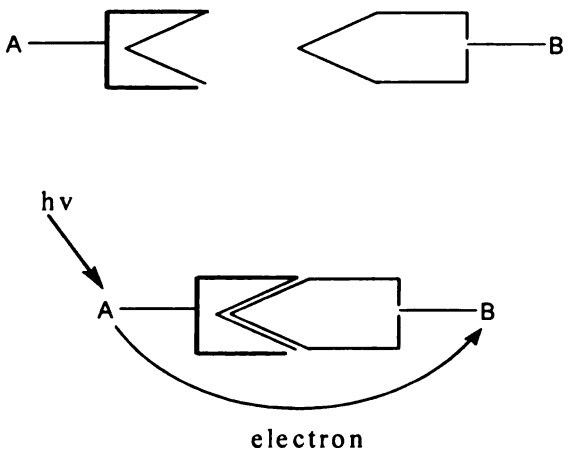


Figure 1.15 Photophysical process prompted by molecular recognition

Electron transfer processes are functions of the donor-acceptor distance, orientation and environment. General descriptions of the processes are:

$$D \text{——} A \rightarrow D^{*} \text{——} A \rightarrow D^{+} \text{——} A^{-}$$

When donor and acceptor get close enough, after donor is excited, there will be an electron transfer between donor and acceptor. A model for the respective orbital interactions is shown in Figure 1.16.

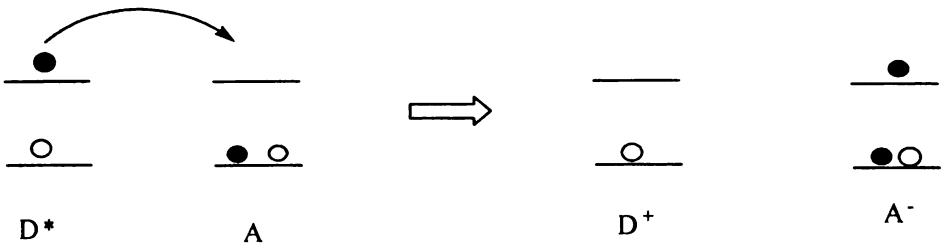


Figure 1.16 Schematic description of electron motion in electron transfer

1.4.2 Photoinduced electron transfer (PET) in Host-Guest Complexes based on Cyclodextrin

As discussed above, cyclodextrins (CDs) can form inclusion complexes with diverse organic compounds. This binding property has been used in the construction of artificial enzymes[40] and molecular machines.[41] The same binding property is also expected to be useful in the assembly of supramolecular PET systems. De Cola et al. used metallocyclodextrins as building blocks in noncovalent assemblies of photoactive units to study the photoinduced intercomponent processes.[42] They functionalized a β-cyclodextrin with a terpyridine unit to get ttp-β-CD by protecting all but one of the hydroxyl groups by methylation and attachment of the ttp unit on the free primary hydroxyl group. Then the metalloreceptor [(β-CD-ttp) Ru (ttp)] $[PF_6]_2$ was synthesized. The resulting system exhibits luminescence in water, centered at 640 nm. After redox-active quinine guests AQS, AQC, and BQ were added to an aqueous solution of $[(\beta\text{-CD-ttp})Ru(ttp)]^{2+}$, there is quenching of the luminescence up to 40%, 20%, and 25%, respectively. Figure 1.17 shows the host-guest complex.

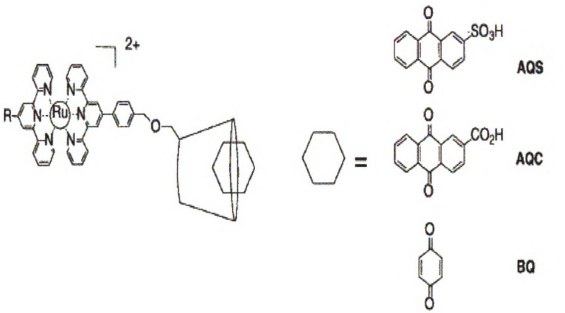


Figure 1.17 Schematic description of luminescence quenching of [(β-CD-ttp)Ru(ttp)]$^{2+}$ by quinones). (Figure adapted from J. M. Haider, M. Chavarot, S. Weidner, I. Sadler, R. M.Willams, L. De Cola, and Z. Pikramenou. Inorg. Chem; 2001, 40 3912-3921)

They also made a metalloguest, [Os (biptpy) (tpy)] [PF_6] which is designed with a biphenyl hydrophobic tail for insertion in the cyclodextrin cavity. It was assembled with the ruthenium cyclodextrins to form a complex. Electron transfer from the ruthenium (II) center appended to the cyclodextrin to an osmium (III) metalloguest in the cyclodextrin cavity has been observed. The photoinduced process between the two metal centers is established via noncovalent bonds in aqueous solutions (Figure 1.18).

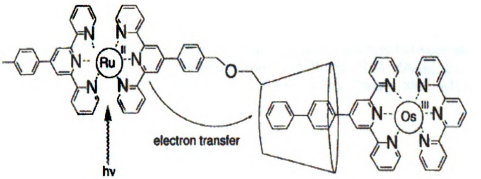


Figure 1.18 Schematic description of electron transfer from the ruthenium center appended to the cy to the cyclodextrin to an osmium metalloguest in the cyclodextrin cavity (Figureadapted from J. M. Haider, M. Chavarot, S. Weidner, I. Sadler, R. M. Willams, L. De Cola, and Z. Pikramenou. Inorg. Chem; 2001, 40 3912-3921)

Yong-Hui Wang et al. designed and synthesized an artificial system for photoinduced electron transfer in which the acceptor (p-nitrobenzoyl-β-cyclodextrin, NBCD) and donor (naphthalene derivatives) were held together via hydrophobic interactions.[42] Fluorescence was employed and efficient photoinduced electron transfer was observed. In this work no metal ions were involved and the whole system is organic. Figure 1.19 shows the pathway of photoinduced electron-transfer reactions between naphthalene derivatives and NBCD.

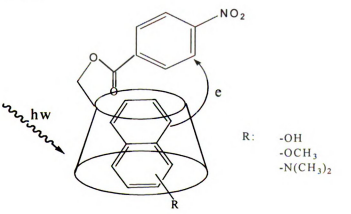


Figure1.19 Pathway of photoinduced electron-transfer reaction between naphthalene derivatives and NBCD (Figure adapted from Y.H. Wang, H.M. Zhang, L. Liu, Z.X. Liang, Q.X., Guo, C.H. Tung, Y. Inoue, Y.C.Liu. J.Org. Chem. 2002, 67, 2429-2434.)

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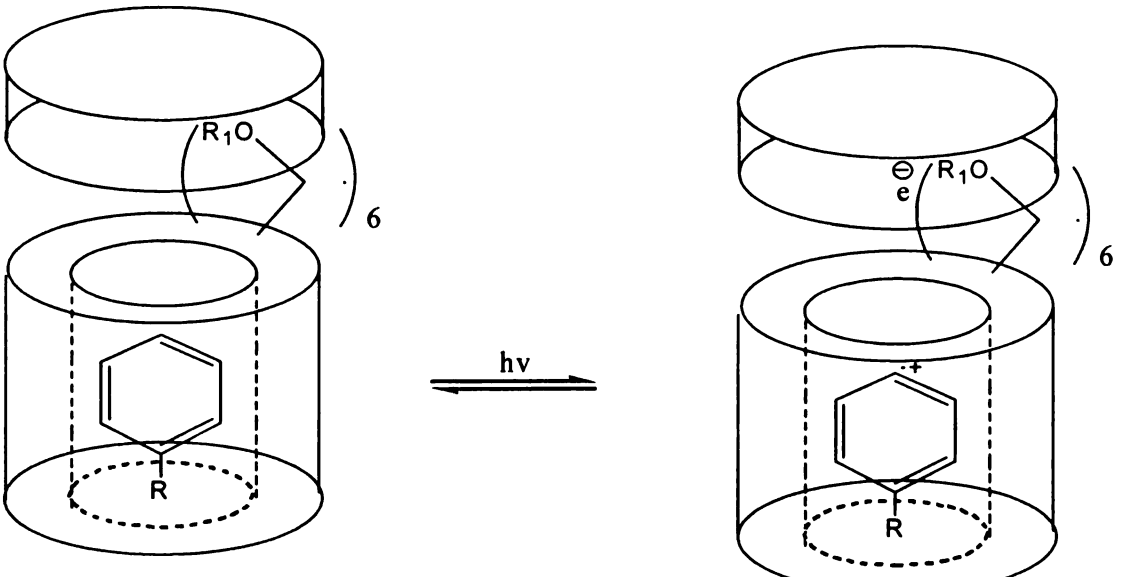
Chapter 2 Design and synthesis of supramolecular host systems

2.1 Rational Design and structure

2.1.1 The design concept

Many artificial systems are developed not only for mechanistic purposes but also for potential device applications. Molecular devices can be defined as structurally organized and functionally integrated systems built into supramolecular architectures. Devices may be photoactive or electroactive.[1-3] The goal of this project is to design and make a supramolecular system that is a potential organic electronic material. Cyclodextrins (CD) are a good molecular template for supramolecule construction because CDs not only have the well-defined microenvironment for molecular recognition but have many hydroxyl groups suited for functionalization. We use modified cyclodextrins combined with some photo-sensitive compounds to form a supramolecular system. The whole idea can be shown in Figure 2.1.



R_1 : pentafluorophenyl
R : -OH, $-OCH_3$

Figure 2.1 Supramolecular system

We functionalize the CD's upper side (primary hydroxyl group's side) with electron-withdrawing groups which function as electron acceptors. We then introduce some photo sensitive compounds which can go to cyclodextrins' cavity as electron donors. If the supramolecular system is irradiated with light, the guest inside the cavity will be excited and there will be an electron transfer event between the acceptor and the donor. The electron-withdrawing groups in the upper region of this supramolecular system can trap this electron to prevent it from escaping or decaying.

One important aspect of this project is how to choose proper guests which can be complexed in the cyclodextrins' cavity. They need to have the following characteristics: First, they need to be a good electron donor; secondly, they should fit into and bind to

cyclodextrin cavity. According to the above requirements, phenols, naphthalene and benzophenone and their derivatives are good candidates for the guest. Figure 2.2 shows the structure of these compounds.

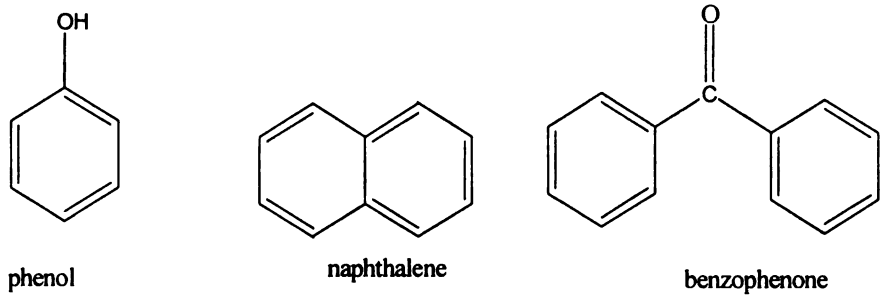


Figure 2.2. Structure of phenol, naphthalene and benzophenone

These compounds all have π bonds and can have $\pi \rightarrow \pi^*$ electronic transition and $n \rightarrow \pi^*$ electronic transition which need less energy. Their ionization potential is low. So they can be good electron donors. They also can fit into the cavity of cyclodextrins. Moreover, they are hydrophobic and have the ability to bind to cyclodextrins to form stable complexes. Researchers have already used naphthalene, benzophenone and their derivatives to bind to cyclodextrins to study photoinduced electron transfer process.[4, 5] The advantage of this system is flexibility. By choosing different electron-withdrawing groups, we can control how strongly electrons can be held in the upper area of the system. We can choose β-CD or γ-CD as the host to make a supramolecular system. Figure 2.3 shows different cyclodextrins.

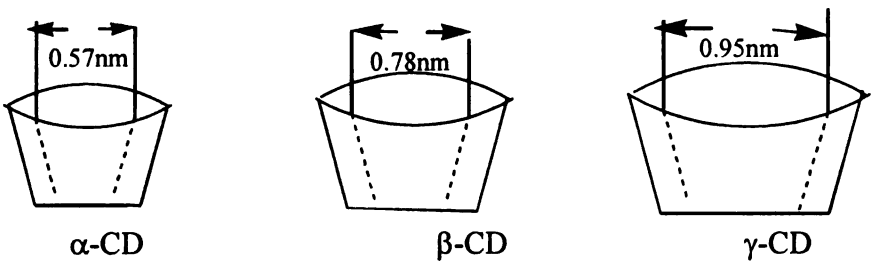


Figure 2.3 Cyclodextrin cups of different size

The size of the intramolecular cavity depends on the number of glucopyranose units. α-CD has six glucopyranose units and the diameter of cavity is 0.57nm. γ-CD has eight glucopyranose units and the diameter of cavity increases to 0.95nm. Because β-CD and γ-CD have a bigger cavity than α-CD, they can provide us more choices to choose proper guests that can be fit into the cavity of cyclodextrins than α-CD.

2.2 The strategy for modifying the cyclodextrins.

We modified α-cyclodextrin to be the host of a supramolecular system. The structure of the system is shown in Figure 2.4.

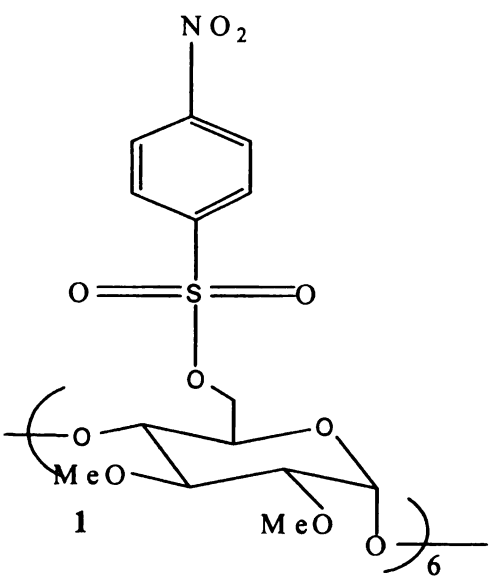
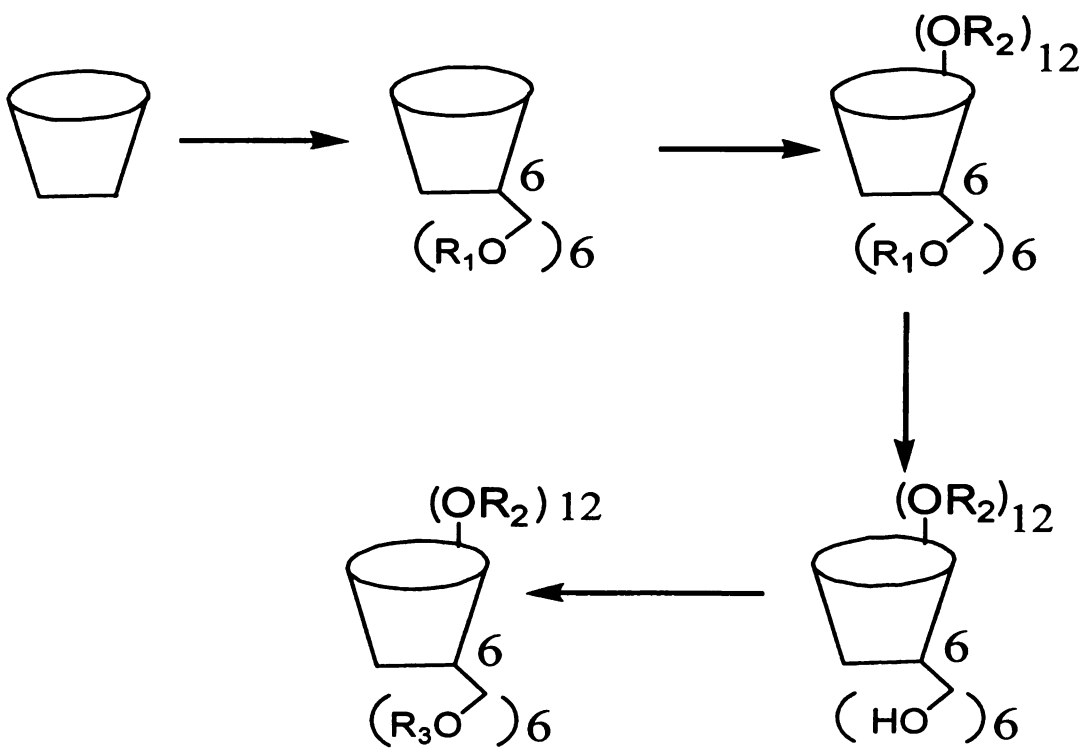


Figure 2.4 The structure of modified α-CD

We used the 4-nitrobenzene sulfonyl group as the electron acceptor to functionalize the six primary hydroxyl groups. Methyl groups were chosen to block all secondary hydroxyl groups. In order to prepare compound **1**, selective modification was chosen. As mentioned in chapter 1, selective modification is full of challenges. We want functional groups to go only to the positions we want them to go to. As discussed before, there are three different types of hydroxyl groups in CDs. They will compete for reactants. We need to choose proper reactants and synthetic route to get the final product. Scheme 2.1 shows the strategy used to modify the cyclodextrin.



Scheme 2.1 Strategy to modify the cyclodextrins

We start to protect all primary hydroxyl groups. The choice of protecting groups should allow only blocking of primary hydroxyl groups and should not react with secondary hydroxyl groups. Tert-butyldiphenylsilyl chloride (TPDPSCl) is an ideal protecting reagent which can protect primary hydroxyl groups only because of its size. After protecting the primary hydroxyl groups, we need to block all secondary hydroxyl groups. Methyl iodide was chosen as a reagent to block the secondary hydroxyls with methyl groups. After deprotection, the primary hydroxyl groups can be functionalized. By several selective protecting and deprotecting steps, we can get the functionalized product.

2.3. Experimental

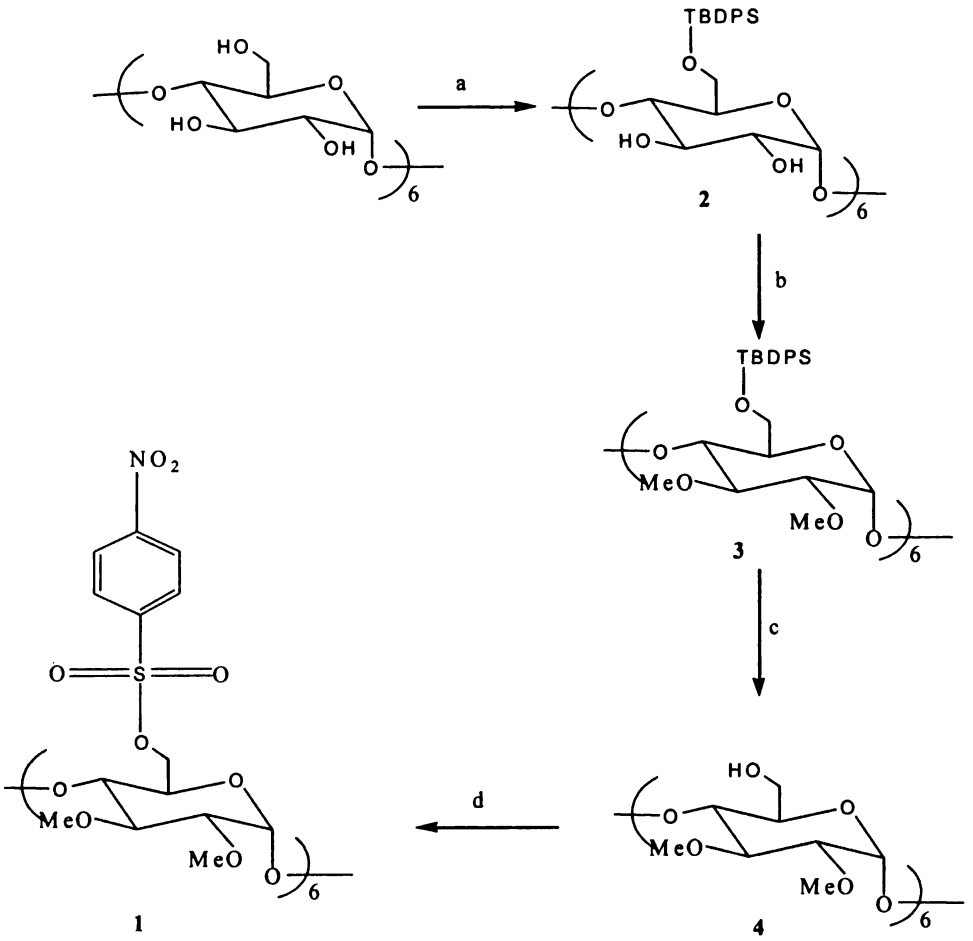
2.3.1 Synthesis

All chemicals were used as received from Aldrich unless otherwise noted.

The α-cyclodextrin was purchased from Cargill, Inc.

NMR spectra were recorded in D-chloroform or Deuterium oxide at room temperature on a Varian VXR 500 MHz spectrometer.

Scheme 2.2 shows synthesis of the goal compound.



Reagents and conditions: (a) TBDPSCl, imidazole, DMF, RT, 91%, 120 hrs; (b) CH_3I, NaH, DMSO, RT, 36 hrs, 86% ; (c) TBAF, refluxing in ethanol, 48 hrs, 51%; (d) 4-nitrobenzene sulfonyl chloride, pyridine, RT, 72 hrs, 65%.

(a) Protecting the primary hydroxyl groups

The reaction was carried out in a 250mL round bottom flask at room temperature. α-cyclodextrin (5g, 0.0051 mol) was dissolved in 30 mL N,N-dimethyl formamide (DMF) followed by addition of imidazole (5.6g, 0.08mol) and TBDPSCl (11.3g, 0.041mol). The reaction mixture was stirred at room temperature for 48 hours. DMF was removed and the crude product was dissolved in ethyl acetate (200 mL) and washed with water

(100 mL) twice. The organic layer was collected and concentrated. The crude product was chromatographed on flash silica (ethyl acetate/methanol/water = 15: 1.4:1 as eluant) to give the final product. ^{1}H NMR (500MHz, $CDCl_3$) δ 7.52 (4H, m), 7.22 (1H, t, J=14Hz), 7.14 (1H, t J=7Hz), 7.10 (2H, t, J=8Hz), 7.04 (2H, t, J=8Hz), 4.92 (1H, d, J=2.5Hz), 4.19 (1H, t, J=9Hz), 3.92 (1H, m), 3.77 (1H, d, J=11Hz), 3.70 (1H, dd, J=17, 3 Hz), 3.58 (1H, d, J=11Hz), 0.88 (9H, s); ^{13}C NMR 135.74, 135.69, 133.83, 133.66, 129.73, 129.68,127.81, 127.68, 101.71 81.79, 74.16, 73.56, 73.24, 63.05, 26.97.

(b) Functionalization of secondary hydroxyl groups

Compound 2 (1g, 0.0004mol) was dissolved in 20 mL DMSO followed by the careful addition of sodium hydride (60% dispersion in mineral oil) (0.4g, 0.24mol). The reaction mixture was stirred at room temperature for 12 h and methyl iodide (1.492g, 0.011mol) was added. The reaction mixture was stirred at room temperature for 24 h. Methanol (5mL) and 15mL water were added to destroy extra sodium hydride. The mixture was extracted with toluene (80mL) twice. The toluene phase was combined and concentrated to produce compound 2. ^{1}H NMR (500MHz, $CDCl_3$) δ 5.10 (1H, d, J=5Hz), 3.86 (2H, m), 3.74 (1H, d, J=15Hz), 3.61 (2H, m) 3.52 (3H, s), 3.46 (3H, s), 3.21 (1H, dd, J=16Hz, 5.5Hz), ^{13}C NMR 97.95, 81.18, 80.28, 79.34, 72.29, 72.12, 60.87,60.29, 57.91, 57.76.

(c) Deprotecting the primary hydroxyl group

Compound 3 (0.54g, 0.001mol) was dissolved in 15 mL ethanol followed by the addition of tetrabutyl ammonium fluoride hydrate (0.501g, 0.002mol). The reaction mixture was refluxed for 60 h and concentrated. The crude product was dissolved in water (50 mL) and washed with toluene (30 mL) twice. The water layer was concentrated and the product was purified by reverse phase column. First, methanol was used as the solvent to pack the column. Water then was added to the column to replace the methanol. One gram mixture was loaded onto the column and water (500 mL) was used as eluent. The mixed solvent (water/methanol = 4:1, 500 mL) then was used to elute. The different solvent systems were used in the following order: water/methanol = 3:1, water/methanol = 2:1, water/methanol = 1:1, water/methanol = 1:2, water/methanol = 1:3, water/methanol = 1:4, pure methanol. The final product was contained in the water/methanol 1:1 solvent system. ^{1}H NMR (500MHz, $CDCl_3$) δ 5.15 (1H, d, J=3.5Hz), 3.89 (2H, m), 3.70 (1H, m), 3.62 (1H, m) 3.53 (3H, s), 3.41 (3H, s), 3.24 (1H, m), ^{13}C NMR 97.95, 81.18, 80.28, 79.34, 72.29, 72.12, 60.87, 60.29, 57.91, 57.76.

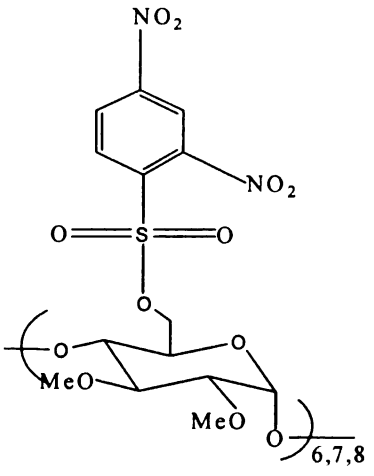
(d) Functionalization of primary hydroxyl groups

Compound 4 (0.05g, 0.00007mol) was dissolved in anhydrous pyridine and 4-nitrobenzene sulfonyl chloride (0.072g, 0.00032mol) was added. The reaction mixture was stirred at room temperature for 72 hours and the solvent was removed. Hexanes (300 mL) was added and decanted. The residue which was not dissolved in hexane was treated with 300 ml toluene and the toluene solution was decanted. The rest of the

material which did not dissolve in toluene was all dissolved in chloroform. All of the organic solutions were concentrated and analyzed by NMR spectroscopy. From the NMR results, the product is in toluene phase; a white solid, 0.06g (65%). ^{1}H NMR (500MHz, $CDCl_3$) δ 8.26 (2H, d, J=9Hz), 8.10 (2H, d J=8Hz), 5.12 (1H, d, J=3Hz), 4.00 (2H, m) 3.65 (3H, s), 3.53 (3H, s), 3.51 (1H, m), 3.48 (1H, m), 3.20 (1H,m) ^{13}C NMR 127.64, 125.12, 123.81, 107.99, 94.15, 77.17, 75.21, 72.25, 68.77, 68.38, 55.99, 55.66.

2.4 Future Directions

We will make several modified cyclodextrins to be host of the supramolecular system. We will modify β-cyclodextrin and γ-cyclodextrin. By doing this, we can use guests with big size that can be fit in the cavity of modified cyclodextrins as electron donor. We will use different electron-withdrawing groups to functionalize the primary hydroxyl groups as electron acceptor, such as dinitrobenzene sulfonyl group, pentafluorobenzene group, and so on. Some target compounds are shown in Figure 2.5.



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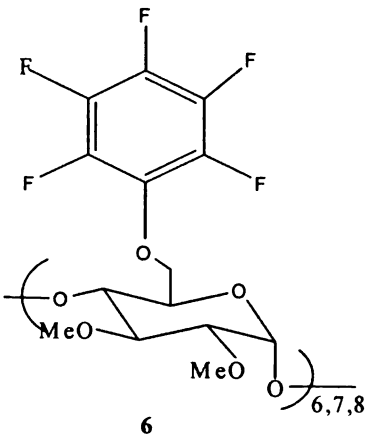

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Figure 2.5 Structure of different modified cyclodextrins

These modified cyclodextrins will be used to form supramolecular systems with electron donor, such as phenol, naphthalene and benzophenone and their derivatives. We can do some test of these supramolecular systems. First, we will test if there is photoinduced electron transfer in these supramolecuar systems in aqueous solution by using fluorescence quenching. If positive results are obtained, we can do some further tests. We can test if these solid supramolecular systems have conductivity when they are irradiated with light by applying a potential to this supramolecuar system.

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CHAPTER 3 Tethered lipid bilayers deposited on gold for bioelectronic applications

3.1 Background

3.1.1 Biological membranes

Biological membranes play important roles in cellular life. They control the transfer of information and the transport of ions and molecules between the inside and outside cellular worlds and take part in many intra- and extra-cellular processes.[1] These complex and dynamic membranes are only a few nanometers thick, consisting of two main components: a bilayer lipid membrane (BLM) and membrane proteins. A lipid bilayer provides a basic structure within which proteins are free to diffuse. Figure 3.1 shows an image of a membranes.

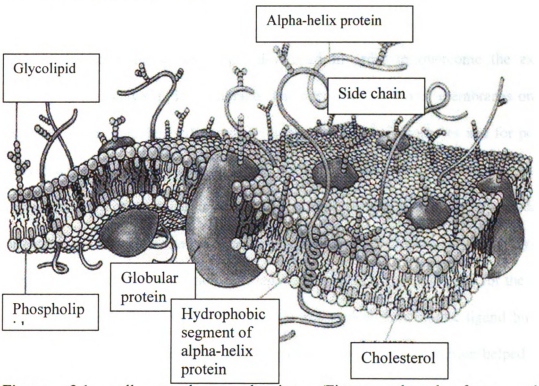


Figure 3.1 cell membrane drawing (Figure adapted from website: www.molecularstation.com/molecular-biologyimages/showphoto.php/photo/17/size/big)

Our current knowledge of the molecular processes which occur at biological membranes is founded on studies both on integrated and reconstituted systems using models of biological membranes. Many of these processes can be reproduced in the laboratory by incorporating proteins into interfaces that facilitate the proteins activities. There is a problem with BLMs. BLMs are not stable. Once formed, they typically survive from minutes to hours and are very sensitive to vibration and mechanical shock.[2] Researchers need to find ways of solving this problem.

3.1.2 Supported bilayer lipid membranes (sBLMs)

Supported bilayer membranes were developed in order to overcome the extreme fragility of the bilayer lipid membrane. The deposition of model membranes on solid supports is very popular both for studying basic membrane processes and for possible biotechnological applications.[3-5] They provide a natural environment for the immobilization of proteins under nondenaturing condition and in a well-defined orientation. They allow the preparation of ultrathin, high-electric-resistance layers on conductors and the incorporation of receptors into these insulating layers for the design of biosensors which are based on electrical and optical detection of ligand binding.[6] The growing interest in confining lipid membranes on surfaces has been helped by the emergence of a multitude of surface-sensitive characterization techniques, advanced surface patterning methods, and liquid handling systems.[7-9]

There are three types of supported membranes which can be assembled as shown in figure 3.2. The first one (A) is integrated bilayers with the inner monolayer which is

fixed to the substrate either covalently or by ion bridges. The second one (B) is freely supported lipid-protein bilayers separated from the substrate by ultrathin water. The third one (C) is bilayer membranes supported on ultrathin, soft hydrated polymer films. This is a completely different type of supported membrane which is used to immobilize monopolar (amphiphilic) proteins. It can be formed by ultrathin films (such as dextran) hydrophobized by coupling of long alkyl chains to the hydrophilic polymer backbone.

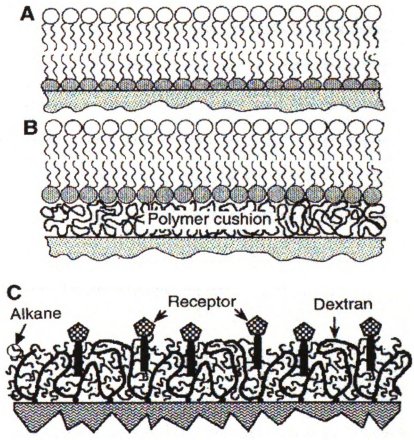


Figure 3.2 Three types of supported membranes (Figure adapted from Sackmann, E Science 1996, 271, 43-48)

Generally, there are two common methods of membrane assembly on surfaces: monolayer transfer (by the Langmuir-Blodgett) and vesicle spreading. For these two methods, the continuity of the supported membranes depends on the smoothness of the substrate. The supports must either be treated briefly by argon sputtering or freshly cleaved mica must be used in order to separate membranes from the substrate by an ultrathin film of water.[10]

Supported membranes have two serious shortcomings. First, there is no cushion between the sBLM and the surface to give space for extramembrane moieties of membrane proteins or other biomolecules and to allow lateral mobility of the membrane components; the second is that unlike most cell membranes, they do not have ionic reservoirs on both sides of the bilayer. Such reservoirs are necessary to achieve protein- or ionophore-mediated transport across the BLM and perform certain bioelectronic applications, such as electrochemical impedance spectroscopy.

3.1.3 Tethered Lipid Bilayer on Gold Surface

3.1.3.1 Tethered ipid bilayer's concept and structure

The tethering of molecular analogues of biological membranes to solid surface has been used in many biomimetic system.[11, 12] Tethered lipid bilayers consist of a lipid tail and a hydrophilic spacer attached to the solid substrate. Because of the simplicity of sulfur-gold tethering chemistry, thiol-and disulfide–labeled compounds have been the basis of

most of these studies. The basic tBLM structure can be seen schematically in Figure 3.3. The mobile lipid (A) forms the bulk of the bilayer membrane. The hydrophilic portion of the reservoir lipid (B) forms the ionic reservoir between the gold electrode and the bilayer membrane. The hydrophobic portion of the reservoir lipid incorporates into the bilayer membrane, so the membrane is tethered to the electrode surface. Spacer molecules (C) are used to further control the lateral spacing between the reservoir lipids. Generally, the spacer molecules are small, hydrophilic disulfide-containing molecules such as dithiodiglycolic acid. D is the potassium specific valinomycin ion carrier which is used to modulate the conductivity of the membrane and investigate the function of the ionic reservoir.

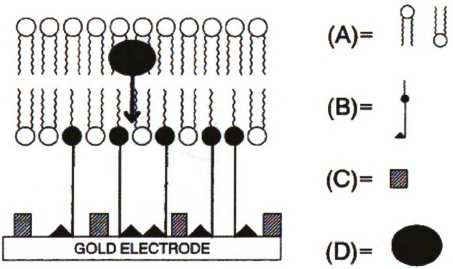


Figure 3.3 Schematic representation of tethered bilayer membrane where (A) is the mobile lipid that makes up the bulk of the membrane, (B) is the reservoir lipid that defines the ionic reservoir and tethers the membrane to the gold surface, (C) is the spacer molecules used to laterally space the reservoir lipids, (D) is the valinomycin ionophore used to modulate the membrane conductivity. (Figure adapted from Raguse, B.; Braach-Maksvytis, V.; Corbekk, B. A,; King, L. G.; Osman, P.D.J.; Wieczorek, L. Langmuir 1998, 14, 648-659)

Compared with the sBLMs, the tethered BLMs give the following advantages: (1) They have a submembrane space that can serve as an ionic reservoir on each side of membrane as well as provide enough space for incorporated membranes proteins; (2) they are robust and have high insulating ability; (3) they have accessibility to electrical measurements.

3.1.3.2 Tethered Lipid bilayers membrane assembly

The method of membrane assembly on surfaces of sBLMS is different with tBLMs. The Figure 3.4 shows how tethered lipid bilayers assemble.

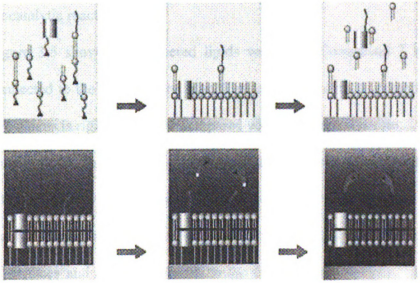


Figure 3.4 tBLMs assembly (Figure adapted from B. A. Cornell, G. Krishan, P. D. Osman, R. D. Pace, L. Wieczorek; Biochem. Soc. Trans. 2001, 29, 613-617)

First, a fresh gold surface is exposed to an ethanol solution of the tethering lipid for a while. This produces the inner and part of the outer leaflet of the membrane. Following an alcohol rinse, a second ethanol solution brings the mobile lipid. Rinsing with water causes a lipid bilayer structure to form spontaneously. Some of the lipids span the

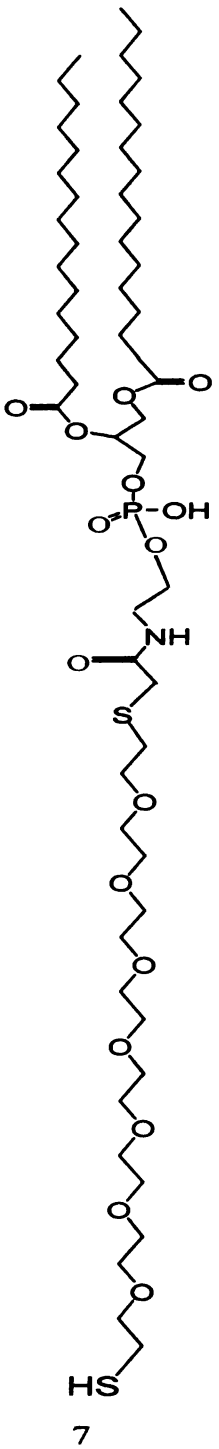
membrane, whereas the remainder is mobile within the two-dimensional plane of the membrane. At the end, the protein can be added in the aqueous solution.

3.1.4 The goal of the project

The goal of the project is to make lipid that can be tethered to gold surface. Some membrane proteins can be bound to the tBLM. The activities of these biomolecules are coupled to an electrical signal and can be expressed and measured. This system can be potentially used for bioelectronic applications. Such applications include devices to characterize the functional properties of membrane proteins, biosensors, and biocatalytic reactors.

Figure 3.5 shows two tethered lipids we made. Compound 7 has two alkyl chains connected to the octaethylene glycol tethered spacer via a glycerol unit. The lipid with two chains is rigid and stable compared with lipid with one chain. The tethering moiety should fulfill the following requirements: it should be hydrophilic and should not interact either with membrane lipids or with membrane proteins. Octaethylene glycol can fulfill these requirements. It is known to prevent non-specific adsorption of proteins to surfaces and does not absorb to the lipid bilayer surfaces.[11] Compound 8 has the same tethering moiety with compound 7. For compound 8, we use two phytanyl chains to replace alkyl chains. Phytanyl chains are stable at high temperatures and the bulkiness of the methyl substituents eliminates temperature dependencies in the membrane disorder around 20-30 °C.[14] Furthermore, the 2,3-di-O-phytanyl-sn-glycerol unit contains only ether linkages to prevent hydrolytic cleavage.[15] This moiety can

form stable biomembranes under the very severe living conditions (e.g., high temperatures) of extremophiles or archaea.[16]



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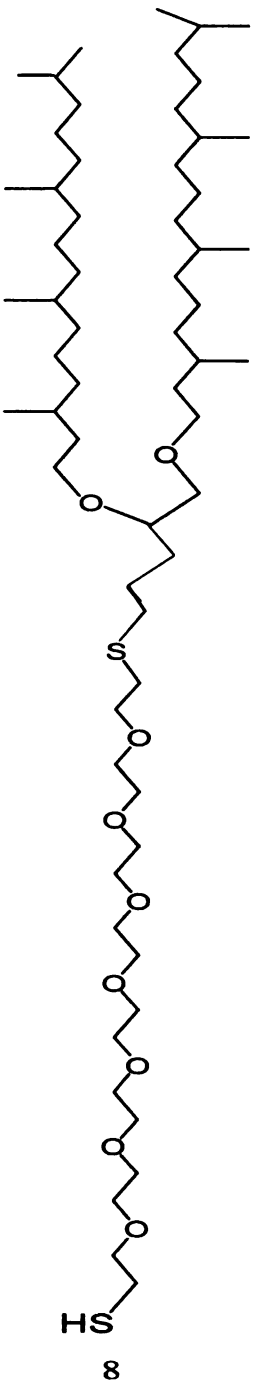

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Figure 3.5 Structure of two tethered lipids

3.1.5 The NMR spectrum of lipids

One of challenges of this project is how to identify the structure of lipids. Lipids generally give poorly resolved NMR spectra. The main reason why structural analyses of lipids by NMR spectroscopy are difficult is because of their asymmetry in polarity. Lipids possess hydrophilic regions that tend to interact with similar regions of other molecules or with each other. There are also hydrophobic regions consisting of long acyl chains which tend to self-associate. This leads to aggregate formation. Because of this molecular association, the motion of lipid molecules in solution tends to be much slower than one would predict based on molecular weights of each molecule. The rotational correlation times are much larger than expected and the NMR spin-spin relaxation times (T_2) tend to be very small. This leads to line broadening and poor signal resolution. The use of a mixed solvent, such as mixture of D-chloroform and D-methanol, can lead to better NMR spectra than those obtained using only D-chloroform. The choice of D-chloroform in the solvent mixture was aimed at preventing the hydrophobic interactions of the acyl chains. D-methanol was used to prevent interactions of hydrophilic regions. There are two NMR spectra of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine in appendix 1. The NMR spectrum obtained in D-chloroform displays signals from the hydrophobic regions only, but the NMR spectrum done in mixed solvent (D-chloroform:D-methanol = 4:1) not only shows signals from hydrophobic regions also shows signals from the hydrophilic regions. In addition to the solvent system, other factors that can affect spectra include concentration and the temperature of the NMR sample.

3.2 Experimental

3.2.1 Synthesis of tethered lipid with alkyl chains

All chemicals were used as received from Aldrich unless otherwise noted.

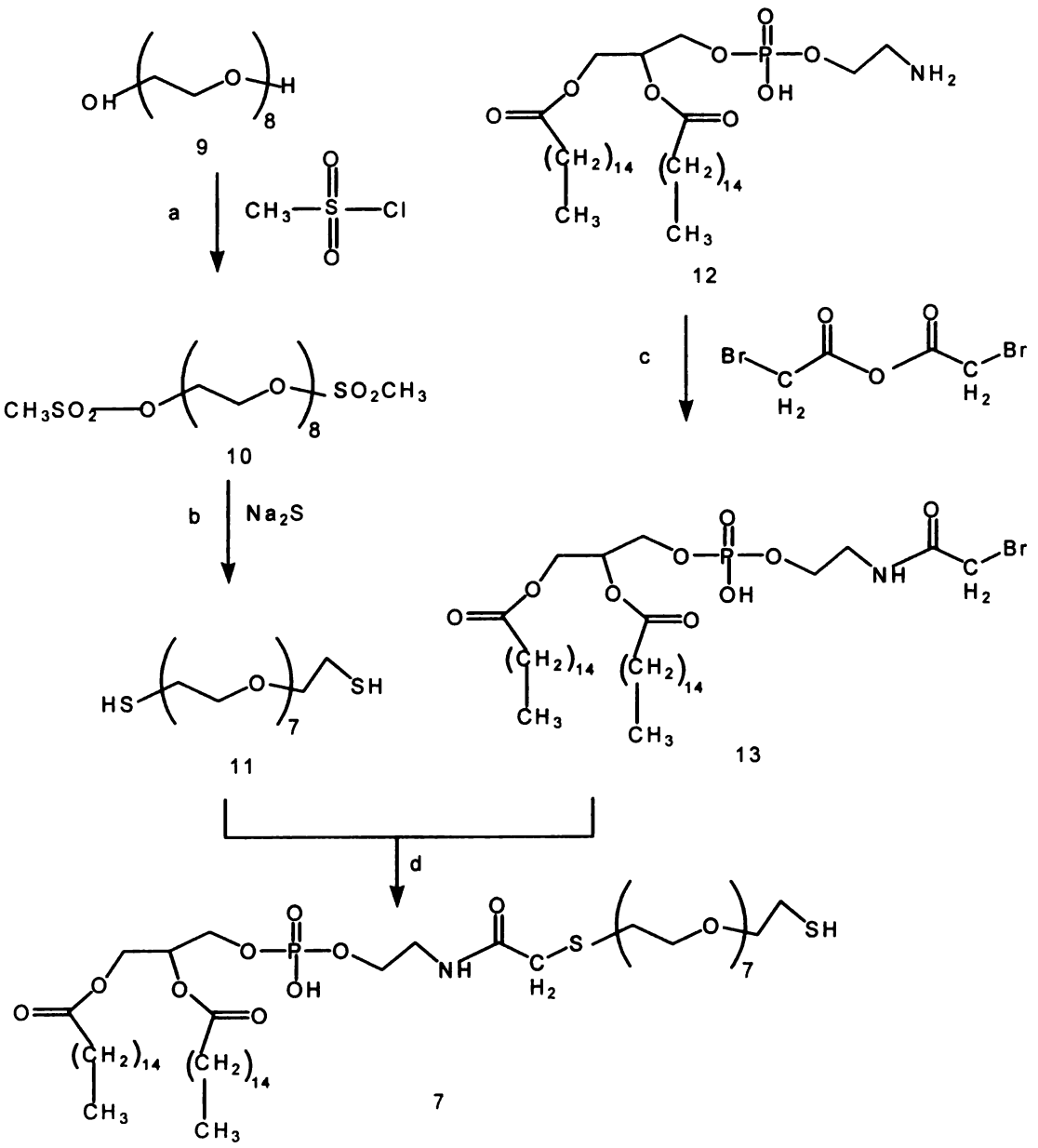
Octaethylene glycol was purchased from polypure.

1,2–Dipalmitoyl–sn–glycero-3-phosphoethanolamine was purchased from Bachem Bioscience Inc.

Lactone was provided by Afid Therapeutic Inc.

The route to tethered lipid is outlined in Scheme 3.1.

Scheme 3.1 Synthesis of tethered lipid



Reagents and conditions: (a) Pyridine and dichloromethane (1:1), 25°C, 6 hrs, 91%; (b) $Na_2S.9H_2O$, ethanol and methanol (1:1), 25°C, 20 hrs, 85%; (c) chloroform and methanol (10:1), 1.5 hrs, 25°C, 70%; (d) methanol and water (2:1), $NaHCO_3$, 25°C, 2 hrs, 62%.

(a) Mesylation of octaethylene glycol to form compound 10

Octaethylene glycol (1g, 0.0027mol) was dissolved in 10 mL mixed solvent (pyridine: dichloromethane = 1:1) in a 100 mL round bottom flask. The mixture was cooled in ice. Methane sulfonyl chloride (0.92g, 0.008mol) was added after 20 minutes and the ice was taken away. The reaction mixture was stirred at room temperature for 6 h. The crude product was dissolved in water (200 mL) and extracted with ethyl acetate (300 mL) twice. The ethyl acetate phase was collected and dried by sodium sulfate. The toluene (100 mL) was added to ethyl acetate phase to help remove extra pyridine. The solvent was removed to give the product. ^{1}H NMR (500MHz, $CDCl_3$) δ 4.39 (m), 3.78 (m), 3.70-3.64 (m), 3.09 (s), ^{13}C NMR 70.09, 69.80, 69.75, 39.01.

(b) Preparation of dithiol compound 11

Sodium sulfide (3.6g, 0.015mol) was dissolved in a 15mL mixture of solvents (methanol:ethanol =1:1) in a 100 mL round bottom flask and was stirred at room temperature. Compound 10 (0.5g, 0.00095mol) was added after 30 minutes. The reaction mixture was stirred at room temperature for 20 h and concentrated. The crude product was dissolved in 300 mL mixture of solvent (chloroform:ethanol =1:1). The precipitate formed was removed by filtration. The filtrate was concentrated to give the product. ^{1}H NMR (500MHz, $CDCl_3$) , δ 3.71-3.62 (m), 2.74 (t, J=7Hz), ^{13}C NMR 71.81, 71.03, 70.97, 70.70, 32.01.

(c) Adding a leaving group to 1,2–Dipalmitoyl–sn–glycero-3-phosphoethanolamine to form compound 13

Compound 12 (0.1g, 0.00015mol) [see appendix 1 for NMR spectra] was dissolved in a 6 mL mixture of solvents (chloroform: methanol = 10:1) and the solution was sonicated 10 minutes. Bromoacetic anhydride (0.31g, 0.0012mol) was added. The reaction was stirred at room temperature for 1 h. The reaction mixture was partitioned between a 100 mL mixture of solvents (chloroform: methanol = 10:1) and 50 mL water. The organic phase was separated and dried with sodium sulfate. The solvent was removed to give the product. ^{1}H NMR (500MHz,) δ 5.21 (b), 4.40 (d), 4.17 (m), 4.08 (b), 3.93 (b), 3.65 (m), 3.19 (b), 2.30 (m), 1.59 (b), 1.3 (b), 0.88 (t). See Appendix 2.

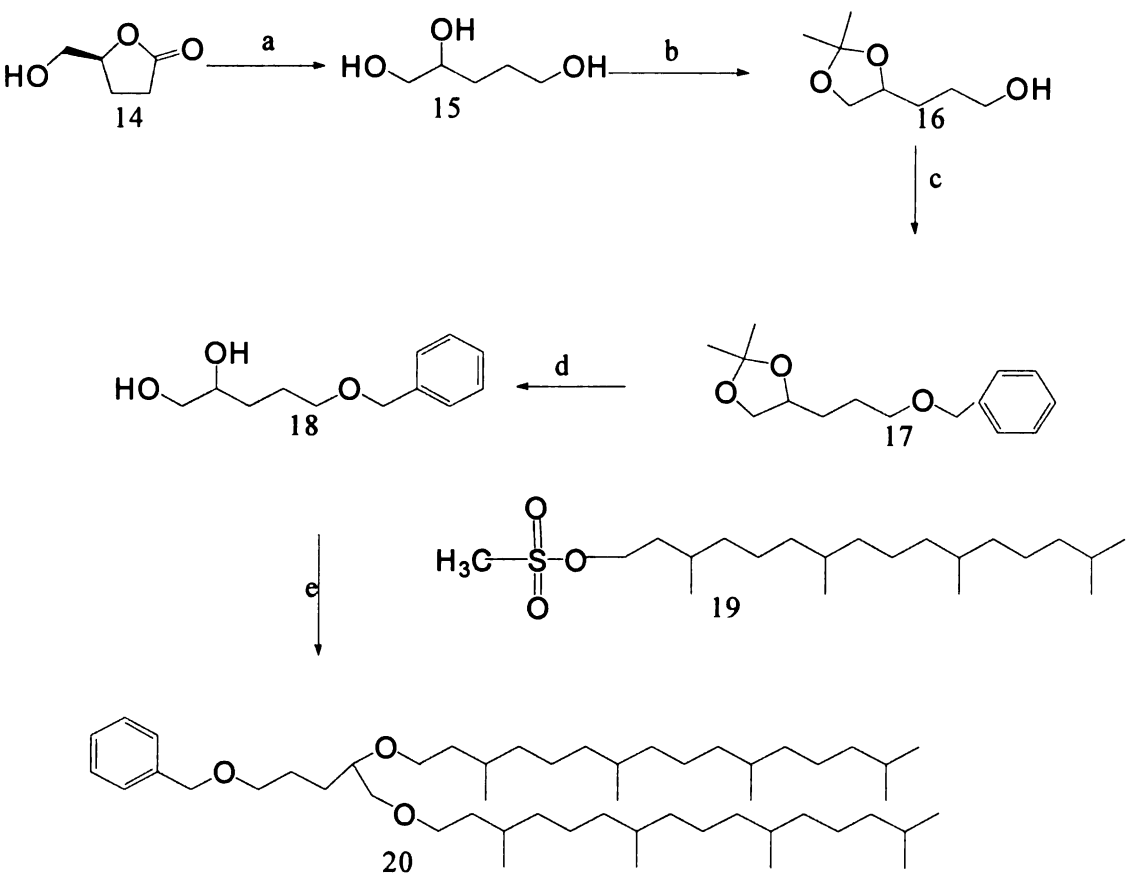
(d) Preparation of final product (compound 7)

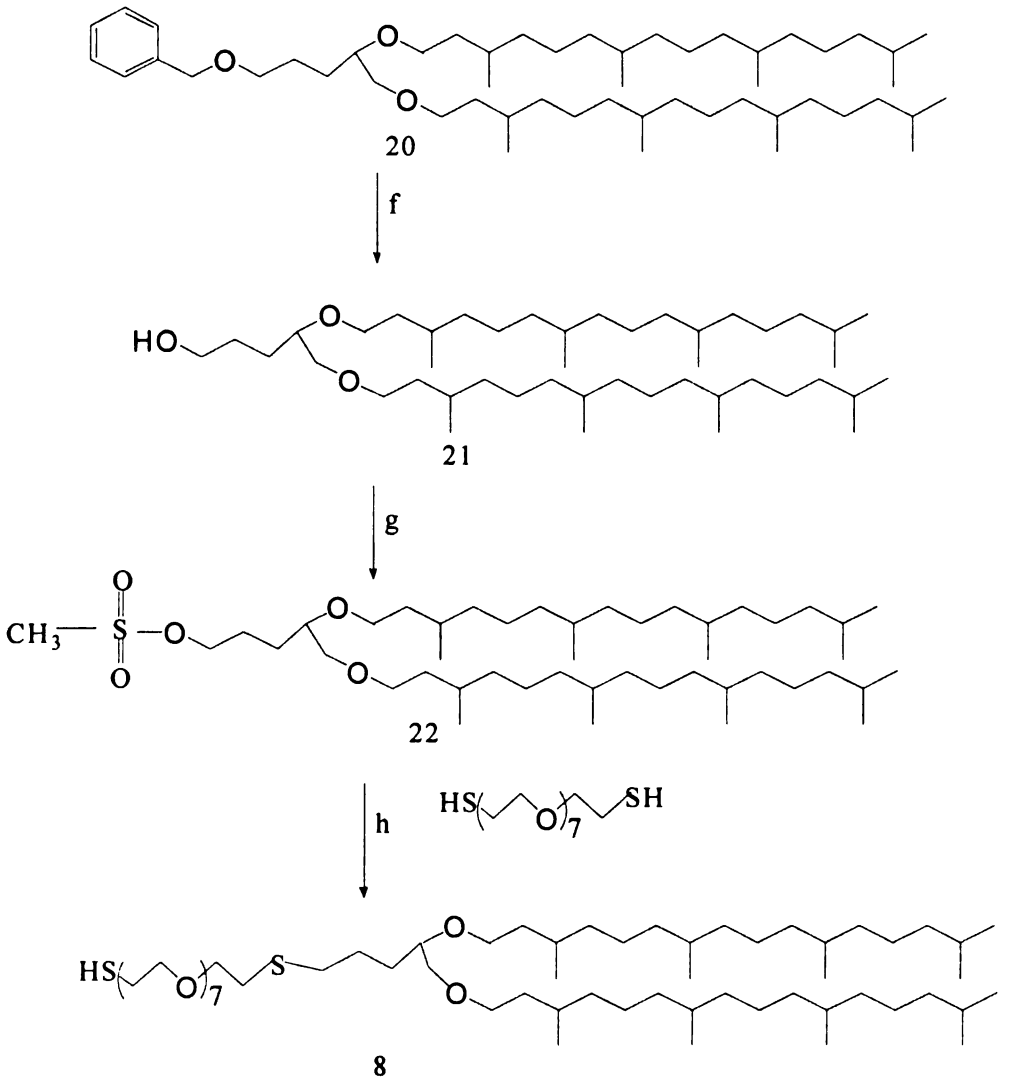
Compound 13 (0.125g, 0.00015mol) was dissolved in a 8 mL mixture of solvents (methanol: water = 2:1) in a 25 mL round bottom flask and the solution was sonicated for 10 minutes. Sodium bicarbonate (0.05g) and compound 11 (0.2g, 0.0005mol) were added. The reaction mixture was stirred at room temperature for 1 h. The solvent was concentrated. The crude product was dissolved in a 50 mL mixture of solvents (chloroform: methanol = 5:1) and washed with 30 mL water. The organic phase was separated and concentrated. The crude product was purified by gel filtration chromatography using Sephadex LH20 (Bead size: 25-100u) to give final product. A mixed solvent (dichloromethane: methanol = 2: 1) was eluant. ^{1}H NMR (500MHz,

$CDCl_3/CD_3OD$ (4:1)) δ 5.22 (b), 4.40 (m), 4.19 (m), 3.82-4.05 (b), 3.50-3.80(b), 2.77-2.88 (m), 2.30(m), 1.59 (b), 1.30 (b), 0.88 (t). See Appendix 3.

3.2.2 Synthesis of tethered lipid with phytanyl chains

Scheme 3.2 Synthesis of tethered bilayer lipid





Reagents and conditions: (a) THF, Methanol, $NaBH_4$, 25°C, 12h, 98%; (b) acetone, dimethoxy propane, H_2SO_4, 25°C, 22h, 95%; (c) THF and DMSO (2:1), sodium hydride, 26h, 25°C, 91%; (d) formic acid, 50°C, 4h, 93%; (e) THF and DMSO (2:1), sodium hydride, 45°C, 48h, 85%; (f) ethanol, Palladium, 12h, 90%; (g) pyridine and dichloromethane (1 :1), 25°C, 20h, 80%; (h) n-propanol, 65 °C, 24h, 62%.

(a) Reduction of lactone to form compound 15

Lactone (5g, 0.043mol) was dissolved in 100 ml THF and cooled in an ice bath for 30 minutes. Sodium borohydride (3.2g, 0.084mol) was added slowly to the reaction. The reaction mixture was stirred at room temperature for 12 h and concentrated. The crude product was dissolved in 90 mL mixture of solvents (methanol:hydrochloric acid = 2:1). After removing the solvent, the residue was dissolved in THF-ethanol (200 mL, 6:1). The solid was removed by filtration and filtrate was collected and concentrated to give the product. ^{1}H NMR (500MHz, D2O) δ 3.47 (1H, m), 3.38 (3H, m), 3.25 (1H, dd, J=12Hz, 7Hz), 1.43 (1H, m), 1.33 (2H, m), 1.22 (1H, m), ^{13}C NMR 71.68, 65.45, 61.70, 28.80, 27.59.

(b) Protecting 1, 2 position hydroxyl groups to form compound 16

Compound 15 (3.1g, 0.0258mol) was dissolved in 40 ml acetone followed by the addition of dimethoxy propane (5.49g, 0.0526mol) and 0.1 mL sulfuric acid. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated to half the volume and poured into 5 mL sodium bicarbonate (1.5g) solution to neutralize the acid. The mixture was concentrated and 40 mL ethyl acetate was added. The solid was filtered off and the filtrate was concentrated to give the product. ^{1}H NMR (500MHz, $CDCl_3$) δ 4.05 (1H, m), 3.98 (1H, t, J=5Hz), 3.58 (1H, m), 3.45 (1H, t, J=5Hz), 1.58 (4H, m), 1.33 (3H, s), 1.28 (3H, s), ^{13}C NMR 109.25, 76.02, 59.75, 62.24, 30.06, 29.25, 27.01, 25.80.

(c) Protecting free primary hydroxyl group to form compound 17

Compound 16 (5g, 0.031mol) was dissolved in 24 ml mixed solvent (THF:DMSO = 2:1). Sodium hydride (2.5g, 60% dispersion in mineral oil) was added followed by benzyl bromide (6.41g, 0.037mol). The reaction mixture was stirred at room temperature for 26 hours. The reaction mixture was added dichloromethane (300 mL) and washed with 100 mL water twice. The organic solvents were combined and concentrated. ^{1}H NMR (500MHz, $CDCl_3$) δ 7.35 (5H, m), 4.52 (2H, s), 4.05 (1H, m), 4.04 (1H, m), 3.52 (3H, m), 1.72-1.65 (4H, m), 1.42 (3H,s), 1.37 (3H, s), ^{13}C NMR 138.78, 128.65, 128.61, 128.02, 127.85, 127.78, 108.95, 76.09, 73.13, 70.26, 69.65, 30.55, 29.96 27.22, 26.27, 25.99.

(d) Deprotecting the 1, 2 position hydroxyl groups to form compound 18

Compound 17 (3.75g, 0.018mol) was dissolved in 16.5 ml mixed solvent (formic acid: water = 1:10). The reaction mixture was stirred at 50°C for 4 hours. Solvent was removed to give the product. ^{1}H NMR (500MHz, D2O) δ 7.28 (5H, m), 4.42 (2H, s), 3.57 (1H, m), 3.32 (3H, m), 1.57 (1H, m), 1.54 (1H, m), 1.42 (1H, m), 1.31 (1H, m), ^{13}C NMR 128.89, 128.71, 128.44, 72.66, 71.68, 70.22, 65.54, 29.06, 25.02.

(e) Adding phytanyl long chains to hydroxyl groups to form compound 20

Compound 18 (2g, 0.01mol) was dissolved in 18 mL mixed solvent (THF:DMSO = 2:1) in a 100mL round bottom flask. Sodium hydride (1.9g, 60% dispersion in mineral oil) was added. Compound 19 (10.77g, 0.029mol) was added after 30 minutes. The reaction mixture was stirred at 45°C for 48 h. The solvent was removed and the residue was loaded onto a flash column packed with silica gel in hexane. Hexanes (1000 mL) were used to run the column followed by 1000 mL dichloromethane. All hexanes fractions and first 300ml dichloromethane fractions was combined and was dried for hydrogenation. ^{1}H NMR (500MHz, $CDCl_3$) δ 7.34 (m), 4.55 (s), 3.70 (m), 3.40-3.51 (m), 1.49-1.69 (m), 0.97-1.59 (b), 0.84-0.87 (m). See Appendix 4.

(f) Hydrogenation to form compound 21

Compound 20 was dissolved in 100mL ethanol, and the hydrogenation was carried out in a parr reactor at room temperature under 100 psi hydrogen with 5% Palladium on carbon as the catalyst. The reaction was done in 12 hours and the mixture was filtered through celite and concentrated. ^{1}H NMR (500MHz, $CDCl_3/CD_3OD$ (4:1)) δ 3.62 (m), 3.30 (m), 1.62-1.74 (m), 0.97-1.60 (b), 0.84-0.87 (b). Positive APCIMS 663.52 ($MH^+ - H_2O$). See Appendix 5.

(g) Mesylation of free primary hydroxyl group to form compound 22

Compound 21 (0.5g, 0.0007mol) was dissolved in 9 mL mixed solvent (pyridine: dichloromethane = 2:1) and cooled in an ice bath for 20 minutes. Methane sulfonyl chloride (0.167g, 0.0015mol) was added. The reaction was stirred at room temperature for 20 hours. Dichloromethane (10 mL) and 8 ml water containing 1g sodium bicarbonate was added. The dichloromethane phase was separated and concentrated to give the product. ^{1}H NMR (500MHz, $CDCl_3$) δ 4.30 (m) 3.46-3.66 (b), 3.03 (s), 1.82 (b), 0.97-1.78 (b), 0.84-0.97 (b). See Appendix 6.

(h) Connecting dithiol long chain to the lipids to form compound 8

Compound 22 (0.23g, 0.0003mol) was dissolved in 16 mL n-propanol. Compound 21 (0.365g, 0.0009mol) and potassium tert-butoxide (0.07g, 0.0006mol) were added. The reaction mixture was stirred at 65 °C for 25 hours. The solvent was removed and the crude product was dissolved in chloroform (60 mL) and washed with water (40 mL) twice. The chloroform phase was separated and concentrated. The final product was purified by gel filtration chromatography using Sephadex LH20 (Bead size: 25-100u). A mixed solvent (dichloromethane:methanol = 2:1) was eluant. In order to further purify the product, dialysis using 8000 MW cut off membranes was performed. The product was dissolved in ethanol and was placed into 4cm long dialysis membranes. The membrane was put into a 4L container containing water. The water was stirred at

room temperature and changed each half hour. After 3.5 hours, the solution was dried by lyophilization. ^{1}H NMR (500MHz, $CDCl_3/CD_3OD$ (4:1)) δ 3.52-3.63 (m), 2.68-2.72 (m), 1.40-1.49 (m), 0.92-1.32 (b), 0.72-0.80 (m). See Appendix 7.

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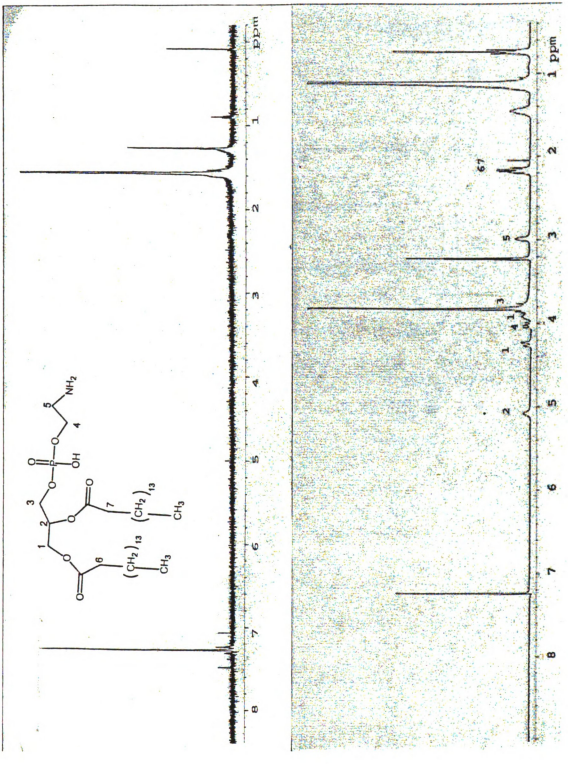
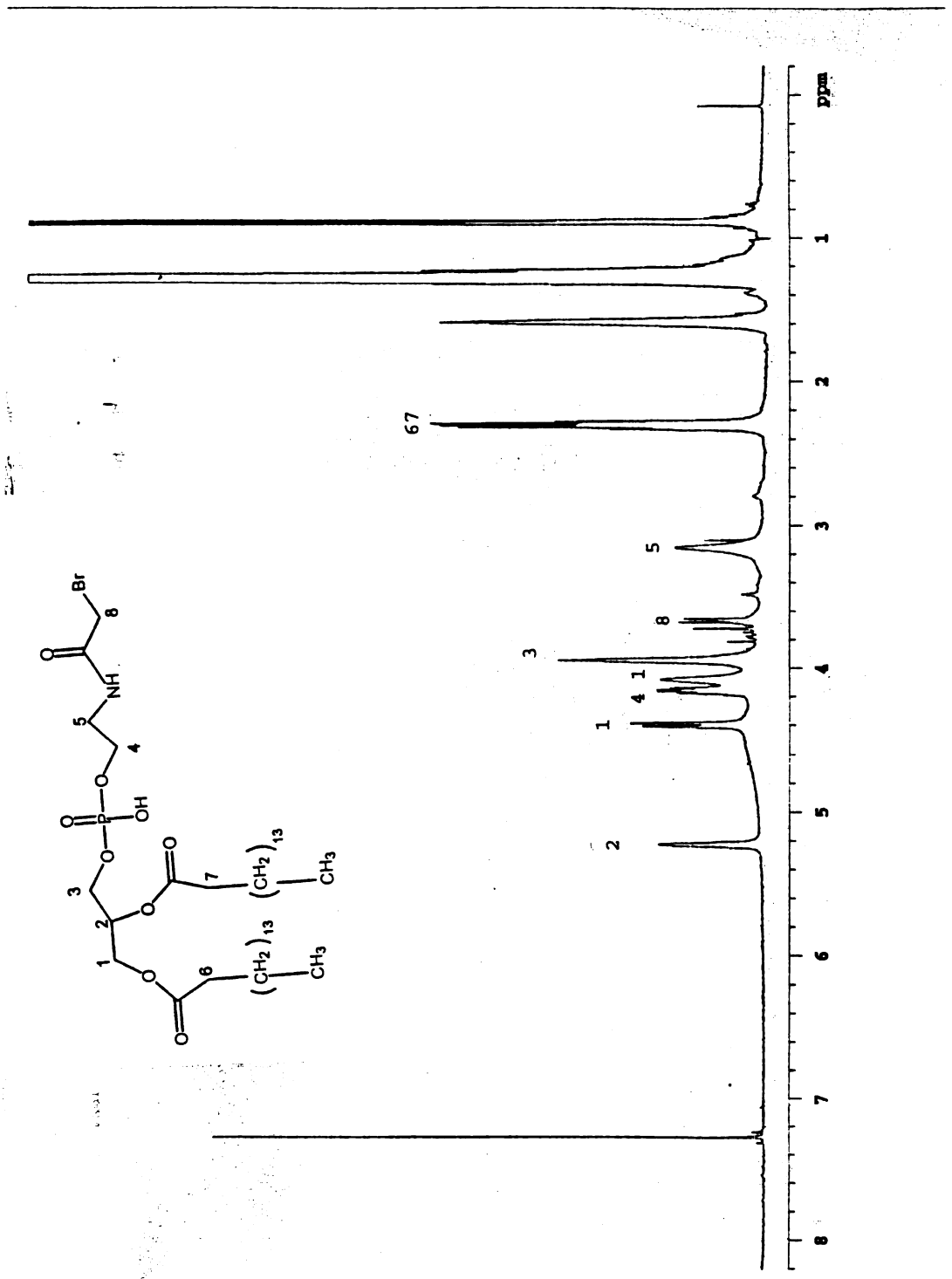
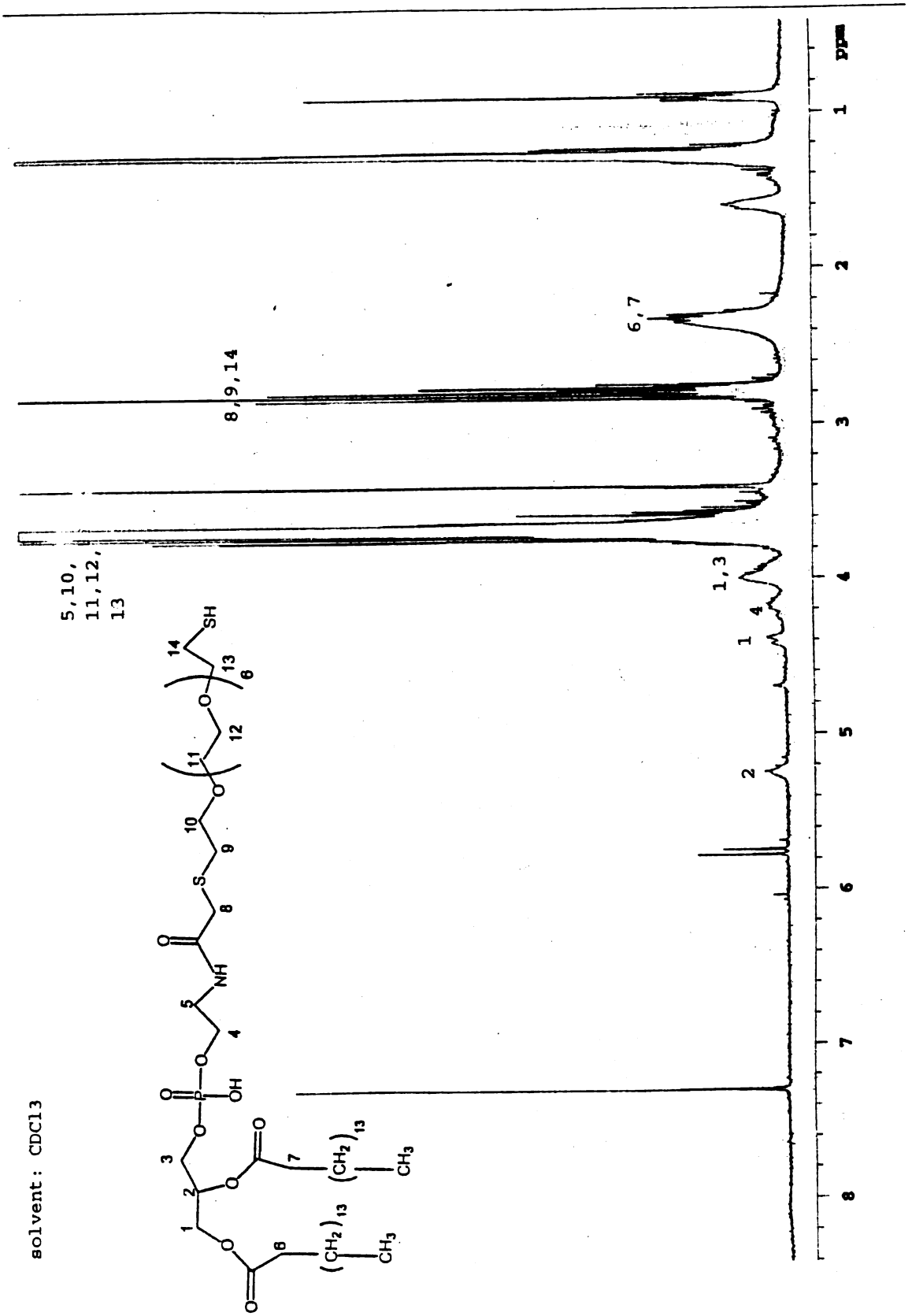


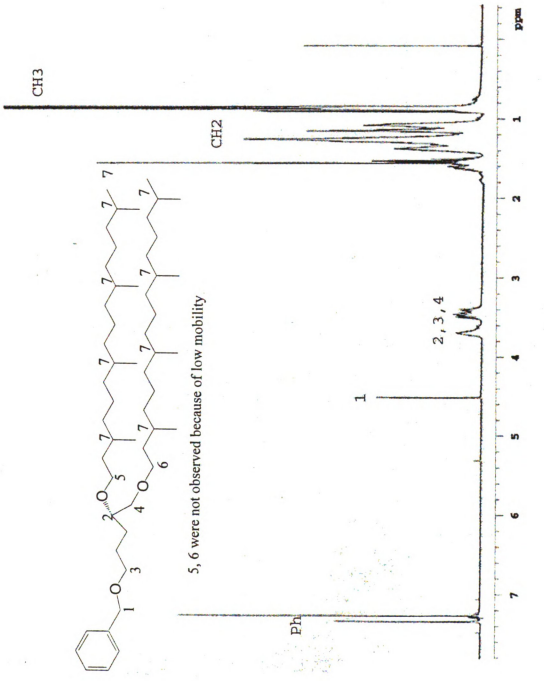
Appendix 1 ^{1}H-NMR spectrum of compound 12 (the left is in D-chloroform; the right one is in D-chloroform:D-methanol = 4:1)



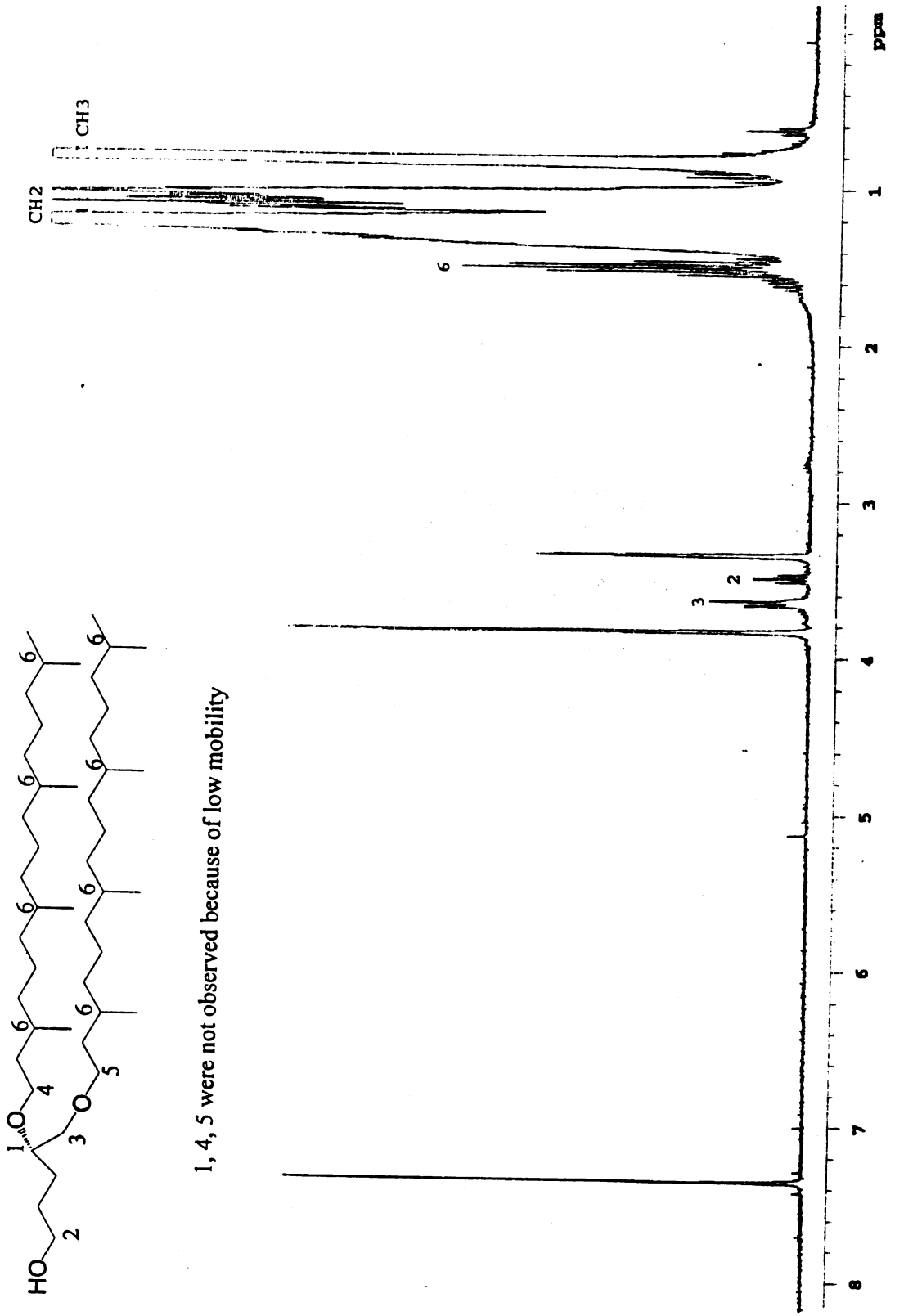
Appendix 2 ^{1}H-NMR spectrum of compound 13



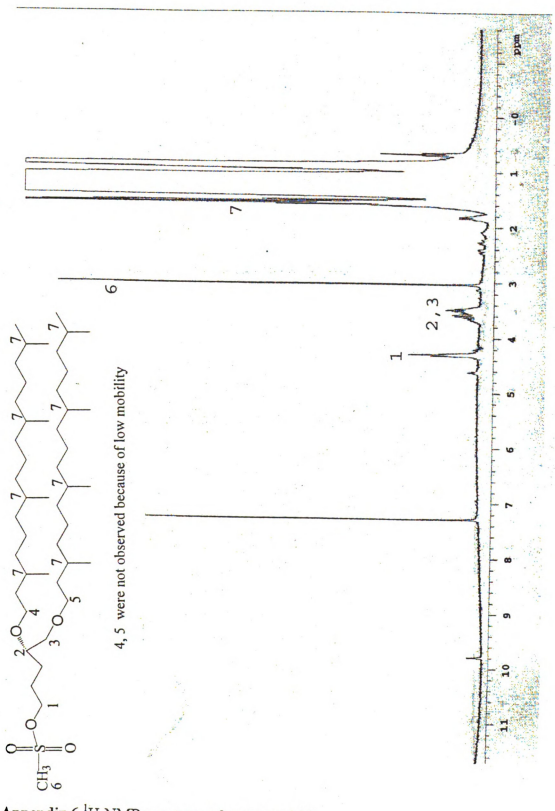
Appendix 3 ^{1}H-NMR spectrum of compound 7



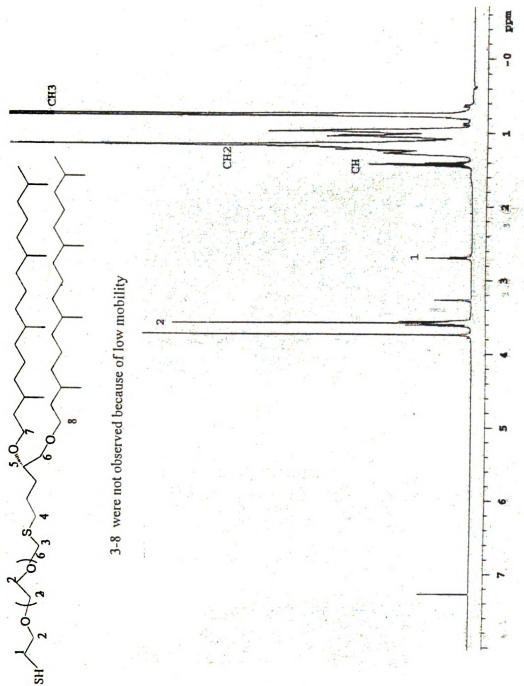
Appendix 4 ^{1}H-NMR spectrum of compound 20



Appendix 5 ^{1}H-NMR spectrum of compound 21



Appendix 6 ^{1}H-NMR spectrum of compound 22



Appendix 7 ^{1}H-NMR spectrum of compound 8