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DEVELOPMENT OF NEW SYNTHETIC ROUTES TO CHIRAL INTERMEDIATES AND SYNTHESIS, CHARACTERIZATION OF NOVEL MEMBRANE BASED ADVANCED MATERIALS

By

Guijun Wang

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ABSTRACT

DEVELOPMENT OF NEW SYNTHETIC ROUTES TO CHIRAL INTERMEDIATES AND SYNTHESIS, CHARACTERIZATION OF NOVEL MEMBRANE BASED ADVANCED MATERIALS

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The main objective of this dissertation is to develop chiral technology for the synthesis of chiral building blocks especially for use in the pharmaceutical and advanced material industries. The two major thrusts are carbohydrate and amino acid based asymmetric synthesis and the design and synthesis of novel, stabilized, membrane lipid based systems which have potential applications in the fabrication of molecular photonic-electronic devices, biosensors, and drug delivery systems.

There are two parts in this dissertation. The first part (chapters 2, 3) describe about the development of new asymmetric synthetic routes to chiral building blocks and chiral drugs for the pharmaceutical industry. This includes the development of new synthetic routes to chiral 3-carbon synthons which are key building blocks for many important compounds such as antiviral drugs, cardivasicular agents, chiral membrane lipids and other glycerol derivatives. It is also describes the development of new asymmetric synthesis of important drugs such as *L*-carnitine, *R*-γ-amino-β-hydroxybutyrate (GABOB) and *S*-beta blockers. New synthetic routes to chiral *cis*-1-amino-2-indanols, key building block for HIV protease inhibitors and important catalysts in asymmetric synthesis are also demonstrated. These new routes have significant advantages over the existing routes, giving high yields and high

optical purity and by simple processes that are highly efficient and applicable to industry. The chirality of the products are conserved from the chiral starting carbohydrate and amino acids.

The second part of the dissertation (chapters 5, 6 &7) is about the design, synthesis and properties of new advanced materials based on membrane mimics. These include stabilized phospholipid analogs and liposomes, chiral multifunctional self assembled 2-dimensional polydiacetylene containing systems, 2-dimensional conducting polyamide conducting thin films and other advanced materials. A tail-to-tail dimer of phosphatidyl ethanolamine was prepared and shown to readily form very uniformly flat self assembled lamellar supramolecular arrays and liposome that are stable at temperatures up to 80°C. This extremely stable and readily functionalizable dimeric phospholipid has potential uses in the fabrication of biomaterials, stable membrane models and liposome drug delivery systems. In chapter 6, two new, very accessible, chiral, self-assembling phospholipid analogs containing diacetylenic units in the middle of their acyl chains were prepared by very simple and highly efficient routes. They formed very uniform, extremely flat, thin films which are readily polymerized to give extensively conjugated systems which absorbed well out into the near infrared region unlike typical polydiacetylenes. The results indicate that this is an excellent approach for preparing ordered polydiacetylene systems for use in designing advanced materials. The last chapter introduces a new approach for obtaining long range order and perfect alignment of long chain polydiacetylene by anchoring them along a polymer backbone.

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Abstract

Chirality or handedness is an important property of many molecules. It has significant impact in biological systems, pharmaceutical industry and advanced materials science. It is of great interest to develop simple and efficient methods to obtain these chiral compounds. Many methods to chiral molecules have been developed including asymmetric synthesis and racemate resolution. In recent years asymmetric synthesis to chiral compounds have been developed rapidly. Several approaches include the use of chiral auxiliaries to induce chirality, biotransformation using fermentation, enzymes, and chiral pool approaches which use optical active raw materials as the chirality source for the desired products. In this chapter, these methods of obtaining chirality especially the chiral pool approaches are discussed. Chiral 3-carbon, 4-carbon molecules are pivotal building blocks of many biological important compounds. Chiral amino indanols are important chiral molecules for drug design and catalysts for asymmetric synthesis. The importance of these chiral compounds have long been recognized, however their syntheses are not very straightforward, the application and synthesis of some of these compounds are reviewed.

1.1. About chirality

Handedness or chirality is an interesting feature of the structure of some molecules including many drug substances. Such molecules are structurally identical but are actually mirror images of each other. They have the relationship that the left hand has to the right hand. They look identical but cannot be interchanged in the same way that a glove that is made for one hand cannot fit the other. There are many molecules that we are familiar with share this left hand / right hand relationship. They have the same molecular weights, densities, boiling points, crystal structure etc and are therefore very difficult to separate. However, they smell differently and taste differently. This is because they impact very differently on living systems. Most biomolecules are chiral or handed, existing in two mirror image forms called enantiomers. Non living systems normally contain equal numbers of the left (L) and right (D) handed forms of a given molecule. Biological systems are sensitive to handedness or chirality, a characteristic hallmark of life is its high degree of homochirality. Hence amino acids are predominately in the L-form and sugars are predominately of the D-form. The designation D or L is based on a set of rules proposed by Fischer at 1919 [1]. There is a set of general rules stipulated by the Cahn-Ingold-Prelog convention which is the currently used nomenclature to describe the stereogenic center in a molecule. It is also known as the sequence rule or the R and S rule [2,3]. This rule can be used to designate configurations rapidly and unambiguously. Chiral molecules are very common in our everyday lives. They are the active constituents not only of many pharmaceuticals but of vitamins, flavours and fragrances, and herbicides and pesticides. One very familiar pair of chiral substances is the right and left handed versions

(mirror images) of carvone. The right hand version smell like spearmint but the left hand version smells like caraway (a spice used in cooking).

The impact of the different chiral forms of chiral molecules often goes far beyond the senses of taste and smell. The consequence of having the wrong chiral form present can be tragic. One well documented case of the awful consequences of this is the story of the use and eventual ban of the drug thalidomide in the early 1960s [4]. The use of a mixture of both left and right handed versions of this drug (it was too costly to separate them) led to severe birth defects in over 10,000 children. It was later found that the left handed molecule was the cause. Another well known case is Ritalin, a drug prescribed for millions of children, and some adults, with attention-deficit hyperactivity disorder (ADHD). ADHD occurs in 3 to 5 percent of school-aged children, it is the most common psychiatric disorder in childhood. The present form of Ritalin is racemic and with side effects such as stomach aches, nervousness, loss of appetite, insomnia and a temporary slowing of growth. It has been found that 50% of the Ritalin in each pill has no effect on ADHD and may cause the above side effects [5]. The importance of optically pure drugs is being realized more and more as it is demonstrated that only one enantiomer accounts for the drug activity and the other form is either inactive or may cause side effects which sometimes are serious. There is increased evidence that the chiral or single -isomer form of the drug often has a better therapeutic profile. Because of tragedies such as the thalidomide one, finding a way of making only one chiral form of a drug or drug intermediate is now one of the most important, challenging and competitive areas for research in chemistry. Nowadays, drugs

in preclinical and clinical development are dominated by chiral compounds. It has been estimated that 80% of all drugs in development are single isomer versions of chiral drugs [6]. Pharmaceutical companies are being forced to take notice of the importance of chiral technology.

Besides its importance in chiral drugs production, chirality is becoming more and more an issue in material science too. Researchers at Molecular OptoElectronics Corporation have reported on developments based on chiral molecules at ACS meeting [7]. It has been found that enantiomerically pure chiral molecules can produce polymers with optical properties four times as stable as those made using conventional precursors. The development of polymers with nonlinear optical (NLO) properties has been hampered by the fact that such properties tend to fade over time, this has made it impractical to make certain fiber optic devices, light source or optical signal processing device out of plastic, it has been proposed that chirality may offer a solution. Some carbon nanotubes have chirality in their atomic arrangements. The chirality may influence the tubule conductivity [8]. In liquid crystal science, chirality is also an important aspect which being utilized more and more by material scientists [9]. In the area of materials related to biomembrane mimics, it has been found that chiral fatty acid vesicles are more stable and able to self reproduce, but the racemic vesicles destabilized during hydrolysis, causing phase separation [10]. Many long chain phospholipids self assemble into spherical bilayer structures, known as liposomes or vesicles, however in one study, researchers found that one class of synthetic phospholipids with polymerizable diacetylenic moieties in the acyl

chains can self assemble into hollow, cylindrical structures, known as tubules [11]. Such tubules have potential for long term release applications such as marine antifouling. Several theories based on molecular chirality have been developed to explain the formation of tubules. They are all based on the principle that chiral interactions cause the molecules to pack at a nonzero angle with respect to their nearest neighbors. This chiral packing induces a twist in the bilayer, which results in the formation of a cylindrical structure [11]. Chirality is an important factor in the assemblies of surfactant too. A long chain L-histidine derivative can lead to a stereo dependent expression of molecular chirality at the supramolecular level [12].

1.2. Methods for obtaining chirality

Given the importance of chirality, the big issue now is how to obtain it. The challenges here are cost and technology. Optical pure molecules are extremely difficult to make thus making the development of some drugs virtually impossible. In one recent case the cost of manufacture of a particular AIDS drug was so high and the manufacturing process so complicated that the price was outside of the reach of most people and the quantities were limited. The company eventually could not keep up with demand and many people had to go without the drug. This is tragic and is a direct result of the cost and difficulty in making chiral drugs. There is a tremendous need for methods and expertise that make this possible.

There are a number of different methods for producing chiral chemicals. The first one is by enantiomer separation. This includes classical resolution, which uses a resolving agent

such as tartaric acid to precipitate salts containing only one isomer. Other methods are crystallization, kinetic resolution, chiral chromatography, etc. Another general way to chirality is by asymmetric chemical synthesis. This includes enantioselective reactions using optically active agents, or catalysts, or by diastereoselective reactions involving chiral auxiliaries. The third strategy is to employ biological agents. This includes biocatalysis, enzymatic and microbial assimilation. The fourth approach is the use of compounds from the natural pool of readily available optically active compounds (such as amino acids, carbohydrates, lactic acids etc) as starting points for synthesis. This last method in which simple chemical reactions are performed on molecules with existing chiral centers to generate optically active building blocks is becoming more and more important and is the most straightforward one.

Racemate resolution still constitutes the main method for the industry synthesis of pure enantiomers. There are three methods for the racemate resolution, direct preferential crystallization, crystallization of diastereoisomeric salts and kinetic resolution. Direct preferential crystallization is a crystallization-induced asymmetric transformation of a racemate or deracemization under suitable conditions which include the type of solvent, temperatures and concentration, one enantiomer will preferentially crystallize while the other remain in solution. If a racemate is a homogeneous solid phase of the two enantiomers that coexist in the same unit cell, then it cannot be separated by the above crystallization method but diastereomer crystallization can be used instead. In this case, the pair of enantiomers are allowed to interact with a pure enantiomer which act as a

resolving agent that allows the formation of a mixture of diastereomers that can be separated by crystallization. The most commonly used resolving agents are based on natural products available from the chirality pool. These include such as L-tartaric acid, D-camphorsulfonic acid and various alkaloid bases. The theoretical yields is 50% unless the diastereomeric salt remaining in solution can epimerize in what is called a crystallization induced asymmetric transformation. In kinetic resolution, the success of which depends on the fact that the two enantiomers react at different rates with a chiral entity. The chiral entity is usually used only in catalytic amounts and can be either a biocatalyst or system such as an enzyme or a microorganism or a chemocatalyst such as a chiral acid or base or a chiral metal complex. The separation of enantiomers by chiral chromatography-chiral HPLC columns is a well established technique. The principle is similar to the above mentioned crystallization methods, which all use the different physical properties of the two isomers to separate them.

Asymmetric synthesis. The asymmetric synthesis strategy for obtaining optical pure materials involve chemical synthesis utilizing enantioselective reactions employing optically active agents, or catalysts, and diastereoselective reactions involving chiral auxiliaries. Catalytic asymmetric synthesis has virtually become synonymous with asymmetric synthesis catalyzed by chiral transition metal complexes [13]. This is a very active field in organic chemistry. Many stereospecific reactions have been developed and many catalysts have been found or designed. Many types of reactions have been explored in this field. These include asymmetric hydrogenation of olefins, carbonyl compounds, and

prochiral ketimines by rhodium complexes or chiral phosphines [14,15] and asymmetric oxidation. One well known example is the Sharpless epoxidation. In this reaction, an allylic alcohol is oxidized to a chiral epoxide with reagents made from ruthenium ions, enantiopure tartaric acid and tert-butoxyl peroxide. There are some other reagents for the Sharpless asymmetric dihydroxylation (AD) based on rhodium complexes with pseudo-enantiomeric ligand [16, 17]. Jacobsen's catalayst, is another well documented one. This catalyst is a cobalt complex of (salen) ligands and can be used for the hydrolytic kinetic resolution of terminal epoxides and for the enantioselective catalytic ring opening of meso epoxides [18,19]. Gregory Fu at MIT has developed a new type of chiral transition metal complex that contains planar dimethylamino pyridine or related structures. The DMAP which can catalyze the kinetic resolution of secondary alcohols [20], the asymmetric synthesis of amino acids [21], and the asymmetric hydrogenation of dehydroamino acids [22]. The catalytic asymmetric aldol reaction is a very useful type of organic reactions effecting enantioselective C-C bond formation [23].

Chiral auxiliaries. The use of auxiliaries is an alternative way towards chirality. One common class of chiral auxiliaries is chiral oxazolidinones (Evans' chiral auxiliaries). They have been used as auxiliaries for a wide range of asymmetric transformations [24]. The use of chiral auxiliaries is different from catalytic reactions in one fundamental way. At least stoichiometric quantities are used. Chiral auxiliaries modify the properties of the reactants such that preferably one diastereomeric product which give an enantiomerically pure entry on removal of the auxiliary is formed. An auxiliary must be inexpensive, easily

removed at the end of the reaction. There are a few chiral auxiliaries that can meet the above four necessary requirements. Even so, the methodology has been highly successful in the stereoselective construction of a number of natural products, antibiotics and other compounds.

Biocatalysis, fermentation, enzyme catalyzed synthesis. A third general strategy for obtaining optically pure compounds is by biocatalysis. This includes enzymatic transformations and microbial assimilation. The use of microorganisms in the preparation of optically active compounds is a promising field with several advantages. One of these advantages is the availability of a diversity of inexpensive raw materials that can be used as substrates for microbial transformations . These range from petroleum hydrocarbons to agricultural waste products. Generally, very high regio and stereo selectivity is obtained. The major limitation to this method is the low product concentration and the large amount of waste biomass produced. The productivity of fermentation methods is considerably low compared to chemical or enzymatic processes. Enzymatic transformations are also a very promising area. Enzymes are very efficient catalysts and no organic solvents are required. The reaction conditions are usually very mild. Most enzymes show high substrate selectivity and very high chemo-, regio- and stereoselectivity. Two widely developed families of enzymes that are used in asymmetric organic synthesis are aldolases and lipases [25-28]. The former one can catalyze asymmetric C-C bond forming reactions and the latter can catalyze the enantioselective of hydrolysis of esters to achieve racemate resolution. Enzymes have been used more and

more in organic synthesis but their availability and stability are still problems. Because of the specificity, they lack general utility in a broad range of organic transformations. These drawbacks limit their applications in industry synthesis of optically pure compounds.

Chiral pool approaches. The easiest and most straightforward approach to the synthesis of optically active compounds is to use an optically active raw material available from the chiral pool. The most abundant source of optically pure compounds in nature is carbohydrates which generally have the D-configuration. Other candidates of the chiral pool are amino acids and α- or β- hydroxy acids such as lactic acid and malic acid. Although carbohydrates have been widely used as chiral building blocks in organic synthesis, very few methods have found industrial applications. This is mainly due to the fact that carbohydrates are over-functionalized with many hydroxyl groups with similar chemical reactivities and many chiral centers, thus making it difficult to use them for synthesis. Natural amino acids are the most important class of compounds in the chiral pool [29]. They have a relative simple structure with one or two chiral centers amenable to variety of chemical transformations. They are readily available in bulk from fermentation and other process. L-Glutamic acid is the least expensive of all the amino acids, and both L and D forms of it is available. Because of the versatility as chiral synthons and its availability, it is the most useful one of the amino acids. L-phenylalanine and L-glutamine are readily available starting material for many organic synthesis as chiral synthons.

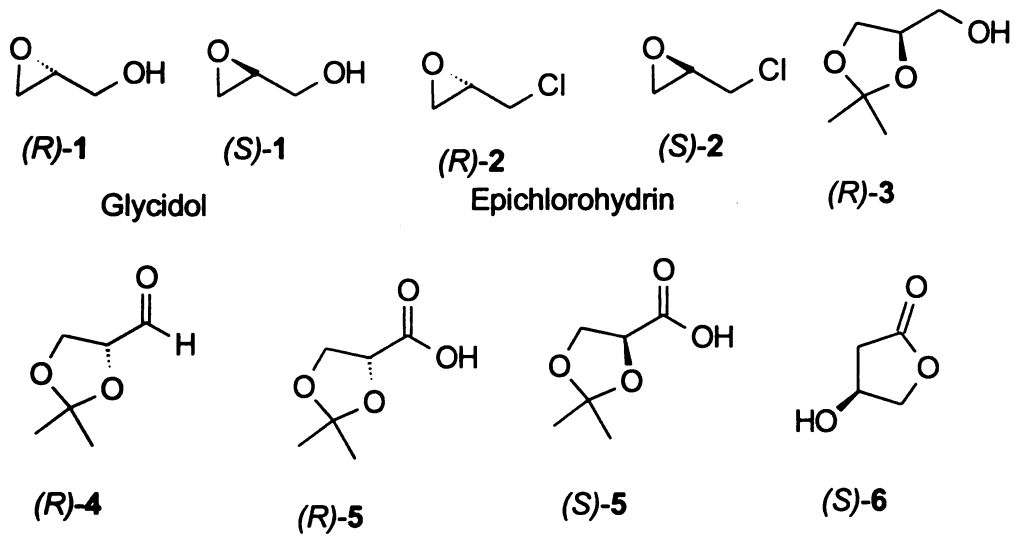
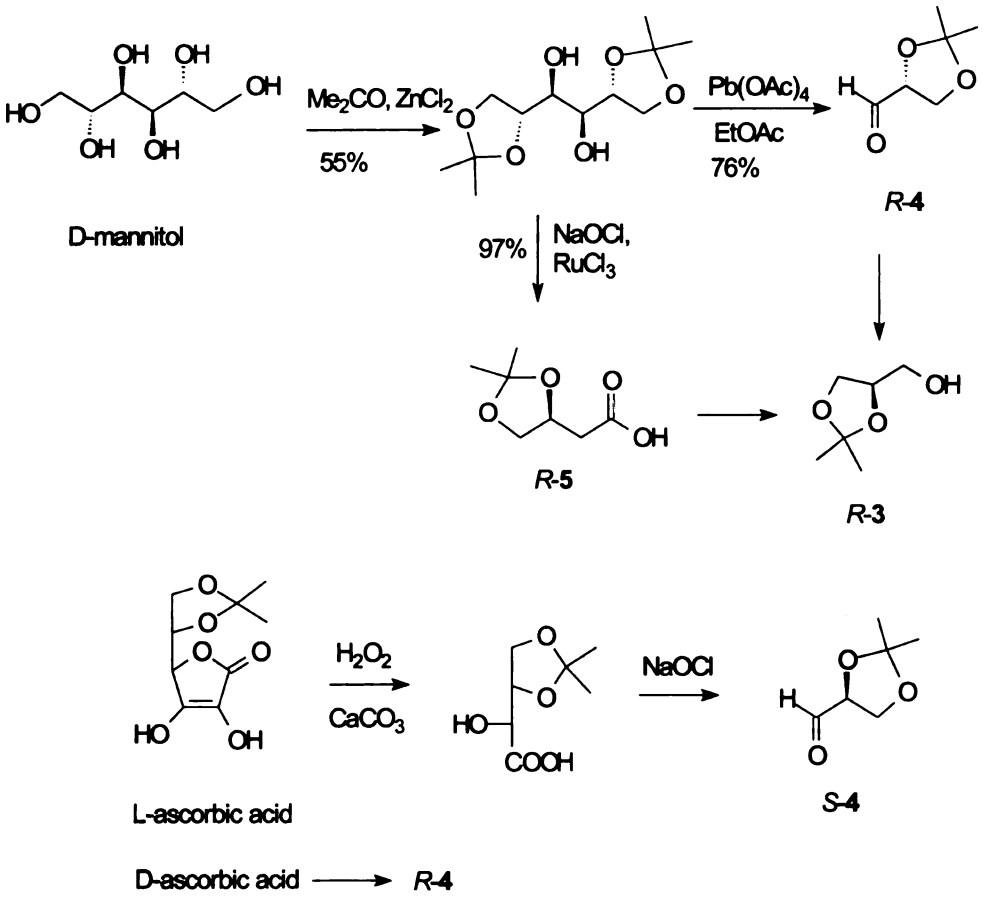


Figure 1.1. The structures of some 3-carbon chiral synthons.

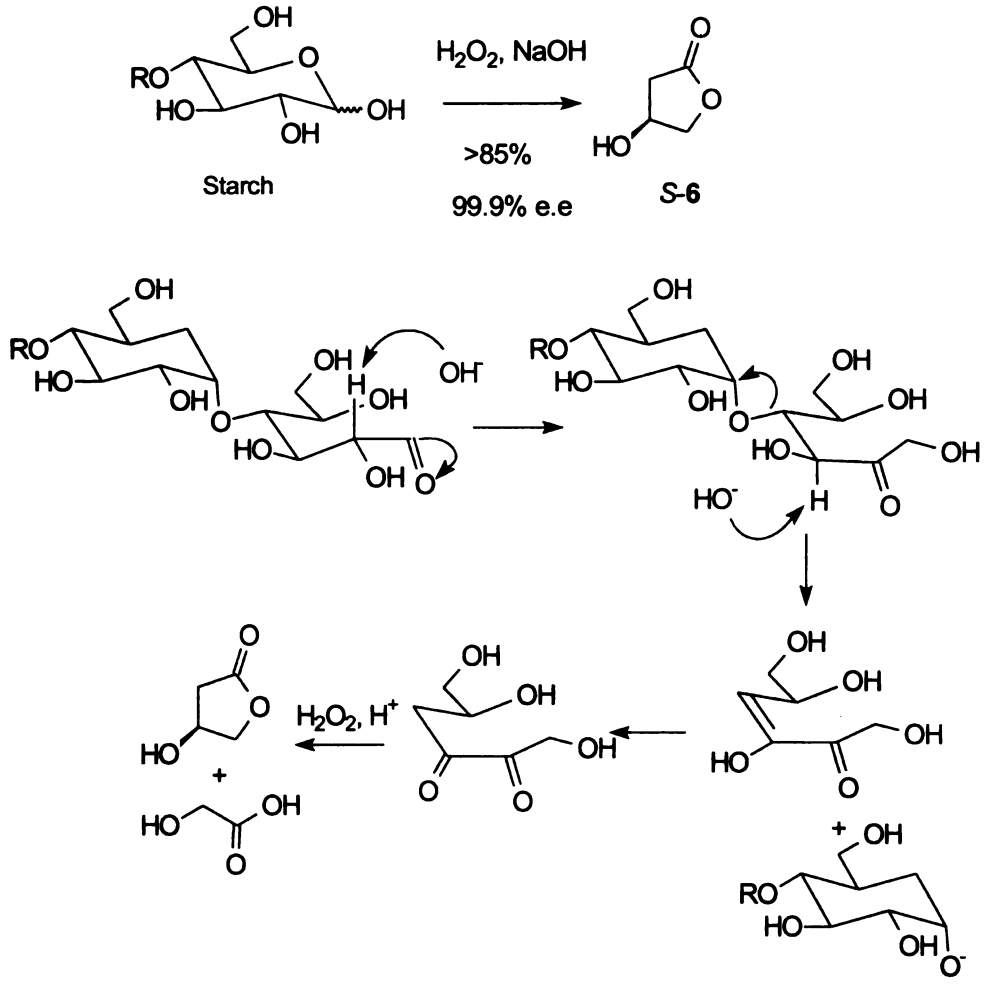
There is an increasing interest in the use of 3-carbon chiral molecules (C3-chiral synthons) that have an asymmetric center at the central carbon atom (Figure 1.1.) for the synthesis of optically active, biologically important compounds. Protected glycerol, glycidol and glyceraldehyde are versatile building blocks to a variety of synthetic transformations. There are many known applications in drug synthesis for R-isopropylidene glycerol. It can be used for the preparation of L-carnitine, S-beta-blockers, phospholipids, antiviral agents etc. Because of its high price, several of these routes are not economically viable. Scheme 1.1 shows some examples of the synthesis of these C3-chiral synthons. One elegant method for [30] for obtaining C3-synthons is from protected D-mannitol. Cleavage between the



Scheme 1.1. The synthesis of C-3 chiral synthons in literatures.

C-3 and C- 4 carbons affords two identical chiral molecules-glyceric acid (**5**). However, the reaction require at least stoichiometric quantities of sodium periodate or lead tetraacetate [31] to give the R-IPG. Another way to chiral C3 synthon from the chiral pool is the oxidation of protected d-or L-ascorbic acids to D-or L- protected glyceraldehyde (**4**). The method has some potential for replacing the above conventional methods but it also has some limitations. The overall yield is moderate (around 60%), it utilizes a Ru catalyst and the reduction of the aldehyde involves the use of either Ru/C or Pd/C catalysts. This approach is inferior to enantiospecific microbial oxidation o other enzymatic transformation methods. Another approach is to degrade unprotected carbohydrates directly to unprotected C-3 chiral synthons. The degradation of glucose to D- glyceric acid in basic media, by sodium anthraquinone-2-sulfonate (AMS) in three steps is an elegant method. The overall yield is about 60% [32].

Though there is abundant research in this area, little of which is applicable to large scale industry synthesis because either the reagents are toxic or expensive, reaction conditions are not mild or the overall yield is too low. In recent years, Hollingsworth has developed a route to obtain chiral 4-carbon synthons from raw carbohydrate feed stock. This is a one step degradation of maltose, lactose, or other 1,4-linked sugar to give optically pure *(S)*-3-hydroxy butyrolactone (**6**) in high yield [33, 34]. The lactone is a very valuable 4-carbon chiral building blocks, leading to a variety of important chiral intermediates. This approach (Scheme 1.2) has many advantages over the other methods discussed above and it has been adopted by industry on the multi ton scale. The advantages include the low



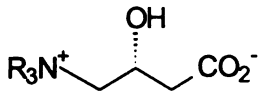
Scheme 1.2. The direct conversion of carbohydrate to *S*-3-hydroxy-γ-butyrolactone.

price of reagents, and the carbohydrate raw materials, ease of implementation and simplicity of isolation of the products.

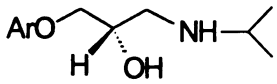
1.3. Chiral 3 and 4-carbon building blocks and their applications.

There is an increasing interest in chiral 3-carbon molecules such as glycidol (**1**), epichlorohydrin (**2**), glycerol protected as the 1,2-O-isopropylidene acetal (**3**) because they are the key intermediates for the synthesis of many biological important compounds such as carnitine, γ-amino-β-hydroxybutyric acid (GABOB), beta-blockers, antiviral agents etc (Figure 1.2). Many methods have been developed for the synthesis of these small chiral molecules. The uses of chiral glycidols were reviewed in 1991 by Hanson [35]. The uses of (R) and (S)-2,3-O-isopropylidene glyceraldehyde (**4**) in stereoselective organic synthesis were reviewed a few years earlier [36]. The C3-synthons can be obtained by oxidative degradation of mannitol, by transformation of serine and also by catalytic asymmetric reactions. Many of the current methods to these 3- carbon compounds currently being practiced require the use of expensive catalysts containing metals such as cobalt, platinum, rhodium or manganese. These metals are either toxic or expensive or both. The two leading technologies were developed in the United States at Scripps (37) and Harvard (38, 39) Both technologies use heavy metals that are harmful to the environment and their use involves expensive metal recovery and / or clean up.

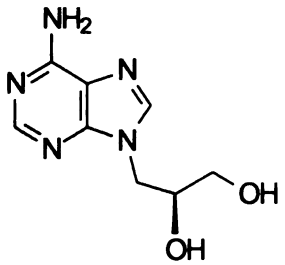
C-3 chiral synthons such as 3-amino-1,2-dihydroxy propane, glycidyl chloride, 1-chloro-2,3-dihydroxy propane have been used in several routes to carnitine. L-carnitine is a



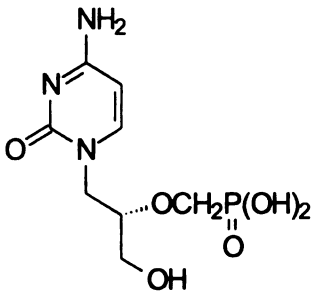
R=Me, *L*-Carnitine
R=H, (*R*)-GABOB



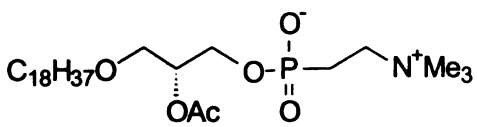
(S)-beta-Blocker
(general structure)



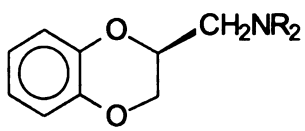
antiviral agent



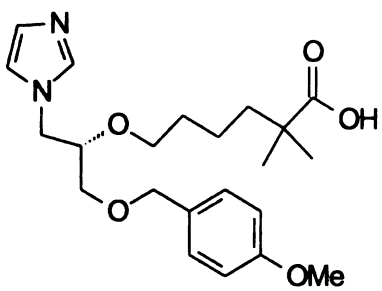
antiviral agent Cidofovir



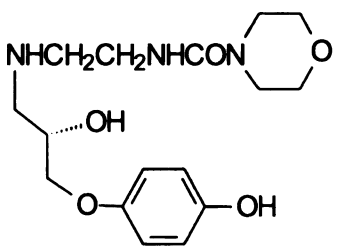
Platelet aggregation factor



alpha-Adrenergic antagonist



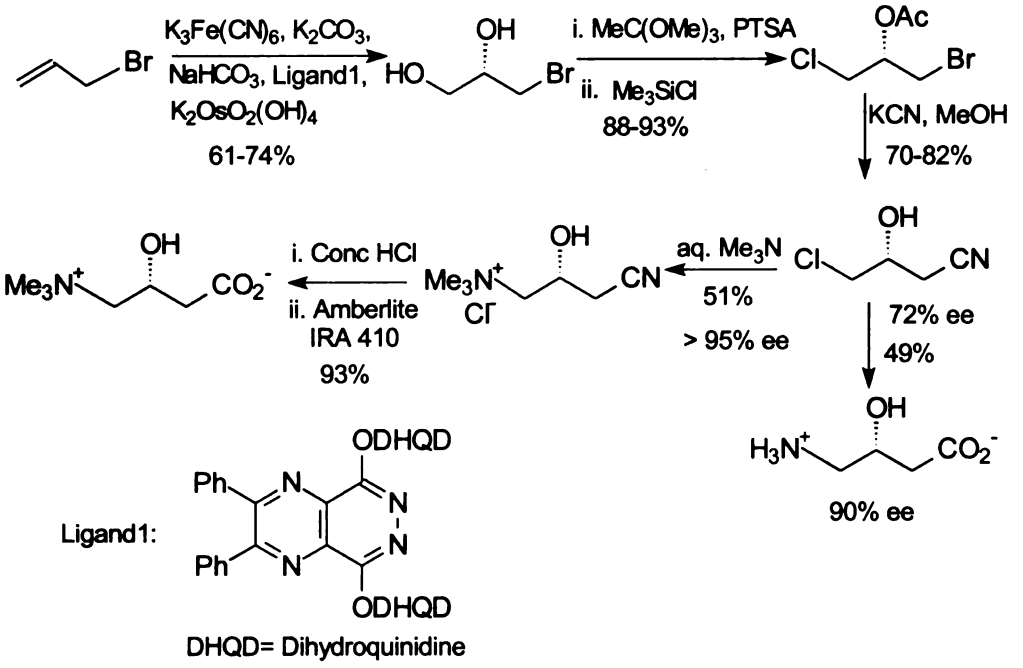
Thromboxane synthase inhibitor



Xamoterol,
beta1-Adrenoceptor partial agonist

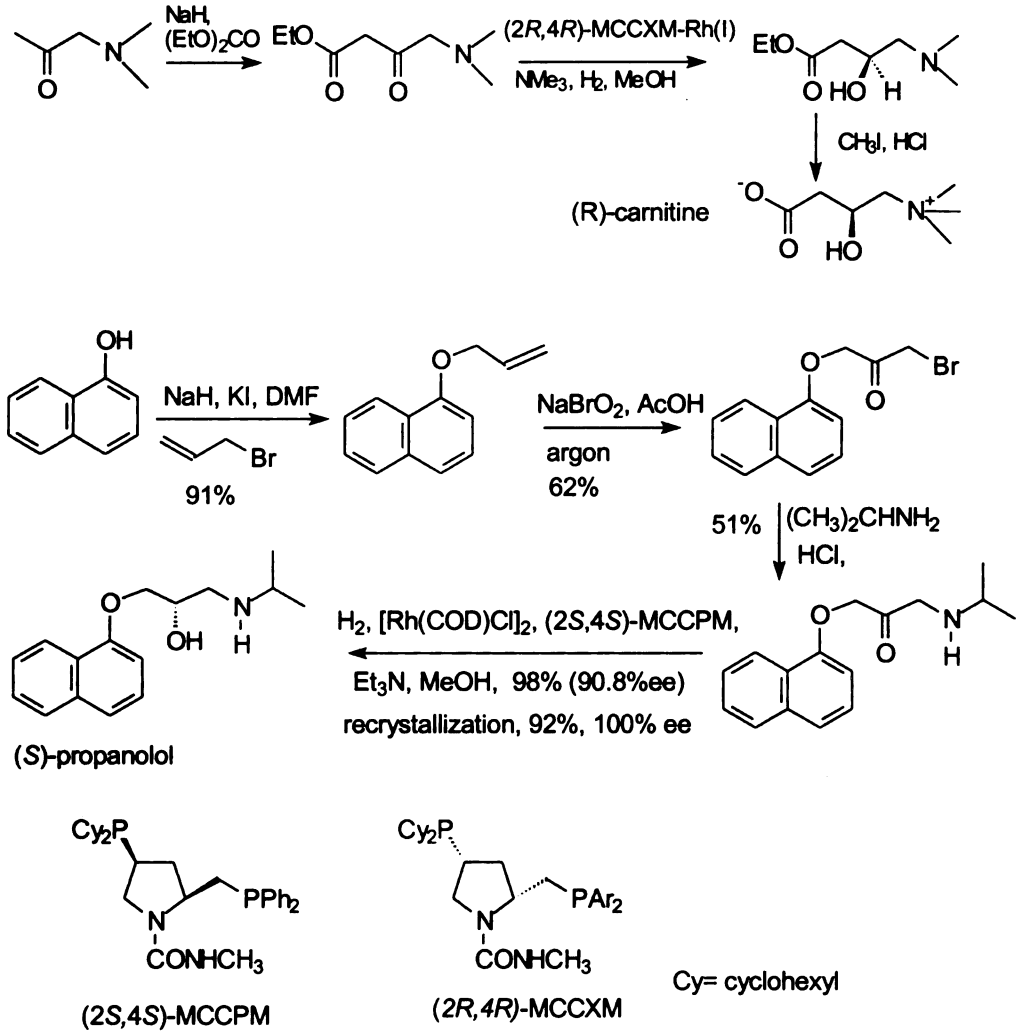
Figure 1.2. The important compounds that can be derived from chiral C-3 synthons.

naturally occurring carrier molecule that transports fatty acids into cellular mitochondria for their conversion into cellular energy. It is also used for the shuttling of acyl groups that are potentially toxic to the cell. Diseases resulting from lack of carnitine lead to severe neurological disorders. Carnitine and its derivatives are therefore very important drugs. Carnitine is also used as a nutrient for life enhancement. The naturally occurring (correct) (L) form is difficult to synthesize. Gamma amino-β-hydroxybutyrate (GABOB) has a very similar structure to carnitine and itself is an important neural transmitter. Many routes to L- carnitine, GABOB and β-blockers are related as they all involve the same C3 synthons as key building blocks. Interestingly, many methods towards the synthesis of L-carnitine start with carbohydrates. For example, there are syntheses from D-ascorbic acid (vitamin C) [40], D-arabinose [41], D-galactono-1,4-lactone [42]. Other chiral pool methods use β-pinene [43], D-malic acids [44]. These routes are not very efficient. They are lengthy and also the starting material is expensive usually despite some improvement over recent years. As noted earlier, many catalytic asymmetric methods have been developed for the preparation of C3-synthons. They include asymmetric epoxidation, selective dihydroxylation of alkenes (Scheme 1.3) [37], kinetically-controlled enantioselective opening of epoxide rings (Jacobson's methods) and asymmetric hydrogenation of α-amino ketone derivative (Scheme 1.4) [45]. There are some other methods involving resolution of precursors of R-carnitine such as resolution of epichlorohydrin by Jocobsen's catalyst [46] and resolution of trimethyl ammonium chloro alcohol intermediate [47]. The Sharpless asymmetric epoxidation is the best known of these methods and provides a viable route to glycidols, but the product optical purity is usually low and recrystallization

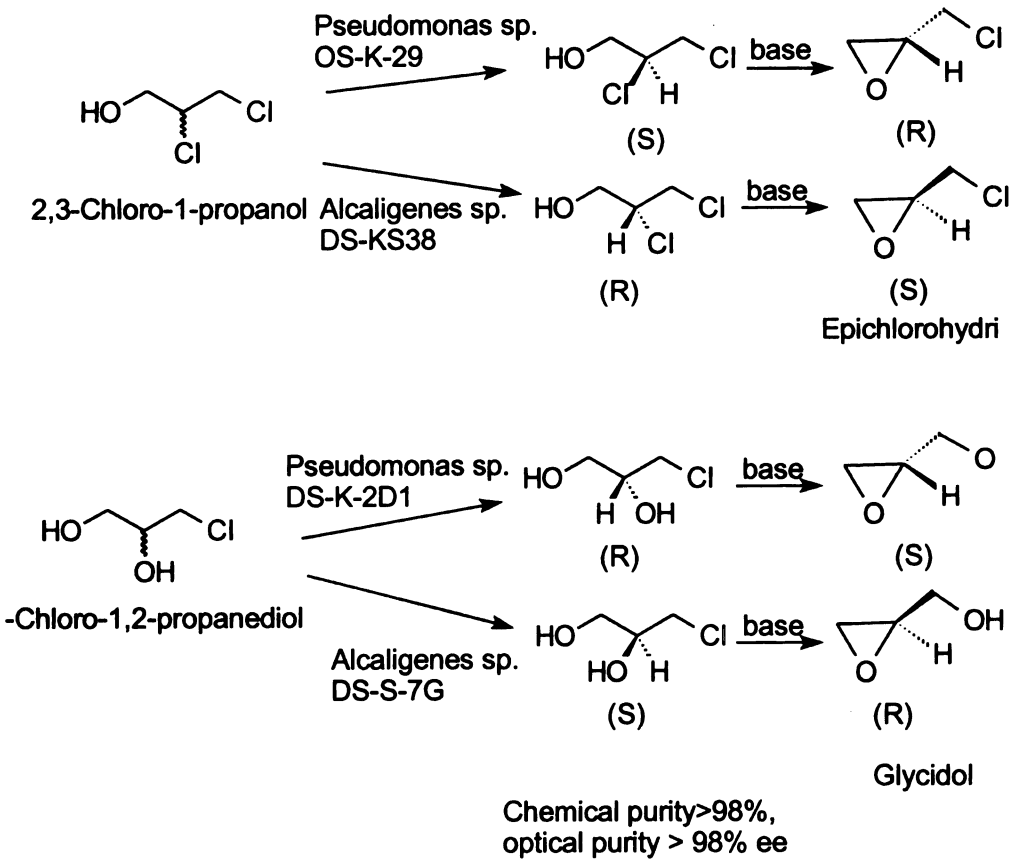


Scheme 1.3. The synthesis of L-carnitine and R-GABOB by asymmetric dihydroxylation.

[48] or HPLC methods [49] are usually necessary for further purification of the product. dichloropropanol and the other S-dichloropropanol as the single source of carbon. If the Daiso Co. developed routes to afford optically active 3-carbon molecules such as epichlorohydrin, glycidol, 2,3-dichloro-1-propanol etc by bioassimilation methods (Scheme 1.5). They use two types of bacteria one of which preferentially assimilates R-dichloropropanol and the other S-dichloropropanol as the single source of carbon. If the bacteria are fed racemic chlorodiols, they utilize one and leave the other stereoisomer as an enantiopure compound [50, 51]. The optical purity is usually very high. This approach

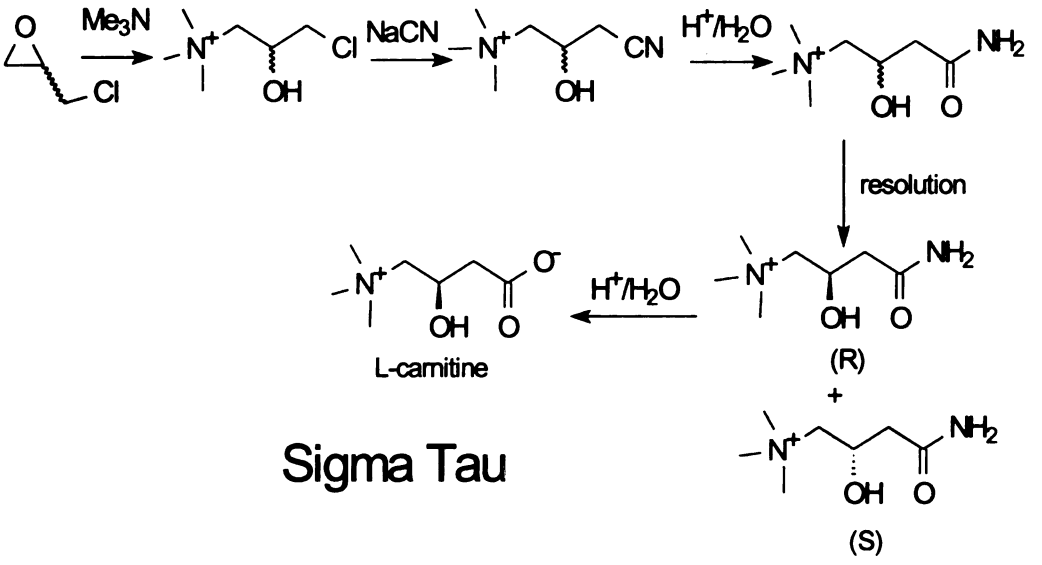


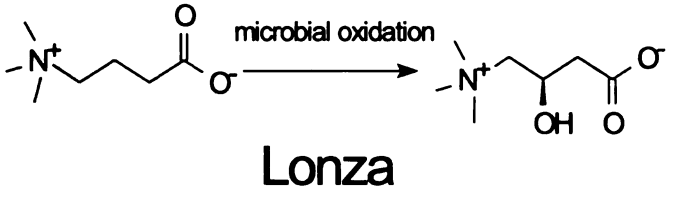
Scheme 1.4 The synthesis of *L*-Carnitine and *S*-beta-Blockers by asymmetric hydroxylation of α-amino ketone derivative.



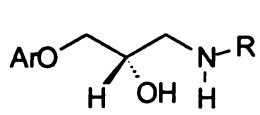
Scheme 1.5. The stereoselective bio-assimilation of C3 building units.

based on microbial resolution has much promise [52]. Despite the number of routes to L-carnitine that have been explored, currently, there are only two commercial routes to L-carnitine [53]. Although many C3- chiral synthons are suitable precursors for R- carnitine, none of them can compete with the existing commercial routes yet. The reasons are either the synthetic route is too lengthy, low optical purity, low yield and or involve the use of toxic heavy metal and the chiral synthons are too expensive. So the production of R-carnitine is dominated by classical resolution and fermentation route. One route is owned by an Italian company called Sigma Tau (Scheme 1.6). Another route is owned by a Swedish concern called Lonza (Scheme 1.6). As the major producer of L-carnitine, Sigma Tau uses racemic epichlorohydrin as the starting material and a classical resolution of racemic carnitinamide as the key step. This key step is very efficient, however it produces equal amounts of the D-enantiomer as a waste material. They have developed some methods for recycling the D-carnitine, including dehydration of the D-carnitine to give intermediate alkene and then carnitine hydrolyse mediated hydroxylation of the intermediate to give the correct product, and also by chemical synthesis to invert the stereocenter in D-carnitine through a β-lactone intermediate to give the correct isomer [54,55].However these need more steps and are obviously not very economic. Alternatively, Lonza commercialized a route based on microbial oxidation of butyrobetaine. There is only one step in the transformation, but the problem they have is the low productivity of the microorganism though they devoted much effort to improving it [56].

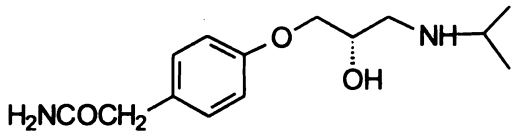




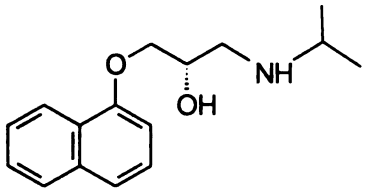
Scheme 1.6. The commercial synthetic routes to L-carnitine, top is by Sigma Tau, bottom one is by Lonza.



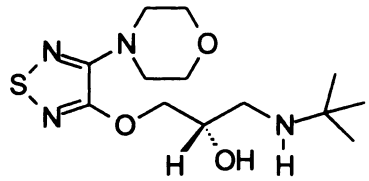
S-beta-Blocker
General structure



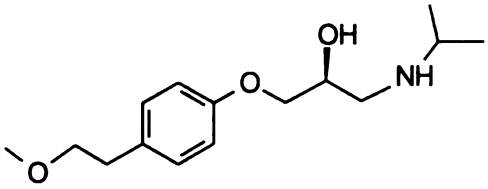
(S)-atenolol



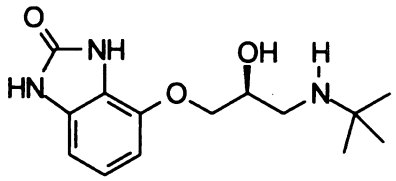
(S)-Propanol



(S)-Timolol



(S)-Metroprolol

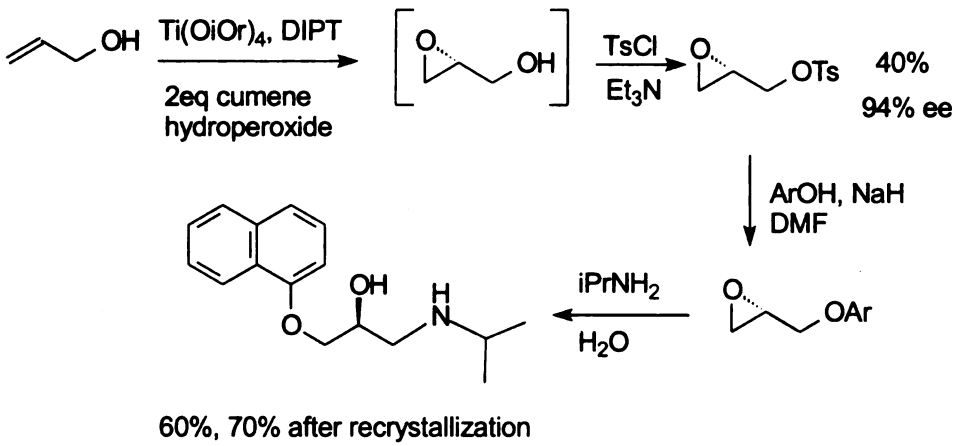


beta-3 adrenergic receptor
for treatment of obesity, diabetes

Figure 1.3. The structures of some important beta-blockers.

Beta-blockers are a type of drug for the treatment of hypertension and heart diseases. They have the general structure shown in Figure 1.3. They reduce the symptoms connected with hypertension, cardiac arrhythmias, migranine headaches, and other disorders related to the sympathetic nervous system. Well recognized trade names are atenolol, metroprolol and propranolol (Figure 1.3). These are currently used as a mixture of both forms (D and L) but there has always been a great interest in the development of methods for preparing only one form. It has been found that the S-form is more than 100 times more effective than the R-form of the drug. The biological active form is the S-form which can be derived from the corresponding 3-carbon synthon. Although many routes [57] have been developed for chiral intermediates of beta-blockers (which are similar as those involved in the synthesis of carnitine) an efficient and economic one is elusive. They are not competitive enough to allow the replacement of the racemate product. The synthesis of racemic beta-blockers is by treatment of racemic epichlorohydrin with substituted aromatic phenoxide. At the beginning, people tried the same route as the racemate process by using optically pure epichlorohydrin, but it didn't give optical pure product because of the activity of epichlorohydrin. There are two paths to opening the epoxide ring leading to a mixture of enantiomer. Certain modifications to the epichlorohydrin had to be made. The chiral intermediates leading to optical pure beta-blockers can be obtained via circuitous routes from D-mannitol [58] and many enzymatic processes including benzylpenicillin acylase and Baker's yeast [59,60] and other routes including biochemical and chemical synthesis [61-63]. Many studies have been devoted to obtain optical pure S-beta-blockers. These include lipase catalyzed reactions [64-72], catalytic asymmetric epoxidation [73, 74] and

kinetic resolution [75], catalytic asymmetric hydrogenation [45, 76], microbial oxidation [77], microbial assimilation [78] etc. Some examples are shown in scheme 1.7. Despite the enormous effort have been devoted by scientists in this area, none of the route to optically pure beta-blockers are competitive enough to replace the racemate product. A better route which is short, efficient and economic is yet to be defined, although asymmetric synthesis or enzymatic kinetic resolution appear to be on the edge, C3-chirons via carbohydrates conversion may offer hope to this matter.



Scheme 1.7. Synthesis of *S*-Propranolol by Sharpless epoxidation method.

Antiviral agents are another class of drugs that can be derived from these three carbon molecules. These are used for treating a number of viral diseases including AIDS. They include drugs such as acyclovir and cidofovir (Figure 1.4). (S)-1,2-Dihydroxy-3-aminopropane is an important substructure in the drug cidofovir [79]. This is an established antiviral agent that is used for the treatment of herpes and is now being promoted for some AIDS indications. Besides S-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir or HPMPC) other nucleotide analogues [80] also have shown important antiviral activity. S-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine (HPMPA) has been found to be active in vitro against a broad spectrum of DNA virals [81, 82] . These antivirals are usually synthesized [83-88] by condensation of nucleic bases such as cytosine, adenine, guanine etc. with the dihydroxychloropropane or other three carbon units (Figure 1.4). A straightforward and cost effective route to the aminodiol will lead to a more efficient route to this and related drugs. It will also enable the development of other analogs as the older drugs lose effectiveness because of build up of resistance.

Besides the above application, 3-carbon chiral compounds are used in the development and manufacture of other drugs. These include platelet activation factors (PAFs), molecules that control the interaction between red cells and stimulate processes such as clotting. They play an important role in the cardiovascular system. PAF or 1-O-alkyl-2-O-acetyl-*sn*-glycerol-3-phosphorylcholine has emerged as one of the most important lipid mediators known [89, 90]. Thromboxane synthase inhibitors are another

class. These molecules are involved in the biosynthesis of prostaglandins [91]. Three carbon chiral synthons are also used in the preparation of lipid analogs [92]. The structures of these important compounds are shown in Figure 1.2.

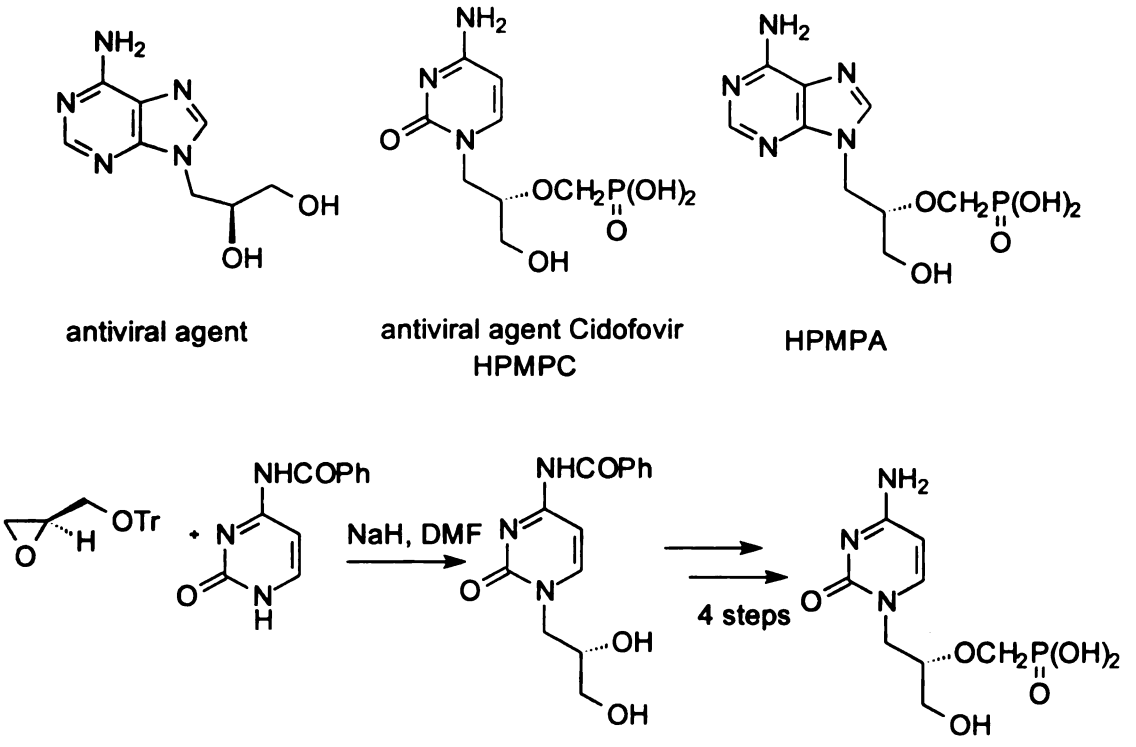


Figure 1.4. The strictures of some antiviral agents and their synthesis from C-3 synthons.

1.4. Introduction of cis-1-Amino-2-indanol

The importance of preparing enantiopure materials is well recognized by the chemical community, and many research groups have devoted a lot of efforts to new asymmetric synthesis methods. However, to find a general useful building block is still challenging. Chiral amino alcohols are important classes of molecules for drug development [93]. One particular molecule of this family is cis-a-amino-2-indanol. This is an important subunit in HIV protease inhibitors and is also for some other applications. This aminoindanol substructure seems to play a crucial role in biological systems and is an important target in the field of asymmetric synthesis [94, 95]. The molecule is a building block for important drugs [96-98] (Figure 1.5). In the synthesis area, it has been used as a chiral resolving agent, asymmetric catalyst and as a chiral auxiliary (Figure 1.5). The aminoindanol derived oxazolidinone **(12)** has been used as a chiral auxiliary in asymmetric syn-aldol reactions [99]. The aminoindanol derivative indane-bis (oxazoline) **(14, 15)** can catalyze the Diels-Alder reaction [100]. The Sepracor group has shown that optically pure cis-1-amino-2-indanol (**7**) is an extremely effective enantioselective catalyst for the borane reduction of some important α-halo ketones [101]. It was found that besides the amino alcohol functionalities, the constrained indane platform is essential for obtaining stereoselectivity [102].

Because of the importance of cis-1-amino-2-indanol in biological systems and in asymmetric synthesis, many method have been explored for synthesizing enantiopure cis 1-amino-2-indanol. Surprisingly, practical syntheses have been elusive until recently when

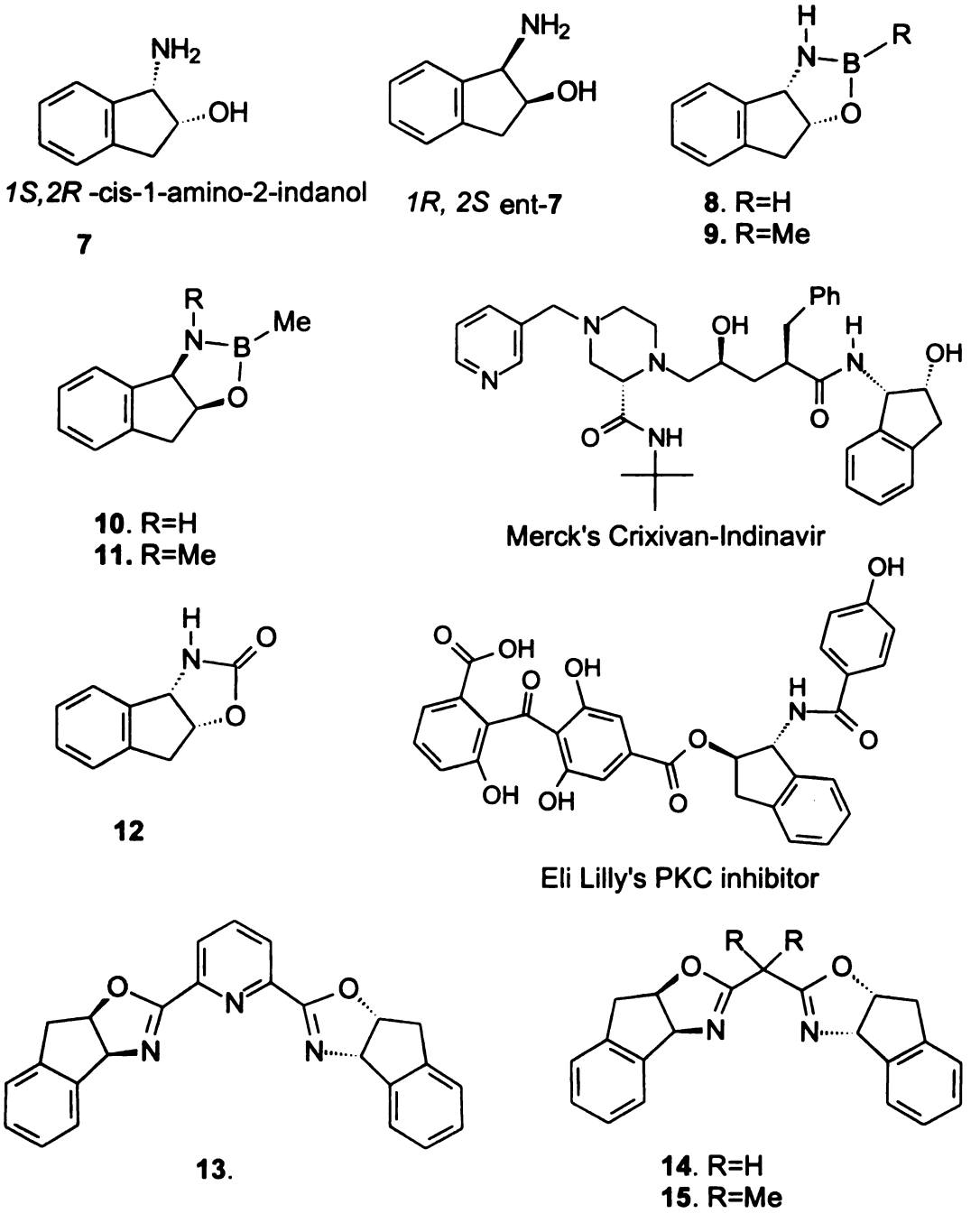
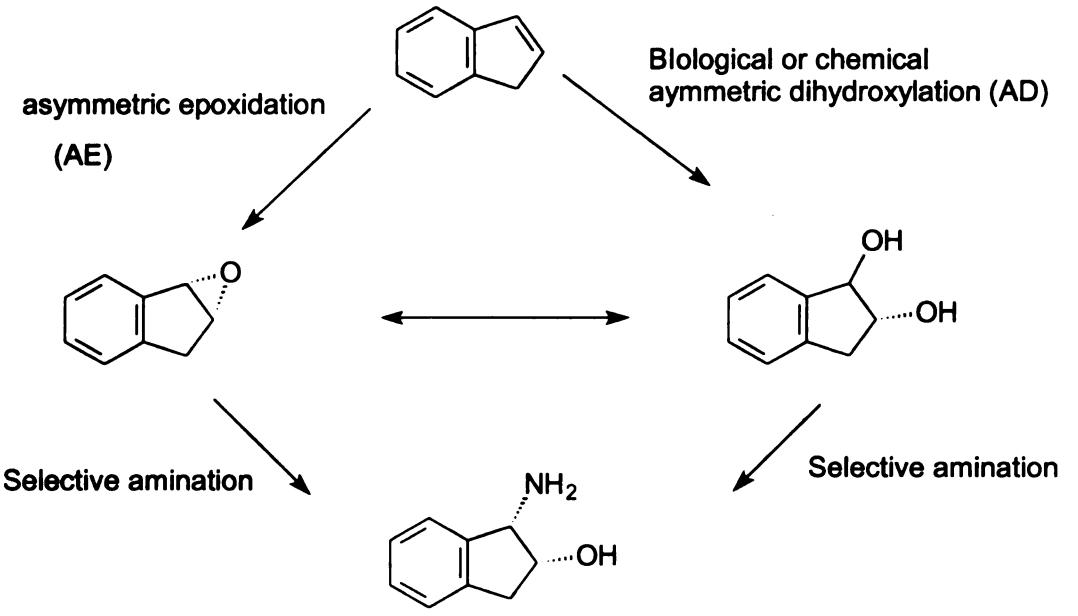


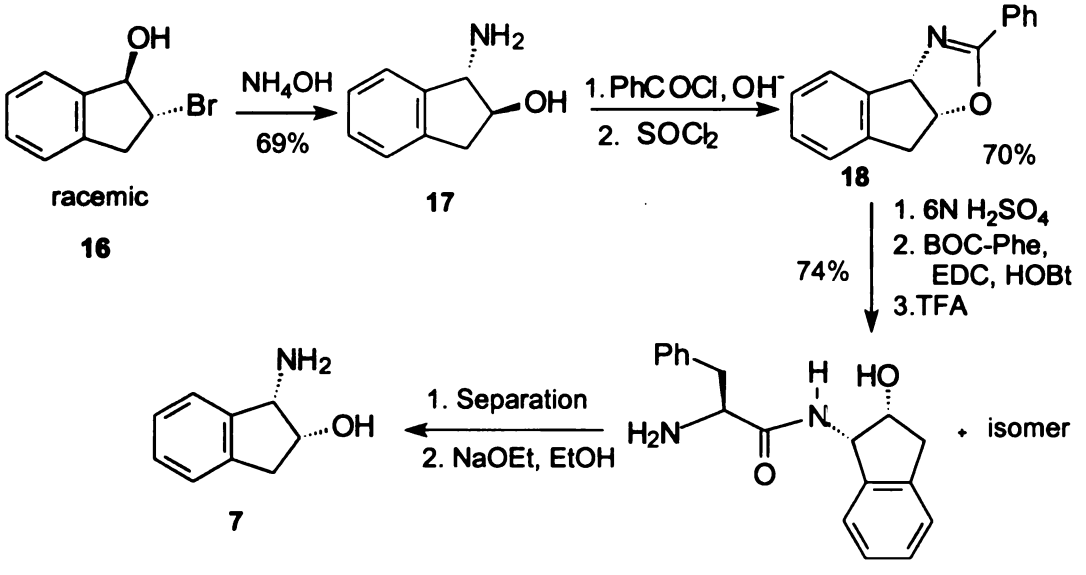
Figure 1.5. The structures of Cis-1-amino-2-indanols, important HIV-protease inhibitors, chiral catalysts derived from the substructure of cis-aminoindanol.

the Merck and Sepracor groups independently developed two relatively practical 1-amino-2-indanol. Surprisingly, practical syntheses have been elusive until recently when the Merck and Sepracor groups independently developed two relatively practical processes for the preparation of chiral *cis* -1-amino-2-indanol. The general synthetic approaches to cis-1-amino-2-indanol are shown in Scheme 1.8. An earlier synthesis [103] by the Merck group is shown in (Scheme 1.9). Racemic 2-bromoindanol **16** was first treated with concentrated ammonium hydroxide to give the trans-aminoindanol **17**, which was then converted to the oxazoline **18**. The oxazoline was hydrolyzed to racemic cis-aminoindanol. Resolution of the racemic compounds was carried out by reaction with BOC-phenylalanine followed by removal of the Boc-group and chromatographic separation of the corresponding phenylalanine amide. Hydrolysis of the amide gave optically pure cis-aminoindanol **7**. Many enzymes such as Baker's yeast [104] and lipases [105,106] have also been used toward the enantioselective synthesis of cis-1-amino-2-indanol.

An example of chemoenzymatic synthesis of cis- aminoindanol is shown in Scheme 1.10. The starting bromoindan **19** was oxidized to enantiopure cis-2-bromo-1-indanol **20**, which was then converted to the optically pure cis-aminoindanol via a Ritter type reaction [107] with sulfuric acid in acetonitrile followed by basic hydrolysis. Senanayake and co-workers have shown that chiral indan-1,2-diols also undergo Ritter -type reactions leading to cis 1-aminoindianol in high yield. In a recent synthesis, indene was converted to the racemic epoxide, this was subjected to with ring opening to give racemic trans-

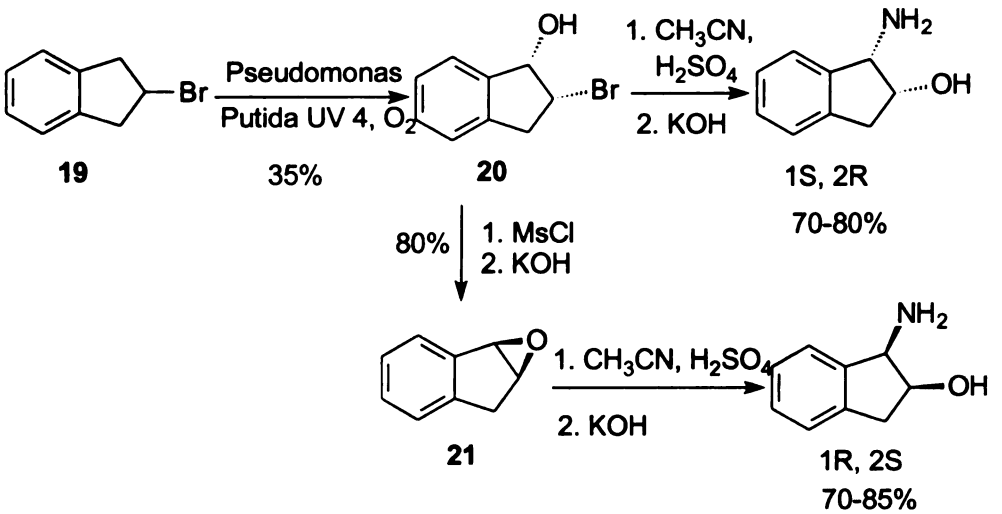


Scheme 1.8. The synthetic approaches to cis-1-amino-2-indanol.



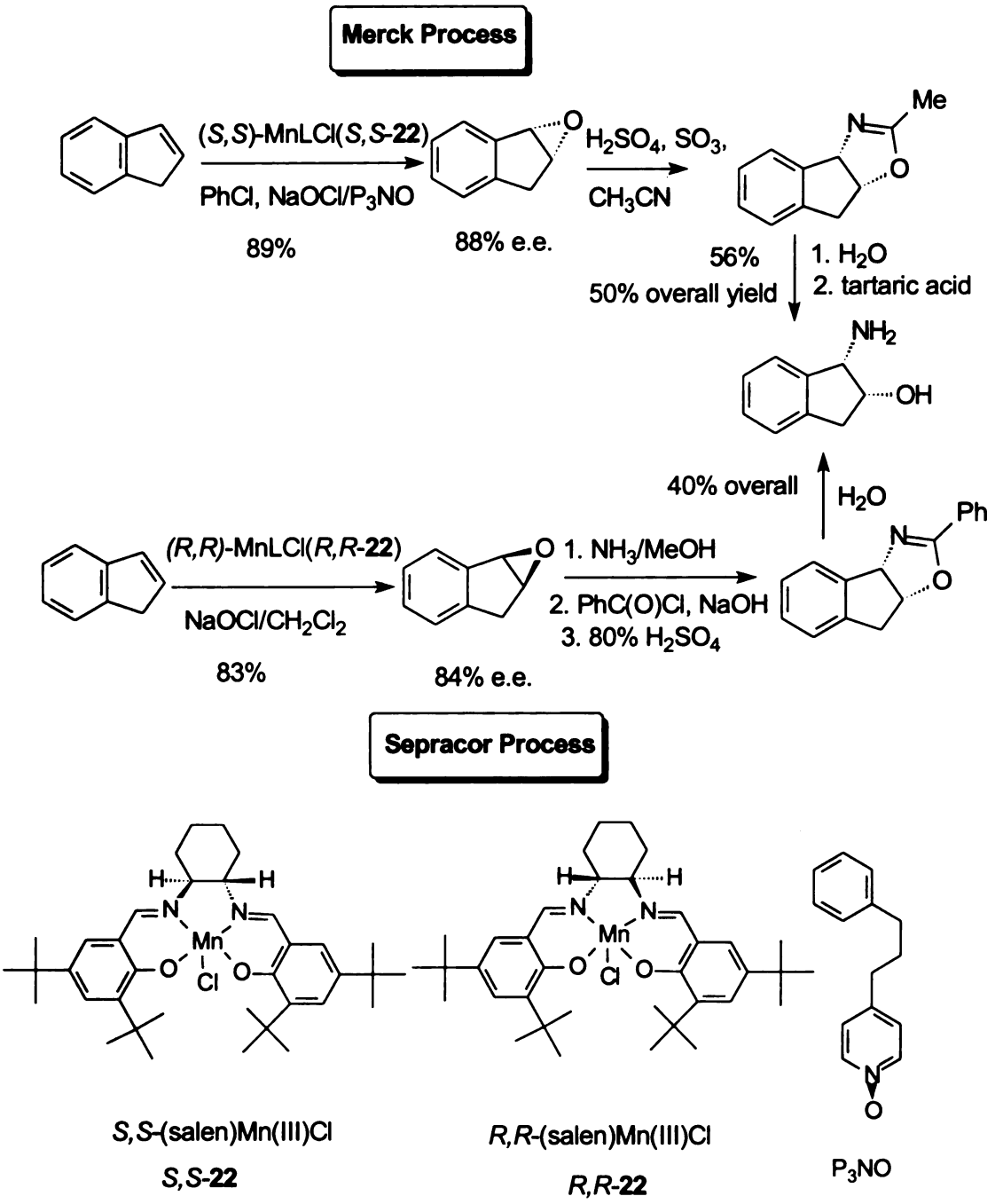
Scheme 1.9. Synthesis of cis-1-amino-2-indanol by Merck's early method.

azidoindanol. A lipase was used to resolve the racemic product. The resulting desired azido acetate was then converted to the desired product by standard transformations. Unfortunately, these methods are either lengthy, low in productivity, have low yields or they suffer some other problems.



Scheme 1.10. The synthesis of cis-1-amino-2-indanol by chemoenzymatic method.

The current most efficient existing routes (scheme 1.11) were developed by Merck and Sepracor [108-110]. Both of these groups rely on Jacobsen's catalyst (chiral Mn-salen complexes) to generate enantiopure epoxide intermediate in the key step [111,112]. Sepracor prepared the indene oxide in 83% yield and 84% ee. The optically active indene oxide was then treated with ammonia to give trans aminoindanol which was transformed to optically pure benzamide **48** by crystallization. The benzamide was transformed to the



Scheme 1.11. The current most efficient synthetic routes to cis-1-amino-2-indanol.

cis amino-indanol. The overall yield from indene is 40%. Merck's approach is similar to Sepracor's, except that they used Jacobsen's catalyst in a combination with the Ritter type reaction. Although these two methods are the most efficient routes currently, it is not the end of the battle. The yield and optical purity of the key-asymmetric epoxidation step are moderate. Right now the requirement of Crixivan by AIDS patients have not been met because of the difficulty in producing the drug. There is still great need for a more economic route that is more environmentally friendly and more efficient.

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Chapter 2

The Direct Conversion of (S)-3-Hydroxy-γ-Butyrolactone to Chiral 3-Carbon Building Blocks and Facilitation of Synthetic Routes to L-Carnitine, (R)-GABOB and (S)-beta-Blockers

Abstract

(S)-3-Hydroxy-γ-butyrolactone is a very valuable 4-carbon chiral synthon for which a simple, 1-step, high-yield route starting from starch, maltose, lactose, maltodextrins and other 4-linked D-pyranoses exists. Because of its ready availability, it is desirable to develop an efficient way of performing a simple chain degradation reaction in which the acyl carbon is lost. This would provide an entry route into chiral 3-carbon synthons via a method that does not involve heavy metal catalysts and utilizes renewable resources. This was accomplished by performing a Hoffman reaction on the isopropylidene acetal of the amide formed by treating the lactone with ammonia. This extends the use of carbohydrates as chiral raw materials for the preparation of important glycerol derivatives. These are important building blocks in the synthesis and development of several classes of drugs ranging from β-blockers to antiviral agents, phospholipids, platelet activation factors and thromboxane synthase inhibitors. A straightforward conversion of the lactone to various chiral 3-carbon building blocks will be discussed. Two routes for converting the lactone to other chiral intermediates which will facilitate the synthesis of L-carnitine, (R)-GABOB and (S)-beta-blockers will also be discussed too.

2.1. Introduction

Many pharmaceutical compounds contain 3-carbon chiral substructures the development of routes to which is often the most difficult aspect of their large scale synthesis. Because of this, there is a constant ongoing effort to identify synthetic routes to these 3-carbon chiral building blocks. Such building blocks include chiral glycidols, halo diols and amino diols. Three key 3-carbon building blocks are (R)-glycidol (**1**), (R)-1-bromo-2,3-dihydroxypropane (**2**) and (S)-3-amino-1,2-dihydroxypropane (**3**). Current routes to (R)-glycidol (**1**) and its (S)-isomer include catalytic oxidations with peroxides and chiral transition metal complexes [1,2], kinetic resolutions by salen Co complex[3,4], enzymatic resolutions of racemic esters by lipases to selectively deacylate one enantiomer [5-7] or using glycerol kinase to selectively phosphorylate one isomer [8]. Another common method is to treat a chiral 1,2-propane diol with a leaving group such as a halide or tosylate ester in the 3-position with base [9, 3]. The chemistries of compound **1** and **2** are interconnected. The availability of an easy route to **2** constitutes a straightforward route to **1** since this transformation is easily effected by treatment of **2** with a mild base such as silver oxide or potassium carbonate. The aminodiol **3** is a substructure that appears in a large number of classes of important drugs. These include the β-blockers such as Propanalol (**4**) Atenolol (**5**), antiviral agents such as (**6**) and the thromboxane synthase inhibitor (**7**). Despite the existence of these methods, there is a need for others that might have certain advantages. For instance, they might have the potential to yield some compounds more directly, give better results at larger scale, do not require high pressures or hydrogen, give superior optical purity, offer some potential price advantages or do not produce certain waste materials especially those containing heavy metals.

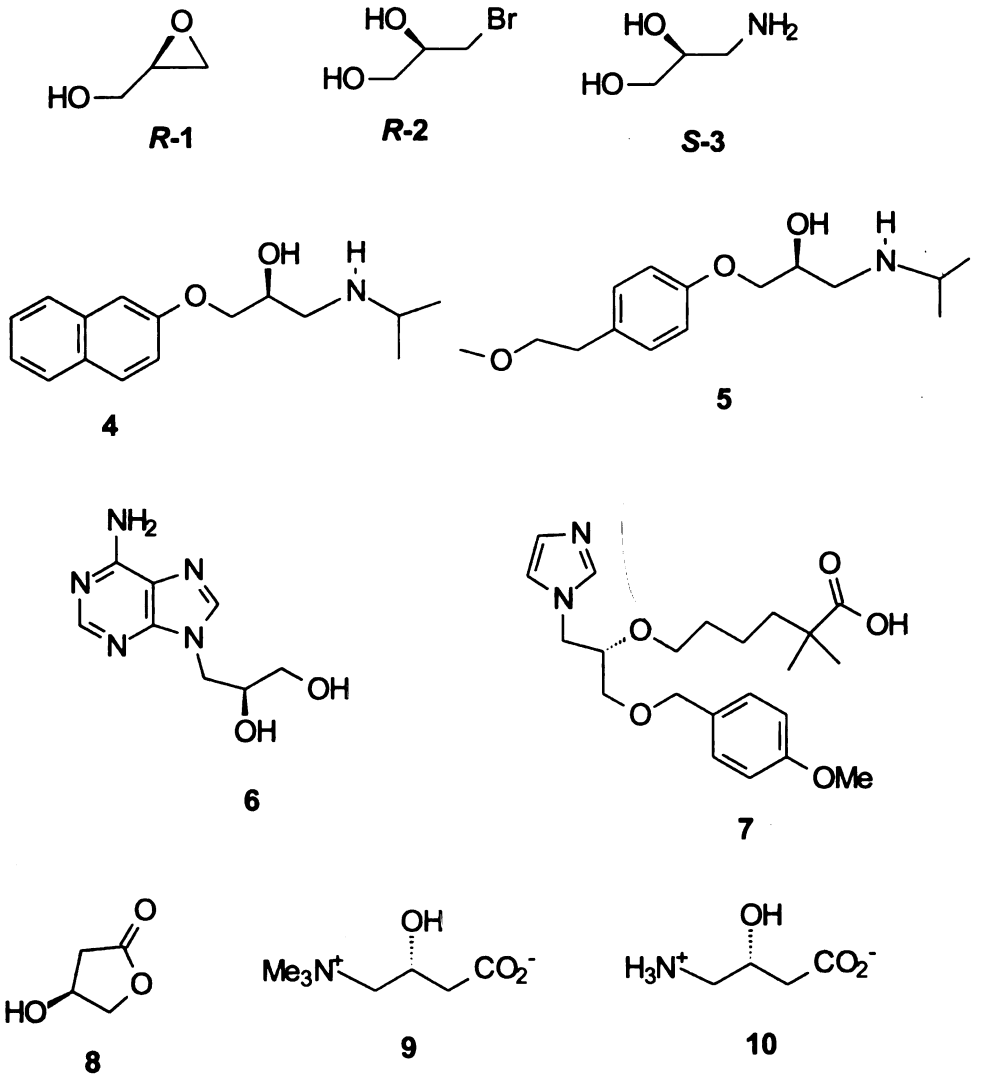
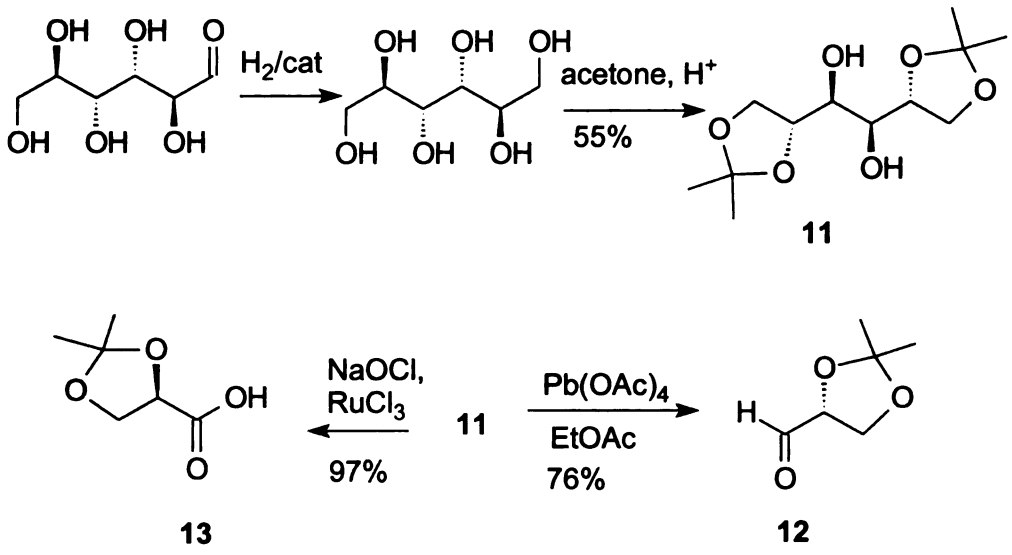


Figure 2.1. The structures of some chiral building blocks and their derivatives.

Another strategy for chiral drug synthesis, the so-called "chiral pool" approach, is to identify natural sources containing these substructures and carve them out to provide the substructures in a form that could be integrated easily into a drug synthesis scheme. Because of the high degree of oxygenation in the desired 3-carbon fragments, carbohydrates are a logical choice for this strategy. They have the highest density of chiral functional groups of all naturally occurring materials. Many of them, such as starch and lactose are readily available and are very cheap, renewable feedstock. It is, however, extremely difficult to convert carbohydrate raw materials into chiral small molecules in good yield by a simple process. This is because several steps of protection and functionalization typically have to be followed. In addition to this, carbohydrates are appreciably soluble only in water. This has proven to be a serious limitation to the chemical transformations they can be subjected to. They are also very sensitive both to acids and bases and therefore pH conditions are also a major constraint. Despite these difficulties, there are some applications where carbohydrates have been used as a source of small chiral building blocks. One of these methods with some practical value is outlined in Scheme 2.1. In this process, fructose or mannose is reduced to mannitol by catalytic hydrogenation[10]. This is then converted to a di-O-isopropylidene acetal which can be oxidized to yield two molecules of D-isopropylidene glyceraldehyde, a valuable 3-carbon synthon, by treatment with sodium periodate[11, 12] or lead tetraacetate[13]. The drawback of this approach is that the carbohydrate has to be modified and protected before the actual reaction to generate the building block is carried out. In addition to this, lead is toxic and periodate is relatively expensive and large quantities are required because of its high molecular weight and the overall yield is unsatisfactory. An alternative set of reagents

for carrying out this oxidation is sodium hypochlorite and ruthenium III chloride (Scheme 2.1). In this case, the protected glyceric acid is the product[14].



Scheme 2.1. The conversion of carbohydrate to C3-chiral building blocks in literature.

There is one significant advance in the use of unprotected carbohydrates as a raw material for producing a chiral building block that could be used to gain access to compounds such as **1-3**. This is the development of a route to (S)-3-Hydroxy-γ-butyrolactone (**8**), a very valuable 4-carbon chiral synthon for which a simple, 1-step, high-yield route starting from starch, maltose, lactose, maltodextrins and other 4-linked D-pyranoses has only recently been introduced into the literature [15-17]. (S)-3-Hydroxy-γ-butyrolactone readily undergoes a variety of chemical transformations to yield chiral substituted tetrahydrofurans, amides, lactams, nitriles and epoxides. This high degree of flexibility as a chiral intermediate makes it a very

valuable synthon for the pharmaceutical industry. A simple 1-carbon degradation method should provide the desired transformation of this intermediate to a 3-carbon structure thus providing ways of obtaining the building blocks of interest. This will provide a straightforward conversion to the Chiral 3 carbon building blocks directly from carbohydrate starting material and it has the potential to reduce the price of the current C3-synthons thus make it more available in large quantities.

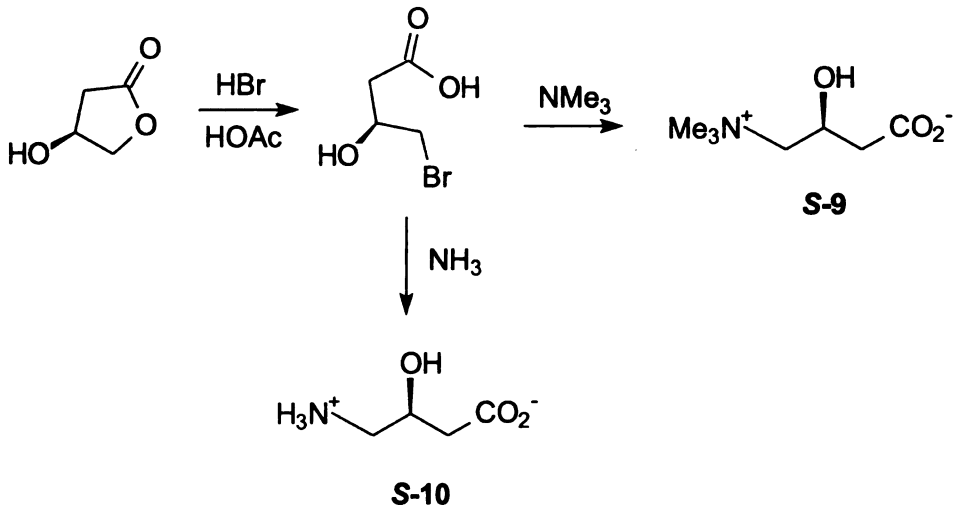
(R)-3-Hydroxy-4-trimethylaminobutyric acid (L-carnitine) **9** and (R)-4-amino-3-hydroxy-butyric acid (GABOB) **10** are two compounds with a very high level of medical significance. L-Carnitine is a very important intermediate in lipid biosynthesis. It functions as a carrier for transporting fatty acids into mitochondria for oxidation. Since fatty acid oxidation is a critical step by which cells derive energy, carnitine is important for cellular energetics. Deficiencies in the biosynthesis of carnitine leads to severe neurological problems. The two major uses of carnitine are in sports medicine and infant nutrition. Supplemental R-carnitine is beneficial to heart patients and also it is effective in treating systematic an myopathic deficiencies. There are several medical indications for which carnitine can be prescribed [18-22].

(R)-4-Amino-3-hydroxy-butyric acid is a well known drug substance that functions as an agonist of gamma amino butyric acid (GABA). It has been demonstrated to be effective in managing a variety of clinical conditions including schizophrenia and other character based illnesses epilepsy and other illnesses that result in severe convulsions [23-25] and its use for the correction of clinical conditions observed in children being treated for DOWN syndrome

has also been explored [26].

Despite the importance of L-carnitine and (R)-GABOB in medical science and the simplicity of the structure of these molecules there are not many straightforward synthetic routes to them. Although many preparative routes including kinetic resolution, enzymatic transformations and asymmetric synthesis have appeared in the literature for the synthesis these molecules, the current existing commercial routes are dominated by kinetic resolution by Sigma-tau[27] and microbial oxidation by Lonza [28] . The drawback of the kinetic resolution is that in the process, half of the product produced is treated as waste, the method using the bacteria is their low productivity. Asymmetric synthesis routes are usually lengthy, and involve the use of expensive and or toxic reagents such as heavy metal catalysts. It is desirable to define an alternative route to L-carnitine by a simple economic asymmetric synthesis starting with the readily available (S)-3-Hydroxy-γ-butyrolactone **8** [15-17].

The functionalities present in this molecule make it potentially easily amenable to conversion to carnitine and GABOB by placing a trimethylammonium group in the 4-position after ring opening the lactone with hydrogen bromide to form 4-halo acid and then displacing the halo group with trimethylamine. However, the stereochemistry at the 3-position is not the desired one. It leads to the undesired D-carnitine, which is thought to be a competitive inhibitor of the L-carnitine (Scheme 2.2). Synthesizing these molecules with the correct stereochemistry from (S)-3-Hydroxy-γ-butyrolactone requires inversion of the 3-hydroxyl group or some



Scheme 2.2. The synthesis of S-carnitine from *S*-3-hydroxy-γ-butyrolactone.

equivalent transformation. Because of its position relative to the carbonyl group, attempts at inverting the 3-hydroxyl group by activation and displacement readily leads to elimination to yield 2-(5H) furanone. Some other inversion is required. One possibility is to switch the priorities of the 1 and 4 positions in the 4-carbon intermediate represented by (S)-3-hydroxy-γ-butyrolactone. This would require removal of the 1-carbon and addition of a new high-priority carbon at position 4. We accomplish this here by carrying out a Hoffman reaction on a suitably protected butyramide to generate a chiral 3-carbon molecule that can readily be converted to the desired products. In another approach, the 1-carbon extension and the Curtius or Hoffmann reaction are performed in that order.

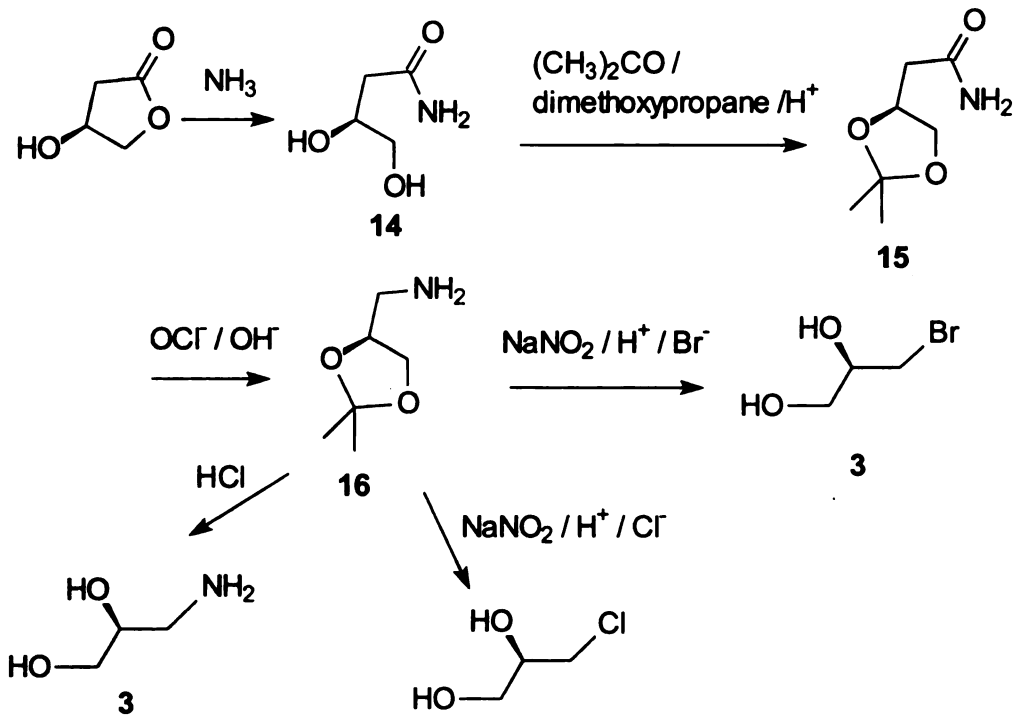
Beta-blockers are a large group of medications that act to block specific receptors in the nervous system. The current drugs are racemic. The effect of beta blockers results in slowing of the heart rate, reduction in blood pressure, and reduced anxiety, they are important drugs for heart diseases and for hypertension and other diseases [29-33]. Though the biological inactive forms are not known to have bad side effects, there is great interest in providing only the biological active S-form of the drug. It is desirable to provide only the effective half form both from the interest of patients (since the dosage is reduced) and of the chemist. Recent development of a family of beta-blockers at Merck which can treat obesity, and a range of other diseases are chiral, many of them also contain the same C3 synthons[34]. Despite numerous synthetic effort [35-40] on this area, the asymmetric synthesis of beta-blockers are still not good enough to supplant the use of the racemic form. The 3-C synthons mentioned above are suitable precursors for synthesis of S-beta-blockers, but they are too expensive and some of them are not available on large scale. The possibility of defining a new synthesis of beta-blockers which may have potential commercial value will be discussed.

2.2. Results and discussions

2.2.1. Preparation of chiral 3-carbon synthons.

In principle, the one carbon deradation of (S)-3-hydroxy-γ-butyrolactone by any one of the classical routes such as the Hoffman, Curtius, Lossen or Schmidt reactions. These involve rearrangement of acyl species with migration to an electron-deficient nitrogen and lost of the acyl carbon. Of these, the Hoffman reaction [41, 42] seemed the most attractive

because the starting compound is a primary amide. This primary amide can easily be obtained by treating the lactone with ammonia. There are potential complications however. Some of these are peculiar to this starting material and include β-elimination and the participation of the γ-hydroxy group instead of migration to re-form the lactone. There are also a myriad of side reactions, such as dialkyl urea formation, that normally attend the Hoffmann reaction. Protection of the hydroxy groups was necessary and the use of an isopropylidene function was explored because of the ease of installation and removal. In addition, confining the β-alkoxy fragment to a dioxolane ring could also restrict the group



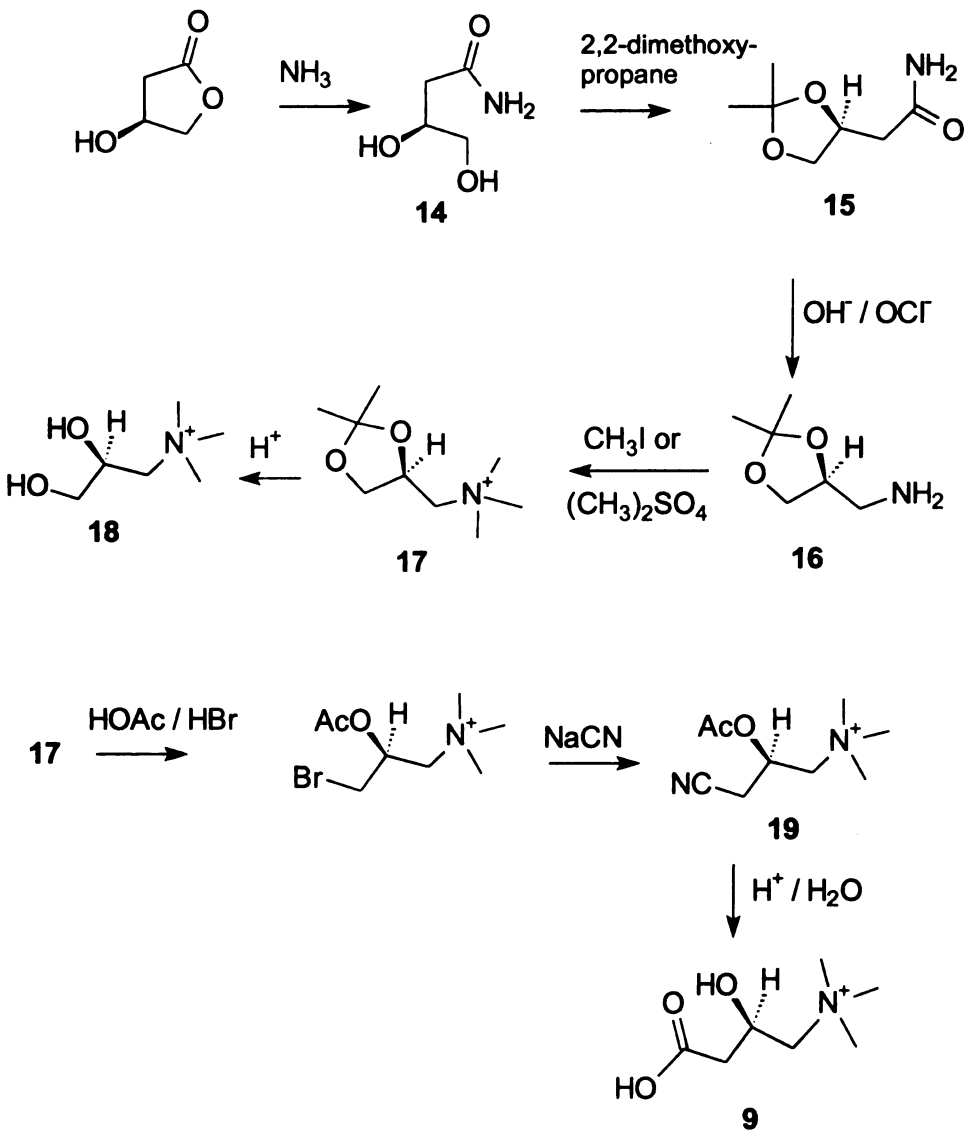
Scheme 2.3. The synthesis of 3-carbon chiral molecules from S-3-hydroxy-γ-butyrolactone

sufficiently to limit access to the conformation required for elimination. The reaction sequence is shown in Scheme 2.3.

The lactone [17] was first converted to a protected amide **15**. The first product of the Hoffmann reaction on the protected amide was the protected aminodiol (**16**). This was formed with virtually quantitative conversion as judged by NMR spectroscopy analysis of the crude reaction mixture although some product was lost on extraction and concentration. The isopropylidene acetal **16** is a very useful form of **3** since it allows modification of the amino group without interference from the hydroxyl functions. It also has a much lower boiling point than **3** and this makes purification by distillation possible even with very modest vacuums. The acetal group was readily removed by treatment of **16** with slightly more than an equivalent of acid to give **3** as a salt. The protected aminodiol (**16**) could be easily converted to the bromodiol **2** by treating it with nitrous acid in the presence of bromide ion. The corresponding chloro compound (**17**) was prepared in good yield by using hydrochloric acid and sodium chloride instead.

2.2.2. Synthesis of L-carnitine and R-GABOB intermediates

There are several routes for the conversion of (S)-2,3-dihydroxypropyl trimethylammonium compounds (**18**) in good yields to L-carnitine (Scheme 2.4) [43, 44]. There has also been significant patent activity around the preparation of these salts [45-47]. The challenge then is to devise a means of transforming (S)-3-hydroxy-γ-butyrolactone to (S)-2,3-dihydroxypropyl



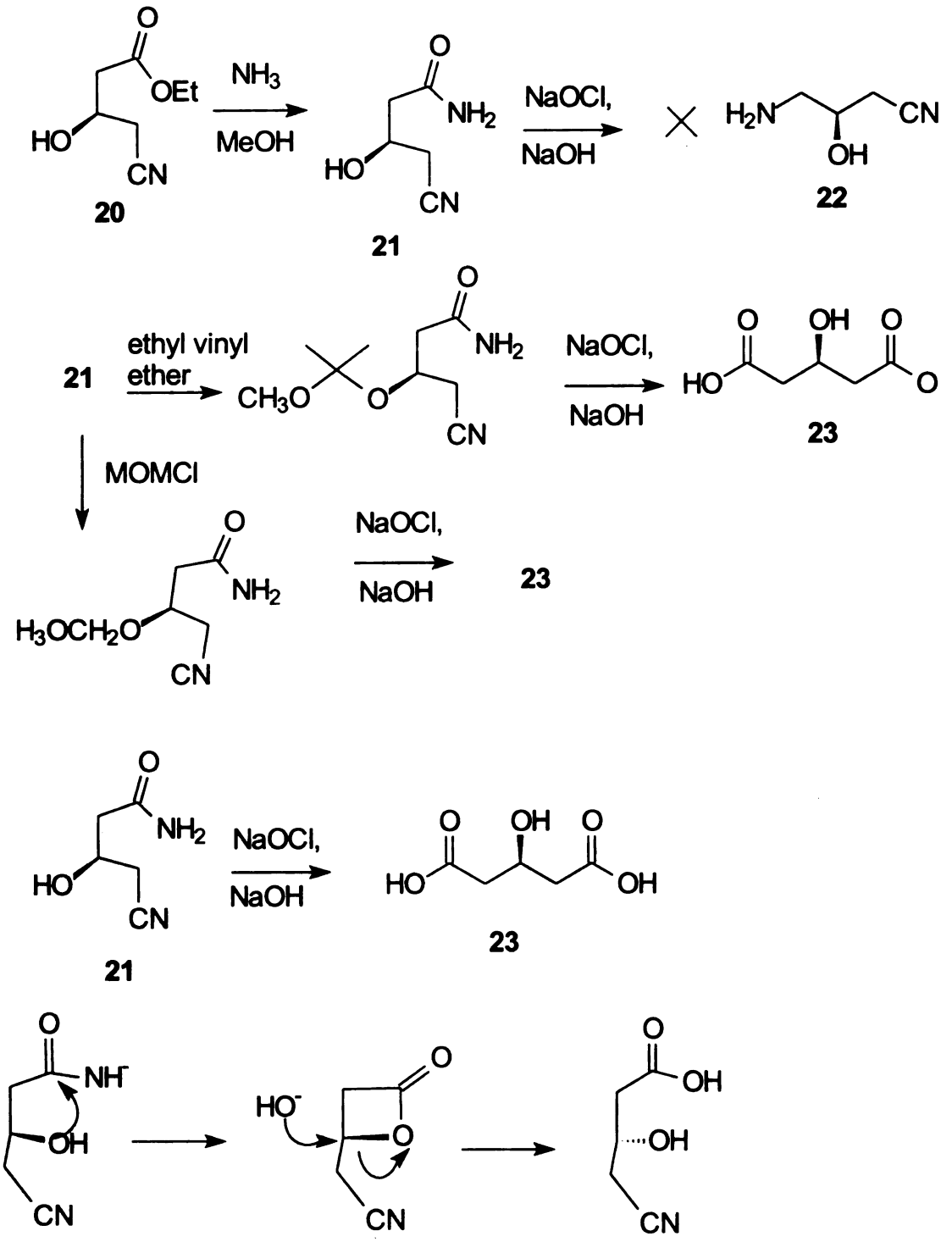
Scheme 2.4. The direct conversion of S-3-hydroxy-γ-butyrolactone to R-carnitine.

trimethylammonium compounds. One way of performing this transformation would be to convert the lactone to the 3,4-dihydroxybutyramide and carry out a Hoffmann reaction to yield the amino-propanediol followed by quaternization of the nitrogen by treatment with a methylating agent. This simple scenario is complicated by two factors. The first is the less than reliable nature of the Hoffmann reaction and the second is the potential for interference by the hydroxyl group in the 4-position. The Hoffmann reaction usually fails on compounds containing a γ-hydroxyl group because of participation to lead to the formation of the starting lactone instead of the migration of the 2-carbon onto the electron-deficient nitrogen. One way around the latter problem would be to protect the diol function in the amide as an isopropylidene acetal. The successful transformation of the resulting 2,2-dimethyl-1,3-dioxolane acetamide followed by methylation of the amino group would result in the formation of (S)-(2,2-dimethyl-1,3-diolan-4-ylmethyl) trimethylammonium salts (**17**). The acetal group can be readily and quantitatively removed by mild acid treatment. This would constitute a formal synthesis of L-carnitine from (S)-3-Hydroxy-γ-butyrolactone.

The trimethylamino diol **18** has been employed in two routes to L-carnitine. The use of this intermediate is not of industrial significance, however, because of the substantial cost of the key optically pure 3-carbon starting materials, epichlorohydrin and chloro-dihydroxy propane. In the first synthesis, (S)-epichlorohydrin is treated with trimethylamine to form (S)-3-chloro-2-hydroxypropyl trimethylammonium sulfate. This is then converted to a nitrile by displacement of the chloro group with cyanide. Hydrolysis yields L-carnitine[45, 46]. In the other synthesis, (R)-1-chloro-2,3-dihydroxypropane is converted to **18** by treatment with

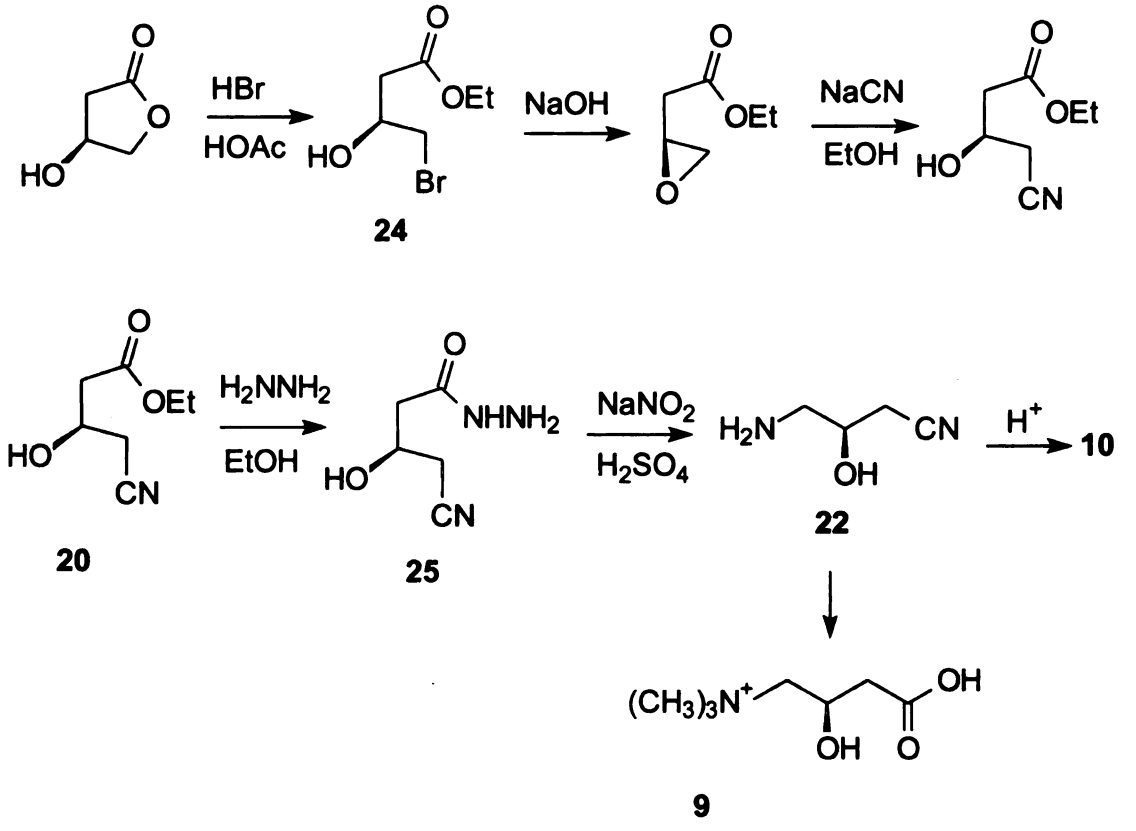
trimethylamine. The diol **18** is then converted to (S)-3-chloro-2-hydroxypropyl trimethylammonium chloride by treatment with thionyl chloride. This is then hydrolysed to yield L-carnitine[47]. This reaction sequence described here has several advantages over the other two. Firstly, it avoids the use of optically pure epichlorohydrin which is very costly. Secondly, gaseous trimethylamine is not used. This synthesis departs from (S)-3-hydroxy-γ-butyrolactone which is available easily, in high yield with high optical purity from starch, lactose and a variety of readily available and cheap carbohydrate raw materials[17]. The (S)-2,3-dihydroxypropyl trimethylammonium salt is obtained cleanly and efficiently from the amide **15** which is, in turn, obtained quantitatively from (S)-3-hydroxy-γ-butyrolactone. This route should therefore constitute an economically viable path to the commercial production of L-carnitine.

In the alternative route to L-carnitine, the lactone is transformed to an (R) 4-cyano-3-hydroxybutyric acid ester which is then transformed to the corresponding amide by treatment with ammonia in methanol. The Hofman reactions using simple reagent such as sodium hypochlorite or sodium hypobromite were carried out on this amide, however the product was not the expected 3-cyano-2-hydroxy amine **22**, instead it gave the 3-hydroxy-pentanedioic acid (**23**) (scheme 2.5). The amide has been synthesized from the corresponding dinitrile[48] and its conversion to to be converted to R-carnitine by other Hofman rearrangement reagents such as I, I-bis-trifluoroacetyloxy-iodobenzene [49] has been demonstrated [50]. The reagents for this transformation are expensive and this method is therefore not very practical. It is our interest to search for some very efficient and economic



Scheme 2.5. The exploration of an alternative route to S-carnitine via Hoffman reaction.

synthetic routes. A simple process using a simple reagent such as bleach is desirable and will have much economic value. The free hydroxy group was therefore protected and a variety of functionalities were tried including acetyl group, methoxymethyl ether, and iopropyl ether. All of these trails gave the diacid as the product. The protecting groups were not able to survive the strong oxidizing conditions. Still bearing our objective in mind, another one carbon degradation reaction, -the Curtius reactions, gave the desired product in a short simple sequence (Scheme 2.6). The conversion was carried out by treating the cyano

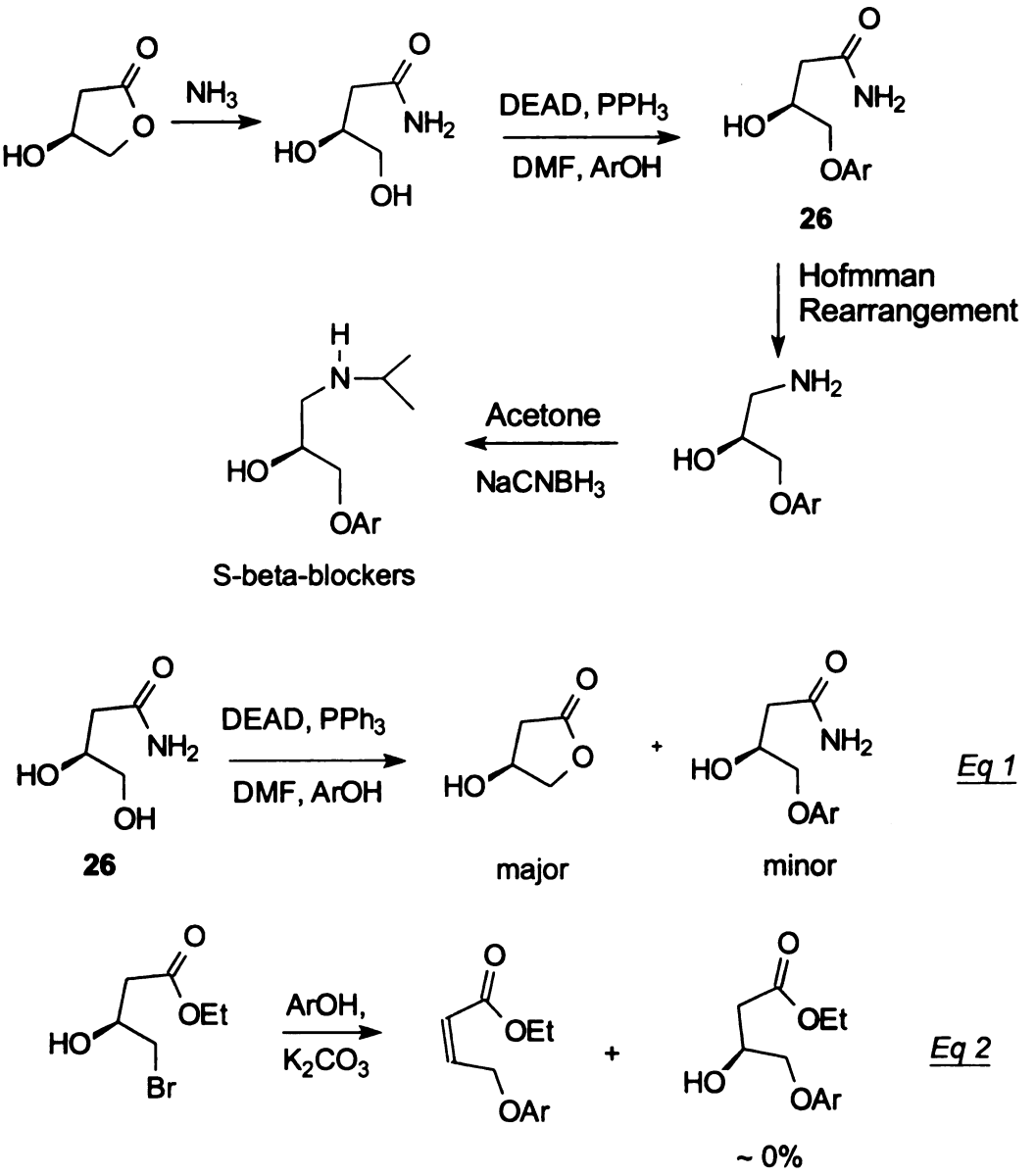


Scheme 2.6. The synthesis of *R*-carnitine and GABOB via Curtius reaction.

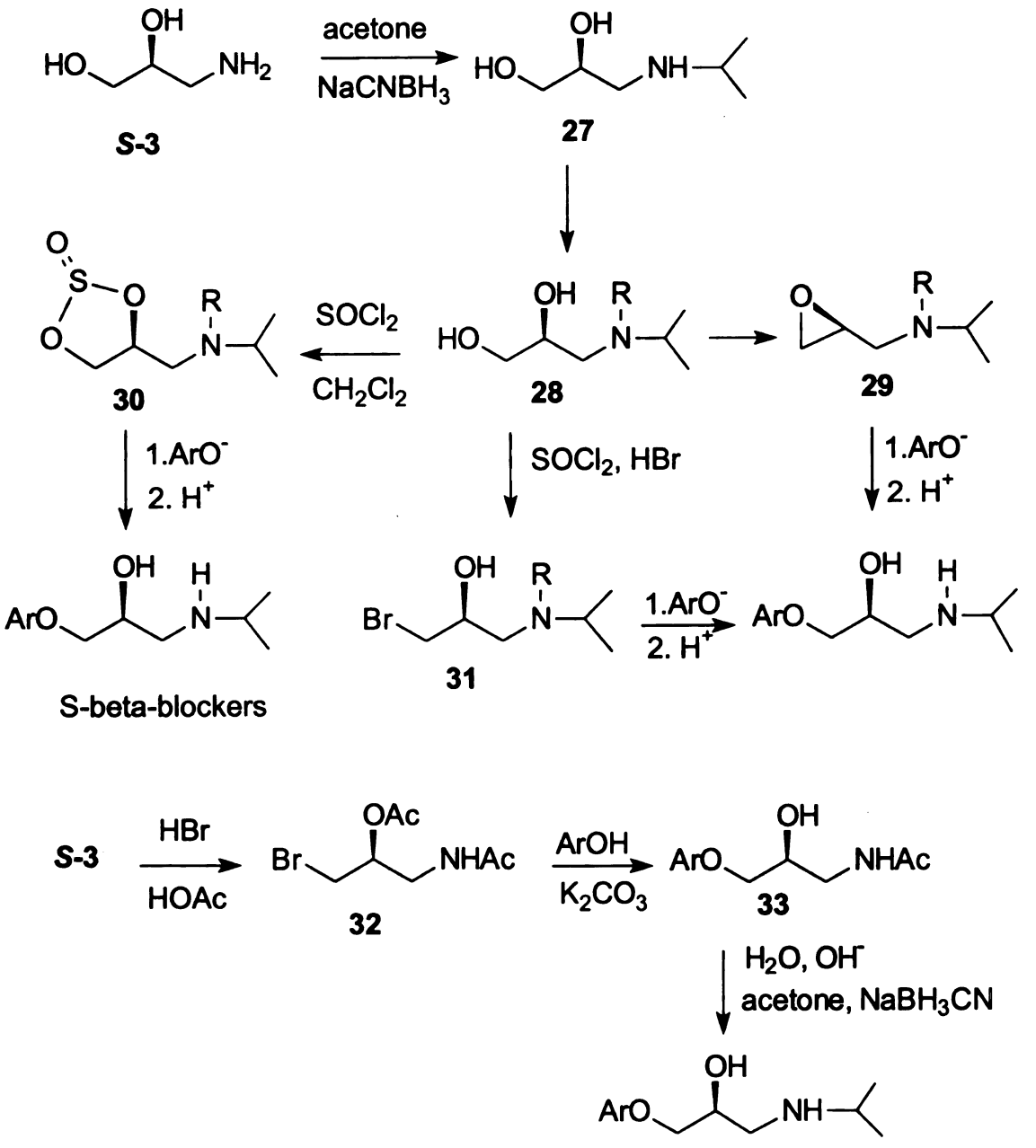
ester with hydrazine and then the resulting hydrazide (**25**) was treated with sodium nitrite and sulfuric acid at room temperature for 24 hours or at 60°C for 10 hours. The reaction was followed by ^{1}H NMR spectroscopy. The conversion from the hydrazide to the amine is nearly quantitative (> 95%). The resulting cyano amine can be converted to GABOB by refluxing it with an acid and also to carnitine by methylation followed by hydrolysis of the cyano group[51,52]. These conversions are straightforward and well documented in the literature. The process just described there for provides another general route to L-Carnitine or (R)-GABOB.

2.2.3. Exploration of new practical routes to chiral S-beta-blockers

In the effort of trying to prepare optically pure S-beta-blockers, several routes for converting the (S)-3-hydroxy-γ-butyrolactone to these products have been explored, one route is shown in Scheme 2.7. The lactone was treated with ammonia, to give the amide and then a direct coupling reaction between the diol and 4-methoxy phenol was carried out under the Mitsunobu conditions[53]. Ideally this should give the product **26**, which can be converted to the corresponding amine by Hoffman degradation. The amine can be transformed into s-beta blockers by reductive amination with acetone. The problem with this reaction is that the amide **14** is not soluble in most organic solvents. Solubility in an organic solvent is a requirement for the Mitunobu reaction[53]. The amide is only soluble in DMF. Another complication arises from the presence of the primary amide function. Under the conditions of the reactions (triphenyl phoshphine and DEAD) the reaction product is mostly the starting lactone instead of the coupling product (Eq1, Scheme 2.7). The mechanism of this reaction



Scheme 2.7. Synthetic explorations to *S*-β-blockers from S-3-hydroxy-γ-butyrolactone.



Scheme 2.8. Synthetic routes to *S*-beta-blockers from C-3 synthons.

to lactone instead of coupling product by the phenol with the primary hydroxy group is probably due the activation of the primary amide by the TTP and DEAD. The primary hydroxy group then attack the activated amide function to give the ester. In another experiment, the coupling of phenoxide with the bromoester **24** made by opening the lactone with HBr and acetic acid lead to elimination products instead of displacement of the bromo group with the phenoxide group (Eq2, Scheme2.7). It seems therefore that to install the aromatic system prior to the Hoffman rearrangement might not be viable. A reasonable route is proposed in *scheme 2.8.* The amino diol can be converted to the S-beta-blockers by several steps. Reductive amination on the **S-3** will give intermediate **27** on which the amine needs to be protected by a t-Boc or similar protecting group such as Cbz. Alternatively, the secondary OH together with the amine can be protected by an oxazolidone function[54] to give compound **28**, which can then be converted to the bromo compound **31**, the epoxide **29**, or the cyclic sulfite[40] **30**. These intermediates **29, 30, 31** can be converted to beta-blockers with the correct stereochemistry. Or the aminodiol can be converted to the direct intermediate bromo compound **32** which can couple with various aromatic phenol to give the precursor of S-beta blockers **33** which upon hydrolysis of the amide and reductive amination will lead to the final products S-beta-blockers.

2.3. Conclusions

A general route for converting the (S)-3-hydroxy-γ-butyrolactone to other useful chiral three carbon building blocks including (S)-3-amino-1,2-dihydroxypropane as its isopropylidene acetal, (R)-1-bromo-2,3-dihydroxypropane and the corresponding chloro compound has been

developed. The latter two compounds are glycidol equivalents. The chemical yields are high and the enantiomeric purities are extremely high. The propensity for the γ-hydroxyl group to participate has been removed by tying it up in a cyclic acetal. No β-elimination was observed. The same reaction schemes can be used to obtain the other enantiomers of the above 3-carbon building blocks by using (R)-3-hydroxy-γ-butyrolactone as starting material. Two general and efficient routes toward the synthesis of biological important compounds such as R-carnitine, GABOB are also developed, the first route is related with the synthesis of three carbon synthons at which the trimethyl ammonia substituted intermediate was the precursor of R-carnitine, the latter by using a Curtius reaction on the extended 5 carbon cyanoester derived from the lactone to give the cyano hydroxy amine which is the direct precursor of carnitine and GABOB. The chiral C-3 synthons are suitable precursors to (S)-beta-blockers. The study provides an entry route from carbohydrate raw materials to chiral C3-synthons, in which the reactions are simple, conditions are mild and no heavy metal waste or other undesirable waste is produced. The process is simple straightforward and efficient. The high optical purity of the lactone was retained through to the final products. This chemistry may prove to be commercially viable paths to the synthesis of C-3 chiral build blocks, R-carnitine, and GABOB. It offers an opportunity to replace the current existing methods or to facilitate the replacement of racemic beta-blockers with chiral beta-blockers.

2.4. Experimental Section

(S)-4-(2,2-dimethyl)-1,3-dioxolane acetamide (14). (S)-3-hydroxy-γ-butyrolactone (204g,

2 mol) was converted to the amide by treatment at room temperature for 14 hours with 440 ml of 30% ammonium hydroxide (3.4 mol). The solution was then concentrated to a syrup at ~ 50° C under reduced pressure until no more water could be removed. Acetone (2 liters) and 2,2-dimethoxypropane (420 g, 2 mol) was added. Sulfuric acid (6 mL) was then added and the mixture protected from moisture with a calcium chloride drying tube, heated at 60 °C for 30 mins and stirred at room temperature for 12 hours. Sodium carbonate (50g) was added and the mixture stirred for 1 hour. Methanol (400 ml) was then added and the mixture filtered and concentrated to dryness. The amide (**15**) crystallized on concentrating. The crystals were washed with hexane and then acetone and was used without further purification. Conversion was essentially quantitative. A small amount when recrystallized from acetone gave white crystals mp, 98-100 °C. $[\alpha]^{23}_{589}$ = -15.4 ($CHCl_3$, c =1), ^{1}H- NMR ($CDCl_3$, 300MHz) δ ppm 6.10 (s, 1H), 5.65 (s, 1H), 4.43 (m, 1H), 4.14 (dd, 1H, J=8.1 and 6.3 Hz) 3.63 (dd, 1H, J=8.1 and 6.8 Hz) 2.55 (dd, 1H, J=15.3 and 7.5Hz), 2.46 (dd, 1H, J=15.3 and 4.8Hz), 1.42 (s, 3H), 1.35 (s,3H) ^{13}C-NMR ($CDCl_3$, 75MHz) δppm 172.86, 109.50, 72.21, 69.05, 40.07, 26.90, 25.50.

(S)-C-(2,2-dimethyl)-1,3-dioxolan-4-yl-methylamine (16) The amide (**15**) (79.5g, 0.5mol) was treated with 10-12% sodium hypochlorite solution (500 ml) and the mixture stirred until all of the solid had dissolved (~ 5 mins). Sodium hydroxide (80 grams dissolved in 500 ml water) was added to the mixture and the solution was warmed to 50-60 °C and the kept there for 24 hours by which time conversion to amine **16** completed. ^{1}H-NMR spectroscopy indicated 100% conversion of **15** to **16**. The amine **16** was isolated as a light yellow liquid

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by extraction of the mixture with ether which upon standing give colorless crystals mp 54-56 °C. The yield was 56.5g (86.3%). This was reported[55] to be liquid, bp 62-65 °C, 15 torr, probably because it was not isolated in as pure a state as we have here. $[\alpha]^{23}_{589}$= +0.9 ($CHCl_3$, c=1), ^{1}H-NMR ($CDCl_3$, 300 MHz) δppm 4.13 (m, 1 H), 4.00 (dd, 1 H, J=8.1 and 6.6Hz), 3.67 (dd, 1H, J=8.1 and 6.3Hz), 2.85 (dd, 1H, J=13.2 and 4.2Hz), 2.78 (dd, 1H, J=13.2 and 6.0Hz), 1.40 (s, 3H), 1.34(s, 3H), 1.31(s, 2H). ^{13}C-NMR ($CDCl_3$, 75MHz) δ ppm 109.10, 77.36, 66.90, 44.71, 26.81, 25.31.

(S)-3-Amino-1,2-propanediol (3) 1g of compound **16** was treated with 2 ml concentrated hydrochloric acid and 2 ml of water, heated on steam bath for 30 minutes, the solvent was removed by rotatory evaporation, a light yellow syrup left in the round bottom flask, which upon cooling to room temperature give white crystals of the hydrochloride salt. The conversion was quantitative. $[\alpha]^{23}_{589}$=-23.3 (H_2O, c=1). ^{1}H-NMR (D_2O, 300 MHz) δppm 3.76 (m, 1 H), 3.47 (dd, 1 H, J=12Hz and 4.8Hz), 3.41 (dd, 1H, J=12 and 5.7Hz), 3.00 (dd, 1H, J=13.2 and 3.0Hz), 2.78 (dd, 1H, J=13.2 and 9.3Hz). ^{13}C-NMR (D_2O, 75MHz) δppm 63.32, 58.64, 37.15.

(R)-3-Bromo-1,2-propanediol (2) The amine **16** (10g) was dissolved in 400 ml water. HBr solution (50 ml, 47% aqueous solution) and 52 g of sodium bromide were added to the solution which was then cooled to 10 °C. Sodium nitrite (70g) was added to the mixture and it was stirred at room temperature for 20 hours after which time NMR spectroscopy indicated complete conversion of the aminodiol to the bromo diol. It was neutralized by sodium

bicarbonate, then most water was removed by rotary evaporation and the residue was taken up in chloroform. The chloroform phase was dried over sodium sulfate and removal of the solvent gave the bromo diol **2** as a yellow liquid. The yield was 10.3g (87.1%). $[\alpha]^{23}_{589}$= 4.00 ($CHCl_3$, c=1). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 3.93 (m, 1H), 3.77 (dd, 1H, J=11.4 and 3.6Hz), 3.66 (dd, 1H, J=11.4 and 6.0 Hz), 3.85-3.46 (m, 2H). ^{13}C-NMR ($CDCl_3$,75MHz) δppm 71.44, 64.27, 34.62.

(R)-3-Chloro-1,2-propanediol (17) The procedure is similar as preparation of bromodiol. The amine **16** 2.62g (0.02mol) was dissolved in 10 ml water, sodium chloride 8.78g (0.15mol), concentrated hydrochloric acid 20 ml (0.2 mol) dilute with 10 ml water were added to the mixtures. Sodium nitrite 10.4g (0.15mol) was added to the reaction mixtures in a period of 10 minutes. Then it was stirred for 24 hours, the reaction checking by NMR spectroscopy indicated complete conversion to the chlorodiol. The mixture was concentrated to dryness, the product was extracted by chloroform 3 or 4 times. The extracts were combined and dried with sodium sulfate, removal of solvent give chlorodiol **16** as a light yellow liquid 1.81g (81.9%). $[\alpha]^{23}_{589}$= -7.16 (H_2O, c=5) ^{1}H NMR (D_2O, 300 MHz) δppm 3.78 (m, 1H), 3.56-3.38(m, 4H). ^{13}C-NMR (D_2O, 75MHz) δppm 66.44, 57.77, 41.08. All products were > 99.5 % optically pure by chiral G.C.

(R)-4-Cyano-3-hydroxy butyramide (21). The cyano ester 15.7 grams (0.10 mol), stirred with 30% ammonia hydroxide 21g (0.18 mol) and 20 ml methanol for 10 hours, after which the reaction is essentially completed, the possible byproduct of the reaction is the acid

instead of the amide at the 1-position, this and other ions were removed by passing through a mix-bed resin by methanol and water as the eluting solvent after removal of the solvent give the amide as a yellow crystalline solid. Yield 10.6g (82.8%) ^{1}H NMR (D_2O, 300 MHz) δppm, 4.25ppm(m, 1 H); 2.71 (dd, J=4.8, 1H 20.4Hz), 2.60(dd, J=6.6, 1H17.1Hz), 2.36 (d, 2H, J=6.6Hz). ^{13}C-NMR (D_2O, 75MHz) δppm 170.72, 114.12, 59.34, 36.79, 20.42.

(R)-4-cyano-3-hydroxy butyrohydrazide (25) 5.2 grams (0.033mol) of the cyano ester **20** was dissolved in absolute ethanol (10ml) and the mixture was added to 1.6g (0.05mol) of anhydrous hydrazine, in absolute ethanol (10ml). The mixture was left stirring for 2 hours over which time a white solid precipitated. The white solid was filtered by vacuum filtration and washed twice with 5ml ethanol. yield: 4.25g (90%), mp. 119.0-120.0°C, ^{1}H NMR (D_2O, 300 MHz) δppm 4.24(m, 1H), 2.70(dd, 1H, J=4.5, 17.1Hz), 2.58(dd,1H, J=6.3, 17.1Hz)2.36 (m, 2H) ^{13}C-NMR (D_2O, 75MHz) δppm 166.72, 114.17, 59.36, 35.67, 20.44.

(R)-4- amino-3-hydroxybutyrocyanide (22) . 1.43g (0.01mol) the hydrazide **25** was dissolved in 10ml water. The mixture was stirring and then 1.2g concentrated sulfuric acid diluted in 10 ml water, was added to the hydrazide solution. The mixture was cooled in an ice bath and then 1.36g (0.02mol) of $NaNO_2$ was added. It was left stirring at room temperature for 24 hours or at 60 °C for 10 hours, after which time the reaction was essentially completed as determined by ^{1}H NMR spectroscopy. The reaction mixture was then concentrated to dryness and then taken up in tetrahydrofuran. It was stirred for 1 hour, filtered to remove salts and other insoluble material and the THF layer was dried with

sodium sulfate. The solvent was then removed by rotatory evaporation to give the product as a brownish semi-solid 0.92g (92%). ^{1}H NMR (D_2O, 300 MHz) 4.90 (m, 1H), 3.73 (dd, J=9.0, 9.9Hz), 3.30(dd, 1H, J=5.7, 9.9Hz), 2.94(dd, 1H, J=4.2,17.4Hz), 2.84 (dd, 1H, J=5.7, 17.4Hz) δppm ^{13}C-NMR (D_2O, 75MHz) δppm 112.78, 67.56, 39.98,18.37.

Trimethyl ammoniium salt (17). The preparation was by the same method used to prepare the protected amine **16** from the lactone, except that at the end of the Hoffman reaction, the amine was converted to the trimethyl ammonium propane diol **17**. Amide (**3**) (0.08g,0.005mol) was treated with 10-12% sodium hypochlorite solution (5 ml) and the mixture stirred until all of the solid had dissolved (~ 5 mins). Sodium hydroxide (0.8 grams dissolved in 5ml water) was added to the mixture and the solution was warmed to 50-60° C and kept there for 24 hours by which time conversion to amine **16** was complete. ^{1}H-NMR spectroscopy indicated 100% conversion of **15** to **16**. The amine **16** was not isolated but was directly converted to the trimethylamino derivative **17** by adding dimethyl sulfate (6 equivalents), sodium hydroxide (0.85g, 0.021 moles) and 2 ml of methanol and stirring for a further (12 hours). Proton NMR spectroscopy (figure 1) clearly indicated complete conversion to the required (2,2-dimethyl-1,3-diolan-4-ylmethyl) trimethylammonium compound. The methine proton signal appeared as a triplet (J= ~ 8Hz) at 4.12 ppm, the methylene protons adjacent to the trimethylamino group was a doublet (J= ~ 8Hz) at 3.4 ppm and the signals for the methylene group on the dioxolane ring were partially obscured by the one for the methylsulfate anion at 3.59 ppm. ^{1}H NMR (D_2O, 300 MHz) 4.14(dd, 1H, J=6.9, 8.7Hz) 3.64-3.58(m, 5H), 3.44-3.38(m, 2H), 3.08(s, 9H), 1.34(s, 3H), 1.27(s, 3H).

S-Bromo diacetyl amino alcohol (32). 6.3g (0.068mol) aminodiol in 250ml round bottom flask was cooled in ice bath, 3 equivalent (48g) of 30% HBr-Acetic acid was added to the round bottom flask at 0°C in a 15 minutes period, it was then left for stirring at room temperature for 30 minutes. 200ml water was then added to the reaction mixture, sodium carbonate was added to neutralize the mixture to pH=7, then the product was extracted with ether 3 times. The ether layer was combined and dried over sodium sulphate overnight, removal of solvent give deep yellow product as a semisolid. Yield was 15.4 g, 95%. ^{1}H NMR ($CDCl_3$, 300 MHz) δppm (s, 1H), 4.99(m, 1H), 3.30-3.60 (m, 4H), 2.10 (s, 3H), 2.06 (s, 3H). ^{13}C-NMR ($CDCl_3$,75MHz) δppm, 177.8, 177.1, 158.1, 81.8, 54.3, 31.1, 28.6, 27.3.

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Chapter 3

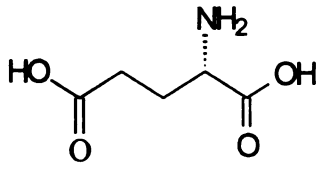
The Exploration of New Synthetic Routes to Important Chiral Intermediates from Amino Acids

Abstract

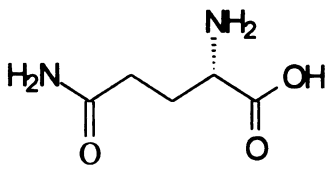
Amino acids are very good candidates in the chiral pool for constructing industrially chiral molecules because they are readily available and have a relatively simple structure with only one or two stereocenters. The two major functional groups (amino and carboxylic) are also very amenable to transformations to other functionalities. Among the family of amino acids, glutamic acid and phenylalanine are two compounds of special interests due largely to their availability and special aspects of their structure. Glutamic acid is the least expensive amino acid available, from this molecule, removal of one carbon is expected to give a series of 4 carbon chiral building blocks which are of great interest for pharmaceutical industry. Phenylalanine is of special interest because it contains an unsubstituted phenyl group. It is also available in very large quantities because it is a component of the popular sweetener aspartame. Many important chiral building blocks can be derived from this molecule by simple transformations. Aminoindanols and aminotetralins are two such types of chiral molecules. In this chapter, the exploration of these two amino acids as the chiral pool starting materials will be described.

3.1. Introduction:

Amino acids are excellent candidates from the chiral pool[1]. They can be converted to a variety of other chiral building blocks because they are readily available and have a relatively simple structure with only one or two stereocenters. The amino and carboxylic acid functions are also easily transformed. Among the family of amino acids, glutamic acid and phenylalanine are two compounds of special interests because of their structure and ready availability.



1. L-Glutamic Acid



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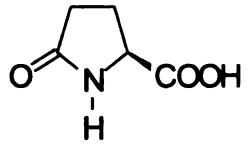

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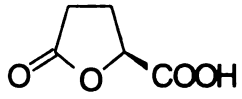

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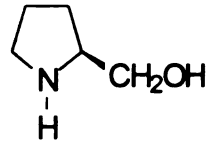

5

Glutamic acid **1** is a proteogenic amino acid with two carboxyl groups of which only one forms a peptide bond and the other gives an acid character to the peptide. Glutamic acid is involved in the transport of potassium ions in the brain and also detoxifies ammonia in the brain by forming glutamine **2,** which can cross the blood-brain barrier. Both the D and L forms of **1** are commercially available. L-glutamic acid is the least expensive of all the amino

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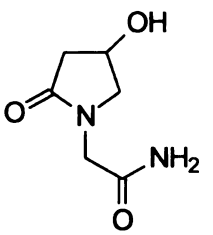
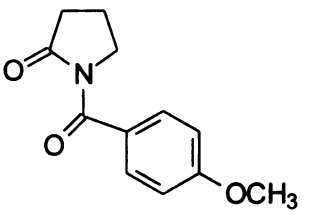
acids and it is also the most useful amino acid as chiral synthon. Many synthetic routes using glutamic acid as starting material have been developed, it is a versatile chiral molecule for many other important chiral compounds. The cyclized form the L-pyroglutamic acid **3** is a versatile chiral synthon too. It can be obtained by refluxing the aqueous solution of **1** with retention of stereochemistry [2]. L-glutamic acid reacts with nitrous acid in aqueous solution to give the α-butyrolactone-α-carboxylic acid **4** with complete retention of configuration. D-prolinol **5** can be obtained by reduction of **4** by $NaBH_4$ [3,4] or borane-dimethylsulfide[4, 5]. The stereocenter in the molecule can be inverted without disturbing the lactone ring and without racemization [6]. This family of compounds are useful building blocks for many biological interesting compounds including pheromones, steroids, terpenes, and drugs [7-9].

One desirable transformation from these 5 carbon chiral molecules is to remove one carbon from the molecule to give a family of 4-carbon multifunctional chiral building units. Some of these such as γ-amino butyric acid derivatives are of biologically interest themselves and they are also intermediates for many other interesting compounds some of which lack chiral centers. For instance, the compounds **6-8** are useful drugs for treatment of human cognitive disorders [10]. The 2-pyrrolidinones such as oxiracetam **6** and aniracetam**7** are non otropic agents that have low toxicity. They have been shown to enhance resistance to brain insults. The dimeric amino acid derivative **8** SUAMM 1221 possesses antiamnesic properties and ability to strongly inhibit mammalian prolyl endopeptidase [11]. Compounds with the common structure **9** are nonsteroidal anti-inflammatory drugs (NSAIDs). They are used in the treatment of a number of arthritic conditions [12]. The N-hydroxy form of 3-amino-2-

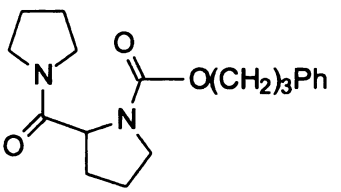
pyrrolidione is a glycine antagonist and occurs in some potent sedatives[13,14]. All these compounds possess the core pyrrolidinone structure.



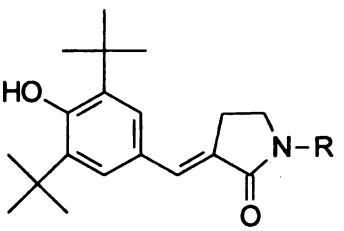
6. Oxiracetam



7. Aniracetam

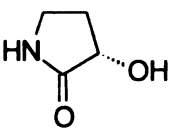


8. SUAMM 1221



R=H, OCH_3, CH_3, etc.

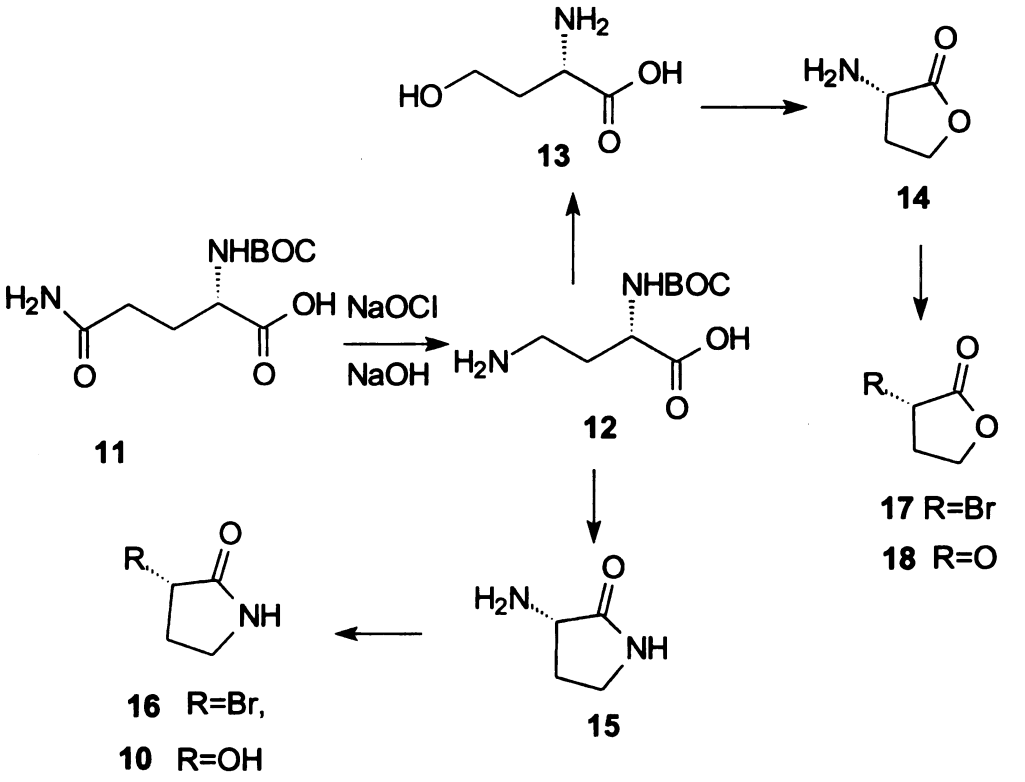
9. NSAIDs



10. 3S-3-hydroxy-2-pyrrolidinone

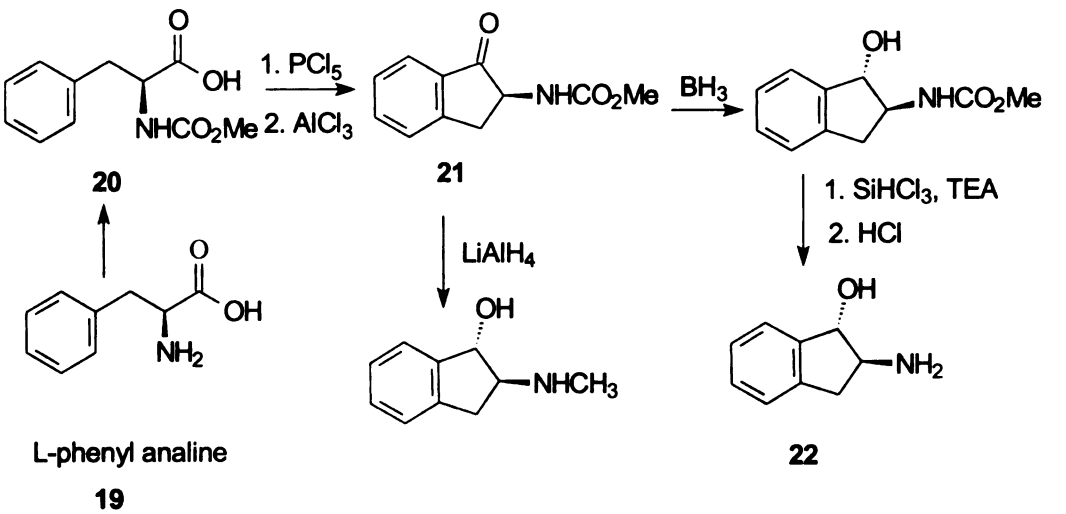
Given the importance of these 5 membered ring compounds, it is of great interest to synthesize the core structure **10** because all the others can be derived from it via standard transformations. The S-3-hydroxy-2-pyrrolidinone **10** has been synthesized by many different routes. It has been synthesized from gamma-butyrolactone [15] , 2-hydroxy-4-phthalimidobutyric acid [16], 2-bromo-gamma-butyrolactone[17], trimethylsilyl-2-pyrrolidinone[18], S-malic acid [10], and by enzymatic methods [19]. A straightforward way of doing so is by the Hoffman rearrangement to convert glutamine to (S)-2,4-diamino-

butyric acid. The 2-amino group can be easily converted to other functional groups (Scheme 3.1). An efficient and straightforward route from glutamine to these 4 carbon building blocks starting from the commercially available compound t-Boc protected glutamine by Hoffman reaction using sodium hypochlorite is discussed.



Scheme 3.1. The conversion to various 4-carbon synthons from t-Boc glutamine.

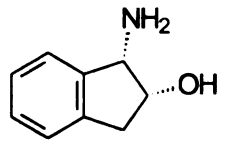
Phenylalanine (**19**) is another useful amino acid that has been explored for chiral synthesis. Many studies have been carried out starting from phenylalanine to give other important chiral molecules such as isoquinoline derivatives [20]. Chiral indanones can be created from phenylalanine by intramolecular Friedel-Crafts acylation (Scheme3.2). The product **21** is produced in 55-75% yield and is greater then 98% enatiomerically pure [21].



Scheme 3.2. The synthesis of chiral indanone from phenylalanine in the literature.

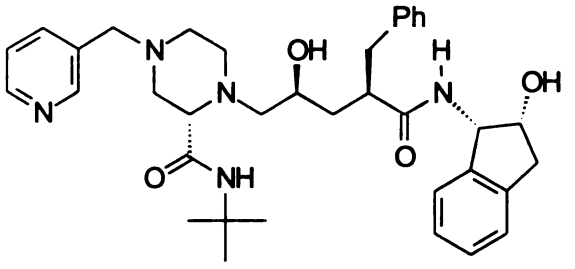
1R, 2S-cis-1-amino-2-indanol **23** and its antipode **24** have gained much attention in recent years [22, 23]. They have been used in the preparation of a series of potent HIV-1 protease inhibitory peptides (**25, 26**) and are also useful in asymmetric synthesis by acting as chiral inducing agents, chiral resolving agents, asymmetric catalysts and as a chiral auxiliary in asymmetric syn-aldol reactions [24]. The aminoindanol derivative indane-bis(oxazoline) can catalyze the Diels-Alder reaction [25]. The Sepracor group has shown that optically pure cis-1-amino-2-indanol **23** is an extremely effective enantioselective catalyst for the borane

reduction of some important α-halo ketones [26]. It was found that besides the amino alcohol functionalities, the constrained indane platform is essential for obtaining stereoselectivity [27].

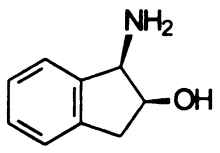


1S,2R -cis-1-amino-2-indanol

23

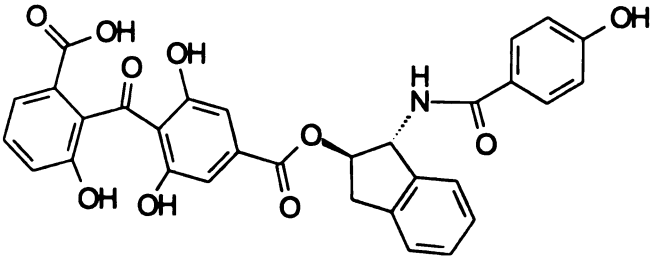


25 Merck's Crixivan-Indinavir



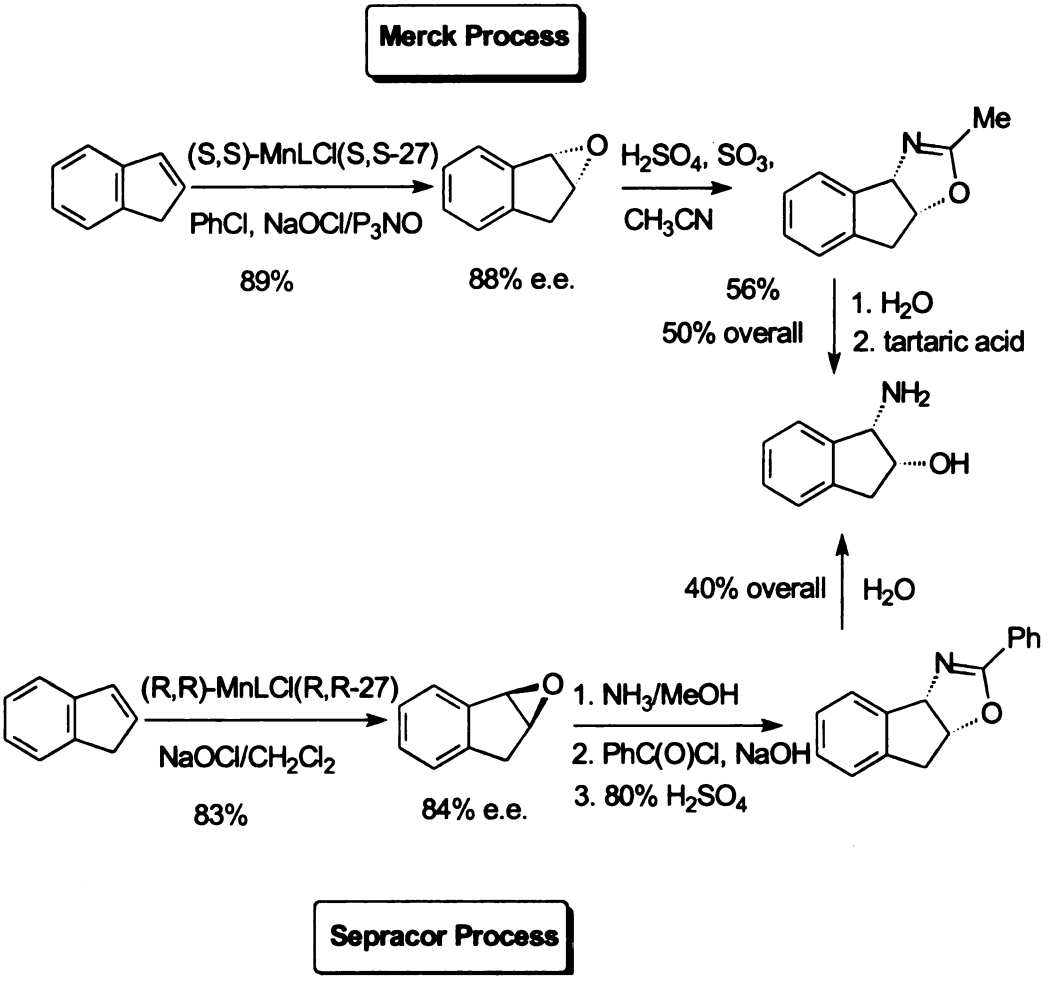
1R, 2S

24

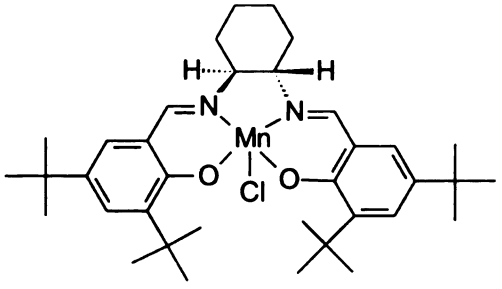


26 Eli Lilly's PKC inhibitor

Because of the importance of *cis*-1-amino-2-indanol in biological systems and in asymmetric synthesis, many methods have been explored for synthesizing it in an enantiopure form including enzymatic resolution[28-30]. Many chiral intermediates such as *cis*-2-bromo-1-indanol, and chiral indan-1,2-diols have been converted to the optically pure cis-aminoindanol via a Ritter type reaction [31] with sulfuric acid in acetonitrile followed by basic hydrolysis. The current most efficient existing routes (Scheme 3.3) were developed

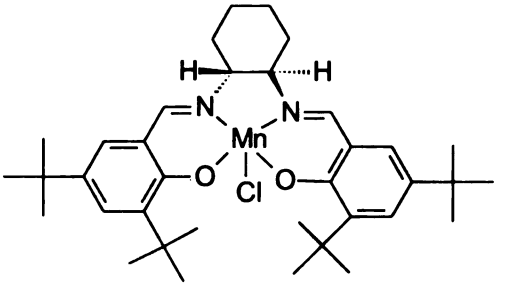


Scheme 3.3. The current most efficient routes to the synthesis of *cis*-1-amino-2-indanol by Merck and Sepracor.



S,S-(salen)Mn(III)Cl

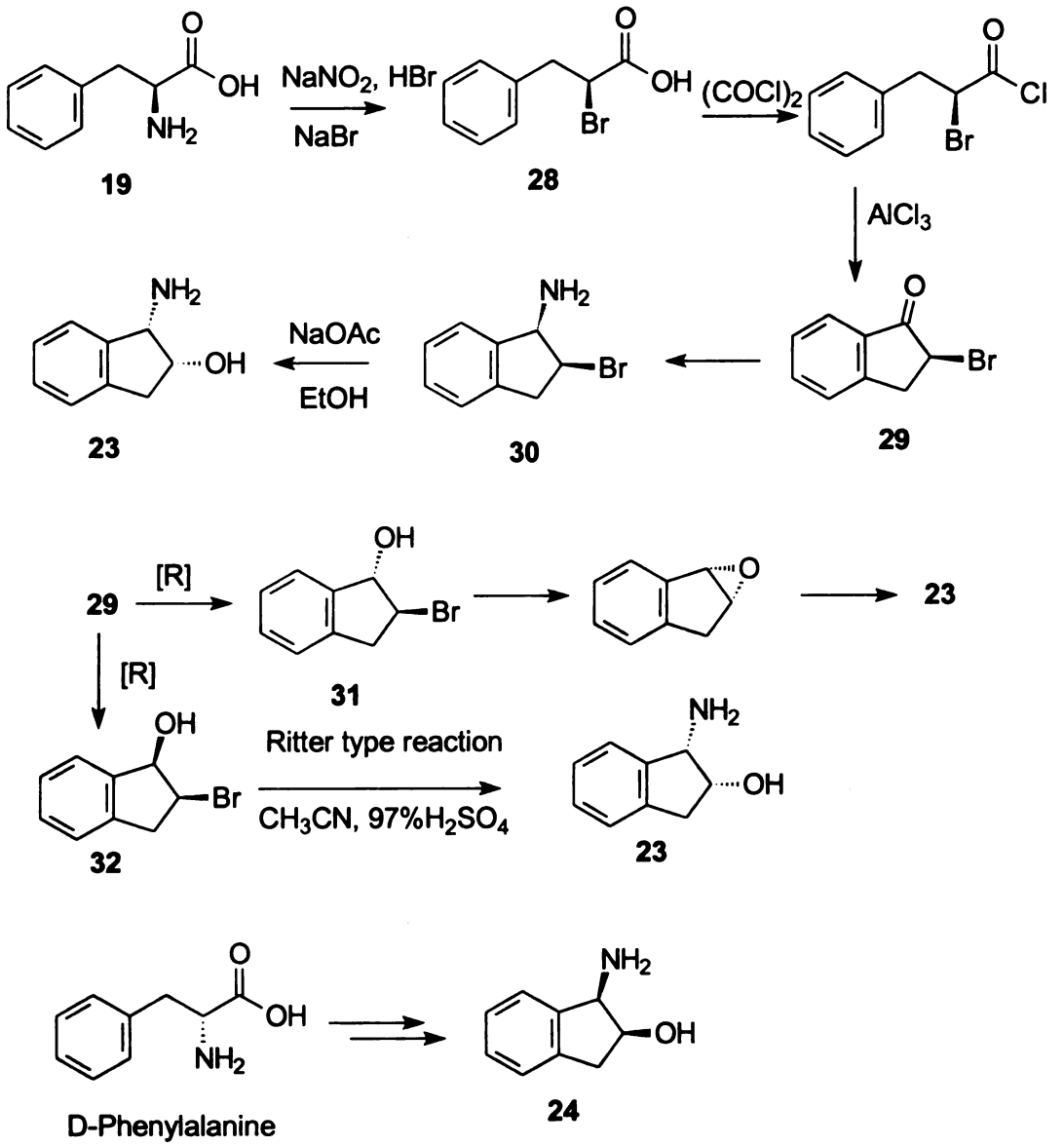
S,S-**27**



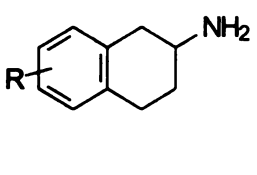
R,R-(salen)Mn(III)Cl

R,R-**27**

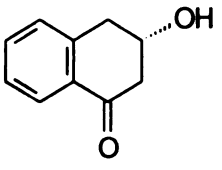
by the Merck and Sepracor's group [32-34]. Both of these groups rely on Jacobsen's catalyst chiral Mn-salen complexes) (**27**) to generate enantiopure epoxide intermediate in the key step [35, 36]. Sepracor prepared the indene oxide in 83% yield and 84% ee. The optically active indene oxide was then treated with ammonia to give *cis* aminoindanol which was transformed to the optically pure benzamide by crystallization. The benzamide was transformed to the cis amino-indanol. The overall yield from indene is 40%. Merck's approach is similar to Sepracor's, except that they used Jacobsen's catalyst in a combination with the Ritter type reaction. Although these two methods are the most efficient asymmetric routes. Thus far they are too inefficient to be used on a commercial scale. The yields and optical purity of the key-asymmetric epoxidation steps are moderate. Right now the requirement for Crixivan by AIDS patients have not been met because of the difficulty in producing the drug which is till manufactured by chiral resolution. There is a dire need for a more economic route that is more environmental friendly and more efficient. Another approach starting from phenylalanine to *cis*-1-amino-2-indanol will be discussed. The reaction routes are outlined in Scheme 3.4.



Scheme 3.4. A new synthetic route from phenylalanine to chiral aminoindanols and other chiral indanol skeletons.



33 Aminotetralins



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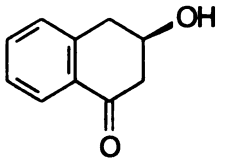
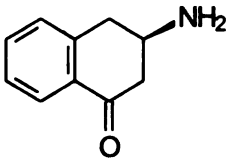


35



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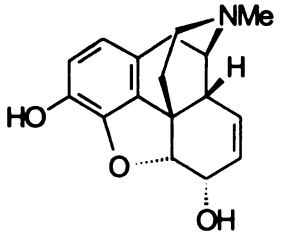
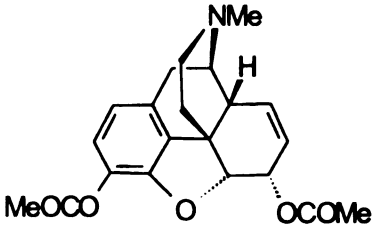
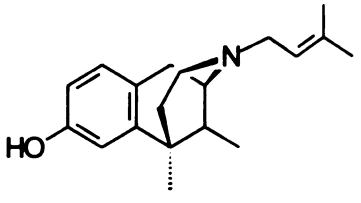


37 Morphine

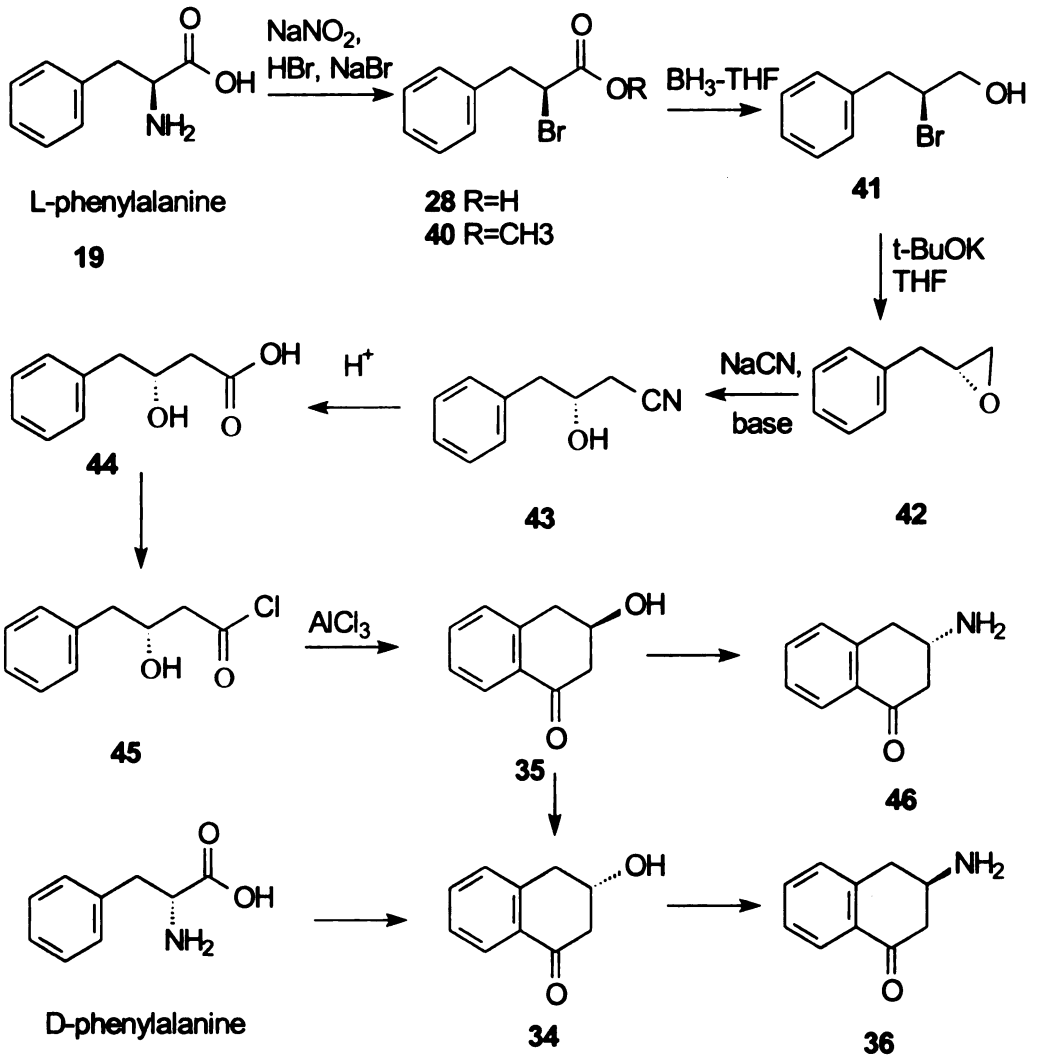


38 Heroin



39 Pentazocine

Aminotetralins (**33**) and tetralinols skeletons are very useful pharmaceutical intermediates. They are used in a variety of important compounds. Aminotetralins are an important class of molecules that are known to have potency in treatment of central nervous system disorders such as Parkinson disease and other brain disorders [37-39]. The aminotetralin skeleton is also found in the very important class of drugs called the morphines. Morphine **37** is the most well studied and most used clinically among all of members of the morphine family. It is used for pain relief acting as powerful analgesic. The problem with morphines or other opioid analgesics is that dependency soon develops. The morphines are very addictive. They are also very toxic. It is of great interest to develop morphine analogs that are devoid of or reduced side effects. Intermediates such as compounds **34-36** are very useful in building the morphine skeleton. The synthesis of these amino or hydroxy



Scheme 3.5. The synthesis of aminotetralins from phenylalanine.

tetralones are very difficult and a general preparation on large scale is highly desirable. In the literature, the syntheses of tetralins usually employ the aryl ketones which are subjected to reductive amination to convert the carbonyl group directly to an amino group[40-42]. In this way, α-aryl ketones are converted to β-amino phenyl compounds[43]. The commercial

routes to these molecules are not well known. In this paper, a new strategy which has the potential to be used in large scale synthesis and with high chiral integrity will be explored. The routes is shown in Scheme 3.5, starting from phenylalanine. In this route the length of the side chain is increase by one carbon, giving the hydroxy acid **44** , which upon Friedel-Crafts cyclization gives the key intermediate **35**. This can be easily converted to the opposite stereochemistry (compound **34**). Starting with D-phenylalanine and using the same scheme also leads to **34**. The hydroxy group can be converted to an amino group by displacement reaction to give compounds **46** and **36**. These are direct intermediates in the preparation of morphine type compounds.

3.2. Results and discussion:

The Hoffman reaction had been carried out starting with S-glutamine, the amino group was protected by p-toluenesulphonyl chloride, and sodium hypobromite was used to yield the N-tosyl-2,4–diaminobutyric acid [44-47]. Deprotection gave the hydrochloride salt. The overall yield for these steps was 63%. The problem with this route is that the tosyl protection is not very easy to remove(treatment with Zn/acetic acid for several days). An alternative route to the four carbon building blocks was therefore explored. The reaction scheme is outlined in *scheme3.1*, the starting material is commercial available t-Boc protected glutamine. Using sodium hypochlorite (10% solution,) the t-Boc protected amino acid **12** was obtained in 75 % conversion based on NMR analysis. The transformation is still being optimized and the yield is expected to be further improved. The resulting compound can be converted to various other building blocks very easily. The t-Boc group has much greater ease of removal

and also are stable to other transformations.

In the synthesis of aminoindanol, the starting material used is L-phenylalanine (scheme 3.4). In this route, the amino group is converted to a bromo group with retention of stereochemistry. This was then converted to the acid chloride which was then cyclized to give the key intermediate compound with the indanol skeleton. In the attempt at obtaining the bromo amine by reductive amination with sodium cyanoborohydride and ammonium acetate, the bromo indanone was converted to cis-bromoindanol exclusively. The stereochemistry of this compound was confirmed from the ^{1}H NMR spectroscopy coupling constant between the two methine protons J=3.6Hz, corresponding to the cis-configuration[48]. Treatment of the bromoindanol with base such as potassium carbonate and sodium carbonate, potassium acetate etc, in ethanol or ethanol water led to predominant elimination of hydrogen bromide. The resulting product was the 1-indanone with a very small amount of trans dihydroxyindanol. An attempt to convert the carbonyl group to an amino group was made by converting the carbonyl group first to an oxime, followed by reduction with sodium borohydride, or sodium cyanoborohydride. The intermediate bromo amine was expected to be obtained. The displacement of the bromo group should give the *cis*-1-amino-2-indanol as the final product. The synthetic sequence is short, and quite efficient. The intermediates are interconvertible and they can be converted to the product by standard transformations[49-56]. The intermediate cis or trans bromoindanol has been converted to the cis-aminoindanol by a Ritter type reaction [31, 49]. The conversion of bromo amine **28** to the stereochemically correct product has many advantages if the reaction

can be carried out with good yields. Further study is being devoted towards these later stages of the synthesis. The strategy shown here is a straightforward and general route to aminoindanol skeleton. The opposite stereochemistry can be obtained by using the other enantiomer , D-phenylalanine, which is also commercial available.

The synthesis of tetralin type of compounds is shown in *scheme 3.5*, the phenylalanine was converted to the bromo acid **28**, then it was reduced by borane to **41** which can be converted to the epoxide **42** by treating with base, the one carbon chain elongation was realized by opening the epoxide with cyanide to give **43**, which can be hydrolyzed to hydroxy acid **44**. The acid **44** itself is a very valuable compound. It can be readily converted to the tetralone skeleton **35** which in turn can be converted to the amino aryl ketone **46**. This is a new compound that can be used in the building of morphine analogs and many other important structures. The other isomer can be readily obtained by starting either form D-phenyl alanine or by converting **35** to the compound with opposite stereochemistry **34**, then to **36**.

3.3. Conclusions:

This chapter described the exploration of new synthetic routes to important pharmaceutical intermediates including chiral 4 carbons and aromatic amino alcohols from readily available amino acids. The chiral diaminobutyric acid was produced by Hoffman rearrangement for m glutamine. The aminotetrolins and aminoindanols are synthesized from phenyl alanine. The approaches explored here have many advantages over the existing methods, including the availability of starting raw material, the reagents used are common and inexpensive, the

reaction sequence is short and with high chiral integrity, the routes are accessible to scale up in industry.

3.4. Experimental Section:

3.4.1. Preparation of C-4 carbon intermediates.

Preparation of compound **12**. L-tert-butoxylcarbonyl glutamine 4.92g (0.02mol), 22ml of 10-13% commercial sodium hypochlorite solution, was mixed and cooled by ice, 3.2 grams of sodium hydroxide in 20ml of water was added to the mixture, it was stirred for 10 minutes, then it was heated at 65 °C for 2 hours. The mixture was neutralized with 3 N hydrochloride acid to pH=3, solvent was removed, the product was isolated by extracting with THF. The yield was: 72% ^{1} H NMR, δppm, 4.10(m, 1H) 3.00(t, 2H, J=7.8Hz), 2.11(m, 1H), 1.94(m, 1H), 1.31(s, 9H). ^{13} C (D2O) NMR, 170.4, 153.0, 77.2, 63.3, 46.8, 32.0, 24.0, 23.1.

Preparation of Compound **15** The t-Boc-diamino acid can be converted to the methyl ester by treating with acetyl chloride and methanol under refluxing condition for 6 hours or so, removal of the solvent give the diamino acid methyl ester, the t-Boc group was removed during this process. The resulting ester. ^{1}H NMR (D_2O, 300MHz) δppm, 4.09(m, 1H), 3.66(s, 3H), 3.04(m, 2H), 2.10(m, 2H), ^{13} C NMR(D_2O, 75MHz), δppm 164.6, 49.4, 45.7, 31.3, 22.8.

The cyclization of the diamino acid can be achieved by treating the above ester with sodium carbonate solution, the free amine react with the ester in situ to give the ring closure form

amino pyrrolidone[44]. ^{1}H (D_2O, 300MHz), δppm, 4.09(m, 1H), 3.20(m, 2H), 2.32(m, 1H), 1.82(m, 1H). ^{13}C NMR(D_2O, 75MHz), 161.6, 48.2, 34.5, 23.9.

3.4.2. Preparation of aminoindanol precursors

Phenylbromoacid 28 50 g(0.297mol) L-Phenylalanine **19** was dissolved in 500ml of water and 90g (3 eq) sodium nitrite, concentrated hydrobromic acid 360ml were added to the solution. The misture was stirred at 5 °C in an ice/water bath. 43g (2eq) of sodium nitrite as added over a 15minutes period. The reaction was stirred at 5 °C for another 1 hour and then at room temperature for 5 hours. Over this time , a light yellow solid settled out, The mixture was extracted with toluene. The toluene layer was concentrated to yield 64 grams (0.279mol) of **28** as a yellow oil. Yield 94%. ^{1}H NMR (300 MHz, $CDCl_3$), δppm, 7.14-7.38(m, 5H), 4.41(t, 2H, J=7.6Hz), 3.45(dd, 1H, J=8.4 and 14.1Hz), 3.23(dd, 1H, J=7.5 Hz, 14.1Hz). ^{13}C NMR (300 MHz, $CDCl_3$) δppm, 175.0, 136.3, 129.1, 128.8, 127.5, 44.8, 40.7.

The brom-indanone 29 20 grams (0.087mol)of above product **28**, treated with 40 grams of oxalyl chloride (after cooling with ice) the mixture was left at room temperature for 24 hours, then it was concentrated, taken up in dichloromethane, cooled in ice and treated with12 grams of anhydrous $AlCl_3$. It was stirred in ice/water for 30 minutes and then at room temperature for 12 hours. Another 300ml of dichloromethane was added, diluted with water, and phase separated, the organic phase was filtered through celite and dried over sodium sulphate, removal of solvent give 16.8 grams of bromoindanone as a dark liquid, upon cooling gave dark brown crystals, no further purification was needed . Yield was 91%.

^{1} H NMR (300 MHz, $CDCl_3$), δppm, 7.83(d, 1H, J=7.5Hz), 7.65(t, 1H, J=7.5Hz), 7.42(t, 1H, J=7.5Hz), 7.25(m, 1H), 4.64(dd, 1H, J=3.4, 7.5Hz), 3.84(dd, 1H, J=7.5Hz, 12.1Hz), 3.40 (dd, 1H, J=3.4, 18.3Hz). ^{13} C NMR (300 MHz, $CDCl_3$) δppm, 199.6, 151.1, 136.0, 133.5, 128.3, 126.4, 125.1, 44.0, 38.0.

Cis-2-bromo-1-indanol 32: In all attempt to do the reductive amination[57,58] on the bromoindanone give the cis-bromoalchol **32** as the major product, with small percentage of the bromo amine. The conditions have been tried are, 2.11g(0.01mol) bromo indanone, 7.7g ammonium acetate (10 mol), 1 eq of sodium caynoborohydride, at pH=4 (by adding acetic acid), and pH=7-8. The mixture was stirred for 24 hours at room temperature, after which the reaction mixture was acidified to pH=2 by concentrated hydrochloric acid, the solvent was removed by rotatory evaporation under reduced pressure. Ether was added to extract most of the organic material in the mixture, after drying the solvent give 1.59g of cis-bromoalcohol **32**.

The direct reduction of the bromoindanone by sodium borohydride give the cis-bromoindanol in excellent yield. 1.05g(0.005mol) of bromoindanone in 100ml round bottom flask was cooled in ice bath, then 0.25g sodium borohydride was added in a 5 minutes period. It was left stirring at room temperature for two hours at which time the reduction is completed indicated by NMR spectroscopy. The reaction mixture was quenched with 1N hydrochloric acid , extracted with diethyl ether. The ether layer was dried and the removal of solvent give the cis-brom-indanol 1.05g as a yellow solid(needle crystalline). Yield was

99%. ^{1}H NMR (300 MHz, $CDCl_3$), δppm, 7.20-7.50 (m, 4H), 4.90-5.05(m, 2H, CH-Br, CH-OH), 3.45(dd, 1H, J=5.0, 17.0), 3.36 (dd, 1H, J=3.2, 17.0), ^{13}C NMR (300 MHz, $CDCl_3$) δppm, 141.6, 139.2, 128.8, 127.4,125.0, 124.6, 76.2, 60.7, 40.2

The acetyl derivative of cis-2-bromo-1-indanol. It was obtained by treating the bromo compound with acetic anhydride, pyridine. ^{1}H NMR (300 MHz, $CDCl_3$), δppm, 7.10-7.45 (m, 4H), 6.02(d, 1H, J=4.8Hz), 4.86 (m, 1H), 3.48(dd,1H, J=6.3, 16.5Hz), 3.38(dd, 1H, J=5.4, 16.5) ^{13}C NMR (300 MHz, $CDCl_3$) δppm, 170.5, 140.3, 138.2, 129.3, 127.4, 125.1, 124.7, 76.7, 51.4, 40.9, 20.9.

Cis 1-amino-2-indanol. The bromoindanol can be converted to the cis amino indanol in one step by Ritter reaction. 2.13 g of the cis-2-bromo1-indanol was dissolved in acetonitrile, it was cooled in acetone-dry ice bath, 3.2 g of fuming sulfuric acid was added to the mixture dropwise. It was left stirring for overnight, 20 ml of water was added, the solvent was removed under rotatory evaporation then the mixture was heated at 60 °C for 4 hours, after which the mixture was concentrated and water removed, the residue was treated with 25% sodium hydroxide solution, the crystals was collected by filtration, and the solid was taken up in methanol or chloroform to remove any salt in the solid, the liquid phase was dried, and give 1.35g cis-1-amino-2-indanol as a white solid (yield: 90%) A small amount of the sample was recrystallized from CH_2Cl_2:hexane, m.p. 119.5-120.5°C. ^{1}H NMR (300 MHz, $CDCl_3$), δppm, 7.21-7.30(m, 4H), 4.36(m, 1H), 4.28(m, 1H), 3.07(dd, 1H, J=16.5, 5.5Hz), 2.92(dd, J= 16.5, 2.7Hz), 2.48(s, 3H, exchange with D_2O). ^{1}H NMR (300 MHz, CD_3OD),

δppm, 7.38(m, 1H), 7.20(m, 3H), 4.41(dt, J=3.3Hz, 5.1Hz), 4.15(d, J=5.1Hz), 3.07(dd, 1H, J=5.4Hz and 16.2Hz), 2.89(dd, 1H, J=3.3Hz, 16.2Hz).

^{13} C NMR (75 MHz, $CDCl_3$) δppm, 143.9, 140.8, 127.9, 126.9, 125.4, 123.9, 72.7, 85.5, 39.3.

3.4.3. Synthetic routes of aminotetrolin precursors.

Synthesis of the bromoalchohol 41. The bromoalchol can be synthesized by reducing the bromoacid **28** by borane-THF complex or by reducing the methyl ester of the **28** by $LiBH_4$[59]. The procedure: 2.43g benzyl bromacid **28** (0.01mol), 14ml of BH_3-THF 1M solution was added to the acid at 0° C in 10 minutes period, then the mixture was stirred at room temperature for 10 hours. At which time proton NMR indicated the reduction of the acid is essentially completed. (The reaction was followed by ^{1} H, NMR and TLC, at 1 hour, about 50% reduction, at 5 hours about 80% reduction, 10 hours, the reduction is completed. About 0.5ml of sample was taken each time for the analysis. Quench with 1N HCl, extract with ether, remove the ether give the white solid for the NMR) The reaction mixture was quenched with 1N HCl, extracted with ether, the ether layer was dried by sodium sulphate, removal of the solvent give the bromoalchohol **41** as a white solid, yield 2.03g (94%) . ^{1} H NMR (300 MHz, $CDCl_3$), δppm, 7.40-7.20 (m, 5H), 4.34(m, 1H), 3.84 (dd, 1H, J=3.9, 12 Hz), 3.75(dd, 1H, J=6.0 and12Hz), 3.28(dd, 1H, J=7.2 and 14.1Hz), 3.18(dd, 1H, J=7.5 and 14.1Hz). ^{13} C NMR (75 MHz, $CDCl_3$) δppm, 139.0(1C), 129.2(2C), 128.5(2C), 127.0(1C), 66.0, 58.6, 41.3.

The epoxide 42: 0.10g bromoalcohol **41** and, 1.5eq of potassium tert-butoxide 0.08g, and 4ml of tetrahydofuran was mixed and stirred at room temperature for 12 hours, after which the mixture was filtered and solvent was removed give the epoxide with good purity and yield . ^{1}H NMR (300 MHz, $CDCl_3$), δppm, 7.38-7.18 (m, 5H), 3.18(m, 1H), 2.92(dd, 1H, J=14.4, 4.8Hz), 2.80(dd, 1H, J= 11.7 and 4.8Hz), 2.77(m, 1H), 2.54(dd, 1H, J=4.8 and 2.7Hz). This is same as the literature vale[60]. ^{13}C NMR (300 MHz, $CDCl_3$) δppm, 137.1(1C), 129.0(2C), 128.5(2C), 126.6(1C), 52.4, 46.8, 38.7.

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Chapter 4

Introduction for Advanced Materials Based on Self-Assembling Lamellar Supramolecular Arrays and Two Dimensional Polymers

Abstract

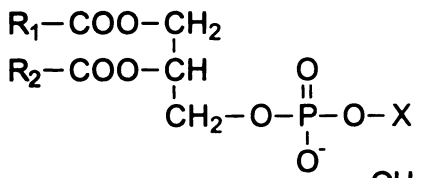
In recent years, the design and fabrication of novel multifunctional advanced materials have gained increasing attention. However, there has been difficulty in finding a simple reliable technique for arranging functional groups in molecules into ordered arrays. Self-assembly which is learned from nature is becoming a key factor and important concept for this type of application. In this approach, the units are made with structures and connecting sites that recognize adjacent units and come together in the correct configuration to form the desired supramolecular (very large) system. In this way, the laborious task of sequentially arranging and fixing each bond is avoided. The information for forming the overall supramolecular structure is built into the individual units. Units may self-assemble to form sheets or tubes or spheres or any other kind of regular object. These molecular assemblies have specific properties that are encoded by chemical functionalities in the constituent units. They are referred to as "smart materials". Nature has her unique and amazing way of organizing living systems. The cell membrane for instance is a perfect example of a natural self assembled system and studies are based upon copying it. Major foci are to design molecules that can assemble themselves into much larger objects to fabricate oriented thin films and other lamellar structures such as stabilized vesicles. The lamellar vesicles are important as models for biomembranes, fabricating biocompatible surfaces, for drug encapsulation, gene delivery and as vehicles for delivering various agents used in cancer therapy. The controlled oriented thin film is useful in developing molecular devices, electronic and photonics materials and other advanced materials which are pivotal for the revolution in communication, information transmission and storage.

4.1. Membrane lipid structure and properties.

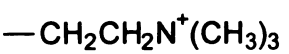
Cell membranes are critical components of the cell surface of living organisms and are responsible for maintaining cellular integrity and communication with the environment and with other cells or organisms. Biomembranes are composed primarily of lipids and protein. The protein is spreaded as an extended sheet over the ionic heads of the phospholipid bilayer or are buried in the hydrophobic core of the bilayer[1]. The lipid membrane provide a matrix for membrane proteins and the structure of the membrane is very important for the biofunctions of cell. There are four main groups of lipids in cell membranes; phospholipids, sphingolipids, glycolipids, and sterols. Some of the examples are shown in Figure 4.1. Phospholipids are the most abundant of the biological membrane lipids.

Many membrane lipids can form the basic bilayer structure. The phospholipids can self assemble into spherical liposomes or lipid vesicles, which contain aqueous solution within an enclosing lipid bilayer. The self organization arises from the inherent properties of lipid molecules and intermolecular interactions between lipids and water. The interactions include hydrophobic, hydrophilic, dipolar, hydrogen-bond, and electrostatics. The combination of these interactions produce the unique characteristics of the assembled bilayers. The ability of lipids to form bilayer structures is mostly due to their amphipathic character. Lipids have a polar or hydrophilic head group region and a nonpolar or hydrophobic part. Such molecules will naturally orient themselves to ensure that the polar head groups associate with water molecules and the hydrophobic tails interact with each other. If the molecule is roughly cylindrical in dimension and have no net charge then the biomolecular planar

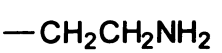
PHOSPHOLIPIDS



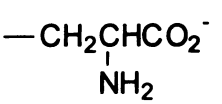
—OH Phosphatidic acid (PA)



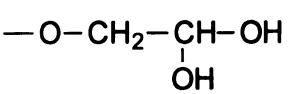
Phosphatidylcholine(PC)



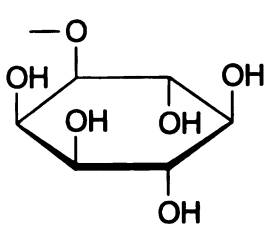
Phosphatidylethanolamine(PE)



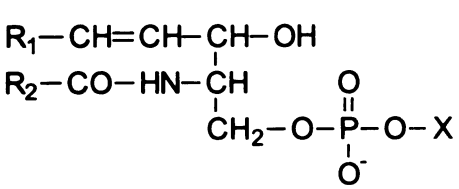
Phosphatidylserine(PS)



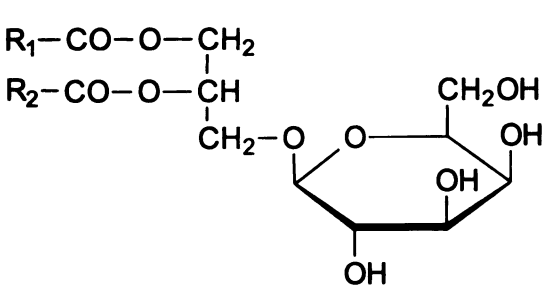
Phosphatidylglycerol(PG)



Phosphatydylinositol(PI)



Sphingolipid



Glycolipid

Figure 4.1. The general structures of some phospholipids.

leaflets will be the most stable configuration in aqueous solution. If they are charged, the presence of an appropriate counter ion will also stabilize the bilayer structure. Besides the planar bilayer structure, some membrane lipids tend to adopt other ordered systems such as micelles and hexagonal II phases[2] (Figure 4.2). The hexagonal packing provide good permeability barriers with the vast bulk of the lipid arranged in a bilayer in vivo. A micelle is a spherical aggregate of lipids and is usually small. Micelles are not as important as lamellar bilayers are in biological systems and for biomaterial based applications. They are, however, important in some other areas such as acting as surfactant in water to dissolve insoluble materials. The bilayer structure is the basis of biological membranes and is very important for cell function. The tendency to form a particular which type of packing structure depends on the structure of the lipids. Among the most important phospholipids, phosphatidylcholine usually forms a bilayer structure, phosphatidyl ethanolamine tends to form micelles or inverted hexagonal structures because of its small head group.

Phospholipids serve as the prototypical materials for the design of thin film materials because of their extremely high packing densities and propensity to form lamellar systems. These 2-dimensional lamellar systems find applications in several areas of research and technology including drug encapsulation [3, 4], gene delivery [5,6], biocompatibility [7,8], bacteriacides [9,10], biosensors [11] and specialized receptor surfaces [12-14] and advanced biomaterials [15-20]. In all of these applications, it is desirable to form a self-assembled lamellar system with some desired functionality on one surface. In the drug encapsulation and gene delivery areas, it is a requirement that the lamellar system have an

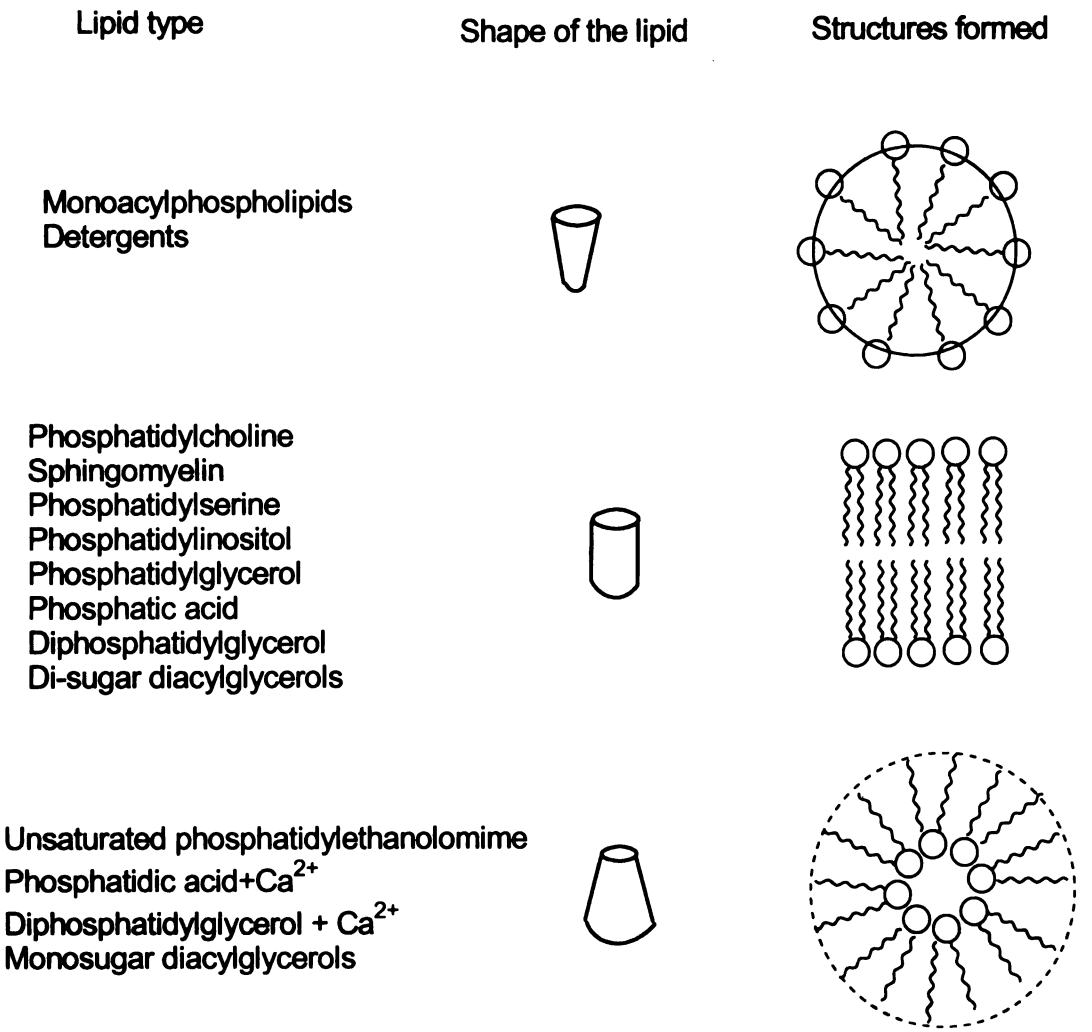


Figure 4.2. Lipid shapes and their packing characteristics (adapted from reference 2).

overall spherical topography to form hollow structures (vesicles or liposomes). Because of its high propensity to form bilayer structures or vesicles, phosphatidylcholine (lecithin) is the most studied phospholipid [21-24]. Potential uses include encapsulating agents in drug delivery [21, 22] and extracting toxic heavy metal from waste water [25-27]. However, the uses of lipid membranes have been limited by difficulties in their fabrication and their poor stability.

4.2. Liposomes, Stabilized Phospholipid Analogs and Their Biological Properties

One of the new and exciting avenues of research that has evolved over the past few years is the preparation of large molecular systems with shapes that allow them to be used as containers for the purpose of trapping and transporting substances such as drugs. These containers (called vesicles or liposomes) are interesting because they are not of the size that can be observed with the naked eye but are of molecular dimension.

They have a multilameller or unilamelar structure and various sizes from 25 nm up to 10 μm (Figure 4.3) [28]. Because liposomes contain both hydrophobic layers and hydrophilic layers alternatively, hydrophobic solutes can be contained within the bilayer and water soluble materials within the aqueous compartments. Highly nonpolar drugs are dissolved in the hydrocarbon core of the micelle, whereas more polar substances are located in the polar head groups near the surface. Inside a liposome, a drug is protected from the cellular enzymes that would destroy it. This increases the lifetime of the drug in the body and,

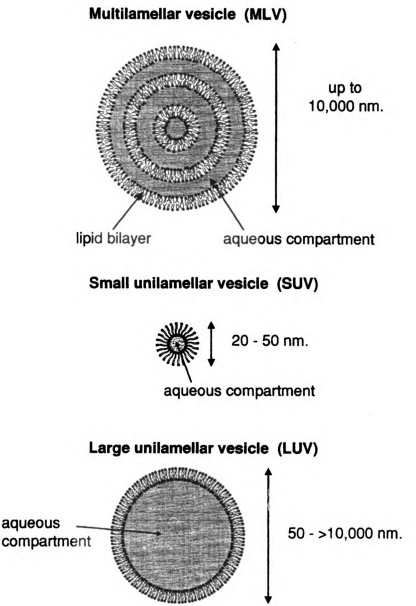


Figure 4.3. The structure and dimensions of unilamellar (SUV and LUV) and multilamellar(MLV) liposome (adapted from reference 28)

therefore, reduces the dosage required to obtain a desired effect. Researchers are developing targeted liposome drug delivery systems. These should be very useful in cancer therapy, because such liposomes should selectively localize anti-cancer drugs at the tumor site thus reducing the toxicity of the drugs to normal cells and improving their therapeutic activity because of the higher drug levels being delivered to the tumor. Liposomes or lipid-based carriers are also being explored as vehicles for carrying DNA into cells for gene therapy. In this therapeutic technique, a defective gene (stretch of DNA) is replaced by a new stretch without defects. Inside of a liposome, the polar DNA molecule can traverse the protective membrane of the target cell. It is also protected from nucleases, enzymes that degrade DNA.

A vast of technology has been developed using liposomes since their discovery by Bangham [29]. Many applications of liposomes as delivery systems have been developed though few of them have reached commercial stage. These include enzyme targeted delivery of anticancer drugs [30-32] bacteriacide [33] , and moisturizers and antiflammatory agents [34] for skin. Cationic liposomes are widely explored for gene therapy [35, 6, 5]. The potential of liposomal delivery seems very promising. But there are some problems with conventional liposomes. Although the idea of using them as drug delivery systems has been around since the early 70s, conventional liposomes have proven not to be good carriers for drugs because they are readily broken by contact with other surfaces (very much like soap bubbles) and because they are easily degraded by cellular enzymes. They can also be easily disrupted by changes in pH, temperature or salt strength. The medical utility of the conventional liposomes is limited by their rapid uptake by phagocytic cells of the immune

system, predominantly in the liver and spleen. This uptake is due to the characteristic nonspecific reactivity of the liposomes, which results in their largely uncontrollable properties upon administration in vivo.

Researchers have sought many ways of stabilizing liposomes and bilayer membranes. These include the preparation of sterically stabilized [36-39] (stealth) or polymorphic liposomes. Synthetic polymers are used for steric stabilization. Another approach is cross-linking membrane components covalently, or the polymerization of polymerizable lipids [40, 41]. Supported self assemble lipid bilayers [13] are also used as self-assemble monolayer stabilized microbubbles [42]. Stealth liposomes contain a phospholipid with polyethylene glycol (PEG) attached to prevent the liposomes from sticking to each other and to the blood cells or vascular walls. They are invisible to the immune system and have shown encouraging results in cancer therapy. Coating liposomal vesicles with a hydrophilic polymer such as PEG reduces uptake by the liver. They can therefore remain in circulation longer than conventional liposomes. Stereo stabilized liposomes have been created in which the lipid bilayer contains glycolipids or are conjugated with ethylene glycol. This can provide a steric barrier outside the membrane. Sterically stabilized liposomes can stay in the blood up to 100 times longer than conventional liposomes thus they can increase the pharmacological efficacy of encapsulated agents. Furthermore they have revived the possibility of ligand-dependent targeting to specific cells by incorporating targeting ligands on their surface, because they are much less subject to nonspecific uptake than are the conventional liposomes [43].

The practical use of self -assembled systems in technological applications requires that the supramolecular assembly to survive and function over a great range. One strategy for obtaining stability is polymerization of the microstructure following self-assembly. There have been intensive studies in this area [44]. Fatty acids have been used to prepare polymerizable vesicles [45-47]. Phospholipids containing diacetylenes in the fatty acyl chains [48-52] and diacetylenic lipids with ammonium or glutamate-based head groups [53] have been studied. Other polymerizable groups that have been explored include butadienes [54], terminal vinyl and methacryloyl functionities [8, 55] and acrylates [56]. These materials have been investigated for producing stabilized microstructures for biosensors and encapsulation applications.

One other methods for forming stabilized systems that has been explored is supported self assembling monolayers and bilayers [57-60] in combination with the Langmuir-Blodgett technique [61, 62]. Supported membranes on solid supports have been widely studied as theoretical models of cell membranes and used as a way of biofunctionalization of inorganic surfaces. These are potential useful for biocompatible surfaces, opto-electronic devices, biosensors and immobilization of proteins. In some cases, hybrid bilayer membrane systems containing both phospholipids and alkanethiol components formed by self assembly supported on a metal surface has been studied and the stability potential applications as sensors and in bioelectronics etc. has been evaluated [63-65]. Self-assembled monolayers are now being used for the oriented immobilization of biomolecules, for functional and

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structural studies of proteins in immobilized biomembranes and on micro-to nanostructured surfaces for biosensor and nanotechnology applications [66].

As noted earlier, one general drawback to the use of phospholipids in the applications discussed above is the general instability of the supramolecular structures they form. Hence vesicles tend to lyse too easily and leak the materials that are trapped therein. Planar lamellar systems on solid supports often lack sufficient intermolecular cohesive power and are too easily perturbed. Some special device such as a Langmuir-Blodgett trough is often required to form them in the first place. It is therefore important to design and synthesize phospholipid variant structures that contain some extra stabilizing features. *Sarcina ventriculi* is a Gram-positive bacterium which is tolerant to extremes of pH (2.0 to 10) as well as moderately high temperatures, and the presence of organic solvents[67, 68]. In recent years, the mechanism of the extreme stability in these bacteria have been established by Hollingsworth etc [69]. The membranes contain very long bifunctional fatty acids spanning the cell membrane and they are synthesized by the tail-to-tail joining of membrane lipid chains between the bilayers. Such organisms live in extremes of environmental conditions such as low pH, high pH and high temperature in addition to the pressure of high concentrations of drugs and organic solvents. These organisms survive by synthesizing membrane lipids that contain very long fatty acid chains that go through the entire membrane instead of just from the middle of the membrane to the outside [70-72]. The fatty acids are very long (28 to 36 carbons) α, ω- dicarboxylic acids that are esterified to a glycerol molecule on both ends. The head groups are phosphatidyl glycerol, monoglucosyl diacyl

glycerols or any of the common functionalities found in the typical membranes [figure 4.4.]. It is clear that much of the stability of the membranes of such extremophilic organisms stems from the presence of the transmembrane fatty acyl group.

The archaebacteria lipids have been used to prepare liposomes, they form single lipid membranes [73] rather than the normal bilayer type because they too contain very long chain lipids twice the length of normal lipids [Figure 4.5]. Membrane lipids extracted from these bacteria have also been used to form liposomes. Physical studies show positive results such as extra stability towards high temperature, high or low pH, and other harsh conditions [74-78]. Because isolation from bacteria only yield material in very small quantities, it is impractical to use them for applications such as targeting drug delivery system, and in the manufacturing of biocompatible surfaces. In recent years due to the importance of these special types of membrane lipids, many synthetic efforts have been made in this area. The total synthesis of these archaebacteria lipids is generally difficult, long winded and not scalable to practical applications [79-82].

Some bolaform amphiphilic models of archaebacteria membrane lipids [83-87] which contain transmembrane alkyl chains and ether linkage to the head groups have been prepared. These tetra ether bipolar lipids have the advantage of forming lamellar systems that are stable to extreme pH [88], high temperatures [84, 89] and high ionic strengths [90]. These models have ether instead of ester linkages connecting the hydrocarbon chains to the head group and lack many of the structural features of biologically important lipids. Another

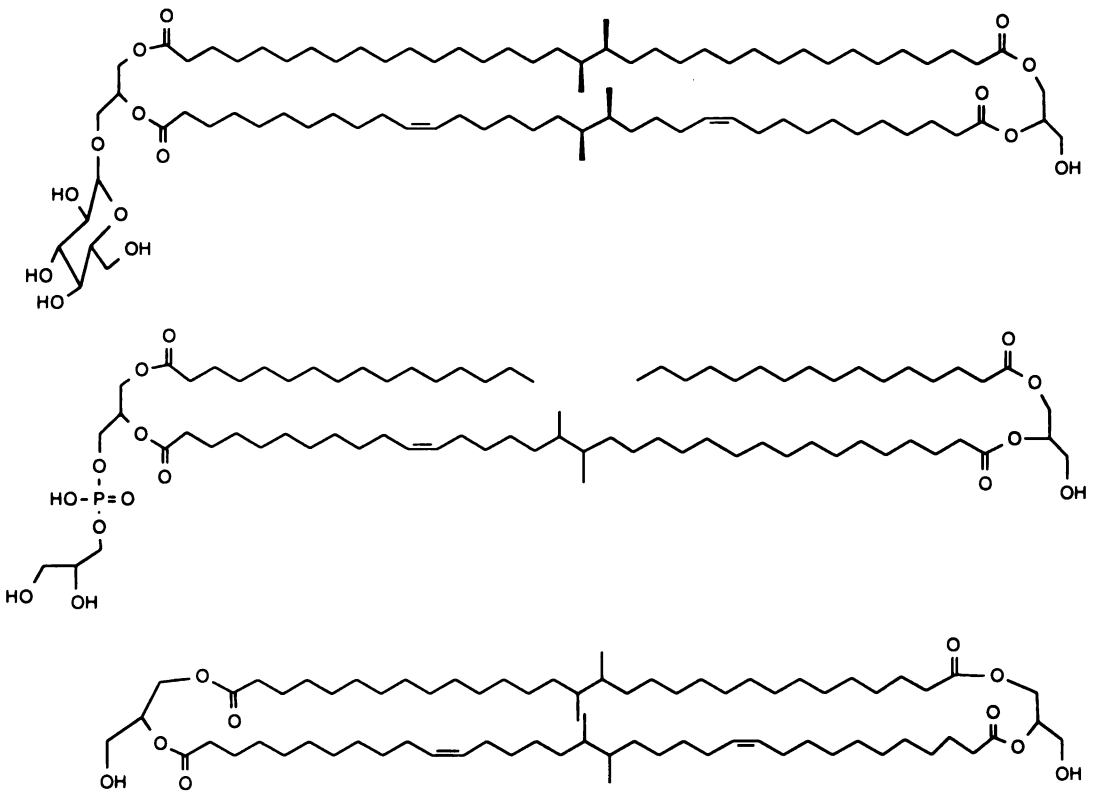
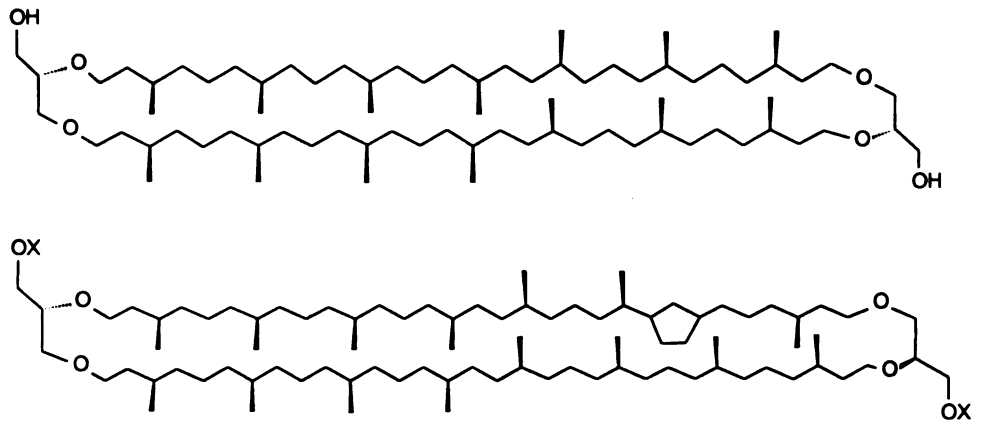
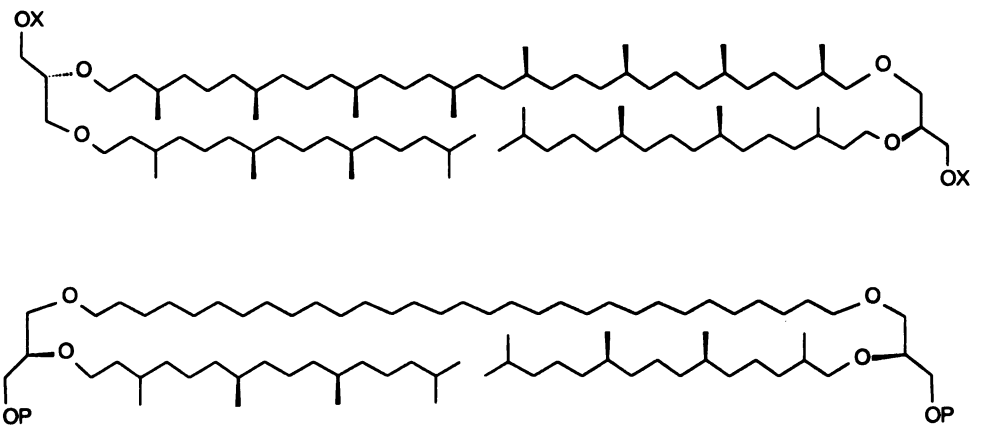


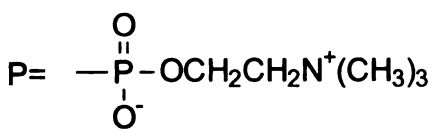
Figure 4.4. The membrane lipids from *Sarcina Ventriculi*



Archaebacterial Lipids Type A



Archaebacterial Lipids Type B



X=H, sugars, phosphoethanolamine, and phosphosugars.

Figure 4.5. The structures of some archaebacteria membrane lipid models.

potential problem of using ether linkage is that they are stable to lipases, so release of encapsulated substances by the action of phospholipase A-type activities (esterases) is not possible. This might limit the practicality of using such liposome systems in applications where host enzymes mediate the release of the trapped substance.

4.3. Mutifunctional Self Assembling Thin Films

It is becoming more and more important to develop synthetic methods for arranging substructures in space for the purpose of preparing new functionalized organic materials where groups with specific structures are organized and arranged to achieve some desired physical effect. These effects can be electrical, optical or mechanical or even a combination of these (electro-optical). Such materials should have broad applications in optical and electronic devices and biosensors. For instance, it is proposed that the can be used as components in pivotal optical devices used in optical computing based on photo-switching and nonlinear optical memory effects. Other areas of potential use are the development of sensors capable of signal amplification, electronic devices such as non-linear resistant components, self-regulating devices, and components in artificial organs. A new generation of intelligent materials with high-level functions is often imagined. It is hoped that such materials can deform or change properties radically by applying external stimuli such as temperature changes, pH changes or electric fields. They can be used as biomimetic membranes which have stimuli-responsive function or as self-oscillation membranes simulating information acceptance and conversion functions, as observed in sensory-organ

cells and neurons. It is hoped that attributes such as perception, judgment, movement, and recognition will eventually be built into such materials.

Molecular self assembly is a recognized strategy for the above applications. Though it is still at too elementary stage to mimic even the most simple process which occurs in biological systems, some success has been made by self assembly on surfaces according to Whitesides[91]. Self-assembled monolayers (SAMs) are well characterized and studied. They are usually prepared by immersing a substrate (gold, silver etc) in the solution containing a ligand (alkanethiol etc.) that is reactive toward the surface or by exposing the substrate to the vapour of the reactive species[92]. Alkanethiol SAMs on gold and silver are usually stable, highly organized and electrically insulating [93, 94] they have been explored for nanofabrication of molecular scale electronic devices [95]. The thickness of SAMs along the dimension perpendicular to the plane of the monolayer can be controlled by varying the structures of the molecules making up the monolayer. They can provide tailorable functions by changing the structures of the organic molecules in straightforward ways. Micropatterning is a powerful method for controlling surface properties, with applications from cell biology to electronics [96-98]. Self assembled monolayers (SAMs) of alkanethiolates on gold and silver [99-101]- the structures most widely used for preparing organic films with specific surface properties are usually patterned by partitioning the surface into regions formed from different thiols [102-105]. The patterning required in microfabrication is usually carried out with photolithography and soft lithography. These techniques have potential applications in the fabrication of microelectronic devices.

Although the alkanethiol SAMs have shown enormous potential in nanofabrication, there are some problems associated with it too. The most promising avenues for self-assembly are presently those based on organic compounds but they are usually electrical insulators. Thus many ideas for information processing and electrical/mechanical transduction will require either fundamental redesign in going from the macroscopic systems presently used to self-assembled systems or the development of new types of organic molecules that show appropriate properties. There are also some fundamental problems with SAMs on metals. For instances, the microscopic roughness of gold and silver surfaces and the presence of steps and kinks in the lattice generate defects in the SAMs. Another problem is the influence of grain boundaries and other defects in the SAMs itself. SAMs of alkanethiols on gold and silver are trans-extended, but tilted respectively by 30° and 11° from the normal to the surface. This tilt results in domains in SAMs even on atomically flat surfaces. The packing of the chains at the domain boundaries is critical [102]. In recent studies, mercury has been used as the substrate[102, 106]. It also has high affinity for thiols and the SAMs on a Hg surface with the thiols chain perpendicular to the surface are reported to be free from domain boundaries. However since mercury is liquid, the surface thus formed will have some fluidity and other means are needed to fix it.

SAMs represent one type of structure that used in molecular self-assembly to build structure and function on the nanometer scale. They are not a general solution to the problem of building functional nanostructures, but the lessons learned from them will be valuable in building more versatile systems.

There is an increasing amount of interest in the design of planar lamellar systems containing conjugated polydiacetylene functions. The polydiacetylenes (PDAs) have very fascinating properties and great potential for applications in materials science, medicinal science, etc. These systems are known to display several interesting properties that could lend themselves to the fabrication of a variety of devices [107,108]. One phenomenon observed in PDAs is the blue to red color transition. These color changes occur in polydiacetylene single crystals, cast films, solutions, and Langmuir-Blodgett films. The transition can be triggered by a variety of environmental perturbations including temperature (thermochromism) and mechanical stress (mechanochromism). They display mechano-optical effects in which compressing the polydiacetylene layers lead to a change in color of the films [109,110]. It has also been observed that biomemetic polydiacetylenes incorporating carbohydrate ligand change color from blue to red upon specific binding of a biological target (biochromism) [107, 111-117]. The liposomes contain diacetylene fatty acids or thin films formed by long chain polydiacetylene containing a receptor binding ligand have been used to detect the binding of influenza virus to surfaces [111, 112, 114] (Figure 4.6), in this case the head group of the fatty acid chain is sialic acid. In another study the molecular recognition and colorimetric detection of chlora toxin has been investigated by polydiacetylene liposomes incorporating Gm1 ganglioside [113]. The charge induced chromatic transition of amino acid derivatized polydiacetylene liposomes was also observed [118]. Polydiacetylene layers also demonstrate color changes in response to alterations in temperature [119-122], pH [118, 123] and on exposure to some solvents [124-125].

Figure 4.6. Schematic structure of the PDA-vesicles modified with a carbohydrate capture molecule, sialic acid. The sugar binds to the influenza virus lectin, hemagglutinin. A variety of capture molecules may be co-assembled with or grafted onto the PDA-vesicle.(Adapted from reference 44).

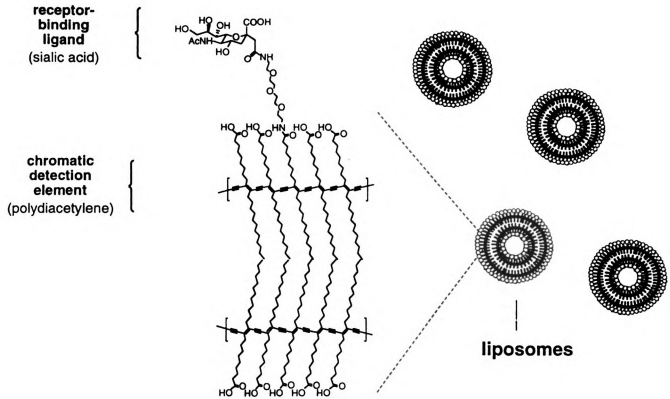
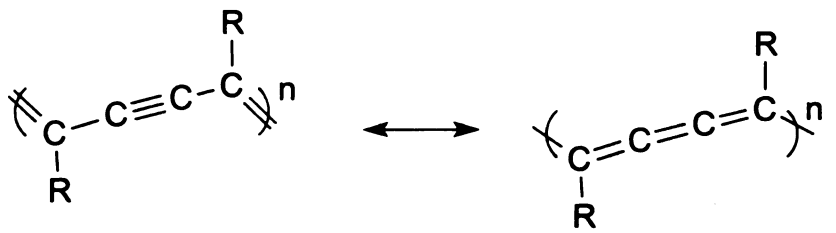

receptor-
binding
ligand
(sialic acid)
chromatic
detection
element
(polydiacetylene)
liposomes

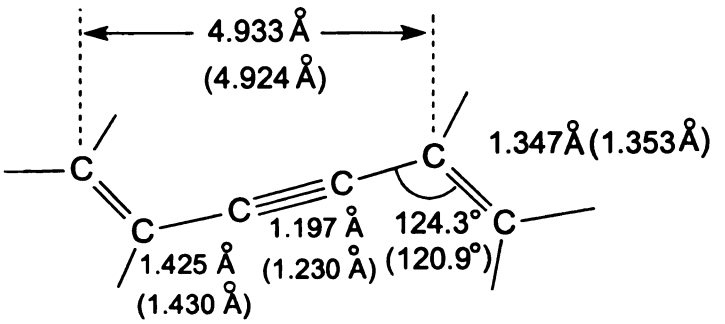
Figure 4.6.

Polydiacetylenes have much potential as new organic materials with potential applications in electrical conductivity, biosensors, non-linear optical applications and near-infrared active applications [126-131]. The fabrication of 2-dimensional systems in which polydiacetylene functions occur as a band within the system are especially of interest [132-135]. Polydiacetylene is well recognized for its high electrical conductivity and high third order non linear optical property due to their extended systems [130, 136-143]. Many studies of the nonlinear optical properties of polydiacetylenes have been done to establish a combination of suitable parameters for useful devices. Materials which do not possess a center of inversion and have oriented dipoles can show so-called second-order nonlinearities like second-harmonic generation or electro optical effects. Third-order nonlinearities which lead to third-harmonic generation or to an intensity-dependent refractive index can appear in every material and do not have the symmetry constraints like the second-order effects. The nonlinear optical response depends on the magnitude of the corresponding material parameters called second- and third-order nonlinear optical susceptibilities respectively [144, 145]. Besides the high third order non linear susceptibility, the second order nonlinear susceptibility of polydiacetylene systems are also being explored. Some aromatic substituted asymmetric PDAs have been prepared and they exhibit second harmonic generation [127, 146 -148]. A variety of systems containing PDAs have been studied, and the possibilities of using them as optical electronic materials and biosensors has been explored.

Despite the vast amount of literature in this area, there are still some fundamental problems associated with polydiacetylene systems. The chromic transition of PDAs is well known



Acetylenic Butatrienic



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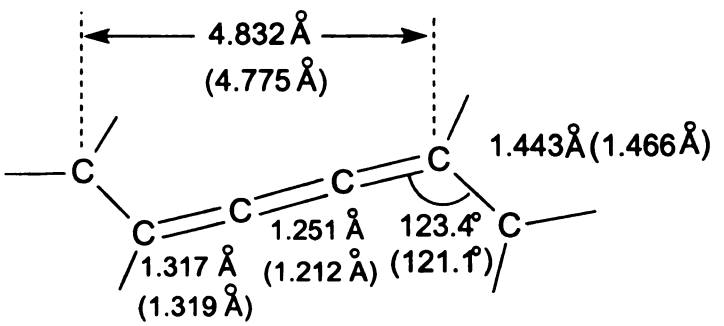


Butatrienic

Figure 4.7. The tow modes of polymerization of diacetylene and the atomic positions of the PDA backbone structures based on the ab initio calculation. The average value from the molecular mechanics calculation is shown in parentheses. (Adapted from reference 154).

and the origin of it has been studied by a number of investigators and attributed to many different phenomena. However, the mechanisms of these phenomenon are not fully established and there are many conflicting reports in this area. General principles have yet to be established [149, 150, 154]. The second issue is the mode of polymerization. There are two types of polymerization modes that currently have been proposed (Figure 4.7). The most common one is the acetylenic form, the triple double bond alternating mode, the other one is the butatriene type, depending on the side chain structure [152-154, 119].

Besides the lack of accurate theoretical explanation of the chromism, the most problematic issue in the application of the polydiacetylene system is that a good method of fabricating them is lacking. The current existing systems are not quite useful for applications such as advanced opto-electronic devices and other materials. The intractability of typical polyethylenes make them difficult to orient. They are insoluble in most solvents, form hard masses or bundles of fibers that are impossible to transform to flat (2D) systems, and they are very sensitive to oxygen and radical species which react with the double bonds and reduce their conductivity. In fact, films can become resistive if exposed to oxygen for a short period. Also the polydiacetylene tend to form fibers instead of films. This makes it unsuitable for many applications especially when lamellar systems are desirable. Under favorable circumstances, the use of amphiphilic molecules with acetylene groups in the hydrocarbon chains leads to film formation [156-157]. However such molecules often contain only one hydrocarbon chain and they have a tendency to form micellar systems.

The alignment of long chain diacetylenes to effect polymerization with the resulting formation of these highly conjugated polydiacetylenes layers or bands is one of the most difficult aspects of the fabrication of these materials. The acetylene functions need to be within 0.5 Å of each other [155]. Several strategies have been tried to obtain good alignment of the diacetylene chains. The most common strategy in recent years is based on the same idea of anchoring alkanethiols on metal surfaces. Thiolated diacetylene chains are anchored on a flat support such as a highly polished gold surface or other metal surfaces [156, 157, 158-162]. However, there are some problems associated with obtaining long range order of alkyl chain containing diacetylenes by this method to. The most severe problem being that, given the large atomic radius of gold compared to carbon, variations in the surface of only a few gold atoms put the acetylene functions in the aligned chains out of register thus terminating polymerization (Figure 4.8). It is therefore very difficult to obtain any high degree of long scale polymerization. It is also an extremely labor-intensive approach. There is also the tendency to form disulfide groups. When this happens, the chains are not aligned and polymerization is not possible [156]. The SAMs of n-alkylthiol-gold show no significant variation in chain crystallinity or long range order with substrate preparation, but for diacetylene containing monolayers the long range order is hard to establish even though a study has show that on special treatment of the gold surface or on a high temperature evaporated gold surface, favorable alignment occurs thus allowing polymerization of the diacetylene units.

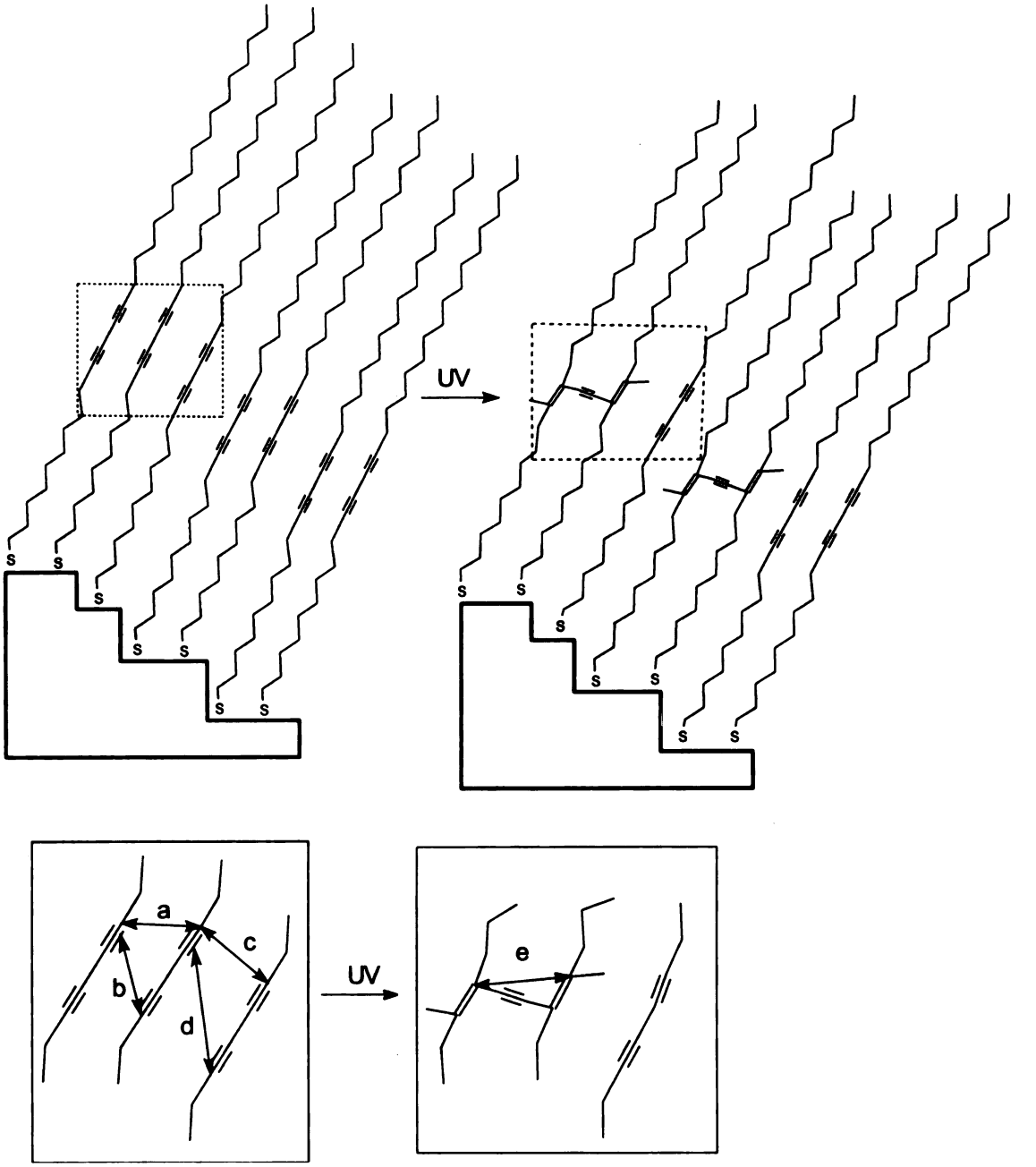


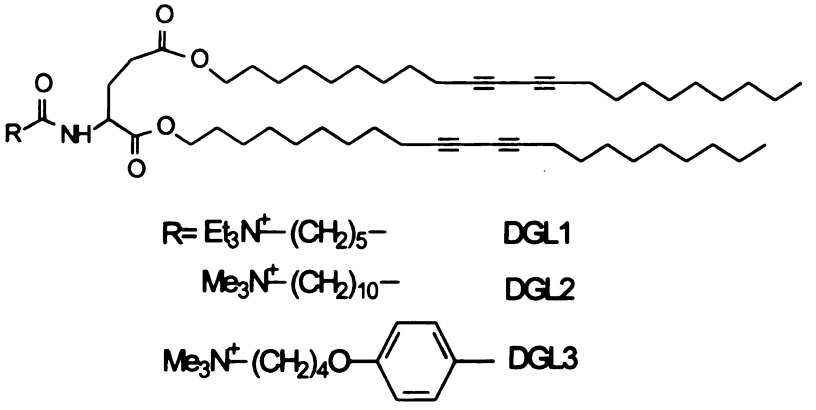
Figure 4.8. Schematic diagram of the structure and polymerization of diacetylene monolayers on gold or other surfaces with a high ration of step to terrace sites. The polymerization requires strict spatial arrangement of the diacetylene groups. a=4.7-5.2Å, b=3.4-4.0 Å. Polymerization across step sites is greatly hindered because the spatial tolerances are exceeded.(c and d are too large). (Adapted from reference 156).

Another approach for aligning diacetylene chains is to generate monolayers using Langmuir Blodgett technology [163-168]. Although it is a good method to prepare highly ordered thin films, it is a labor-intensive method and LB films are not stable enough to remain intact under a variety of chemical or physical conditions. This restricts their utility. This method is also not suitable for large scale preparations.

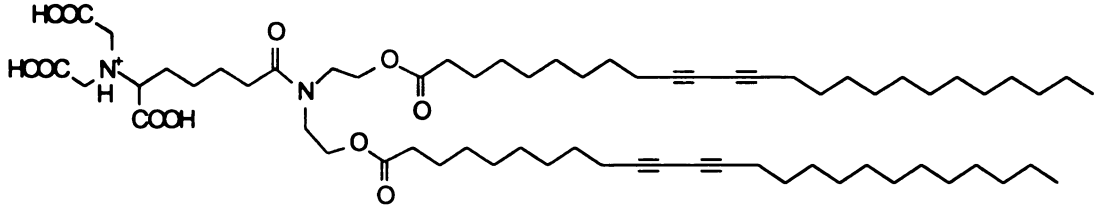
Because phospholipids readily form stable lamellar systems or other highly ordered structures, another possible approach is to synthesize diacetylene lipids that are biomembrane mimics. The inclusion of conjugated diacetylenic groups at the same position in each acyl chain of a phospholipid chain [39, 40, 169-170] (Figure 4.9) should give ideal self-assembling units which can be polymerized to form highly organized, stable, 2-dimensional systems containing a conducting polydiacetylene layer. Such phospholipids have been prepared and the physical properties characterized. These phospholipids containing diacetylene function in the middle of the chains were reported to form tubules under certain conditions [171-172]. Another approach that has been tried is to use microorganisms to carry out the integration of fatty acids containing diacetylenic functions into phospholipids. Using this strategy, as much as 90% integration of diacetylenic fatty acids into microbial phospholipids was obtained [173]. There are some problems with this approach however. Because the membrane is only two molecules thick and just surrounds the cell, the actual amount of material recovered per gram of cell mass is extremely small. In addition to this, the lipid species made by any one microorganism are extremely diverse and may include neutral, and negatively charged headgroups with different structures. It is

a challenge to separate species with only one type of headgroup and, even then, there is a tremendous amount of diversity in the fatty acid species that are derived from the normal microbial metabolism. Also microorganisms contain a myriad of membrane-associated enzymatic activities that can reduce or oxidize the diacetylenic functions. The problems with diacetylenic phosphatidyl choline are not only that they are difficult but that are also it has been found that these DAPC have problems on photopolymerization. Some other double chained diacetylenic lipids with a fairly flexible head group such as glutamate [132, 174, 175] and amidobutyl-nitrilotriacetic acid derivatized chelator lipid [134, 176] (Figure 4.9) are also being synthesized and characterized. The photopolymerization of diacetylenes is acutely sensitive to the molecular order of crystals and supramolecular assemblies. It was found that diacetylenic fatty acids are polymerizable only in close-packed solid-like monolayers [177-178]. Bilayer membranes of diacetylene lipids are neither photopolymerizable above the lipid phase transition temperature of the membrane nor in small sonicated vesicles where the lipid chain packing is disordered by the sharp radius of curvature of the membrane. The absorption maximas of PDAs are indicative of the length of the polymer chain and the order of the polymer structure. Longer conjugation and higher ordered PDAs exhibit absorption maxima at 650 nm, while shorter and less ordered PDAs such as phosphatidylcholine diacetylene bilayers show absorption at 540 nm.

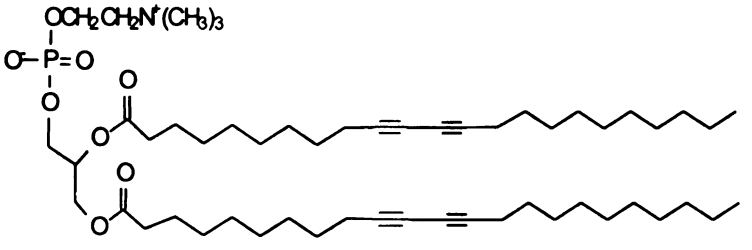
It is unclear why the DAPC systems could not afford extensive polymerization [179]. It probably has something to do with the way that they were prepared. It might also be related



A. The structure of some diacetylenic glutamate lipids (DGL)



B. The structure of a chelater lipid.



C. Structure of a diacetylenic glycerophosphocholine (DAPC)

Figure 4.9. The structures of three types of diacetylene containing lipids in literature.

to the structure of the head group. In any event, this approach of using biomembrane mimic lipids to achieve long range order in polydiacetylene systems is very promising. Some other systems are with simple head group but which possess the self assembly properties of phospholipids are desirable.

In summary, polydiacetylenes are very promising conductive polymers for many applications, including biosensors, optical electronic devices, mutifunctional materials, etc. However, many problems need to be solved before they can reach the actual application stage. The major one is to obtain high long range order of the alkyl diacetylene systems.

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Chapter 5

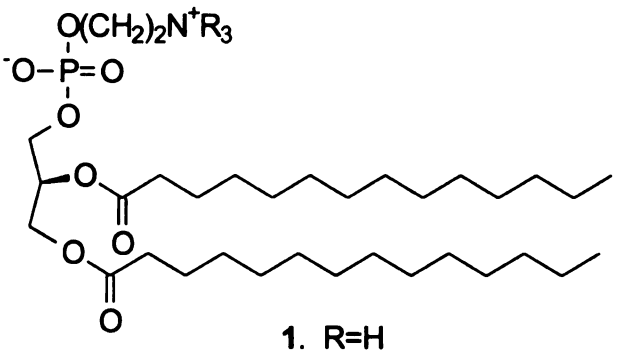
Synthesis and Properties of a Bipolar, Bis-Phosphatidyl Ethanolamine that Forms Stable 2-Dimensional Self-Assembled Bilayer Systems and Liposomes

Abstract

Phosphatidyl ethanolamine is one of the most common naturally occurring phospholipids. The presence of an amino group also makes it one of the most straightforward to functionalize. Unfortunately however, it tends to form micelles and hexagonal clusters instead of forming lamellar systems such as sheets and vesicles (liposome), key supramolecular structures for a variety of biomedical and other technical applications. This has been overcome by synthesizing a dimeric phosphatidyl ethanolamine molecule in which the acyl chains at the 2-position of glycerol are joined at their termini by a carbon-carbon bond thus resulting in a trans-membrane type fatty acyl linkage. Such tail-to-tail bipolar transmembrane lipids are found in the membranes of thermophilic and other extremophilic bacteria. The dimeric phosphatidyl ethanolamine readily forms very uniformly flat self assembled lamellar supramolecular arrays and liposome that are stable at temperatures up to 80°C. The planar systems formed by the new lipid were characterized by atomic force microscopy and the uniformity of the vesicles were observed by laser scanning confocal microscopy. The thermal stability of the phospholipid and the vesicles formed was examined by NMR spectroscopy including proton T_1 measurements as a function of temperature. Fourier transform infrared spectroscopy indicated a much greater order of the alkyl chain in the dimer than in phosphatidyl ethanolamine. This extremely stable and readily functionalizable dimeric phospholipid has potential uses in the fabrication of biomaterials, stable membrane models and liposome drug delivery systems.

5.1. Introduction

There has recently been much interest in the use of phospholipids, phospholipid analogs and other systems that form 2-dimensional lamellar systems in several areas of research and technology including drug encapsulation[1,2], gene delivery [3,4], biocompatibilty[5,6], sensors and specialized receptor surfaces [7,8] and advanced biomaterials [9-14]. Phospholipids serve as the prototypical materials for the design of such surfaces because of their extremely high packing densities and propensity to form lamellar systems. In all of these applications, it is desirable to form a self-assembled lamellar system with some desired functionality on one surface. In the drug encapsulation and gene delivery areas, it is a requirement that the lamellar system have an overall spherical topography to form hollow structures called vesicles or liposome. From the standpoint of chemical functionality, of all of the naturally occurring phospholipid systems, none is more suited for such applications than phosphatidyl ethanolamine **1**. This is because of the presence of the easily derivatizable primary amino group. Unfortunately, because of the small size of its head group, phosphatidyl ethanolamine tends not to form lamellar systems but forms micelles or hexagonal phases instead[15]. It is therefore not possible to make pre-formed lamellar systems and then functionalize them.

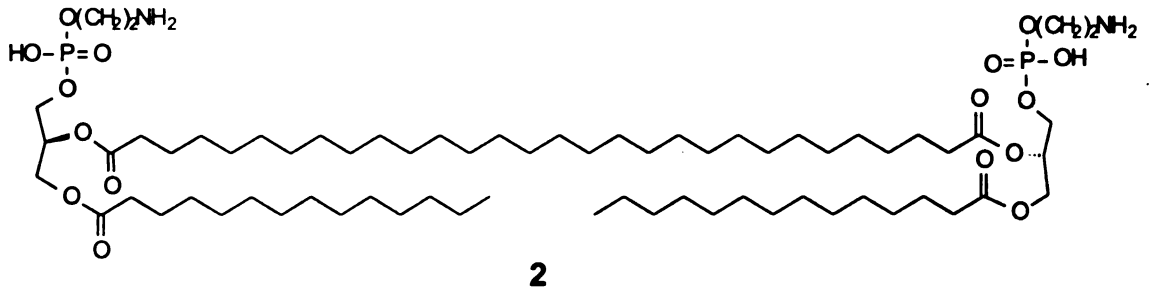


1. R=H

3. R=CH_3

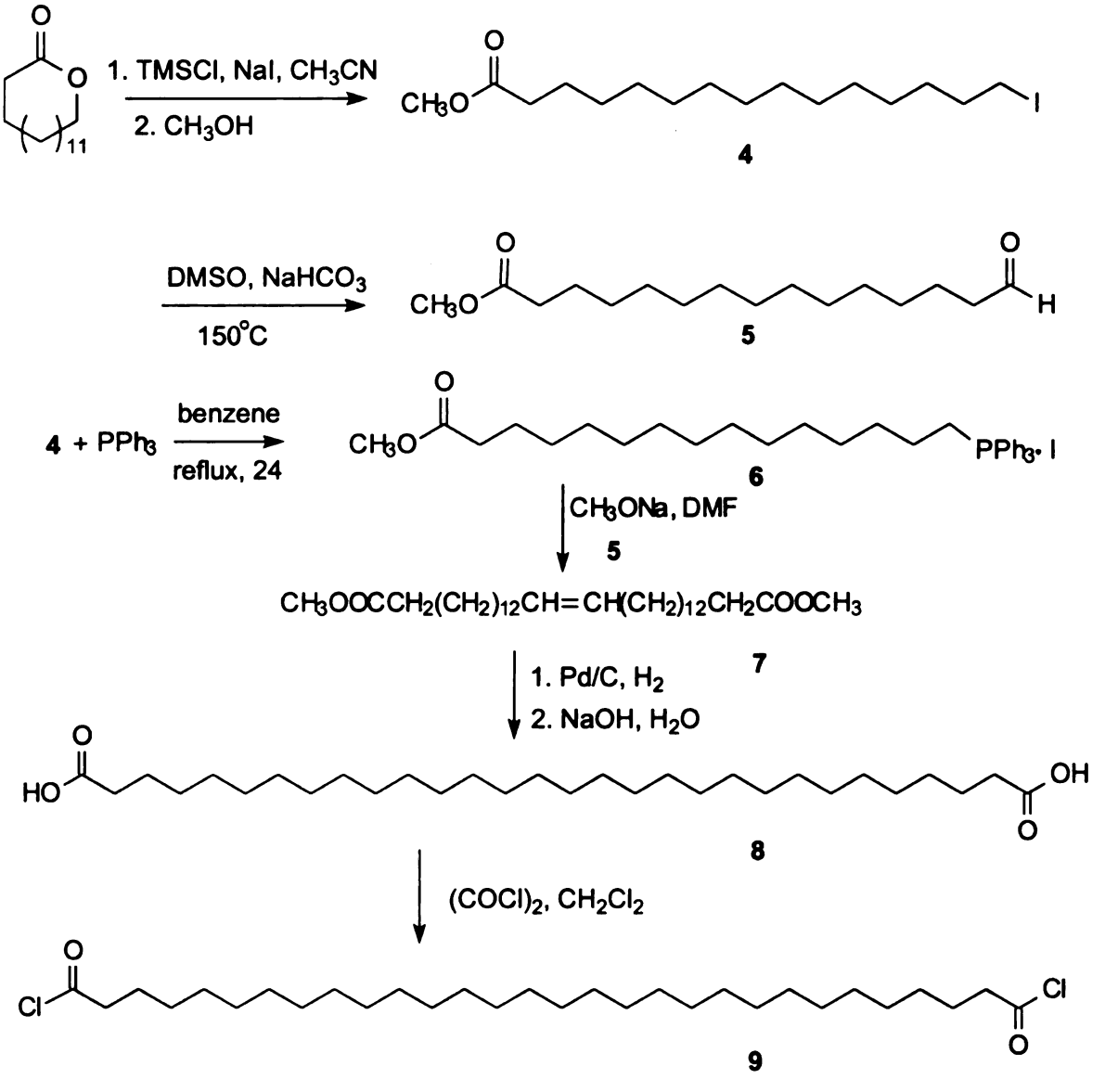
One general drawback to the use of phospholipids in the applications discussed above is the general instability of the supramolecular structures they form. Hence vesicles tend to lyse too easily and leak the materials that are trapped therein. Planar lamellar systems on solid supports often lack sufficient intermolecular cohesive power and are too easily perturbed. Some special device such as a Langmuir-Blodgett trough is often required to form them in the first place. It is therefore important to design and synthesize phospholipid variant structures that contain some extra stabilizing features. The preparation and use of such materials is of special significance to several very different fields.

There are good clues as to how to stabilize the lamellar bilayer structure of phospholipids if one examines the structures of biomembranes of organisms from habitats where extremes of temperature, pH or the presence of deleterious chemical substances persist. Such organisms can flourish at temperatures in excess of 100 °C in geothermally active sites at the bottom of the ocean. For such organisms, one common structural feature of their membrane lipids is the presence of transmembrane fatty acyl components[16-18]. These lipids contain very long (28 carbons or more) α-ω-dicarboxylic acids formed by tail-to-tail joining of lipid fatty acyl chains between the two leaflets of the bilayer[18-19]. The synthesis of lipids such as **2** with the phosphoethanolamine head groups is an excellent target. Such molecules are not known but would combine the good thermal and chemical stability associated with the presence of the very long α-ω-dicarboxylic acid group with the ease of modification of the primary amine group. In addition, any tendency to form non-lamellar systems such as micelles would be suppressed. Because of the stabilization by ionic forces at both ends and the extra

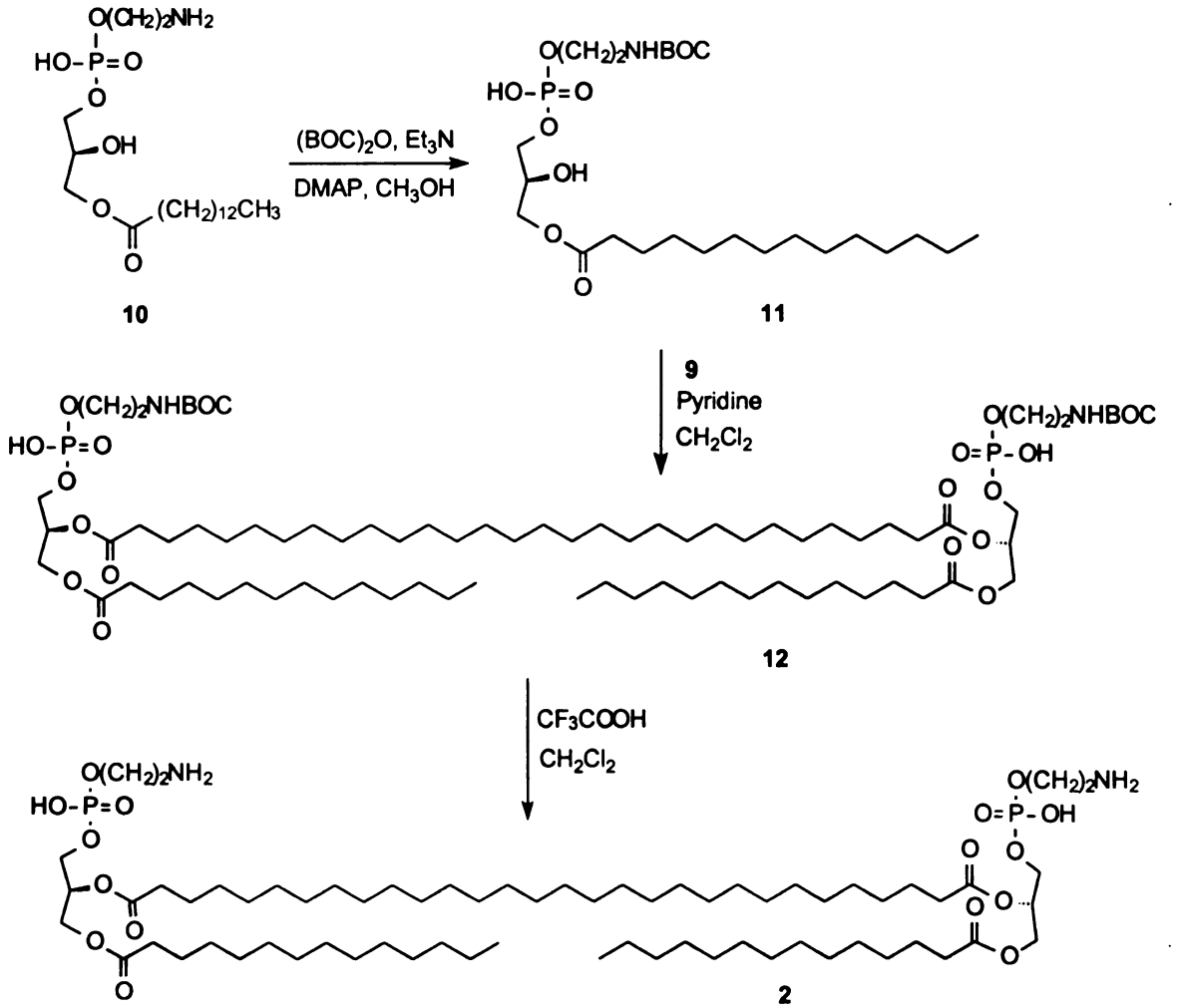


2

stabilization by the very long hydrocarbon chains, it should not be necessary to compress films of these lipids molecules into compact layers using Langmuir-Blodgett troughs. This approach has been used with synthetic bolaform amphiphile models of archaebacteria membrane lipids[20-24] which also contain transmembrane alkyl chains except that they are ether linked to the head groups. These tetra ether bipolar lipids have the advantage of forming lamellar systems that are stable to extreme pH[25], high temperatures[21, 26] and high ionic strengths[27]. However, these models have ether instead of ester linkages connecting the hydrocarbon chains to the head group and lack many of the structural features of biologically important lipids. This might be critical for medical applications. We describe herein the preparation and characterization of the transmembrane stabilized triacotanedioic acid - containing phosphatidyl ethanolamine **2**. The lipid contains normal phosphatidyl ethanolamine head groups and ester linkages. Since archaebacteria lipids contain ether linkages between the hydrocarbon chains and the head groups, release of encapsulated substances by the action of phospholipase A-type activities(esterases) is not possible. This may limit the practicality of using such liposome systems in applications where host enzymes mediate the release of the trapped substance.



Scheme 5.1 The synthesis of triacotanedioic acid **8**.



Scheme 5.2 The synthesis of dimeric phosphatidyl ethanolamine **2**.

5.2. Results and discussion

Synthesis of the transmembrane phospholipid analog 2. The synthesis of **2** involved firstly the preparation of triacotanedioic acid followed by its coupling to a protected *lyso*-lipid. Triacotanedioic acid was prepared from pentadecalactone according to Scheme 5.1. The lactone was converted to the 15-iodoacid **4** and a portion of the acid oxidized to the aldehyde **5** . The aldehyde and iodoacid (protected as esters) were joined by Wittig reaction to form triacotan-15-ene dioic acid dimethyl ester which was converted to triacotanedioic acid dimethyl ester by catalytic hydrogenation. Saponification and acidification yielded the diacid which was converted to the acyl chloride **9** with oxalyl chloride. The transmembrane stabilized phospholipid **2** was then constructed as shown in scheme 5.2. Two molecules (**11**) of amino-protected (t-butyloxycarbonyl) *lyso*-phosphatidyl ethanolamine with a tetradecanoyl group in the 1-position were joined by one molecule of the acid chloride of triacotanedioic acid. The desired compound **2** was obtained after removal of the t-butyloxycarbonyl group with trifluoro acetic acid.

Supramolecular structure, chain packing, and film forming properties. The fully hydrated films formed by compound **2** and a typical phospholipid that forms lamellar systems and vesicles easily (phosphatidyl choline **3**) were studied by laser confocal scanning microscopy. The images were observed using polarized light, dark field, and phase contrast optics. The results are shown in Figure 5.1. The lipid **2** exhibited very different behavior compare to the normal phospholipid **3**. Whereas the latter formed sheets, ribbons and vesicles with a large variation in size, lipid **2** formed only vesicles with a very narrow

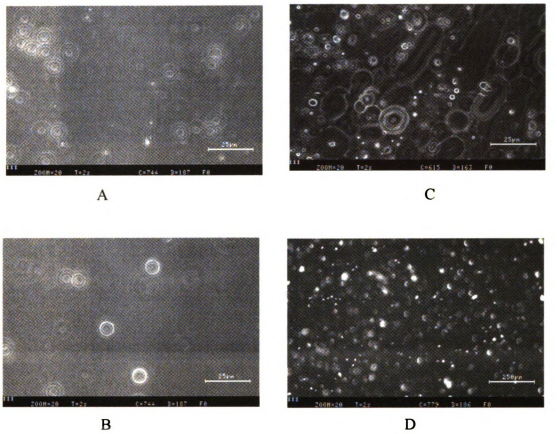
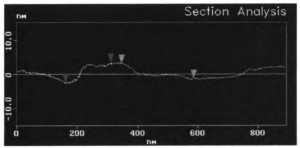
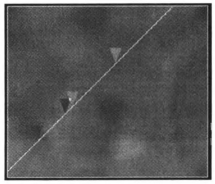


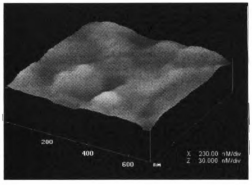
Figure 5.1. Laser scanning confocal microscopy images of hydrated compound **2** and **3**. The images to the left A and B are phase-contrast pictures of **2.** The circular structures are liposomes. Note the uniformity of their size. Image D is a dark field image of compound **2** showing the spherical outline of the liposomes. The image C is a phase contrast picture of **3**. Note the variation in size of the liposomes as well as the presence of several other lamellar structures corresponding to sheets and ribbons.



a



b



c

Figure 5.2. The atomic force micrographs of the thin film formed by compound **2**. (a) is the section analysis over an 800 nm range, (b) is a top view of the same area from which the section analysis was taken and (c) is the surface plot of the same area as above two images.

distribution in size.

The atomic force micrographs (Figure 5.2) of thin films formed by compound **2** on mica plates demonstrated that they were extremely flat and uniform with a maximum surface variation of only 5.8 nm over a distance of 800 nm (0.7%). This ability to form 2-dimensional systems with such low surface variation has much significance for the potential use of lipid **2** in a variety of applications. The presence of the free primary amino head group should allow surfaces modified with this lipid to be further functionalized with a variety of reagents especially peptides and proteins with specific biological functions. The amino groups can also be functionalized with various optical probes and a myriad of other structures. Such surfaces have potential value in nano-fabrication, surface patterning, advanced material science and the construction of biological membrane mimics.

Information about intermolecular chain packing and intramolecular chain conformation of phospholipids and other membrane forming amphiphiles can be obtained by vibrational spectroscopy methods such as Raman and infra-red spectroscopy[28-31]. It is known that for hydrocarbon chains, the intensity ratio of the methylene asymmetric ($2918cm^{-1}$) over symmetric ($2850cm^{-1}$) vibrations is sensitive to the intermolecular chain packing order and the ratio increases with increasing order[31]. Fourier transform infrared spectroscopy experiments were carried out on lipid **2** and on di myristoyl phosphatidyl ethanolamine **1** in both the dry and the hydrated states. The peaks were deconvoluted and quantified by Fourier analysis in conjunction with a curve-fitting routine using a mixed Laurentian and Gaussian

line shape and a peak width parameter that could be adjusted to give optimum fitting. Analysis on the dry films of di myristoyl phosphatidyl ethanolamine gave an area ratio (asymmetric to symmetric) of 1.83 while the value obtained for the dimer was 2.58. The hydrated samples (prepared by forming the films from water by slow evaporation onto a calcium fluoride window) gave a ratio of 1.37 for di myristoyl phosphatidyl ethanolamine and 2.33 for compound **2**. These results demonstrated a substantially higher ordering of the alkyl chains in the dimeric phospholipid compared to di myristoyl phosphatidyl ethanolamine in both dry and hydrated states. In the hydrated state, both lipids showed a small decrease in order but the dimer was still significantly better packed than the normal phospholipid.

Self assembly properties, stability, and liposome formation. The structural order and stability of the supramolecular systems formed by lipid **2** were probed by two different nuclear magnetic resonance (NMR) spectroscopy experiments. Nuclear magnetic resonance spectroscopy is a very useful tool for studying biomembrane systems [16, 17, 20]. The motional freedom of specific groups in the lipid can be determined by measuring the parameter referred to as T_1 or the spin lattice relaxation time. It is also known as the longitudinal relaxation time. This parameter describes the rate of magnetization transfer from a nucleus at its high energy state to its environment via a dipolar coupling mechanism. If the system is rigid and / or ordered, this transfer is effective and T_1 is short. When the system is less rigid or structured, the nucleus may not be close to any other one for long enough (on average) to facilitate good transfer and the value of T_1 is then larger. If T_1 is evaluated as a

function of temperature, a sudden, simultaneous change in its value at any given temperature for more than one signal would indicate a change in phase of the system. T_1 relaxation times of **2** in deuterated DMSO at various temperature were measured. The curves for T_1 dependence on temperatures (1/T) are shown in Figure 5.3. As expected, the terminal

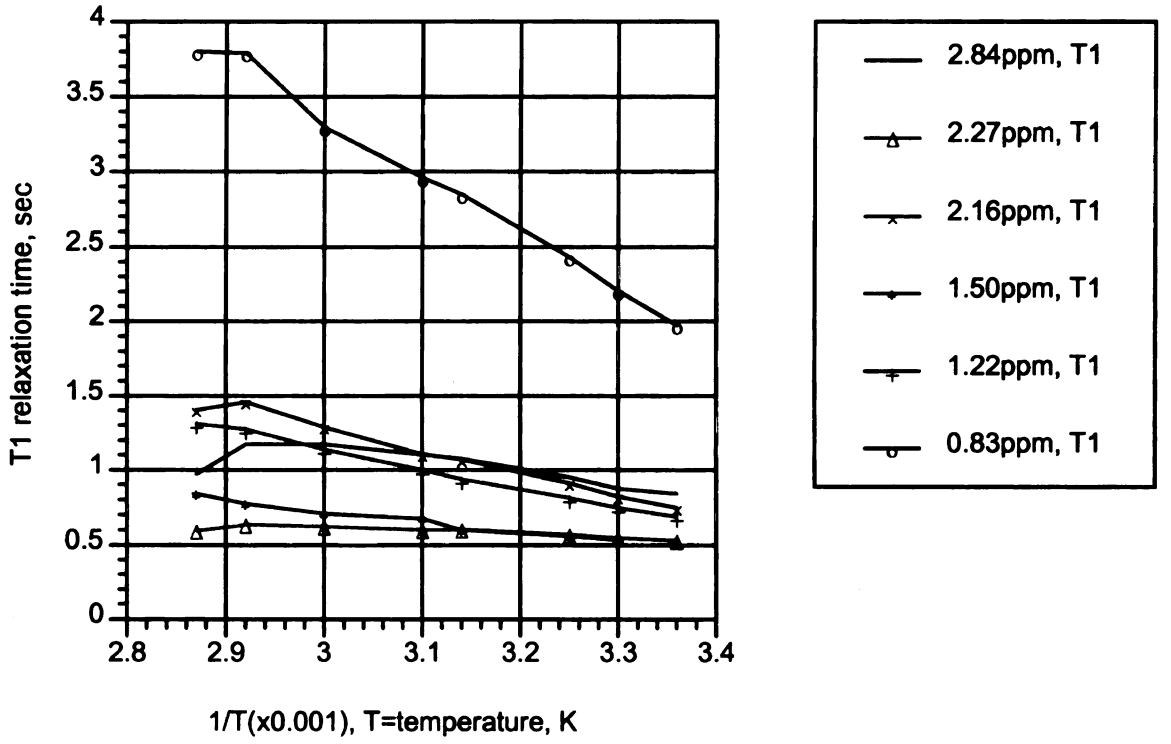


Figure 5.3. The relationship of T_1 relaxation time versus the reciprocal of temperature(1/T) for bulk methylene (1.22ppm), methyl (0.83ppm), methylenes next to amino group (2.84ppm), methylene α to carbonyl groups (2.27ppm and 2.16ppm) and methylene β to carbonyl groups (1.55ppm).

methyl groups displayed the largest T_1 values among all the groups present in the lipid. In general, the value of T_1 increased with temperature. There was a discontinuity in the plot of T_1 vs temperature for the methyl signals at 70°C indicating a phase transition. No discontinuity was observed for the curve corresponding to the bulk methylene groups up to a temperature of 75°C. Two signals corresponding to the methylene protons adjacent to the ester carbonyl groups were observed. One appeared at 2.16 and the other at 2.27 ppm. The nuclei giving rise to the first signal had a significantly larger T_1 value indicating that it was most likely due to the protons on the more mobile 14 carbon chain. A discontinuity was observed for both signals at 70°C. In the head group region, the signals for the methylene group adjacent to the amino group displayed a larger T_1 value indicating that it was more mobile than the other methylene group. A discontinuity at 70°C was also observed. The concerted abrupt change in T_1 at 70°C is indicative of a significant change in phase at this temperature. It indicates that there is a high degree of supramolecular organization in lipid **2** even in dissociating solvents and at temperatures way above those that normal lipids function .

Liposome or vesicles have been explored for drug delivery models because of their ability to encapsulate various component inside. Because of their instability, liposome made from typical lipids are not good drug carriers and many methods have been developed to improve the stability of liposome [32,33]. Lipids isolated from thermophilic bacteria were used to prepare liposome which proved to have increased resistance to leakage[34-36]. In order to explore the stability of liposome formed from the stabilized phospholipid dimer **2** as a drug delivery

system, the ability to trap water (H_2O) and ferric ions (paramagnetic relaxing agents) inside such liposome was studied by proton-NMR spectroscopy. The spectra of a suspension of these liposome were obtained in D_2O with increasing temperature with the expectation that when the temperature was high enough to cause leakage, the ferric ions would leak out and cause a sudden increase in line width of the bulk external water line. The liposome were prepared according to literature methods [37-40] with modifications as described in the experimental section. There was one striking feature of the NMR spectra (Figure 5.4). The signal for the water line at 20°C was broad and triangular in shape indicating that the water was restricted in motion and confined to a region with significant chemical shift anisotropy. This was very reminiscent of the peak shape for phosphorous signals in phospholipids [41, 42] where the shape stems from the same cause. Closer inspection of the peak revealed that there were several envelopes superimposed on each other. These signals were due to trapped water in different environments inside of the vesicles. As the temperature was increased, the envelopes became more discreet as the individual peaks narrowed because of increased mobility of the water molecules. As the temperature was increased, there was also a gradual exchange of H_2O from inside for D_2O from outside of the vesicles. This was indicated by a gradual disappearance of some of the signals. The presence of multiple water peaks even at 80°C indicated how resistant to breakage and leakage the liposome were even at that temperature. Breakage would have led to an immediate reduction of all of the signals to one broad signal. It was clear from the experiments that the ferric ions were still trapped inside of the vesicles even at this temperature.

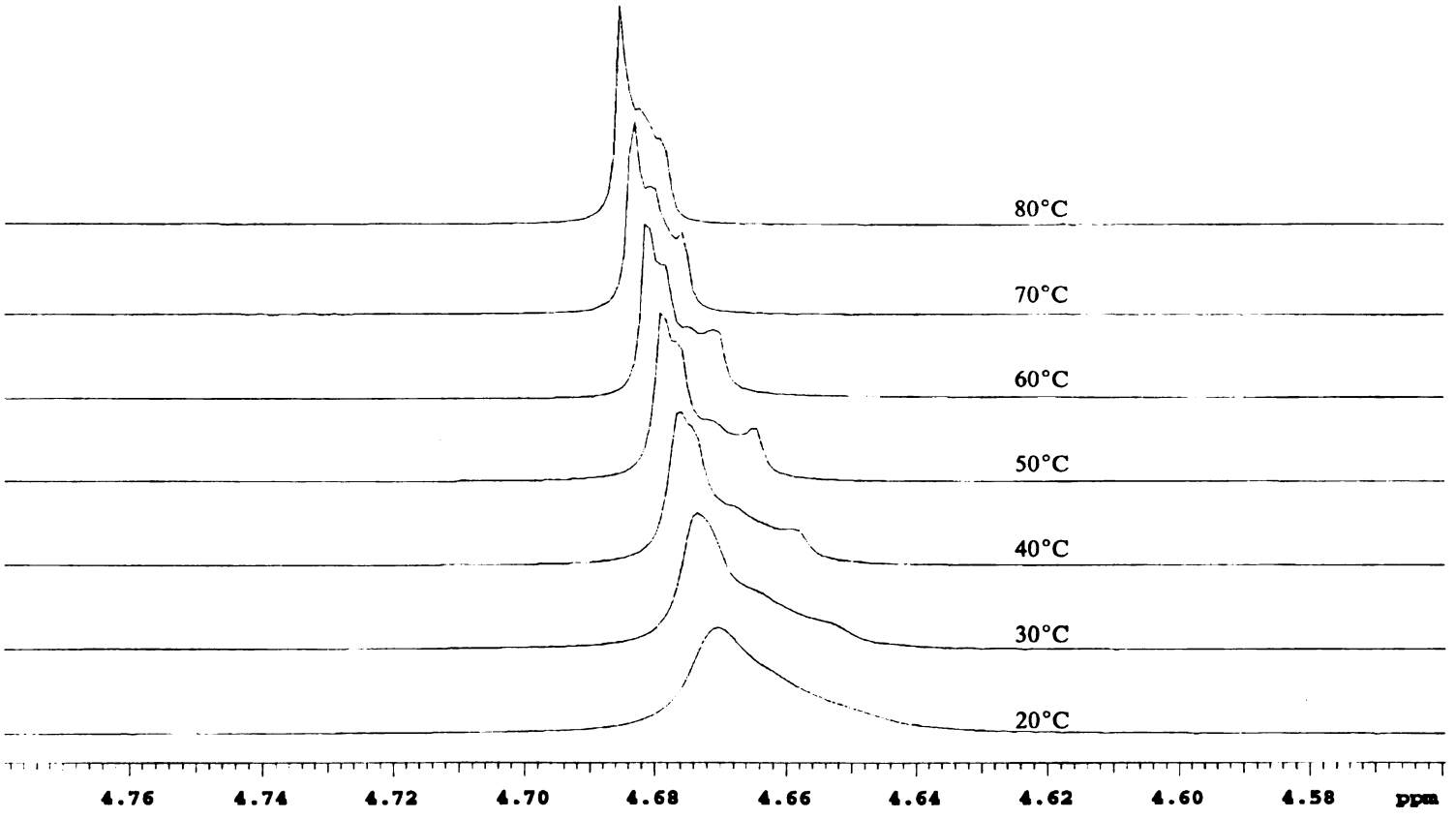


Figure 5.4. The 300MHz ^{1}H NMR spectra of the water signals in a liposome suspension of compound **2** in PBS-deuterium oxide at different temperatures.

5.3. Conclusion

In these studies, we have synthesized a new membrane-spanning dimeric phosphatidyl ethanolamine molecule that readily forms stable vesicles of uniform size as well as flat thin lamellar film with very small variation in surface features. The motion of the lipids in these systems is considerably more restricted and the alkyl chains are more ordered than those in typical phospholipid systems in both the dry and hydrated states. The lipid has good thermal stability and high phase transition temperature. The vesicles fabricated from this tail-to-tail dimer lipid are extremely uniform in size and very stable to high temperatures. The results we obtained here are important because phosphatidyl ethanolamine does not form vesicles on its own. The synthesis of phospholipids in general is difficult and the synthesis of ether-containing archaebacteria-type lipids even more so. Lipids from archaebacteria are not generally available in significant quantities. Naturally occurring phospholipids are available in substantial quantities and the strategy we adopt here can be employed if the *sn*-2 fatty acyl groups were removed with a phospholipase A enzyme. The approach we describe here is generally applicable to other lyso- phospholipids provided that the head groups can be protected. These ultra-stable membranes can be further developed for fabricating stabilized drug delivery systems. They also have potential for the preparation of biocompatible surfaces and the fabrication of molecular devices through modification of the primary amino group perhaps with metal-binding functional groups. There are also possibilities for other surface chemistry applications in material science and biotechnology.

5.4. Experimental section

5. 4. 1. Characterization of physical properties

Atomic force microscopy. These analyses were performed using a Nanoscope III instrument operating in contact mode. For these measurements, the compound **2** was dissolved in chloroform- (0.5-1% solutions) and ~ 10 μL used to coat freshly cleaved mica plates spinning at 200 rpm to effect uniform coating.

Laser scanning confocal light microscopy. These experiments were performed on a Zeiss 210 instrument with a 488 nm laser. Images were obtained in the dark-field, phase contrast and polarization modes. For the polarizing mode experiments, an analyzing cross-polarizer was placed on the objective lens and rotated until light cancellation. For film preparation, The compound was dissolved in chloroform, and deposited on a microslide, after the solvent evaporated, a drop of water was added on top of the film, the film was hydrated for two-three days. Before the measurements, a drop of water was added on top of the film which was then covered with a round cover glass. For comparison, a sample using PC (compound **3**) was prepared and observed by the same methods.

Vesicle stability experiment. The vesicles were prepared according to methods in the literature[37-40] with modifications. About 1-2 mg of compound **2** was suspended in 0.4 ml phosphate buffer solution (PBS) (Dulbecco, pH=7) in a small vial, about 50 micro liter of 0.1M $MgSO_4$ solution and 3-4 mg of ferric citrate were added to the vial. The mixture was then sonicated and vortexed alternatingly for about 45 minutes. It was then allowed to stand

at room temperature for 20 hours before being dialyzed against PBS. Just before the NMR spectroscopy measurements, the water was exchanged with PBS made up in deuterated water. About 50 μL of this liposome suspension was added to 0.6 ml of PBS in deuterated oxide for the NMR study.

FT-IR experiment. The FT-IR experiments were performed on Nicolet 710 spectrometer. For the dry state measurements, the sample was dissolved in chloroform and the solution was transferred to a sodium chloride window and the solvent allowed to evaporate. For measurements in the hydrated state, the sample was dissolved in water which was sonicated to form a suspension, a sample of which was placed on a calcium fluoride window. The suspension was air dried for 3-4 hours to remove the excess water. Heating and reduced pressure is generally required to remove the hydration water. The deconvolution of the methylene symmetrical and asymmetrical absorptions and the calculation of peak area were performed using Galacitic Peaksolve program by Galactic Industries Corporation.

T_1 relaxation experiments. Proton T_1 measurements were performed on a Varian 300 MHz NMR spectrometer. Measurements were made at temperatures of 25, 30, 35, 40, 45, 50, 60, 70, 75°C. The parameters for each temperature were the same. 14 points were collected for each relaxation curve at each temperature. The pulse sequence used was π-τ-$\pi/2$, pw 90=12.7 msec. The solvent used was deuterated-DMSO.

5. 4. 2. Synthesis

15-iodo-pentadecanoic acid methyl ester 4. To a 1000 ml round bottom flask was added pentadecalactone 22.0 g (0.915mol), trimethyl silyl chloride 23.2 ml (0.183mol), sodium iodide 41.2 g(0.275 mol), and 220 ml of acetonitrile. The mixture was left stirring at 50 °C for 14 hours, 220 ml of methanol was added to the reaction mixture, the heat was turned off and the mixture left for another 3 hours. The dark brown mixture was filtered and the liquid was concentrated by rotatory evaporation to remove all solvents. The residue was dissolved in chloroform, washed with 50 ml of saturated sodium thiosulfate and the organic layer dried with sodium sulfate. Upon removal of solvent, a white to light yellow solid was obtained. 34.6 g (99%). Mp. 44-45 °C. ^{1}H NMR($CDCl_3$, 300 MHz), δ 3.64 (S, 3H), δ 3.17 (t, 2 H, J=6.9-7.2 Hz) , δ 2.28 (t, 2 H, J=7.5-8.4 Hz), δ 1.80 (q, 2H, J=7.2 Hz), δ 1.59 (q, 2H, J=7.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 174.29, 51.40, 34.09, 33.55, 30.49, 29.56, 29.55, 29.51, 29.41,29.39, 29.23,,29.13,28.52, 25.72, 24.93, 7.27. IR (NaCl, $CHCl_3$, wavenumber, cm^{-1}), 2916.7, 2851.2, 1734.2, 1473.8, 1457.2, 1250.0, 1202.2, 1169.0, 756.2.

15-oxo-pentadecanoic acid methyl ester 5. A mixture of 4 g sodium hydrogen carbonate and 60 ml of dry dimethyl sulfide was heated to 150°C under dry nitrogen. To this mixture 5.32 g (13.9 mmol) of compound **4** was added and heating and stirring continued for 4 minutes. The flask was cooled quickly and the mixture was poured into water which was stirred for several hours. The white solid that formed (the aldehyde) was filtered out or recovered by extraction 4 times with ether. The yield of aldehyde **5** was 3.70g (98.6%). The aldehyde can be purified by chromotography (solvent Hexane: acetate =7:1) The yield

after purification is 3.20 g (86.5%). ^{1}H NMR($CDCl_3$, 300 MHz), δ 9.74 (t, 1H, J=2.0 Hz), δ 2.40 (d't, 2 H, J=7.2 Hz, 2.0Hz), 2.28 (t, 2H,7.5Hz). ^{13}C NMR ($CDCl_3$, 75 MHZ), δppm 202.84, 174.28, 51.36, 43.86, 34.06, 29.52, 29.37, 29.29, 29.19, 29.09, 24.90, 22.04.

Synthesis of the triacontanedioic acid 8. Preparation of the triphenyl phosphine salt **6** : The methyl ester iodide **4** 7.64g (0.02 mol) and 10.48 g (0.004 mol) triphenyl phosphine were stirred in benzene under refluxing condition for 24 hours after which the solvent was removed by vacuum distillation. The residue was taken up in diethyl ether and the solution stirred vigorously for at least one hour. The mixture was filtered to remove the triphenyl phosphine in ether and the white solid was collected and washed with ether again to ensure all the excess triphenyl phosphine to be removed. The product **6** was dried under vacuum oven for 24 hours and the yield was 11.6 g (90%). The salt **6** (6.4 g) and anhydrous dimethylsformamide 20 ml were mixed in a round bottom flask. Sodium methoxide (0.6g) was added very quickly to the solution which was stirred and cooled in an ice bath for 10 minutes. After this time, then 0.45 g of freshly prepared aldehyde **5** dissolved in 10 ml of dry DMF was added to the mixture in a dropwise fashion under dry nitrogen. The temperature was maintained at 0-5°C during the addition. The reaction mixture was stirred for 24 hours, after which TLC analysis indicated that the reaction was essentially completed. The reaction mixture was diluted with water and extracted with hexane several times. The crude product in the hexane extract was purified by flash column chromatography (hexane: ethyl acetate= 9:1). The product was a white solid. Yield: 0.74 g (87%). ^{1}H NMR($CDCl_3$, 300 MHz), 5.40-5.28ppm (m, 2H), 3.66ppm (s, 3H), 2.30ppm (t, 4H, J=7.5Hz), 2.07-

1.91(m, 4H) 1.68-1.54ppm (m, 4H), 1.25(s, broad, 40H). ^{13}C NMR ($CDCl_3$, 75 MHz), 174.32, 129.85, 51.41, 34.08, 29.75, 29.63, 29.56, 29.43, 29.29, 29.24, 29.12, 27.17, 24.93. The ester was hydrogenated (50-200 psi) using pd-C (10%) in ethanol to give the saturated compound, which upon soaponification and acidification of the sodium salt, gave the diacid **8** (0.56 g) as a white solid. The overall yield was 80% for these two steps. ^1H NMR (DMSO, 300MHz) 2.149(t, 4H , J=7.2Hz), 1.52-1.36(m, 4H), 1.32-1.12(s, broad, 48H). ^{13}C NMR (DMSO, 75MHz), 175.65, 34.50, 29.70, 29.52(m), 29.32, 25.30. IR($CHCl_3$, NaCl) 3046(broad), 2915, 2848, 1694, 1471, 1462, 1434,1408, 1284, 1109, 897.6, 718.5. MS Calc $C_{30}H_{58}O_4$ 482 ES(+) 505 ($M+Na^+$).

Preparation of compound 2. The Boc protection of lipid **9**. 0.55g (1.29 mmol) phospholipid 1-myristoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine **11** dissolve in 100 ml of a 1:1 chloroform and methanol mixture. $(Boc)_2O$ 0.6 g and 0.02 g of dimethylamino pyridine, 0.3 ml of triethylamine was added to the reaction mixture, stirred overnight. The solvent was removed, the colorless liquid was washed with 0.1N HCl once , saturated sodium bicarbonate solution once, then by brine. The organic layer was dried overnight by sodium sulfate. After removal of the solvent, and dried in vacuum oven for 24 hours yielded a light yellow semi-solid 0.65g (95 %). ^1H NMR(CD_3OD, 300 MHz), δ 4.07ppm (dd, 1 H, J=4.5 Hz, 11.7Hz), 3.99 ppm (dd, 1H, J=5.7Hz,11.7Hz), 3.85 (m, 1H) (3,01-3.89), 3.72-3.80 ppm (m, 4H), 3.16 ppm (2H, t, J=5.4Hz) 2.24ppm(2H, t, 7.5Hz), 1.44-1.57ppm (2H, m), 1.32ppm (s, 9H), 1.18(s, broad, 20H), 0.79 (t, 3H, 6.6Hz).

Dicarboxylate acid (0.12g, 0.25mmol) **8** in 10 ml of oxalyl chloride and 5 ml of dichloromethane, was stirred for 12 hours. The solvent was removed quickly and the resulting acid chloride **9** was dissolved in 5 ml dry dichloromethane and added to a round bottom flask which contained phospholipid **11** (0.32 g, 0.61 mmol), 5 ml dry pyridine and 10 ml dichloromethane. The mixture was stirred for 24 hours protected from moisture by a drying tube. The solvent was removed, the crude product was taken up in chloroform and washed with sodium bicarbonate solution. The organic layer was dried and the solvent was removed. The crude product was purified by flash column chromatography. (chloroform:methanol=7:3). The purified compound **12** was obtained as a white solid 0.24g (65%). ^{1}H NMR(CD_3OD, $CHCl_3$= 5:1) ppm , 5.22 (s, 2H), 4.25-4.10(m, 4H), 4.10-3.82(m, 8H), 3.40-3.25(4H, overlap with D-methanol absorption), 2.35(t, 8H, J=7.5Hz), 1.61(m, 8H), 1.44(s, 9H), 1.29(s, 88H), 0.90(t, 6.8Hz). ^{13}C NMR($CDCl_3$, DEPT) 68.8, 66.9, 64.5, 41.0, 34.0, 31.9, 29.7, 29.5, 29.4, 29.2, 28.9, 28.4, 24.8, 22.7, 14.1. IR($CHCl_3$, NaCl) 3380, 2929, 2856, 1743, 1670, 1524, 1464, 1365, 1246, 1173, 1106, 1067, 954.1.

A sample of **12** (~50mg) was deprotected by treatment of a solution in 3ml of dichloromethane with 3 ml of trifluoroacetic acid. This solution was stirred at room temperature for 4 hours and the solvent and other volatile material formed during the reaction was removed on the rotatory evaporator. Compound **2** was obtained as an off-white solid. The removal of the protecting group was completed as judged by NMR spectroscopy. ^{1}H NMR(CD_3OD, $CHCl_3$= 5:1) 5.24 (m, 2H), 4.30-3.68(m, 12H), 2.40-2.24 (m, 8H), 1.61(m, 8H), 1.27(s, 88H), 0.88(t, 6H, 6.6Hz). ^{13}C NMR($CDCl_3$, DEPT) 33.9, 31.9,

29.7, 29.3, 29.2, 24.8, 22.6, 14.0. IR($CHCl_3$, NaCl) 3397, 3240, 2919, 2851, 1738, 1682, 1468, 1207, 1140, 1078, 1024, 800.0, 771.8, 723.4. MS: Calc. MW for $C_{68}H_{134}N_2P_2O_{16}$ 1297 (ES-) 1296(M-1)

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Chapter 6

Synthesis and Properties of Chiral Self-Assembling Lamellar Polydiacetylene Systems with Very Long Range Order

Abstract

Two new, very accessible, chiral, self-assembling phospholipid analogs containing diacetylenic units in the middle of their acyl chains were prepared by very simple and highly efficient routes. Films were cast and then polymerized by activation with UV light or doped with iodine. X-ray diffraction, NMR spectroscopy measurements and molecular mechanics analyses using the MM3 force field indicated that the acyl chains are closely packed in a parallel fashion. The new molecules and the polymerized films derived from them were characterized by a variety of physical methods including confocal polarized light microscopy, atomic force microscopy, and near infrared spectroscopy. These analytical methods showed that the un-polymerized molecules are able to form lamellar systems with very long range order. They formed very uniform, extremely flat, thin films which are readily polymerized to give extensively conjugated systems which absorbed well out into the near infrared region unlike typical polydiacetylenes. The propensity of the new molecules to form such well-packed structures arises from their resemblance to diacyl *glycero*- lipid with the π-stacking interaction from the diacetylene units giving extra stabilization. The results indicate that this is an excellent approach for preparing ordered polydiacetylene systems for use in designing advanced materials.

6.1. INTRODUCTION

There is an increasing amount of interest in the design of planar lamellar systems containing conjugated polydiacetylene functions. These systems are known to display several interesting properties that could lend themselves to the fabrication of a variety of devices[1, 2]. For instance, they display mechano-optical effects in which compressing the polydiacetylene layers lead to a change in color of the films[3, 4]. In other experiments, attaching a carbohydrate molecule to a polydiacetylene layer resulted in a change in color when viral particles bound to the carbohydrate[5-7]. Polydiacetylene layers also demonstrate color changes in response to alterations in temperature[8-10], pH[11] and on exposure to some solvents[12, 13]. Polydiacetylene is well recognized for its high electrical conductivity and high third order non linear optical property. Unfortunately, it forms fibers not films and its high insolubility and general physical intractability makes it unsuitable for many applications especially when lamellar systems are desirable. Under favorable circumstances, the use of amphiphilic molecules with acetylene groups in the hydrocarbon chains leads to film formation[14, 15]. However such molecules often contain only one hydrocarbon chain and they have a tendency to form micellar systems. In order to obtain suitable films, the chains then have to be anchored to surfaces [16-19] or Langmuir-Blodgett troughs have to be employed [20-24]. The problem with the ordering of acetylenic thiols on gold and other metal surfaces is the difficulty in ensuring that the chains are aligned so that the alkyne groups are in a proper orientation and close enough to allow the polymerization process . This is difficult because imperfections on the metal surface of only a few atoms in dimension force adjacent chains to be at different heights thus separating the acetylenic groups by too great a distance. A

substrate-independent way of ordering alkyl chains with diacetylenic functions is therefore highly desirable. Molecular self-assembly has much promise in this area.

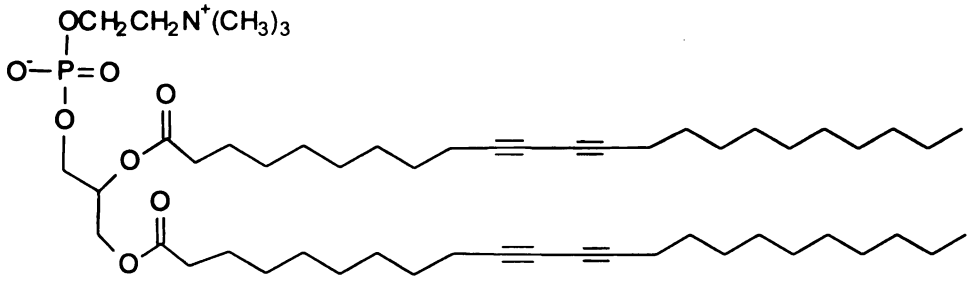


Figure 6.1. The structure of a typical phospholipid (phosphatidyl choline) containing diacetylenic fatty acyl groups.

Phospholipids readily form stable lamellar systems or other highly ordered structures. The inclusion of conjugated diacetylenic groups at the same position in each acyl chain of a phospholipid chain[25, 26] (figure 1) should give ideal self-assembling units which can be polymerized to form highly organized, stable, 2-dimensional systems containing a conducting polydiacetylene layer. Such phospholids have been prepared and the physical properties characterized. However, the synthesis of phospholipids is extremely laborious. These phospholipids containing diacetylene function in the middle of the chains were reported to form tubules under certain conditions[27, 28]. Another approach that has been tried is to use microorganisms to carry out the integration of fatty acids containing diacetylenic functions into phospholipids. Using this strategy, as much as 90% integration of diacetylenic fatty acids into microbial phospholipids was obtained [29]. There are some problems with this approach however. Because the membrane is only two molecules thick and just surrounds

the cell, the actual amount of material recovered per gram of cell mass is extremely small. In addition to this, the lipid species made by any one microorganism are extremely diverse and may include neutral, and negatively charged headgroups with different structures. It is a challenge to separate species with only one type of headgroup and, even then, there is a tremendous amount of diversity in the fatty acid species that are derived from the normal microbial metabolism. Also microorganisms contain a myriad of membrane-associated enzymatic activities that can reduce or oxidize the diacetylenic functions.

Based on the above discussion, it is clear that only synthetic approaches have the potential for producing phospholipids or phospholipid analogs containing diacetylenic functions in the fatty acyl chains and which have a high degree of chemical integrity. Because of the difficulty in preparing phospholipids and hard to prepare on large scale, simpler analogs which still contain the critical structural elements of phospholipids, a chiral 1,2-diacyl moiety and a polar headgroup, are desirable. Even more desirable are phospholipid analogs that have the general structure of the lipids found in bacteria that inhabit environments with extremely high temperatures or extremes of pH. The lipids of such organisms contain two transmembrane hydrocarbon chains that are linked to a headgroup at either end. In some bacteria the linkages are ether linkages but in others[30, 31], they are ester functions (figure 2). Such molecules should self-assemble to form extremely stable lamellar systems without the aid of devices such as Langmuir-Blodgett troughs. They would be excellent targets for the preparation of planar polydiacetylenic systems. We therefore embarked on the synthesis and characterization of compounds **1** and **2**. Like typical phospholipids, they have two long acyl

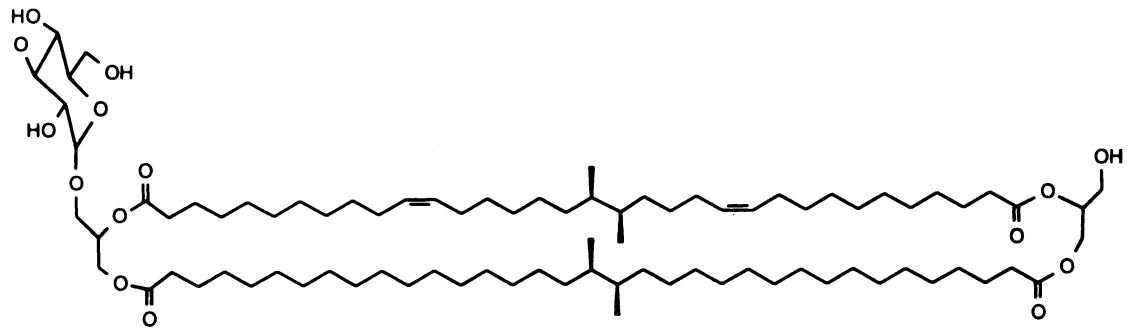
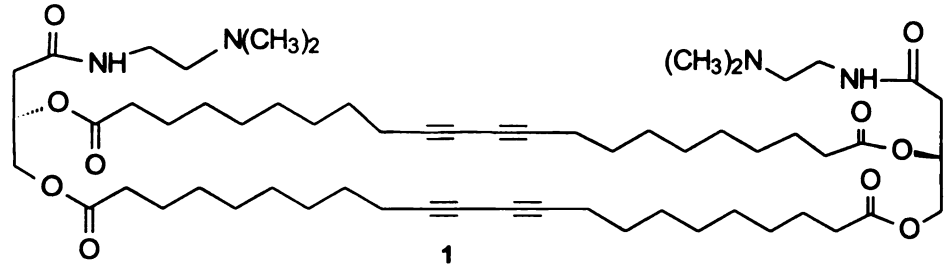
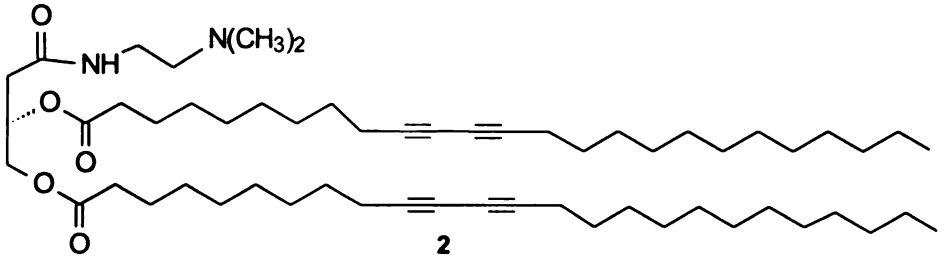


Figure 6.2. The structure of a membrane lipid from a thermotropic bacterium.

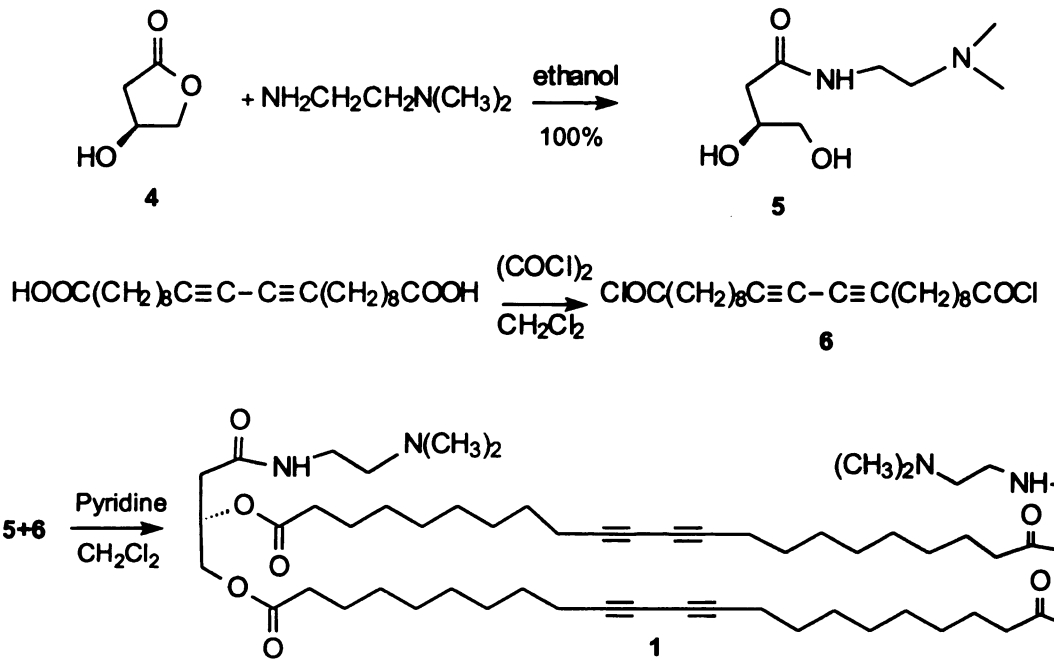
chains attached to a chiral 1,2-diol. They also have a dimethylaminoethane group similar to the one found in phospholipids such as N, N-dimethylphosphatidyl ethanolamine. When the diacetylenic units polymerize, chiral two dimensional polymers will be obtained and they are expected to be stabilized by polymerization and self organization. (S)-3-Hydroxy-γ-butyrolactone was the source of chirality for compounds **1** and **2**. The synthetic routes are outlined in schemes 1 and 2 respectively.



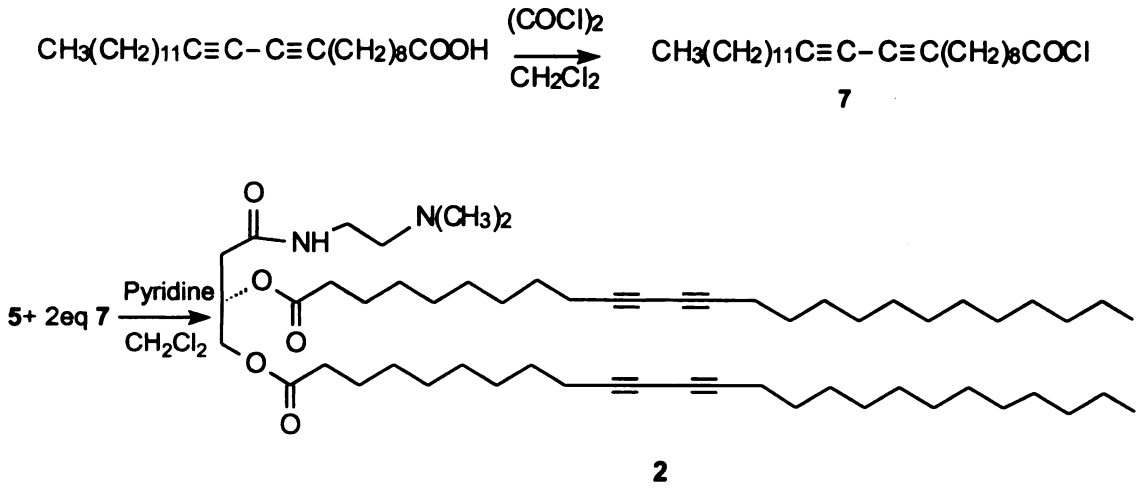
1



2



Scheme 6.1. The synthesis of bilpolar molecule **1**.



Scheme 6.2. The synthesis of lipid analog **2**.

6.2. RESULTS AND DISCUSSION

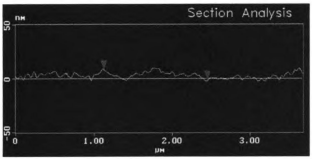
Preparation of compounds 1 and 2. The chiral, diacetylenic phospholipid analogs **1** and **2** were obtained in very good yield . In the case of **2** the preparation necessitated only three steps all of which proceeded in good yield. The first step to form the N, N-dimethylaminoethyl dihydroxybutyramide was essentially quantitative. In the case of compound **1** the only possible complication was the formation of the isomeric structures in which the acyl chain was linked to the primary hydroxyl group on one side and to the secondary group on the other. This did not occur since only the intermediate species (**3**) in which a single chain was linked to the primary position of an N-alkyl dihydroxybutyramide on each side was detected under conditions in which the reaction was only partially complete. The identity of the intermediate species as **3** was readily determined by ^{1}H-NMR spectroscopy. The signals for the methylene protons adjacent to oxygen appeared at 4.30 and 4.10 ppm, substantially downfield from their original positions in the starting diol. The methine proton signal was not shifted from 4.06 ppm. There were no signals corresponding to un-esterified primary alcohols in the spectrum although a substantial degree of under-esterification of the secondary position was evident from the spectra of the total mixture.

Supramolecular Structure, Stability and Long-Range Order. The parallel orientation of the two acyl chains of both **1** and **2** was quite evident by NMR spectroscopy from the coupling constants of the methylene protons adjacent to the acyloxy group. The coupling constants between these protons and the neighboring methine proton are indicative of the relative orientation of the acyloxy groups on these two carbons. The splittings were similar

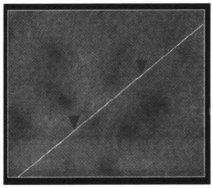
to those observed in a similar molecule where the acyl chains bore pyrenyl substituents at their termini and were known to be parallel by virtue of the fact that the pyrenyl groups displayed excimer emission[32]. For compound **2,** the signals for these methylene protons were two mutually coupled doublet of doublets . One appeared at 4.30 ppm (3.6Hz and 12.0 Hz) and the other at 4.12 ppm (5.8 Hz and 12 Hz). These values were also similar to those observed for the coupling constants for the 1 and 1' protons with the 2-proton of the glyceryl moiety of diacyl glycerols. Similar results were observed for **1** although the signals were considerably broadened in this case because of slower rotational averaging in this latter system. The parallel nature of the acyl chains was also confirmed by X-ray diffraction analysis of the lipid systems in a water / alcohol system. Reflections at 4.0 Angstroms in the case of lipid **2** and 3.4 Angstroms in the case of **1** were observed. These correspond to alkyl chain separations and the smaller value for **1** is expected because in this molecule, the chains are held together at both ends. These results in conjunction with molecular mechanics calculations supported the conformation of the molecules as shown in Figure 6.3.

The long-range order of the 2-dimensional systems formed by **1** and **2** was examined by a combination of atomic force microscopy and laser scanning confocal microscopy using phase-contrast, dark field and polarizing optics. As was mentioned earlier, X-ray analysis of the fully hydrated systems indicated that the hydrocarbon chains were arranged in a stacked parallel order as is expected in lamellar systems. A reflection at 60 angstroms corresponding to slightly less than twice the width (34 Å) of a monolayer as measured from the molecular models was observed. This indicated that compound **2** formed slightly interdigitated bilayers.

Figure 6.3. Space filling models showing the conformations of compounds **1**(left) and **2** (right) as indicated by NMR spectroscopy and molecular mechanics calculation. Red is oxygen, blue is nitrogen, gray is carbon and light blue is hydrogen.



A



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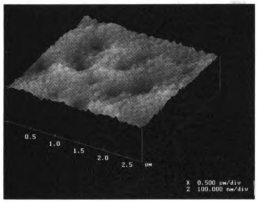
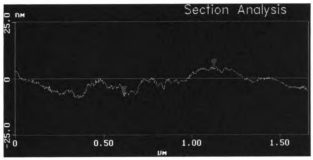
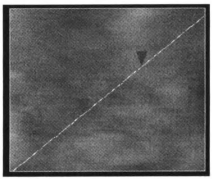


C

Figure 6.4. AFM images of a film formed by compound **1** on a freshly-cleaned mica surface. A. Section analysis over a 4 μm length. B. The top view of the same area as in A. C. Surface plot of the same area.



A



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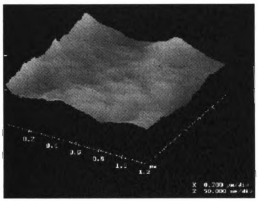

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Figure 6.5. AFM images of a film formed by compound **2** on a freshly-cleaned mica surface. A. Section analysis over a 2 µm length. B. The top view of the same area as in A. C. Surface plot of the same area.

Atomic force micrographs (Figures 6.4 and 6.5) of layers of compounds **1** and **2** respectively prepared on mica plates demonstrated they formed flat films with a surface variation of only 9.85nm over a distance of 3μm (0.3%) for **1**, and 12nm over a1.5μm range (0.8%) for layer **2**.

Information on the order and supramolecular organization of the two systems could also be obtained by analyzing images obtained from optical laser scanning confocal microscopy. The most useful information is obtained from images using polarizing optics. If the layers are ordered relative to the plane of the slide and the polarized laser light is blocked by a cross polarizer after going through the layer then only a black background would be observed. If, however, there are regions of disorder in the film, domains within the layer where the molecules are oriented differently or the layers buckle, then the plane of polarization of the laser light is rotated and is no longer canceled by the cross polarizing filter. Areas of brightness are then observed in these defect regions. As can be seen in figure 6, the polarized light micrographs from both systems (6a, 6c) indicated a very high degree of order with only a few point defects. The dark field images (6b, 6d) show the film edges and the topological properties of the films. Significant defects were only observed at the edge of the layers where there was a discontinuity or curvature as the film bent to contact the glass.

Based on the 3-dimensional structures proposed for **1** and **2**, a packing model which is consistent with their long-range stability and high packing densities of these systems could be derived. The model is one that maximizes the contact between the hydrocarbon chains

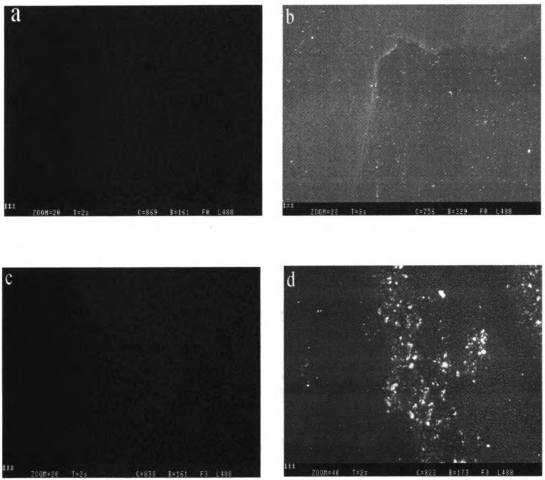

Figure 6.6. Laser scanning confocal micrograph of hydrated films of compound **1** (top, a, b) and compound **2** (bottom, c, d). The images on the left (a, c) were acquired using cross polarizers and the images to the right (b, d) were obtained using dark field optics. The films are the areas to the right of the fields.

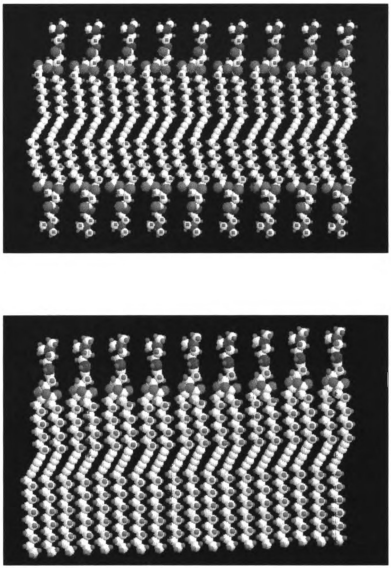

Figure 6.7. Proposed packing models from compound **1** (top) and **2** (bottom) based on X-ray powder diffraction information and molecular modeling.

such that the π-interaction is maximized and the alkane chains are in contact along their entire length (Figure 6.7). The π-overlap is expected to be one of the dominant forces between the chains of these molecules. Based on the enthalpies of vaporization of a series of alkanes and conjugated and unconjugated diacetylenes [33, 34] , this component could be estimated at 5.1 kcal/mol for chains just at the point of separating into the gas phase. This is a lower limit and a much higher value is expected for closely packed chains. The dispersion forces between methylene groups in hydrocarbons is an area that has obtained much attention Of special importance has been problem of calculating the dispersion energy between extended hydrocarbon chains in isolated molecules and large extended arrays[35, 36]. The van der Waals interaction energy between alkane chains scales linearly with chain length and varies as the inverse fifth power of chain separation[35]. In the case of compound **1** where there are 16 methylene groups in contact and the chains are separated by 3.42 Angstroms (based on the X-ray data) the total interaction energy is 45.2 kcal/ mol. In the case of compound **2** there are 20 methylene groups in contact, but at a greater separation, this energy is expected to be 31.5 kcal/ mol. This gives a lower limit for overall inter-chain interaction energies for **1** and **2** of 50.3 and 36.6 kcal/mol respectively. These are substantially higher than the interaction energies of lipid molecules in biomembranes of comparable acyl chain length. In the case of **1**, since the hydrocarbon chains are tethered at both ends, the average inter-chain separation is even less. The attraction between the hydrocarbon chains in this case is even greater since it is known to increase by two fold if the two chains move one Angstrom closer from a distance of 5 Angstroms[35]. This dramatic increase in van der Waals energy with reduced chain separation will also be true for the π-stacking component which is likely to be

severely underestimated here.

Formation, Characterization and Properties of Polydiacetylene Films. Two methods were used to prepare materials with the desired optical and spectroscopic properties that are characteristic of conductive polydiacetylene layers. Such systems exhibit intense electronic transitions at long wavelengths. These go beyond the visible spectrum and well into the infrared region. The optical properties of the polydiacetyenic systems were characterized by near IR spectroscopy experiments. The samples were prepared as described in the experimental section and spectra were measured over two ranges, from 600-1050 nm and 900-1700 nm. The results shown in Figure 6. 8 demonstrate that the optical behavior of the two compounds polymerized by the same method are similar. Both formed blue films on exposure to UV radiation. In the case of compound **1**, there was a strong absorption at 848.3 nm, and this extended to 1700 nm. The maximum for UV- irradiated compound **2** appeared at 817.8 nm. Both spectra displayed a small maximum at ~ 700 nm. These polymerized blue films turned red on exposure to solvents such as chloroform. Films that were treated with iodine, were bright orange. The absorption maximum for iodine doped **1** was appeared at 823.1 nm and the maximum for **2** was at 772.8 nm. Again there was a small maximum at ~700 nm in both spectra. The occurence of such intense maxima in the region of 800 nm and beyond with significant absorbance over 1600 nm in the films we have prepared by this method is very unique. Typically, no significant absorbance beyond 600-680 nm is observed in these systems[1, 20, 22, 37]. This might indicate either an exceptionally long statistical length for the chain that makes up an exciton unit, an alternative mode of polymerization to the

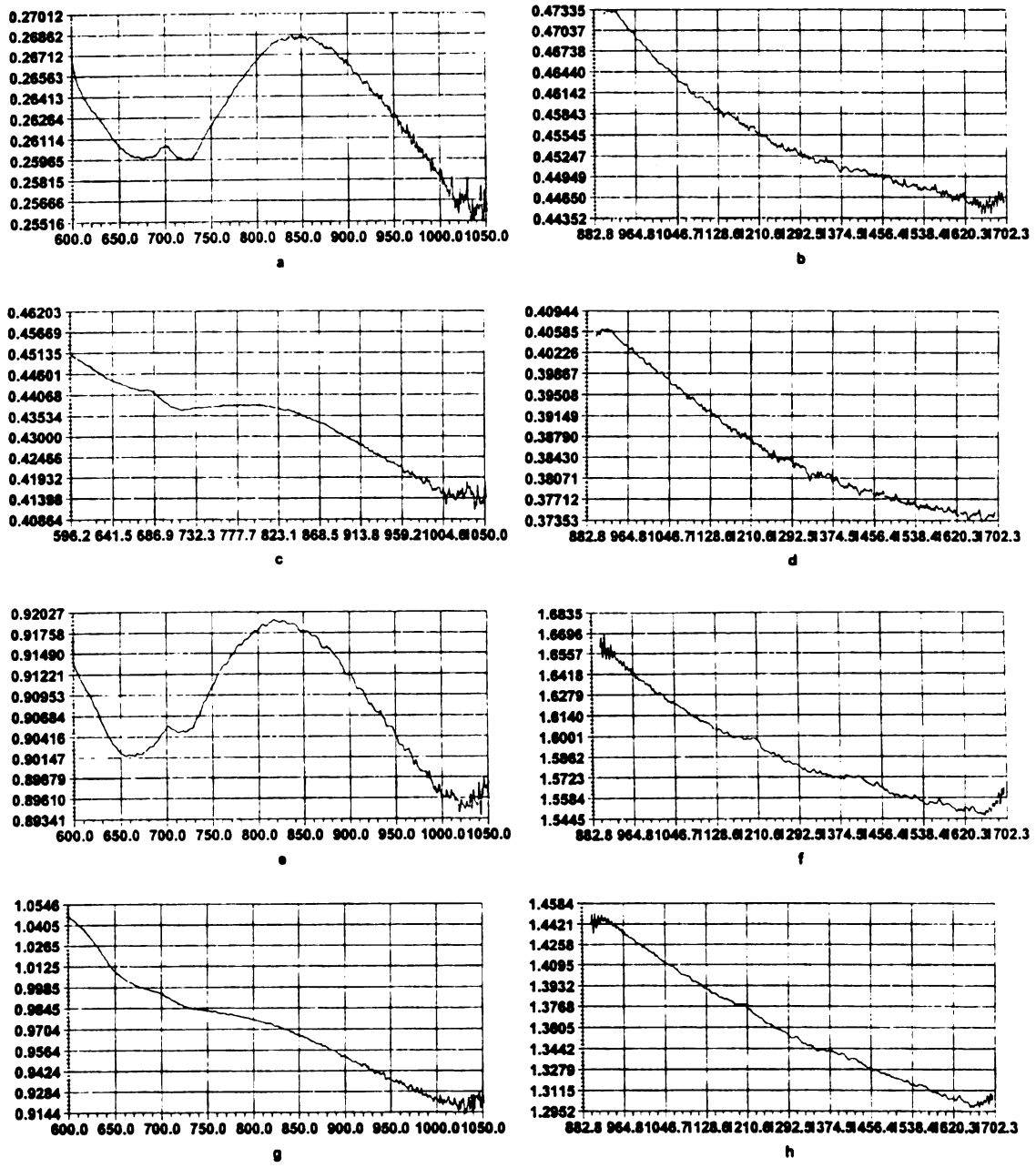

Figure 6.8. Near infrared spectra of films formed from **1** (top 4) and **2** (bottom 4). In a, b, e, f, the film was polymerized by UV irradiation and in c, d, g, h, the films were iodine doped. Spectra on the left were aquired in the range of 600-1050nm, and those to the right were acquired between 880 and 1700 nm.

typical 1,4-addition mode or a local excimer or charge transfer from juxtaposition of conjugated systems.

6.3. Conclusions

In summary, the method we describe here allows the assembly of hydrocarbon chains containing diacetylene functions with the packing densities, orientation and long-range order necessary for forming highly conjugated 2-dimensional polymer systems. Our strategy was to prepare molecules which are analogs of bio-membrane lipids and use their self-assembling propensity to direct and achieve the regularity of packing and the high 2-dimensional order that is required. The syntheses are characterized by brevity and very high efficiency. A series of instrumental analyses indicated that these membrane lipid mimics have the desired properties. X-ray diffraction, laser confocal polarized light microscopy and molecular modeling indicate that they form well oriented lamellar films. Atomic force microscopy experiments prove that they are able to form very flat thin films. Near IR study confirms that the UV polymerized or doped films have the desired optical properties. The conjugation is exceptionally high with electronic transitions occurring far into the infrared spectrum. These membrane mimics are excellent building units for cast films. There is no need for an external boundary to confine monomers, the behavior of the system is built into the structure of the monomer. What we have described here is a simple way of generating 2-dimensional molecular networks containing diacetylene function in the system. This should prove to be of important utility in the design of advanced materials such as conducting materials and drug delivery systems and electro-optical devices.

6.4. EXPERIMENTAL

Near IR experiments. The compounds **1** or **2** were dissolved in chloroform to give approximately 1% solutions. A few drops of each clear solution were transferred by a Pasteur pipette onto clean microscope slides; the liquid spread and evaporated to form a thin film. Films were either polymerized by irradiation with UV light (254 nm, 6 Watts, 350 μWatts per cm^2 for 3 minutes) or the layers were doped by holding the slide in a horizontal position about 10-15 cm above some iodine crystals at room temperature. In the UV polymerization experiment, the light yellow to colorless film turned to blue after UV irradiation. The films turned to yellow-orange after doping with iodine. Near IR experiments were performed on diode array spectrometers (CDI SPEC) from Control Data (South Bend, Indiana). The films were irradiated using a tungsten source and the slides were placed directly in the light path.

Atomic force microscopy. These analyses were performed using a Nanoscope III instrument operating in contact mode. For these measurements, compounds **1** and **2** were dissolved in chloroform-methanol (0.5-1% solutions) and ~ 10 μL transferred to freshly cleaved mica plates spinning at 200 rpm. This facilitated even film spreading and evaporation.

Laser scanning confocal light microscopy. These experiments were performed on a Zeiss 210 instrument with a 488 nm laser. Images were obtained in the bright field, dark-field, phase contrast and polarization modes. For the polarizing mode experiments, an analyzing cross-polarizer was placed on the objective lens and rotated until light cancellation. For film preparation, the compounds were dissolved in a 4:1 ethanol : water mixture, and a few drops

of solution were deposited on clean glass slides which were left in a horizontal position at 30-40° C for two hours to allow the solvent to evaporate.

X-ray diffraction. These studies were performed on a Rigaku instrument with a Rotaflex rotating copper anode operating at 45 kV with a current of 100 mA. The X-ray beam was collimated with a 1/6 slit and the Kα line was selected. The compounds were dissolved in a minimum volume of ethanol and 1/4 to 1/3 the volume of water added so that an overall 20% cloudy but uniform dispersion of sample was obtained. The samples were sonicated and vortexed several time to ensure uniformity of distribution and then sealed in glass capillaries and diffraction data obtained.

Molecular Mechanics Calculations. These were performed using the MM3 forcefield[38] as implemented in the program Alchemy (Tripos Inc. St. Louis, MO 63144 USA) Minimizations were performed using the conjugate gradient method. The parameters were used without modification since the MM3 forcefield is parametrized to very accurately reproduce the geometries and heats of formation of hydrocarbons.

Synthesis. *(S) 1-N,N-Dimethylaminoethyl-3,4-dihydroxybutyramide* **(5).** The synthetic route utilizes the stereocenter in (S)-3-hydroxybutyrolactone as the source of chirality. The preparation of the lactone has been described earlier and the steps described here have been demonstrated to preserve the stereocenter[32]. (S)-3-hydroxybutyrolactone 51g (0.5mol), N, N-dimethylethylenediamine 44g (0.5mol), and 100 ml absolute ethanol were mixed and

stirred for 24 hours. The solvent was removed by rotatory evaporation under reduced pressure. The product was dried by vacuum oven for 24 hours to yield a heavy brown syrup 95 g (100%) .^{1}H NMR (300 MHZ, $CDCl_3$) δ 6.19 (s (broad), 1H), 4.05 (m, 1H), 3.65 (dd, 1H, J=11.4, 3.9 Hz), 3.52 (dd, 1H, J=11.4, 5.1Hz), 3.49-3.39 (m, 1H), 3.33-3.21(m, 1H), 2.45-2.35 (m, 4H), 2.23 (s, 6H). ^{13}C NMR (75 MHZ, $CDCl_3$) 172.34, 69.12, 65.89, 57.83, 44.95, 39.60, 36.58. IR (NaCl window, $CHCl_3$ as solvent), 3297, 3090, 2944, 2865, 2824, 2780, 1647, 1555, 1462, 1190,1040 cm^{-1}

Preparation of compound **1**. All reactions and workups relating to diacetylenic compounds were conducted with exclusion of light. Amberized glassware was used and samples covered with aluminum foil. A photography safe light was used for illumination when conducting chromatographic separations. 10,12-Docosadiynedioic acid 1.81 g (0.005 mol), oxalyl chloride 15ml (0.172mol), and dry dicholoromethane 10ml, were mixed and stirred under a dry atmosphere overnight at room temperature. The solvent and excess oxalyl chloride were quickly removed under reduced pressure by rotatory evaporation. The product was taken up in 5ml hexane and rotatory evaporated to dryness to remove the last traces of oxalyl chloride. The crude, freshly prepared amide 5 were used directly for the next reaction step. A mixture of dried amide **5** (0.95g, 0.005mol), 5 ml dry pyridine and 5 ml of dry dichloromethane was cooled to 0 °C in an ice bath under dry nitrogen. The acid chloride **6** (0.005mol) dissolved in 5 ml of dry dichloromethane was added to the mixture with a dropping funnel over a 10 minute period. The reaction mixture was then stirred for 24 hours. The solvent was removed by rotary evaporation, and the residue was taken up in chloroform, washed

sequentially with 0.1 N HCl, saturated sodium bicarbonate solution, and then brine and the organic phase was separated and dried with anhydrous sodium sulfate. Removal of solvent yielded product **1**, 2.3 g (89%) as a red solid which was homogenous by thin layer chromatography. ^{1}H NMR (300 MHZ, $CDCl_3$) 6.41 (s (broad), 2H), 5.40 (m, 2H), 4.31 (m, 2H), 4.13 (m, 2H), 3.31 (m, 4H), 2.47 (m, 4H), 2.35-2.15 (m , 32H), 1.64-1.40 (m, 16H), 1.27 (b, 32H) ^{13}C NMR (75 MHZ, $CDCl_3$) 173.25, 172.73, 168.63, 68.54, 65.20, 64.27, 57.53, 44.88, 37.82, 36.51, 34.17, 33.98, 29.01, 28.86, 28.73, 28.24, 24.76, 19.12 IR (NaCl, $CHCl_3$) 2932, 2855, 1738, 1653, 1547, 1462, 1159, 621.2 Fast atom bombardment mass spectrometry (FAB MS) 1033.9 ($C_{60}H_{96}N_4O_{10}$, MH^+)

Preparation of compound **2.** The method was essentially the same as described above for **1** with a slight difference in the procedure. 10,12-Pentacosadiynoic acid (2.24g, 0.006 mol), 10 ml oxalyl chloride (0.115mol), and dry dicholoromethane (10 ml) were stirred overnight at room temperature. The workup was the same as above. For the esterification, the amide **5** 0.475g (0.0025mol) was used for the reaction. The crude product was purified by flash column chromatography on silica gel, (chloroform, acetone, methanol; 1:1:1) Product **2** was obtained as a purple solid 1.97g (87%). ^{1} H NMR, (300 MHZ, $CDCl_3$), 6.92 (s, 1H), 5.39 (m, 1H), 4.30 (dd, 1H, J=3.6, 12.0 Hz) 4.12 (dd, 1H, J=5.8, 12Hz) 5.35 (m, 2H), 2.55-2.44 (m, 2H), 2.32-2.18 (m, 24H), 1.64-1.42 (m, 12H), 1.40-1.10 (m, 2H), 0.85 (t, J=6.6 Hz). ^{13}C NMR (75 MHZ, $CDCl_3$), 173.30, 172.78, 168.90, 68.58, 65.27,65.18, 64.38, 57.52, 44.50, 37.75, 36.20, 34.23, 34.03, 31.89, 29.61, 29.46, 29.33, 29.09, 28.91, 28.85, 28.78, 28.33, 24.80, 22.67, 19.18, 14.11. IR (NaCl, $CHCl_3$) 2919, 2851, 1734, 1653, 1470, 1246, 1175,

718. cm^{-1}. FAB MS 903.7 (MH^+, $C_{58}H_{98}N_2O_5$). Because of their high liability to oxygen and instability to light, combustion analyses could not be obtained on these materials.

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Chapter 7

Preparation of 2-Dimensional Polydiacetylenes by Monomer Chain Alignment Along a Polar Polyamide Backbone: A General Strategy for Preparing Highly Ordered, Stabilized 2- Dimensional Systems

Abstract

A new approach to obtain long range order and perfect alignment of long chain polydiacetylene by anchoring them along a polymer backbone is introduced. Both the oligomer and the polymer which arrange the long alkyl chain containing diacetylene function in the middle of the chain were prepared and characterized. In addition to the optical properties and chromism of typical polydiacetylenes, they exhibit some fascinating properties such as the absorption spectra are different from all current existing systems, they absorb into the near IR range. The IR spectra and X-ray powder diffractions suggest a novel mechanism of polymerization occurring in these systems. The polymer can self assemble and have a bilayer packing, the system has very high order, high packing density and it is a new method to fabricate 2-dimensional ordered functional materials and expect to be used in optical-electronic devices, chemosensors and new multifunctional smart materials.

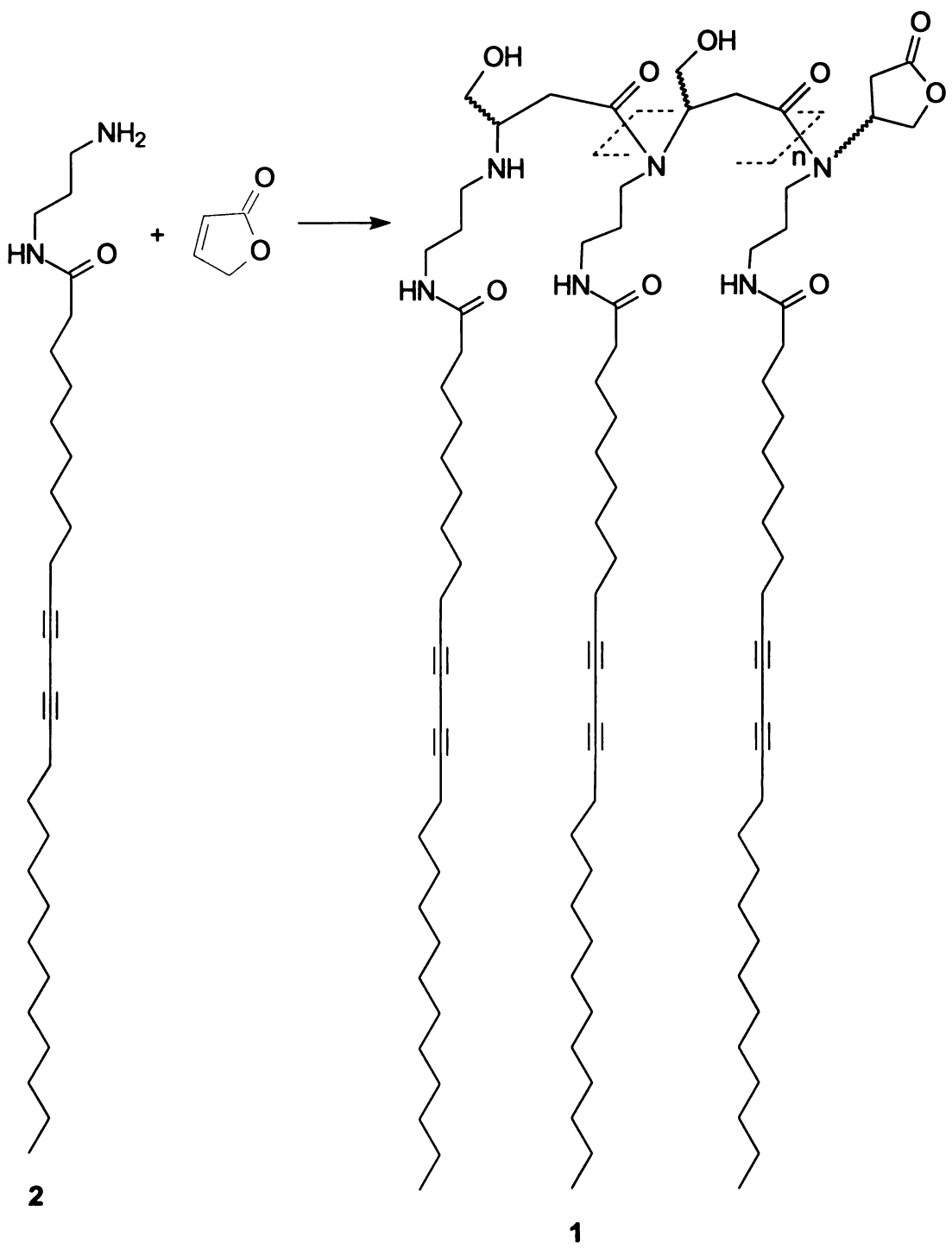
7.1. Introduction:

One of the newer and more exciting goals of modern organic chemistry is the fabrication of large regular molecular arrays that approach nanoscale dimensions in size and that have special optical, electronic or other physical properties. Such materials, it is hoped, will form the basis of an emerging molecular technology that will facilitate the construction of devices such as optical switches, light emitting diodes, light dependent conductive or resistive devices and frequency doublers. (1-5). The construction of such large molecular systems and ensembles requires a method for accurately and reproducibly aligning the molecular units in close proximity with proper position of the functional groups from which the desired physical properties are desired. A general , reliable way of doing this has tremendous implications for the new field of advanced materials. We demonstrate such a method here. It should allow the precise alignment of groups that interact by dipoles, charge transfer, electron transfer and that can be subjected to chemical polymerization. All of these yield materials with exciting new physical properties.

The system of polydiacetylenes are a case in point. These have much potential as new organic materials with possible applications in electrical conductivity, non-linear optical applications and near-infrared active applications (6-11). The fabrication of 2-dimensional aligned hydrocarbon systems in which the polydiacetylene functions occur as a band or layer that is sandwiched between two application layers are especially of interest (12-17). Unfortunately, the alignment of long chain diacetylenes to effect polymerization thus forming of these highly conjugated polydiacetylenes layers or bands is one of the most

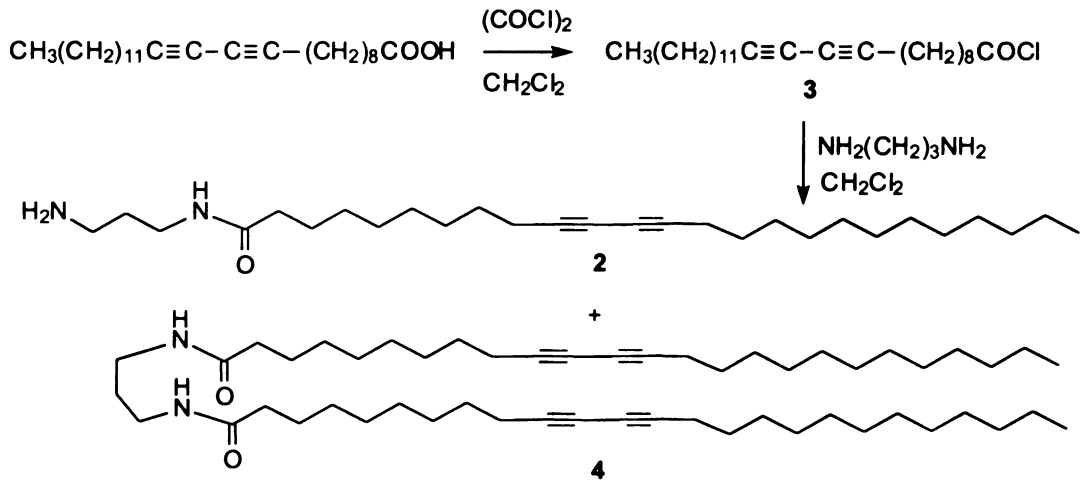
difficult aspects of the fabrication of these advanced materials. The acetylene functions need to be within 0.5 Angstroms of each other (18). Several strategies have been tried the most common one being to align the chains on a flat support such as a highly polished gold surface (19-22). The molecules are generally anchored to the surface via thiol groups. Unlike the case for self assembling monolayers (SAMS) formed by saturated alkanethiols (23, 24) , there are some problems associated with this method for obtaining long range order of alkyl chains containing diacetylenes functions that are suitably positioned for polymerization. The most severe of these is that, because of the large atomic radius of gold compared to carbon, variations in the surface of only a few gold atoms put the acetylene functions in the aligned chains out of register thus terminating or inhibiting polymerization. It is also an extremely labor-intensive approach. There is also the tendency to form disulfide groups. When this happens, the chains are not aligned and polymerization is not possible (19). Another approach to solving the problem is to generate monolayers of hydrocarbons containing polydiacetylenes using Langmuir Blodgett technology (25-29). Although it is a good method for preparing highly ordered thin films, it is a labor-intensive one. In addition to this, LB films are not stable enough to remain intact under a variety of chemical or physical conditions. They are also not suitable for large scale preparations.

One possible approach is to align the long chain alkyl groups containing diacetylene functions along the backbone of a polymer to form a comb structure.This approach has spawned several strategies for the preparation of side-chain polymers(30-33).The problem here is to arrange for there to be a group attached to each monomer unit in the chain. This



Scheme 7.1. The formation of the polymer by reaction of the long chain amine with 2(5H)-furanone.

is generally not a straightforward goal if the polymer is pre-formed because of steric reasons. It is, however, possible if the monomer contains the desired alkyl chain with the diacetylene function. Such side-chain polymers have not been synthesized as yet. We demonstrate here that such a monomer can be easily prepared by a Michael-type addition of a primary amine to an ene-lactone such as 2(5H)-furanone. This generates a β-alkylamino-γ-butyrolactone which immediately polymerizes to form a material with the desired properties (scheme7.1). If the alkyl chains are aligned in a parallel fashion, the overall superstructure is one in which the actual system is a 2-dimensional, chemically connected monolayer system with polar amide and hydroxyl groups on one side and hydrophobic hydrocarbon groups on the other. This arrangement is highly desirable for forming monolayers. One important advantage is that such monolayers do not have to be mechanically compressed because the units are held together at very close range and in uniform register by covalent bonds in the polyamide chain region.



Scheme 7.2. The synthesis of the primary long chain amine containing diacetylene group.

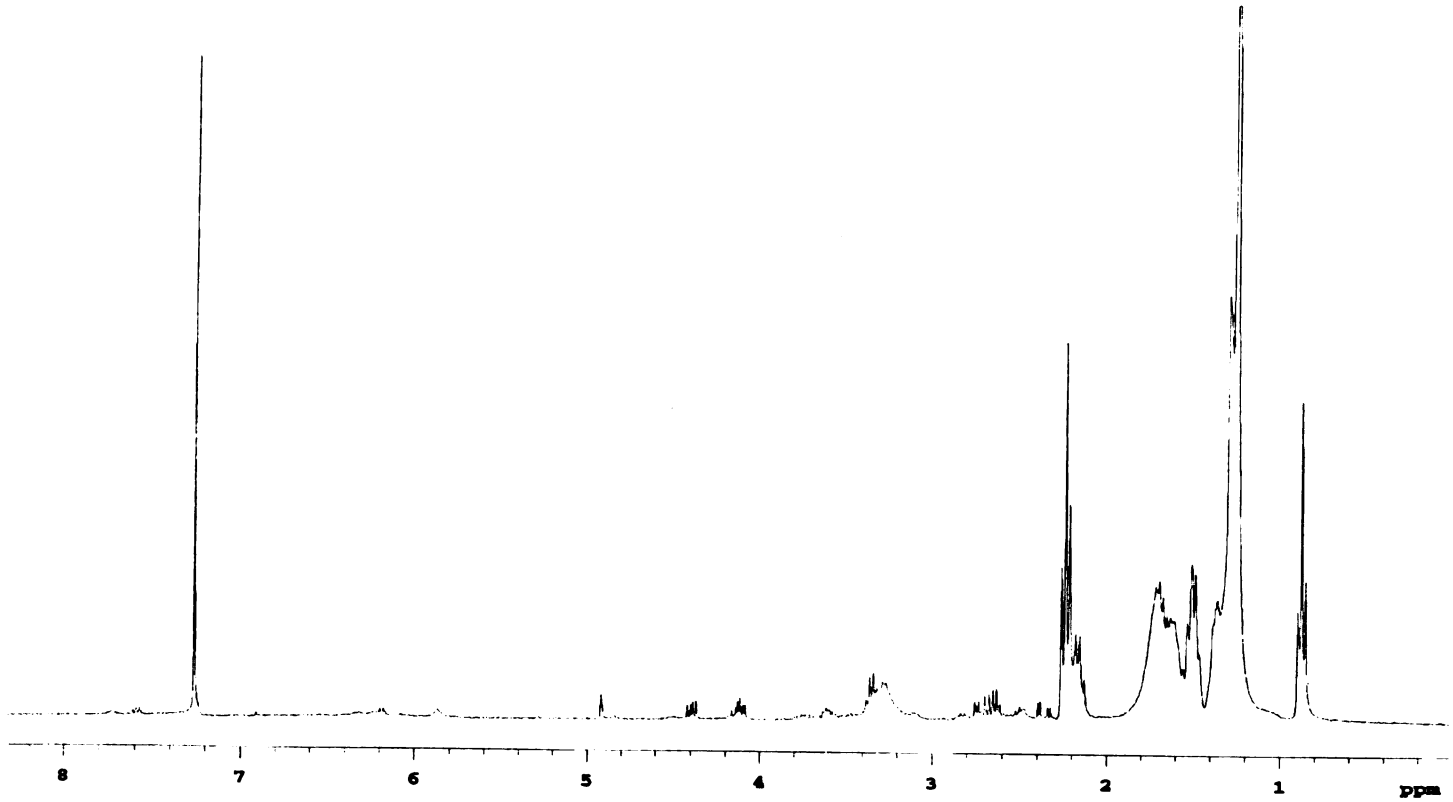


Figure 7.1. ^{1}H-NMR spectrum of the oligomers formed in the reaction shown in Scheme 1.

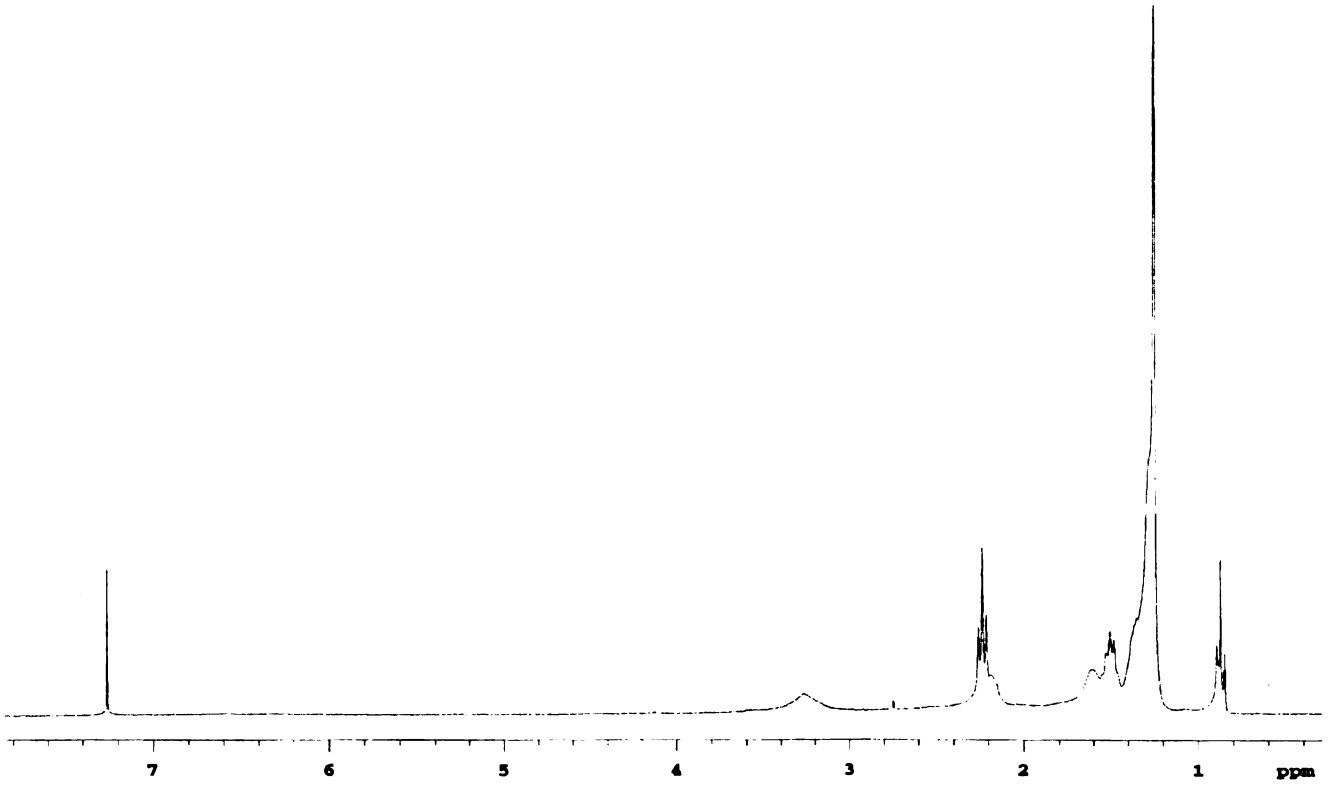


Figure 7.2. ^{1}H NMR spectrum of the high molecular weight polymer formed by heating of the oligomer to further the polymerization along the amide backbone.

7.2. Results and discussion:

Synthesis of the polymer .Primary long chain amines with conjugated diacetylene groups were not readily available but an appropriate surrogate could be easily prepared from a commercially available acid and a diamine (Scheme 7.2). This amine readily reacted on gentle heating with 1 equivalent of 2(5H)-furanone in chloroform / ethanol at 50 °C to give oligoamides. NMR spectra at this stage showed signals for unchanged furanone and for the intermediate β-alkylamino-γ-butyrolactone (Figure 7.1.).This material could be further polymerized by heating in the absence of solvent at 80-90°C after which all of the furanone signals had disappeared and the resonances for the polyamide backbone and diamine linker were broad and ill-defined as is typical for polymers (Figure 7.2.). Preliminary analysis using matrix assisted desorption mass spectroscopy (MALDI-MS) indicated that the degree of polymerization was well in excess of 100 repeating units (>50,000 Daltons) but an upper limit could not be established .

Optical properties The oligomers and polymers both exhibited the typical thermo-and solvatochromatic properties of polydiacetylenes. The green-blue oligomer product (Figure 7.3) was colorless when placed on glass slide as a thin film and it turned to deep blue on irradiation with U.V. light and red on dissolution in chloroform, treatment with acid or on heating (Figures 7.4). The blue color was restored on cooling or on removal of the solvent. If the material was polymerized completely by heating at 90°C to form the high molecular weight polyamide, a bright red product was formed which turned to an amber solid on cooling. This product turned to a bright golden color (Figure 7. 5) on doping with iodine.

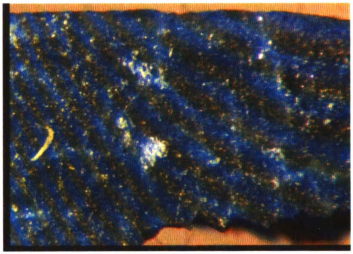


Figure 7.3. The photograph of the oligomer solid after UV irradiation of 10 minutes.

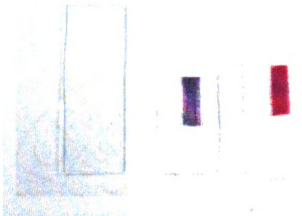


Figure 7.4. The film formed by the oligomer amide by dissolving in chloroform, left (colorless strip) is the film prepared by casting the material on to the glass slide by solvent evaporation; the middle (blue) is the film prepared as the left one being exposed to UV light for 7 minutes, the right (red) is the blue film prepared as the middle one then was heated at 80 °C for 1 minute.

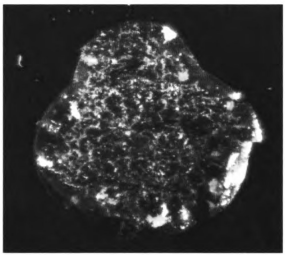
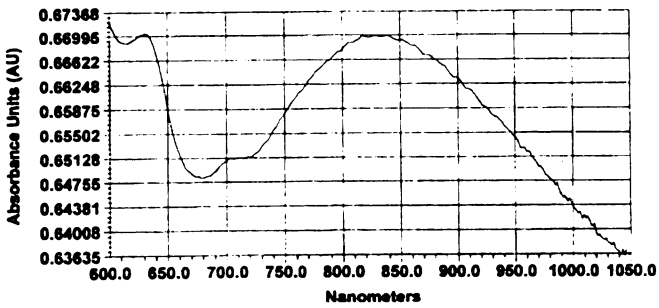
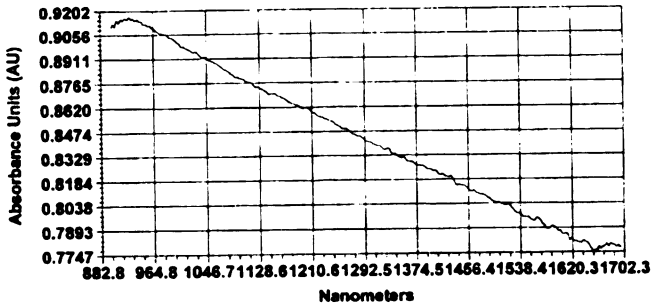


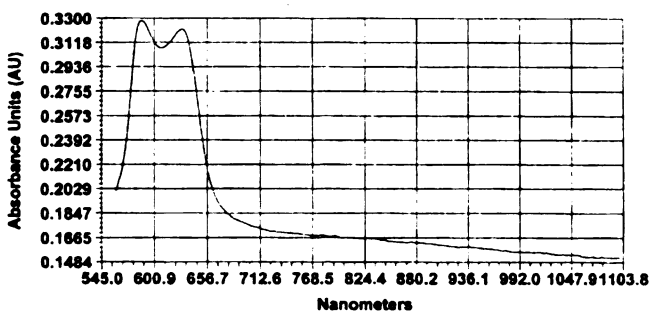
Figure 7.5. The photograph of the polymer upon treating with UV for 10 minutes and then doped with iodine.



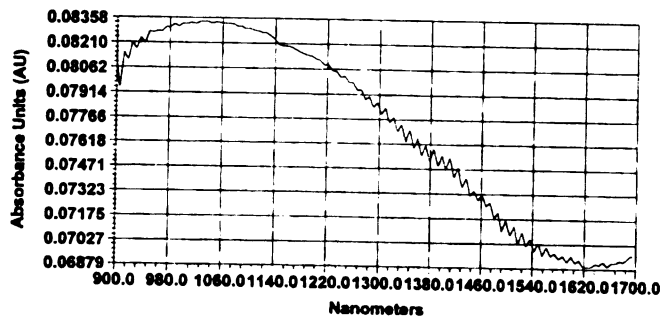
A



B



C



D

Figure 7.6. The near IR spectra of the oligomer cross linked by UV light(A, B) and the Near IR spectra of the polymer cross linked by UV light (C, D).

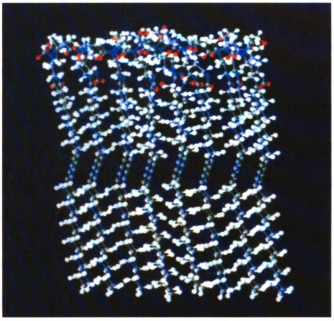
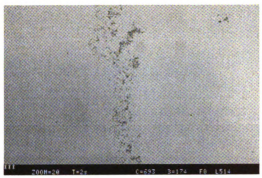


Figure 7. 7. The proposed model of packing structure of the polymer.

Both the doped and un-doped polymers absorbed strongly in the near infrared region of the electromagnetic spectrum indicating a new mode conjugation by the polydiacetylene cross linking. The oligomer also show strong absorption in the near IR region (Figure 7.6 a, b). Material that was polymerized by heating for 10 hours at 90 °C and UV cross-linked displayed an absorption maximum at 1038 nm. The beginning of another absorption band with a tail at 1700nm was also evident (Figure 6 c, d). Absorption in this region of the spectrum is generally not observed and certainly have not been reported for typical polydiacetylenes. This indicates that this method of chain alignment makes some other mode of polymerization in addition to the typical 1,4 addition mode possible. Bands that are normally attributed to the 1,4-mode were also visible at 587 nm and 640 nm.

High Long Range Order Both single crystal X-ray diffraction and powder diffraction data experiments indicated the high degree of order of these polymer systems. The X-ray Powder diffraction data was consistent with a model of the polymer as shown in Figure 7.7. There was a strong reflection at 2θ corresponding to 58.9 Angstroms, a distance equivalent to the height of the 2-D system. There were also reflections in the wide angle region at 4.4, 4.3, 3.9, 3.7 Angstroms corresponding to four different inter-chain separations of alkyl chains. These are significantly shorter than the alkyl chain separation of 4.9 Å in the classical 1,4 addition ene-yne mode , and also for that of the butatriene mode which is 4.7 Å (34, 35). The most likely mechanism is by 1, 2 addition. This gives a polyene backbone with pendant acetylene groups (36). The pending acetylenic group further polymerize to give the polyacene type structure.

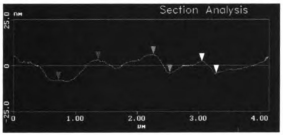


A

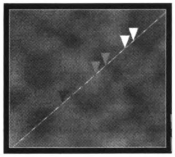
B

C

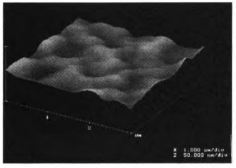
Figure 7.8. The laser scanning confocal micrograph of the oligomer. A, by polarized light; B, by phase contrast method, C, by fluorescence.



A



B



C

Figure 7.9. The atomic force microscopy of the oligomer. A, section analysis; B. The top view of the cross section area the same with A, C. the surface contour plot of the same area as above.

Characterization of the thin film formed by the oligomer. The laser scanning confocal images of the oligomer shows that they formed very uniform flat thin film (Figure 7.8), by the polarized light microscopy. This is also further demonstrated from the atomic force microscopy (AFM) measurement (Figure 7.9). The AFM images show that they formed extremely flat, highly ordered thin film. The surface variation is only 11 nm over a range of 4000 nm (0.3%). These experimental results indicated that the oligomer can self assemble and form highly organized supramolecular structure.

7.3. Conclusion

A new method of obtaining high long range arrangement of long polydiacetylene containing alkyl chain was described. The two dimensional (or 3-D) comb polymer show some remarkable new properties in addition to the well known properties of polydiacetylene systems. The oligomer can self assemble into highly ordered extremely flat thin films, both the oliogmer and the polymer are readily polymerized. The absorption spectra of the two systems are from the UV till way out in the near IR region. This might suggest very high level of conjugation and or a new mechanism of polymerization occurred in this type of system. This method offers a solution to the problem of arranging long chain polydiacetylene in perfect order, provides the material obtained and the approach applied here are expected to be useful for fabricating advanced multifunctional material such as optic-electronics and chemosensors.

7.4. Experimental Section

Near IR experiments. The green oligomer and the further connected polyamide were dissolved in chloroform to give approximately 1% solutions. A few drops of each clear solution were transferred by a Pasteur pipette onto clean microscope slides; the liquid spread and evaporated to form a thin film. Films were either polymerized by irradiation with UV light (254 nm, 6 Watts, 350 μWatts per cm^2 for 3 minutes) or the layers were doped by holding the slide in a horizontal position about 10-15 cm above some iodine crystals at room temperature. In the UV polymerization experiment, the colorless oligomer film turned to blue after UV irradiation after 30 seconds and it turned deeper with longer irradiation time. The polyamide thin film was brownish at room temperature it turned to different color as the normal blue, upon doping with iodine, it turned to golden metalic color. Near IR experiments were performed on diode array spectrometers (CDI SPEC) from Control Data (South Bend, Indiana). The films were irradiated using a tungsten source and the slides were placed directly in the light path.

Atomic force microscopy. These analyses were performed using a Nanoscope III instrument operating in contact mode. For these measurements, the oligomer was dissolved in chloroform-methanol (0.5-1% solutions) and ~ 10 μL transferred to freshly cleaved mica plates spinning at 200 rpm. This facilitated even film spreading and evaporation.

Laser scanning confocal light microscopy. These experiments were performed on a Zeiss 210 instrument with a 488 nm laser. Images were obtained in the bright field, dark-field,

phase contrast and polarization modes. For the polarizing mode experiments, an analyzing cross-polarizer was placed on the objective lens and rotated until light cancellation. For film preparation, the compounds were dissolved in a 4:1 ethanol : water mixture, and a few drops of solution were deposited on clean glass slides which were left in a horizontal position at 30-40° C for two hours to allow the solvent to evaporate.

X-ray diffraction. This study was performed on a Rigaku instrument with a Rotaflex rotating copper anode operating at 45 kV with a current of 100 mA. The X-ray beam was collimated with a 1/6 slit and the Kα line was selected. The green oligomer solid was grinded to fine powder and suspended in water ethanol (10:1 mixture), then the slurry was transferred to the glass slide, air dry for one day, the solid deposited on the slide was used for the measurement. After the measurement the part under x-ray beam turned to black, the part without X-ray irradiation is still green.

Molecular Mechanics Calculations. This was performed using as implemented in the program Biograf. Minimizations were performed using the conjugate gradient method.

Synthesis of polyamide

Preparation of compound **2**. All reactions and workups relating to diacetylenic compounds were conducted with exclusion of light. Amberized glassware was used and samples covered with aluminum foil. A photography safe light was used for illumination when conducting chromatographic separations. 10,12-Docosadiynedioic acid 0.90 g (0.0025 mol), oxalyl

chloride 5ml (0.054 mol), and dry dicholoromethane 10ml, were mixed and stirred under a dry atmosphere overnight at room temperature. The solvent and excess oxalyl chloride were quickly removed under reduced pressure by rotatory evaporation. The product was taken up in 5ml hexane and rotatory evaporated to dryness to remove the last traces of oxalyl chloride. The crude, freshly prepared amide 5 were used directly for the next reaction step.

To the mixture of Diaminopropane (0.6g, 3 eq) in dichloromethane, the acid chloride **3** (0.0025mol) dissolved in 5 ml of dry dichloromethane was added to the mixture with a dropping funnel over a 10 minute period. The reaction mixture was then stirred for 24 hours protected from moisture. The residue was taken up in chloroform, washed sequentially with 0.1 N HCl, saturated sodium bicarbonate solution, and then brine and the organic phase was separated and dried with anhydrous sodium sulfate. Removal of solvent yielded product **2** with a small portion of byproduct **4** . The desired product 2 can be separated from the dimer by product **4** by flash column chromatography (eluting solvent: methanol: chloroform=1:3. Product **2** was obtained as white powder yield 0.86g (80%), ^{1}H NMR (300 MHZ, $CDCl_3$) 6.22 (s (broad), 1H), 3.34 (q, J= 6.0Hz, 2H), 2.78 (t, J=6.3Hz, 2H), 2.24-2.10 (m, 6H) , 1.64-1.20 (m, 34H), 0.86 (m, 3H) IR (NaCl, $CHCl_3$) 3296, 2918, 2849, (2176, 2140 weak), 1700, 1639, 1553, 1463, 722 cm^{-1} . Compound **4**, ^{1} H NMR (300 MHZ, $CDCl_3$), 6.18(s, 2H), 3.25 (q, 4H, J=6.0Hz), 2.26-2.10 (m, 12H), 1.60 (m, 4h), 1.47(m, 8H), 1.40-1.18(m, 66H), 0.86(m, 6H). IR (NaCl, $CHCl_3$). 3345, 2918, 2850, 2716(w), 2140(w), 1728, 1636, 1525, 1471, 1423, 1255, 1192, 956, 717 , 660 cm^{-1}.

Preparation of compound **1.** The amine **2** 0.59 g was added to 0.12g furanone, 3ml

ethanol, 7ml chloroform, the mixture was stirred for 12 hours at room temperature, then at 50 °C for another 6 hours until all the solvents were evaporated, the oligoamide was formed as a green crystalline solid. The charaterization of the compound, ^{1} H NMR, (300 MHz, $CDCl_3$), the signals are broadened, 4.38(m), 4.10(m), 3.70(m), 3.40-3.20(m), 2.80-2.60(m), 2.24-2.10(m), 1.80-1.40(m), 1.40-1.18(m), 0,86(m). IR (NaCl, $CHCl_3$) 3309, 3090, 2819, 2849, 2178(w), 2141(w), 1772, 1638, 1544, 1470, 1418, 1065 cm^{-1}. The polymer after heating the oliogamide for 10 hours: ^{1} H NMR, (300 MHz, $CDCl_3$), the signals are significantly broadened, and the terminal functional groups' signals disappeared. 3.25ppm (m, broad), 2.40-2.20(m), 1,59(m), 1.49(m), 1.23(m), 8,86(m). IR (NaCl, $CHCl_3$), 3310, 3082, 2919, 2850, 1776, 1645, 1544, 1467, 1620, 720. Because of their high liability to oxygen and instability to light, combustion analyses could not be obtained on these materials.

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