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By

Jie Song

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ABSTRACT

STUDIES OF CHIRALITY, SUPRAMOLECULAR STRUCTURE, ORDER AND REACTIVITY IN AND FROM BIOLOGICAL SYSTEMS

By

Jie Song

Glycosyl diacylglycerols are excellent lipids for the formation of both bi and monolayer lamellar systems but they are generally not commercially available or chemically easily accessible. Several glycolipids designed to be easily accessible structural surrogates of monoglucosyl diacylglycerol (MGDG) have been synthesized. The general strategy of replacing the natural glycerol linker with a chiral 1,2,4-butanetriol simplified the synthesis significantly. A novel acetal linkage was introduced in one of the design, which brings chemically and physically tunable new properties in a glycolipid. These include base stability, resistance to the degradation by phospholipase and other esterase activities present in all cellular systems, increased mobility of the headgroup, possibilities of new packing arrangements and the potential for use in encapsulation strategies using liposomes where a decrease in pH is used as the environmental cue for release. Another two MGDG analogs were close structural precursors of potent anti-HIV agent sulfoquinovosyl diacylglycerol (SQDG). The quick access to a close SQDG analog from these MGDG analogs was attempted.

It is known that biomembranes play a crucial role in living cells by serving both as selective barriers for transport and as sites for molecular recognition and catalysis. I am

interested in whether and how the packing order, symmetry properties, steric and proximity factors of membrane systems or other highly ordered supramolecular ensembles encode the regio- and stereo- regularities of reactions occurring on their surfaces. A series of glycolipids were designed, synthesized, and the predominant supramolecular structures they form were characterized. By initiating transglycosylation reactions among these systems and analyzing the regio- and stereo-regularity of the transglycosylation products, I tried to shed some light on the encodement of 1-D linkage and sequence information in the 2-D organization of supramolecular ensembles.

Chiral β-hydroxy acids and γ-hydroxy acids are important pharmaceutical intermediates. General routes towards alkenyl-containing 5 and 6-carbon chiral β-hydroxy acids and γ-hydroxy acids using carbohydrates as chiral synthon were developed. These intermediates are also logical precursors of corresponding amino acids. An increasingly important pharmaceutical target Vigabatrin® (γ-vinyl-γ-amino-butyric acid) was synthesized in a short synthetic route from its chiral γ-hydroxy acid precursor using the developed strategy.

DEDICATION

To Mom and Dad.

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The past four years is a very tasteful period of time in my life. I appreciate all the lessons I have learned, no matter they came in a pleasant or a disturbing way. I am glad to

be put into such a challenging environment and meet so many unusual characters. All I can say is that life is so much better than any fiction and it serves as a good mirror for everyone.

As always, the past four years' journey will not be enjoyable without those whom I love deeply. First and foremost, my dearest mom, dad and brother. I can never tell you all how much your love means to me. You have taught me the most important and invaluable lesson in life: what to love and embrace, what to despise and ignore. With you, my faith in the virtues of human nature will never die. Mom, I am so proud to have you as my role model!

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Finally, my deep thanks to Hong, for being a considerate and supportive husband. There is not an easy way to tell you my appreciation of the love, encouragement and trust that you have translated in a unique way. I am so glad that we will take the many new journeys ahead together.

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LIST OF ABBREVIATIONS

Ac:	Acetyl
Ac_2O:	Acetic anhydride
AIDS:	Acquired immune deficiency syndrome
BBB:	Blood-brain-barrier
Cat.:	Catalyst / Catalytic
CNS:	Central nervous system
d:	Doublet
DA:	Dopamine
dd:	Doublets of doublet
d.d.:	Double distilled
d.e.:	Diastereomeric excess
δ:	Chemical shift
DEAD:	Diethyl azodicarboxylate
DMF:	N,N-Dimethylformamide
DMSO:	Dimethyl sulfoxide
2-D NMR:	Two-dimensional nuclear magnetic resonance
DQF-COSY:	Double quantum filtered J-correlated spectroscopy
DSC:	Differential scanning calorimetry
e.e.:	Enantiomeric excess
EI:	Electron impact ionization
eq.:	equivalent

ESI:	Electrospray ionization
ether:	Diethyl ether
FAB:	Fast atom bombardment
GABA:	γ-Aminobutyric acid
GABA-T:	GABA-aminotransferase
GAD:	Glutamic acid decarboxylase
GC-MS:	Gas chromatography-mass spectrometry
Gly:	Glycerol
HBA:	Hydrogen bromide in acetic acid
HIV:	Human immunodeficiency virus
HMQC:	Hetero-nuclear multiquantum coherence spectroscopy
HRMS:	High-resolution mass spectrum
Hz:	Hertz
LAH:	Lithium aluminum hydride
LRMS:	Low-resolution mass spectrum
m:	Multiplet
MG:	Methyl-β-D-glucoside
MGDG:	Monoglucosyl diacylglycerol
MM:	Molecular mechanics calculation
MMTS:	Methyl methylthiomethyl sulfoxide
m.p.:	Melting point
Ms:	Methanesulfonyl
NBA:	N-Benzyl alcohol

NBS:	N-Bromosuccinimide
NIS:	N-Iodosuccinimide
nOe:	Nuclear Overhauser effect
NOESY:	Nuclear Overhauser effect spectroscopy
OG:	Octyl-β-D-glucoside
PTC:	Phase transfer catalyst
Py:	Pyridine
q:	Quartet
r.t.:	Room temperature
s:	Singlet
SQDG:	Sulfoquinovosyl diacylglycerol
t:	Triplet
TFA:	Trifluoroacetic acid
THF:	Tetrahydrofuran
TLC:	Thin layer chromatography
TMOF:	Trimethyl orthoformate
TMSCl:	Trimethylchlorosilane
TOCSY:	Total correlation spectroscopy
*p*TSA:	*p*-Toluenesulfonic acid
UDP:	Uridine 5'-diphosphate

CHAPTER 1

INTRODUCTION

I. Smart self-assembling systems with novel chemical, physical and biological properties

Besides water, and along with proteins, nucleic acids and carbohydrates, lipids are essential biomolecules in the structure and function of living matter. Apart from the abundant non-polar lipids, or fats, which are important as major fuels in cell metabolism, there are polar lipids and sterols that function as building blocks of cell membranes. Polar lipids have amphiphilic properties. They consist of water-soluble polar headgroup and insoluble hydrocarbon tails. The polar headgroups can be charged (positively or negatively), zwitterionic, or noncharged polyhydroxylated moieties (e.g. carbohydrates), in which the polar character is due to hydroxyl groups' H-bonds forming ability with water, or the combination of these. The water insoluble part consists normally of one or two fatty acid chains. Chain lengths in these lipids range from 8 to 24, mostly from 14 to 18 carbons, and they can be fully saturated or unsaturated with 1 to 4 double bonds.[1] Natural lipids may exhibit, as opposed to synthetic ones, a great diversity in the composition of fatty acid chains.

Amphiphilic lipids have broad applications in both basic sciences and in pharmacological, medicinal and material industry. For instance, they have been used in fundamental studies of surfaces with different topologies[2-5], the understanding of phase transition of liquid crystals[6,7], model studies of the biophysics of biomembranes and cells[8-10], construction of biocompatible films[11,12], studies of the evolution of life (eg. the origin[13] and role[14] of amphiphilic lipids in cell communication, lipid asymmetry[15], unusual lipids in primitive life forms[16], chirality in amphiphilic systems[17], helicity[18], etc.), various areas in supramolecular chemistry[19] (e.g. studies of molecular recognition[20,21],

molecular information processing[22], models of self-organization[23], etc.), membrane mimetic chemistry[24], catalysis[25], chemical separation and purification[26], drug delivery[27-29], cosmetic applications[30], and many others that preclude an exhaustive listing.

Glycolipids as one of the very important components of membrane lipids have received increasing amounts of attention in recent years. Traditionally, not many of the aforementioned applications were based on glycolipids. This is mainly because it is more difficult to have quick access to pure synthetic glycolipids in large quantity than other non-carbohydrate based amphiphilic lipids such as phospholipids. However, the inherent chirality and multifunctional groups that exist in glycolipids make them unmatchable sources in the fabrication of ferroelectric or other chiral liquid crystal materials, biocompatible films and in other applications that require surface modification. Moreover, with the deeper understanding of the critical role of carbohydrates in many cell surface communication (recognition) processes[31], it becomes clear that glycolipids would bring many improved properties to applications where the proper carbohydrate headgroups' specific interactions with certain biological agents are highly desired. These include biosensor design[32-34] and targeted drug delivery using vesicles.

Lipid vesicles, or liposomes, are spherical, self-closed structures composed of curved lipid bilayers which entrap some of the surrounding solvent into their interior. They are made predominantly from amphiphiles and may consist of one or several concentric membranes. As one specific type of drug carrier, vesicles have several advantages[35] compared to other carriers. 1) They are made from natural constituents and thus are biodegradable and relatively nontoxic. 2) They can be made, relatively easily in many cases, from a wide variety of components. This leads to different biological

properties for different types of vesicles. In principle, these properties can be modified in accordance with the goals of the specific therapeutic application. 3) For basic liposome-drug preparations no chemical bond formation is required, although covalent bonding, such as the coupling of protein, can be done to modify the basic liposome structure. In general, drugs are trapped without chemical modification either in solution in the internal aqueous spaces of the liposome (water-soluble drugs) or in the lipid bilayer (lipid-soluble drugs). Thus, when the drug is released at the appropriate site, it is usually released in the form in which it was entrapped.

Two key issues (or difficulties) in gene or targeted drug delivery using vesicles are: 1) how to specifically reach the targeted tissues or cells; and 2) how to deliver the encapsulated content into the target cells in a controllable fashion.[36] A successful drug delivery vehicle has to have both features to function satisfactorily. In most cases, there are several components that carry out individual functions. First of all, there are natural or unnatural lipids that serve as the structural components of vesicles; then there are ligands associated with the surface of the vesicles for recognition; and finally there are specific components or some built-in functionality which can respond to triggering factors (which include enzymatic, thermal binding or pH cues) and cause release of encapsulated content after fusion of the vesicles with the targeted cell membranes. Some recent novel endeavors include using synthetic peptides' conformational changes in response to pH variation to mediate controlled release[37-39] and inducing desired phase change of cationic liposomes to trigger release[40].

Although there have not been any precedents, I think it should be possible to combine these functions in one single molecule. With the maturity of today's synthetic

organic chemistry, it should be practical to design and synthesize lipids that carry multi-functional groups responsible for more than one job and therefore are much more efficient in terms of their role in targeted delivery. There are many functionalities, sometimes not present in natural lipids, that are sensitive to environmental changes, both physical and chemical, and therefore can be used as gate-keepers of liposome contents once they are incorporated into the constituent lipids. For instance, acetals are labile under acidic conditions while stable under basic conditions; ester groups can be modified to be sensitive to either basic or acidic conditions; properly positioned alkenyl groups can be used as efficient cleavage/cyclization triggers under Fraser-Reid conditions when there are intramolecular nucleophiles present. Some neutral basic functional groups on lipids can also be used as potential conformational changing factors to initiate leakage. For instance, upon the protonation or ionization of such groups, electrostatic repulsion may lead to phase change or reorganization of the vesicle structure and if such reorganization is dramatic enough, leakage could be induced during the process. There is also much that can be done in designing lipid polar headgroups to enable recognition of certain cell surfaces. Apart from attaching macromolecular ligands on liposome surfaces as a conventional method for recognition, one can also attach small molecules such as oligosaccharides that could be recognized by certain receptors on targeted cell surfaces to lipids through a variety of linkers. With the many glycosylation techniques available, synthesis of such glycolipids is certainly possible.

The first part of Chapter 2 (§ 2.1) touches on this area. The possibility of designing, synthesizing and characterizing such a multi-functional lipid on a practically useful scale is demonstrated. Instead of the fatty acyl moieties found in most

diacylglycerol glycolipids, a 2,2-diakyl-1,3-dioxolane function provides the hydrophobic moiety of the molecule. This brings base stability, possibilities of new packing arrangements, and the potential for use in encapsulation strategies using liposomes where a decrease in pH is used as the environmental cue for release. The acetal linkage also makes the molecule unsusceptible to degradation by phospholipase A and other esterase activities found in biological systems thus further extending their utility. It also affords synthetic simplicity since only one dioxolane acetal is formed on reaction of butane-1,2,4-triol with long chain symmetric ketones, whereas in the case of glycerol, one hydroxyl group has to be selectively protected to avoid the formation of both enantiomers[41-44]. Besides its promising potential as a candidate for a drug delivery vehicle with controlled release feature, this molecule's stable lamellar phase behavior and the conserved conformational preference of a typical double-chain glycolipid also makes it a potential substitute for the important naturally occurring glycolipid monoglucosyl diacylglycerol (MGDG).

MGDG are excellent lipids for the formation of lamellar systems and can be used in many of the broad applications discussed earlier. They are also structural precursors of many biologically important substances, such as sulfoquinovosyl diacylglycerol (SQDG). Unfortunately, such naturally occurring compounds as MGDG are commercially available in only very small quantities with heterogeneous lipid chain length distribution and various alkyl chain modifications. Traditionally, the chemical synthesis of optically pure MGDG inevitably involved protection and deprotection of glycerol linkers and suffered the problem of selective deacetylation at the end of the synthesis if peracetylated sugar was introduced as the glycon[41-44]. Simple structural surrogates of MGDG are not

known. Simple, efficient routes to glycolipids that closely match them and meanwhile have versatile chemical and physical properties that enable broad applications are therefore highly valuable.

The strategy of replacing the glycerol linker with (*S*)-1,2,4-trihydroxybutane to quickly access to the novel MGDG analog that described in § 2.1 was further expanded into the second part of Chapter 2 (§ 2.2). Two other close structural analogs of MGDG as well as the biologically important target SQDG, which is known for its anti-HIV activity, were synthesized.

It is important to understand that the conformational properties and the characterization of the phase behavior of such self-assembling system has proven to be challenging due to their almost unavoidable mesomorphism nature[45]. Historically, advances in the understanding of lipid mesomorphism have been closely tied to the development and use of techniques for probing molecular structure. Many different physical techniques – X-ray and neutron diffraction, NMR spectroscopy, electron spin resonance, electron microscopy, infra-red and visible light spectroscopies, calorimetry, polarized-light microscopy, etc. – have been useful in studying lipid phases[46]. Of these, X-ray diffraction, NMR spectroscopy and calorimetry have been most central to our understanding of lipid phases. X-ray diffraction is directly sensitive to the relative positions of the atoms in a mesomorph and is, therefore, a fundamental tool for the identification of the structural rearrangements which characterize different lipid phases. Unfortunately, X-ray diffraction patterns typically require long exposure times (although this is changing with the advent of intense synchrotron X-ray sources) and so the information which is derived is time-averaged over the exposure interval. NMR, on the

other hand, probes the local environment of the excited nuclei over time scales of, typically, tens of microseconds, and may be used to gain information on the dynamics of the mesomorph. The drawback of NMR is that long-range structure, which is very readily determined by X-ray diffraction, can only be indirectly inferred via NMR. Calorimetry has been the most important as a simple identifier of the temperature at which phase transitions occur, since many lipid phase transitions involve heat changes which can be straightforwardly measured. However, calorimetry does not, itself, yield structural information. Finally, optical microscopy, especially polarized-light microscopy pattern provides empirical information of phase determination as well.

It is well beyond the scope of this chapter to thoroughly discuss the principles of any of the major physical methods used to study lipid phases. However, it is important to understand the basic uses and limitations of these methods that were employed in the characterization of the self-assembling systems in chapter 2 and chapter 3.

In general, 2-D NMR homo-nuclear and hetero-nuclear correlation spectroscopy together with nuclear Overhauser effect experiments and molecular mechanics calculation were used to obtain information on the dynamics and conformational preference of these amphiphiles (e.g. the headgroup orientation, the configuration and flexibility of the linker connecting the hydrophobic and hydrophilic regions, and sometimes the lipid chain arrangement as well). Careful analysis of 1-D ^{1}H NMR data such as the signal broadening, unusual coupling constants, etc., was carried out to extract important information on both the dynamics and the packing behavior of such molecules as well. X-ray powder diffraction, differential scanning calorimetry (DSC) and optical microscopy experiments were performed to characterize the phase behavior. It is

important to point out that any conclusions drawn were based on the overall consideration of all lines of information available.

II. Role of templating effects in the regio- and stereo-control of reactions in highly ordered supramolecular ensembles

One of the most significant challenges presently facing chemists and biologists is to define the 2-D structure and function of biological membranes. It has long been known that biomembranes play a crucial role in living cells by serving both as selective barriers for transport and as sites for molecular recognition and catalysis. Although a considerable amount of information has emerged concerning the composition and dynamics of biomembranes, their 2-D organization still remains to be fully defined[47]. Many questions closely related to this are therefore still to be addressed at a higher level. For instance, do lipids organize themselves into nonrandom supramolecular structures or domains? If they do, what is their functional importance? And how do they 'intimately' become involved in basic membrane processes such as fusion, transport, recognition and catalysis? Do changes in domain structures induce the formation of a diseased state, for instance, the malignant transformation of cells? Such questions are not only of high theoretical interest, but have far-reaching practical implications.

We are particularly interested in asking the fundamental question of whether and how the packing order, symmetry properties, steric and proximity factors of membrane systems or other highly ordered supramolecular ensembles encode the regio- and stereo-regularities of reactions occurring on their surfaces. Such a question is obviously of

critical importance, but are also of considerable complexity as well. As a first step towards seeking the answer, our attention has focused on simple model systems.

In chapter 3, a series of glycolipids were designed, synthesized, and the predominant supramolecular structures they form were characterized. Transglycosylation reactions were then initiated among some of these systems, the regio- and stereo-regularity of the transglycosylation products were then analyzed. Our goal is to shed some light on the encodement of 1-D linkage and sequence information in the 2-D organization of supramolecular ensembles. This would not only ultimately help us understand many important membrane-associated processes, such as the regio- and stereo-selective biosynthesis of oligo- and polysaccharides, but help us transform this secret of nature into practical applications, such as regio- and stereo-selective organic synthesis based on non-covalent ordering.

III. Synthesis of 5 and 6-carbon chiral intermediates using carbohydrates as chiral synthons

Optically active compounds are ubiquitous. An overwhelming majority of naturally occurring medicinal agents, flavors and fragrances are chiral molecules. And they almost all exist in nature as the single, active enantiomer. Indeed, the essential components of life - proteins, DNA and carbohydrates - are constructed from optically active building blocks. The legendary pioneer of chiral chemistry Louis Pasteur made the insightful remark on the predominance of chirality in nature back in the nineteenth century:

The universe is dissymmetrical; for if the whole of the bodies which compose the solar system were placed before a glass moving with their individual movements, the image in the glass could not be superposed on reality... Life is dominated by dissymmetrical actions. I can foresee that all living species are primordially, in their structure, in their external forms, functions of cosmic dissymmetry.

With the recognition of the universal phenomenon of enantioselectivity in biological activity and lessons learned from many tragic incidents of administrating racemic drugs, the common practice of synthesizing racemates is rapidly outdated both in industry and in research. Nowadays, chirality has become an important factor in the design of many new compounds with improved properties. A growing number of pharmaceuticals, flavors, fragrances, and agrochemical products possess elements of chirality, and the demand for an even broader range of chiral intermediates will continue to escalate.

Chiral hydroxyl acids constitute an important class of compounds that have been employed as chiral auxiliaries and as building blocks for the synthesis of a wide variety of biologically active molecules. They are also logical precursors of corresponding amino acids. Five and six-carbon chiral β- and γ-hydroxyl acids are no exceptions. However, despite their well-recognized value as both chiral auxiliaries[1-4] and as synthetic building blocks[5-8], economical manufacture of these enantiomerically pure compounds has proven difficult, and thus commercial access is limited to relatively few of these useful chiral intermediates. Simple access to these molecules is therefore still highly desirable.

Another related class of compounds is chiral amino acids. Besides their fundamental significance as building blocks of proteins and other essential natural

substances, amino acids are also important intermediates for the production of a whole spectrum of pharmaceuticals, food additives and agrochemicals.[9-11] β- or γ-Amino acids are not only important signaling molecules (e.g. γ-Amino butyric acid, or GABA, is an important neurotransmitter[12,13] that mediates many central nerve system activities.) and critical substructures of many naturally occurring molecules[14], but also widely used building blocks of various β-peptides[15], β-lactam antibiotics[16] and enzyme inhibitors[17]. Whereas the proteinogenic amino acids can be produced by extraction from natural raw materials or by fermentative processes, the preparation of unnatural and non-proteinogenic amino acids such as γ-amino acids requires chemical synthesis or enzymatic processes.

Despite the major advantage of high selectivity and mild reaction conditions of biocatalysis, the economical viability of a chemo-enzymatic route can only be assured after achieving several additional technical and economical requirements. These include availability and cost of starting material and biocatalyst (enzyme), selectivity and productivity of the enzyme and the process, and the ease of workup and isolation of the product. These requirements can not always be met due to the limitation of most enzymes' stability in extreme pH and temperature, their high cost and sometimes unsatisfactory selectivity, the difficulty of efficient recycling of some Redox cofactors, the substrate and product inhibition, etc.

There are several synthetic approaches to obtain chiral intermediates in general. As it is usual in life and especially in chemistry, there is never a superior technology in general. It always depends on the nature of the pursued chiral intermediate. In cases where racemic compounds are readily and cheaply available, the resolution of those may

be the method of choice. This approach gains additional advantages if both enantiomers are needed or the undesired enantiomer can be recycled efficiently. Several disadvantages of resolution process are: 1) It requires extra steps to recover the desired isomer, and the maximum theoretical yield is 50%; 2) Perfect resolutions are rare. Separation of the product from racemic substrates in a kinetic resolution may be problematic; and 3) Very often it could lead to the waste of half of the product due to the decomposition of the other isomer during the process.

Direct obtainment of optically pure forms can be realized through enantioselective reactions using either chiral reagents, chiral auxiliary or transition metal catalysts with chiral ligands. From many research chemists' point of view, asymmetric synthesis is by far the most elegant one, especially if one succeeds in developing a 'true' catalytical process. But, in many cases these processes are not as cost-effective as alternative methods or are not easy to scale up. Such approaches tend to be costly due to the often expensive noble transition metals and/or hard-to-prepare chiral ligands. The stereoselectivity of these processes is not always impressive. Another big disadvantage is the toxicity of most of the transition metal catalysts used in such processes, which poses serious environmental problems.

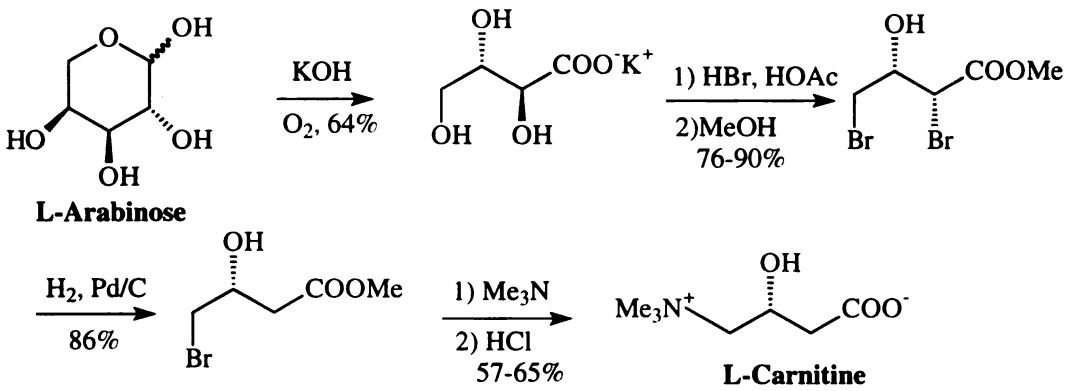
An alternative approach towards asymmetric structures in enantiomerically pure form takes advantage of the readily available optically pure starting materials of known absolute configuration.[18] In many cases, it may be best to start with a raw material from the chiral pool, as there are rich natural sources of carbohydrates, amino acids, terpenes and alkaloids readily available. The challenge is to transform the starting material with as few steps as possible to the desired chiral compound without affecting the chiral centers.

The optical purity of the product is typically very high if no racemization of the original stereocenters occurs during the transformation. Two major limitations for this approach are: 1) It is sometimes difficult to recognize the hidden links between the chiral pool and the final target especially when the starting material does not resemble much of the final target in structure; 2) It is not always easy to design a concise route to convert the selected chiral synthon into the desired structure.

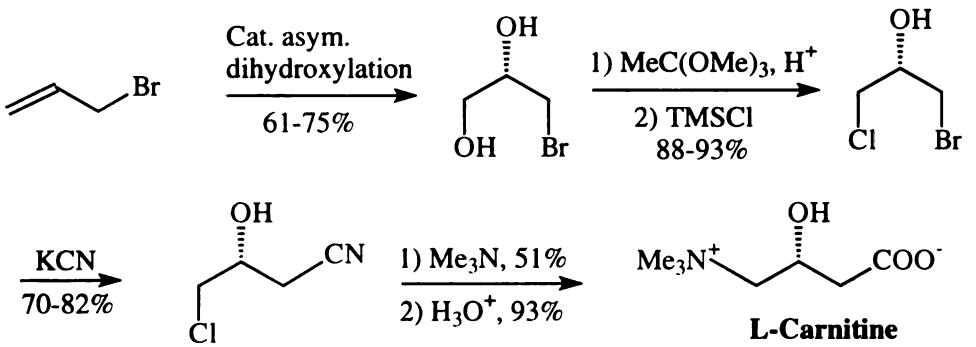
The readily available carbohydrates and their numerous derivatives make an unequaled source of enantiomerically pure starting materials.[18,19] They are generally inexpensive. And there are rich choices of sugars with different combinations of stereocenters. More importantly, the stereo-control obtained in small rings (furanoses or pyranoses) is typically very good. However, despite the greater awareness of carbohydrate synthons in recent years, the full potential of the carbohydrate chiral pool is still little exploited. Carbohydrates, as multihydroxyl compounds, often discourage chemists to select them as chiral pool by its seemingly unavoidable tedious selective protection deprotection procedures and the removal of unwanted functionalities in the end of the synthesis. The lack of knowledge of some less common naturally occurring sugars and their inexpensive availability by synthetic chemists is another barrier. These compounds have usually been prepared by carbohydrate chemists who do not often venture outside their field and attempt to synthesize non-carbohydrate compounds. Finally, the potential of handling carbohydrates without protection deprotection chemistry has not yet been fully explored and taken advantage of by the majority of synthetic chemists. It is important to point out that some of these limitations could also be transformed into advantages, provided it is handled wisely. For instance, the drawback of

carbohydrates' overfunctionalization also gives them great potential and versatility. Where chiral centers in other areas of chemistry are usually preserved with great care, this need not be the case in carbohydrate chemistry. The synthesis of a noncarbohydrate compound using the carbohydrate chiral pool where unwanted chiral centers are quickly eliminated are probably more desirable than approaches where the introduction of new chiral centers require extreme caution. Even for the synthesis of compounds with only one chiral center, use of a carbohydrate building block can often compete with other strategies. In the case of the synthesis of L-carnitine (shown in Schemes 1-3), the approach using L-arabinose as chiral synthon[20] (Scheme 1) was neither longer nor less practical than either transition metal catalyzed enantioselective dihydroxylation[21] (Scheme 2) or the one starting with more carnitine-like, but more expensive, (+)-malic acid[22] (Scheme 3).

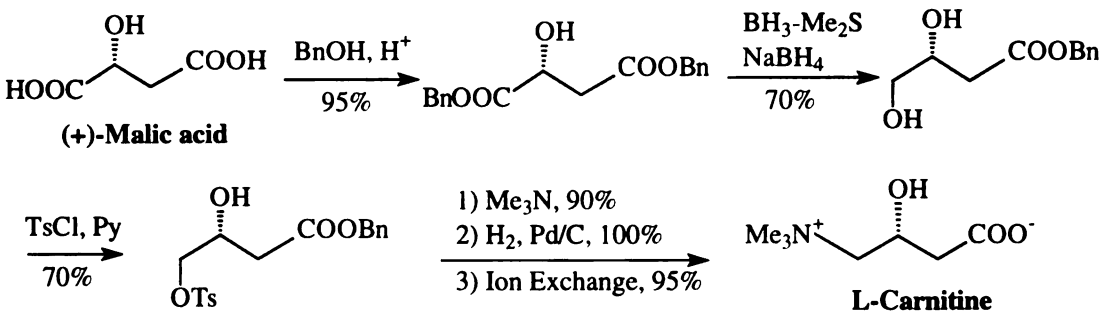
It is the intention of this part of my thesis to explore the possibility of using carbohydrates and their lactone derivatives as chiral synthon to quickly access to important chiral intermediates such as β-hydroxyl acids, γ-hydroxyl acids and γ-amino acids. The development of general and concise routes towards these compounds is our goal. Much of the emphasis is therefore directed towards the chemistry that is free from traditional carbohydrate protection and deprotection procedures. To make effective use of carbohydrates in synthesis, it is important to benefit from the vast practical experience in the field and look for opportunities to combine such experience with specific targets to simplify the solution.



Scheme 1. L-Carnitine synthesis from L-arabinose.



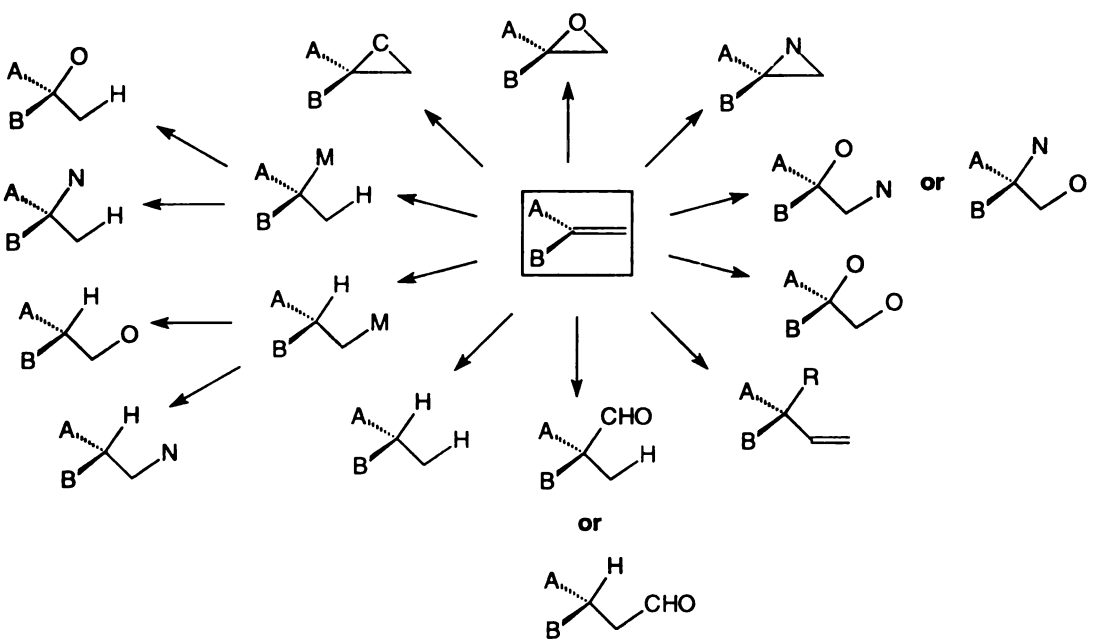
Scheme 2. Catalytic asymmetric synthesis of L-carnitine.



Scheme 3. L-Carnitine synthesis from (+)-malic acid.

A very important while still not fully explored area in carbohydrate chemistry is the chemistry of aldonolactones. Many aldonolactones are readily available, either commercially or by oxidation of the parent sugar with bromine. As the oxidative form of sugars, lactones possess many unique chemical properties which when exploited could be very useful in simplifying the chemical transformation based upon them. Much of the pioneering work using aldonolactones as chiral synthons was done by Pedersen, Lundt and their colleagues.[23] It is well known that both the primary hydroxyl and the α-hydroxyl group are more reactive than other hydroxyl groups due to steric and electronic reasons. In the meantime it is not difficult to differentiate the reactivity of these two since the primary hydroxyl is more reactive towards sterically hindered reagent while the α-hydroxyl is more reactive towards electron-rich reagent. This opens the opportunity to carry out a series of transformations without the usual protection chemistry. Indeed, it is well established that reaction of aldonolactones with hydrogen bromide in acetic acid result in the introduction of bromine to either α-position, or the primary position, or both positions with no need of protecting other hydroxyls.[24]

When this is combined with the versatility of transformations of bromo-group to other functionalities, such as via alkylation, substitution and elimination, a wide range of chiral intermediates could be quickly accessed. One of the useful functionalities could be easily introduced by such process is the alkenyl group. By controlling the regioselectivity of bromination and the subsequent stereoselectivity of elimination (syn- vs. anti-), one can have access to α, β-unsaturated lactone, vinyl lactone or doubly eliminated system at will. From there, the well-established alkene chemistry makes further functionalization in stereoselective fashion possible. Some examples of such transformations are illustrated in



Scheme 4. Reactions of prostereogenic alkenes.

Scheme 4, including hydrogenation[25], cycloaddition[26], cyclopropanation[27], epoxidation[28], aziridination[29], Michael addition[30], dihydroxylation and hydroxyamination[31], Heck reaction[32], hydroboration and hydrosilylation[33], hydroformylation[34], etc.

Therefore, it seems to be practical to develop concise and general routes from carbohydrate lactones towards highly functionalizable alkenyl containing chiral intermediates. 5- and 6-carbon chiral β- and γ-hydroxyl acid intermediates are of our particular interest because of their obvious practical importance and the current lack of efficient and general access to them in literature.

The essence of our strategy is to introduce vinyl group using selective bromination chemistry of aldonolactone and the subsequent elimination. This approach is free from usual protection and deprotection chemistry and is very easy to carry out (usually at room temperature or 60°C) and scale up. This is a major advantage comparing to the more commonly employed while much more difficult to process Wittig Reaction. In the existing literature of syntheses of anticonvulsant drug vigabatrin, almost all methods rely on Wittig Reaction to introduce the crucial vinyl group.[35-41] Another challenging issue is how to selectively retain the desired chiral center (β- or γ-position) while quickly get rid of the others.

In the first part of Chapter 4 (§ 4.1), the development of a concise and general route towards 5-carbon chiral β-hydroxyl acid using 2-deoxyribose as chiral synthon was presented. Two important pharmaceutical intermediates (*S*)-β-hydroxy-4-pentenoic acid and (*S*)-4-pentene-1,3-diol were synthesized in high yield and e.e. via an efficient 3-step sequence. The second part of Chapter 4 (§ 4.2) demonstrated the approach towards both enantiomers of chiral 4-hydroxy-5-hexenoic acids using inexpensive δ-gluconolactone

and *L*-mannonic-γ-lactone as chiral synthons. And as a demonstration of one of its many potential applications, the increasingly important pharmaceutical target Vigabatrin® (γ-vinyl-γ-amino-butyric acid) was synthesized in a short synthetic route from this chiral γ-hydroxyl acid precursor.

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Chapter 2

Design, Synthesis and Characterization of Self-assembling Glycolipids with Defined Chemical, Physical and Biological Properties

§ 2.1 Design, Synthesis, Conformational Analysis, and Phase Characterization of a Versatile Self-assembling Monoglucosyl Diacylglycerol Analog that Respond to pH Changes

(Adapted from the paper entitled 'Synthesis, Conformational Analysis, and Phase Characterization of a Versatile Self-assembling monoglucosyl Diacylglycerol Analog', Jie Song and Rawle I. Hollingsworth, *J. Am. Chem. Soc.* **1999**, *121*, 1851-1861.)

Abstract

Glycosyl diacylglycerols are excellent lipids for the formation of both bi and monolayer lamellar systems but they are generally not commercially available and the synthesis of optically pure glycosyl diacylglycerols inevitably involves protection and deprotection of glycerol linkers. A novel glycolipid designed to be an easily accessible structural surrogate of monoglucosyl diacylglycerol (MGDG) has been synthesized. In this molecule, glycerol is replaced with (S)-1,2,4-trihydroxybutane. Instead of the fatty acyl moieties found in MGDG, a 2,2-dialkyl-1,3-dioxolane function provides the hydrophobic moiety of the molecule. This different functionality affords chemically and physically tunable new properties in a glycolipid. These include base stability, increased mobility of the headgroup, possibilities of new packing arrangements and the potential for use in encapsulation strategies using liposomes where a decrease in pH is used as the environmental cue for release. The acetal linkage also makes the molecule unsusceptible to degradation by phospholipase A and other esterase activities found in biological systems thus further extending their utility. The choice of the butane triol linker removes the common problem of racemization of protected glycerol by acetal and ester migration. It also affords synthetic simplicity since only one dioxolane acetal is formed on reaction of butane-1,2,4-triol with ketones, whereas in the case of glycerol, one hydroxyl group has to be selectively protected to avoid the formation of both enantiomers. 2-D NMR homo-nuclear and hetero-nuclear correlation spectroscopy together with nuclear Overhauser effect experiments and molecular mechanics calculation were used to obtain information on the headgroup orientation and on the configuration of the trialkoxybutane backbone. These supported a structure in which the alkyl chains were extended in a

parallel fashion and the headgroup, although free to rotate along C2-C3 and C3-C4 of the trialkoxy butane substructure, extended away in the other direction in a manner similar to that observed in the case of MGDG. X-ray powder diffraction and optical microscopy data both supported a lamellar phase behavior of this amphiphilic molecule in water. ^{1}H-NMR experiments monitoring the rate of acetal cleavage of this amphiphile revealed its tunable acid susceptibility. The unique structural feature, phase behavior and controllable acid susceptibility of this glycolipid is potentially useful in many applications.

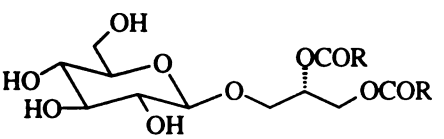
Introduction

Lipids are excellent molecules for use in the design and fabrication of highly ordered 2-dimensional molecular systems. They find many applications ranging from the preparation of biocompatible films and drug delivery vehicles to biosensor design and nanotechonology.[1] Phospholipids and simple alkyl species with polar headgroups have been extensively studied in this context. Glycolipids, especially those with two hydrocarbon chains, also have great potential in the preparation of self-assembled lamellar systems. Molecules with single alkyl chains tend to form micelles. Glycolipids are typically uncharged (unlike phospholipids) and this not only gives them more chemical flexibility from the standpoint of synthesis and solubility, but also makes them better candidates in applications where charged species would have undesired properties or lead to complications. For instance, it is known that as drug delivery vehicles, charged vesicles are usually more toxic than neutral ones.[2] Moreover, glycolipids have several hydroxyl groups giving them great potential for use in applications that require surface modification. If a surface is modified with a glycolipid array, the headgroups can be

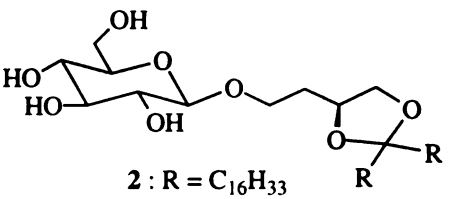
halogenated, acylated, alkylated, oxidized, phosphorylated or modified by a whole spectrum of chemical or biological processes. The correct carbohydrate headgroup can serve as an acceptor for other glycosyl groups either by enzymatic or traditional chemical means. Hence a glycosylated surface that interacts in some special manner with some biological agent or cell can be fabricated. Finally, glycolipids that are able to form lamellar systems are excellent models for studying templating effect and regio- and stereo-chemistry of oligosaccharide synthesis upon ordered surfaces, such as membranes.

One excellent example of a glycolipid with potential for use in the applications mentioned above is the naturally occurring compound monoglucosyl diacylglycerol (MGDG) **1**. This is one of the common glycolipid components of the membrane of cellular systems, especially bacteria.[3-5] Unfortunately, MGDG and other double-chain glycolipids are commercially available in only very small (milligram) quantities as isolation products from various natural sources. Such products have a heterogeneous lipid chain length distribution and various alkyl chain modifications such as unsaturation and branching. The enzymatic synthesis of membrane glycolipids is achievable,[6] but it would be very expensive especially if large quantities are needed. The chemical synthesis is therefore the solution to large-scale production of homogeneous products. However, this is not trivial, either. This is especially due to the inevitable problem of the enantioselective protection and deprotection of the glycerol linkers in order to prepare optically pure glycosyl diacylglycerols.[7-10] Simple structural surrogates of glycolipids such as MGDG **1** are not known. A simple, efficient route to glycolipids that closely match them and meanwhile have versatile chemical and physical properties that enable broad applications is therefore highly desirable. Such molecules should have two alkyl

chains, and the carbohydrate headgroup should be linked to the hydrocarbon chains via a substructure that closely approximates the glycerol moiety of typical glycolipids. The orientation of the carbohydrate headgroup relative to the hydrocarbon chains should also be conserved. The glycolipid surrogate should pack and form stable lamellar systems. The new functionality introduced should have useful chemical properties that lend themselves to practical applications. In addition to all of these, the new lipid should be optically pure and easy to assemble in only a few steps. In this work, we describe the synthesis and properties of the glycolipid **2**, which bears a close resemblance to MGDG **1** in terms of both its ability to form lamellar system, its headgroup orientation and linker flexibility. Its preparation is simple and requires only three key steps. This new glycolipid, however, also has several new physical and chemical properties that lend themselves to a variety of important applications including the preparation of base and lipase stable liposomes and liposomes that can be cleaved below a specific pH.



1 : R = $C_{15}H_{31}$



2 : R = $C_{16}H_{33}$

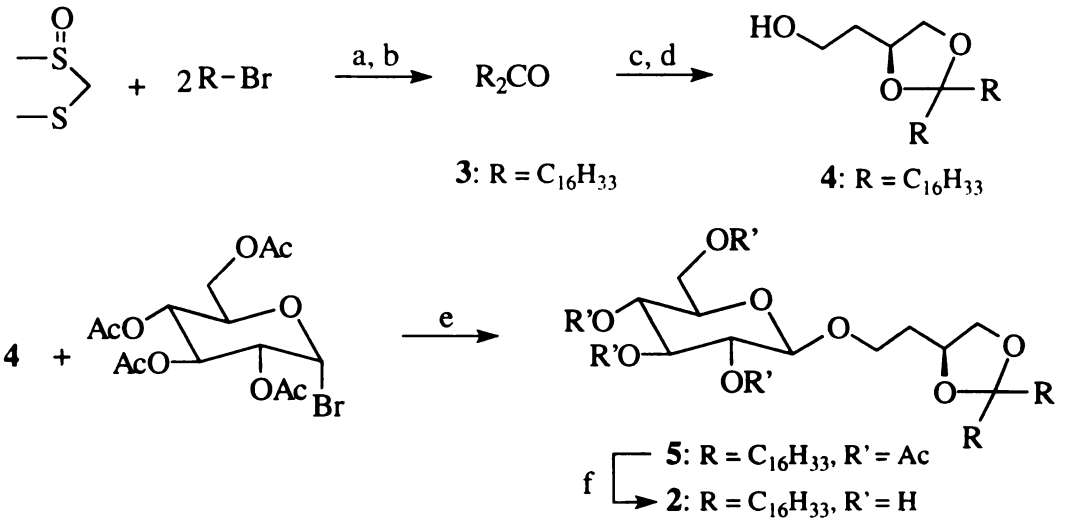
Results and Discussion

Design and Synthesis of Glycolipid 2

In order to maintain a close resemblance to the glycerol moiety of MGDG **1**, an optically pure (S)-1,2,4-butanetriol was chosen as the chiral linker connecting the sugar headgroup and the hydrocarbon tails. Two 16-carbon hydrocarbon chains were attached to the linker via an acetal linkage. Acetals are stable to base and this would allow the de-

esterification of the per-acetylated sugar at the end of the synthesis without fear of losing the hydrophobic chains in the aglycone. In the case of the typical acyl glycerols, this deprotection is problematic because of the risk of cleaving the fatty acyl groups. The acetal function should also give some extra ordering to the headgroup once the lamellar array is formed because of its rigidity. Another advantage beyond these is that the acetal linkage cannot be degraded by the esterolytic lipase activities (especially phospholipase A type activities) that are present in all cellular systems. This is a problem with conventional liposomes and leads to leakage. Finally, the acid-sensitive acetal linkage could be utilized as a way to obtain sudden or controlled release in response to a pH cue if this glycolipid is incorporated in the construction of liposomes. Such liposomes would be clinically useful if they enable drugs to be targeted to areas of the body, such as primary tumors or sites of inflammation and infection,[11] where pH is lower than the normal physiological value. Another potential application is the controlled release of drugs to the stomach to fight ulcers and buffer acidity. In the latter application, once the pH drops, more vesicles would be cleaved releasing the buffering component at a greater rate. The resulting increase in pH would stop further hydrolysis and release until the pH drops again. This property may also be useful where cleavable surfactants under mild acidic conditions are desired. The resulting (S)-2,2-di-hexadecyl-4-hydroxyethyl-1,3-dioxolane was then β-linked to a glucose moiety to give glycolipid **2**.

The synthetic route is summarized in Scheme 1. A thirty three-carbon symmetric ketone **3** was prepared using methyl methylthiomethyl sulfoxide (MMTS) as carbonyl donor.[12] MMTS was dialkylated with hexadecyl bromide, and direct treatment of the crude dialkylated intermediate with hydrochloric acid in tetrahydrofuran gave symmetric



Scheme 1. Synthesis of Compound **2**. *Reagents, Conditions and Yields:* (a) NaH, THF, 50°C, 24hr; (b) 6N HCl, THF, r.t., 24hr, 80% (for 2 steps); (c) 3 equiv of TMOF, MeOH-THF (1:2, v/v), cat. *p*TSA, reflux, 12hr; (d) 1.5 equiv of (S)-1,2,4-butanetriol, DMF-THF (1:1, v/v), reflux, 24 hr, 69% (for 2 steps); (e) 0.4 equiv of HgO, 2 equiv of $HgCN_2$, PhH-CH_3NO_2 (1:1, v/v), 65°C, 12hr, 58%; (f) K_2CO_3 (s), MeOH, r.t., 6hr, quantitative. TMOF = trimethyl orthoformate; *p*TSA = *p*-toluenesulfonic acid.

ketone **3** in 80% overall yield. Ketone **3** was converted to dimethoxy acetal and then trans-acetalized with (S)-1,2,4-butanetriol. The more stable acetal, the 1,3-dioxolane **4** (as opposed to the six-membered cyclic acetal), was obtained in 69% yield from ketone **3**. The coupling of the chiral acetal linker **4** to α-acetobromoglucose in the presence of mercury (II) salts[13] gave stereospecifically the desired β-anomer **5** in 58% yield, which after quantitative deacetylation afforded target molecule **2**.

This synthetic route is short and efficient. No protection of the triol linker is needed prior to the acetalization as is the case with the glycerol linker where acetal migration would give the enantiomeric dioxolane leading to racemization. Syntheses employing chiral isopropylidene acetals of glycerol require special steps to avoid this problem.[8] The deprotection of the peracetylated glycolipid is also much more straightforward compared to the same reaction in the synthesis of diacyl glyceroglycolipids[14] since no selective deacetylation condition for preserving the long hydrocarbon chains needs to be employed.

The melting point ranges for both compound **5** (158.0-164.0°C) and **2** (140-145.5°C) were quite broad and they both went through a waxy phase before finally turned into clear liquid. This behavior is expected for such glycolipids since they form liquid crystalline phases just above the melting point.

Conformational Analysis

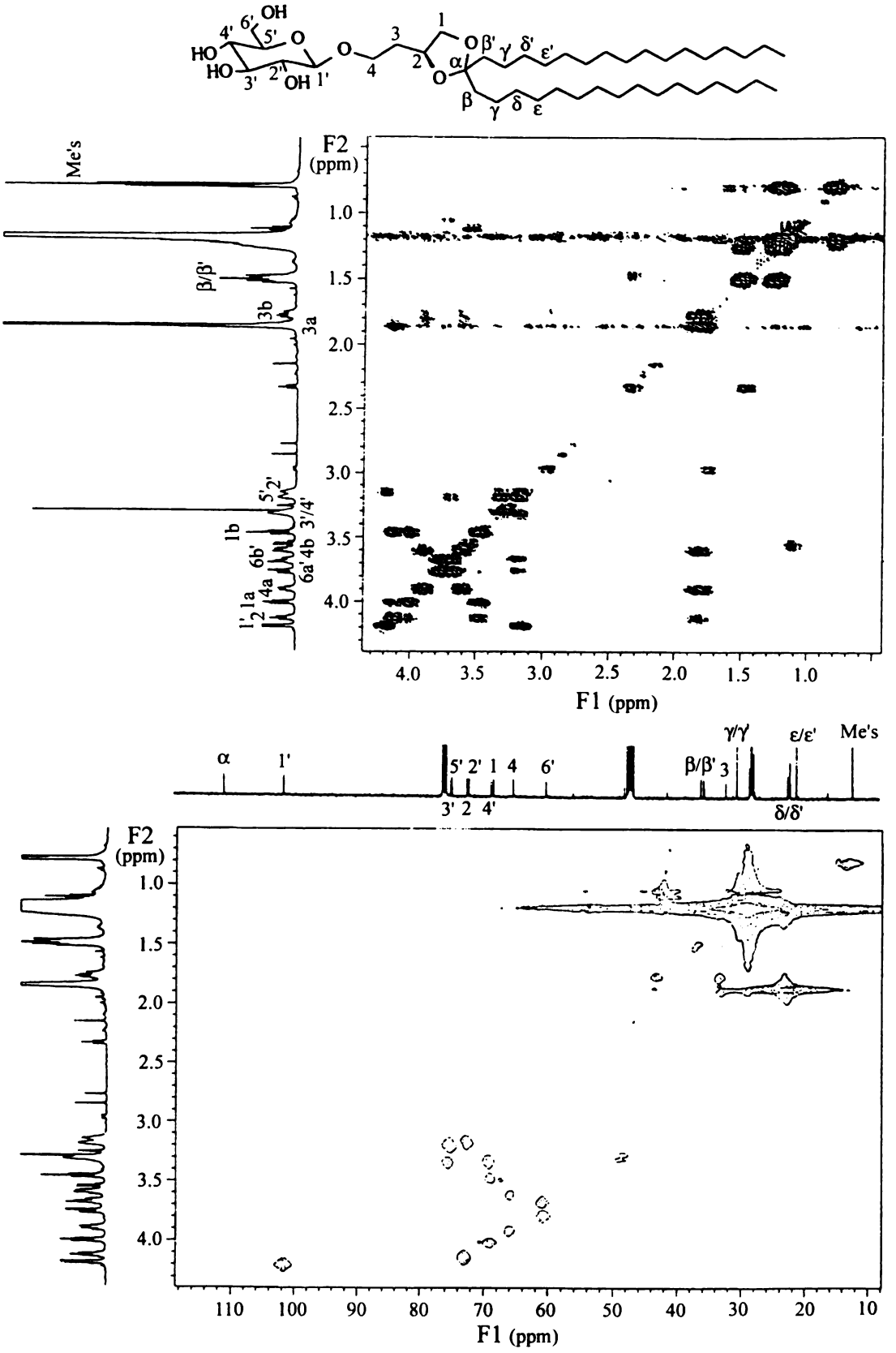
When lamellar systems crystallize, they often form thin leaflets at best. This makes X-ray structure determination difficult because of lack of information in one dimension. A variety of physical methods then have to be employed to gain information on the conformation and packing arrangement[15]. The average solution conformation,

packing and phase behavior of glycolipid **2** were characterized by a combination of NMR spectroscopy, molecular mechanics (MM) calculations, polarized-light microscopy, x-ray diffraction, and differential scanning calorimetry (DSC).

In order to get well-resolved ^{1}H and ^{13}C NMR spectra of glycolipid **2**, a solvent system of 2:1 / CD_3OD:$CDCl_3$ was used to balance between the substrate solubility and the solvent polarity. This ensured the free tumbling of the substrate in order to obtain acceptable spectral resolution.[16] A trace of pyridine was also added to stabilize the acetal linkage. All of the ^{13}C and ^{1}H NMR signals of compound **2** were assigned unequivocally with the aid of double quantum filtered J-correlated spectroscopy (DQF-COSY) and gradient ^{1}H-^{13}C hetero-nuclear multiquantum coherence spectroscopy (^{1}H-^{13}C HMQC) experiments (Fig.1).

The solution 3-dimensional structure of glycolipid **2** was determined by nuclear Overhauser effect spectroscopy (NOESY) experiments and molecular mechanics calculations. The nOe volumes and the calculated internuclear distances are listed in Table 1. The nOe volume and the known distance between sugar ring protons 1' and 5' were taken as references. Strong nOes between protons 1a/1b/2 and protons β/β' were also observed (Fig. 2), although the volumes could not be accurately measured because of the overlapping of the four β/β' protons. Semi-quantitative information could be obtained for these. The residual acetone peak also masked nOe information of protons 3a and 3b. Despite the severe overlapping of the hydrocarbon methylene signals at 1.18 ppm, we still observed nOe peaks between β/β' protons and γ/γ' protons on the downfield edge of the large methylene peak envelope. Although this information cannot be used

Figure 1. DQF-COSY (top) and ^{1}H-^{13}C HMQC spectra of compound **2**.


F2 (ppm)
F1 (ppm)
Me's
β/β'
3b
3a
5'
2'
1b
6b'
6a'
4b
3'/4'
1'
2
1a
4a
α
1'
5'
2'
1
4
6'
3'
2
4'
β/β'
3
γ/γ'
ε/ε'
Me's
δ/δ'

Proton Pairs	nOe volumes	Distances in Å
1'- 5'	229519	2.51
1'- 4a	137267	2.73
1'- 4b	240664	2.49
2 – 4b	68272	3.07

Table 1. Distances calculated from NOESY experiment for compound **2**.

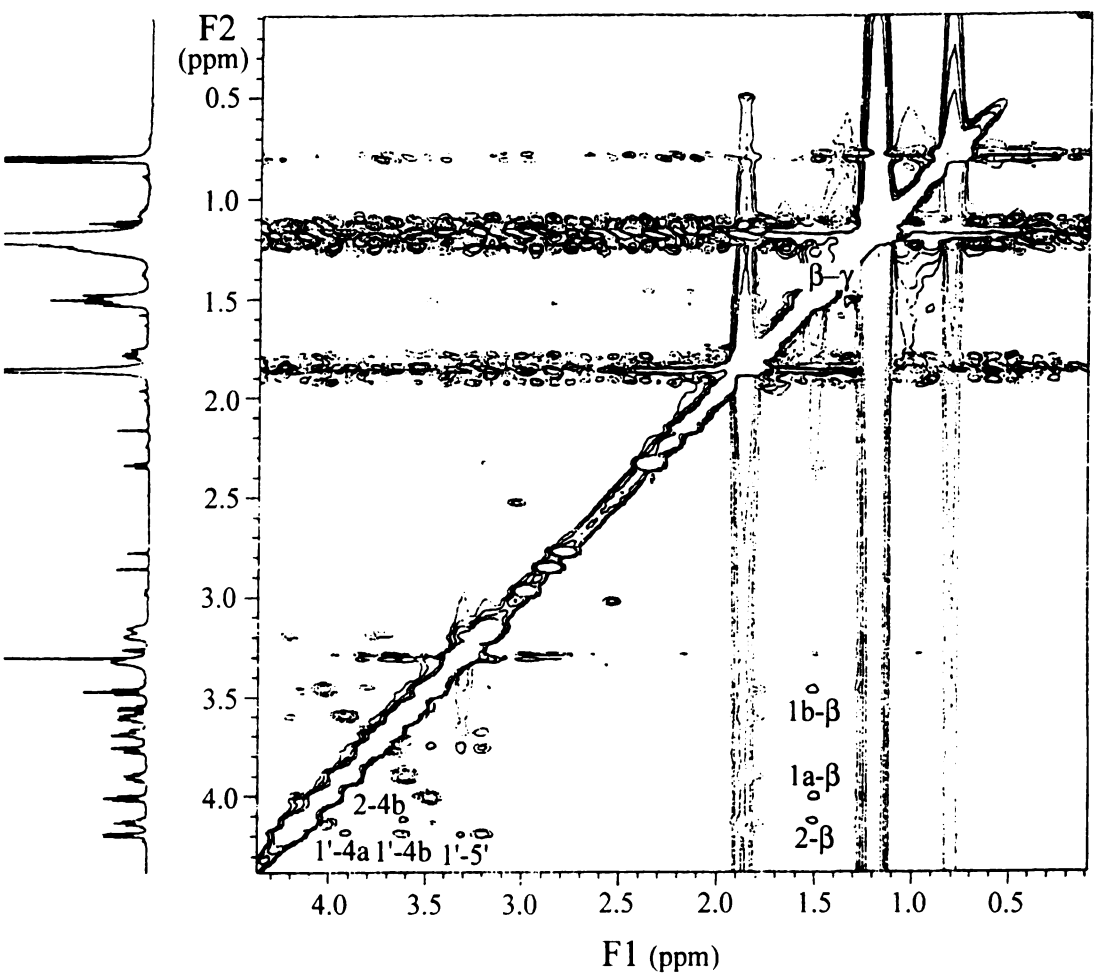


Figure 2. NOESY spectrum of compound **2.** A mixing time of 500 ms was used.

quantitatively, it provides very important evidence for the conclusion that the two chains are parallel. This (in addition to several other lines of information) allows the distinction between the proposed conformation and one in which the two alkyl chains are extended away from each other.

Molecular mechanics calculation using distance constraints listed in Table 1 gave the lowest energy conformer shown in Figure 3, in which the two hydrocarbon chains stay close together, with a *cis* turn at the beginning of each chain. This suggests that the gain from van der Waals interaction by packing two lipid chains close together is greater than the penalty for having the *cis* turns. The full length of this molecule was measured as 31 Å. The torsion angle ϕ and φ ($\phi = H_1'\text{-}C_1'\text{-}O\text{-}C_4$; $\varphi = C_1'\text{-}O\text{-}C_4\text{-}C_3$), which describe the relative orientation of the sugar headgroup, were 51.4° and 166.8° respectively, in this lowest energy conformer in which the glucose ring extends away from and along the same line as the hydrocarbon chains. These values matched very well with those obtained from MGDG conformational studies (ϕ = 47°; φ = 169°).[17-19] This demonstrates that the orientation of the sugar headgroup relative to the hydrocarbon chains is well conserved from MGDG to its structural surrogate **2**. The torsion angles describing the 4-carbon linker conformation were defined as θ_1 and θ_2 ($\theta_1 = H_{4a}\text{-}C_4\text{-}C_3\text{-}C_2$; $\theta_2 = C_4\text{-}C_3\text{-}C_2\text{-}C_1$) and they were measured as –173.8° and 78.0° in this lowest energy conformer.

Admittedly, in this study we did not take into account the surface interactions of this molecular ensemble, and also no solvent box was included during the calculation. Some recent studies of headgroup orientations of isotope-labeled glycolipids at lipid bilayer interfaces incorporated a membrane interaction term to the potential energy function in computer models. These studies offered important insight into the degree and

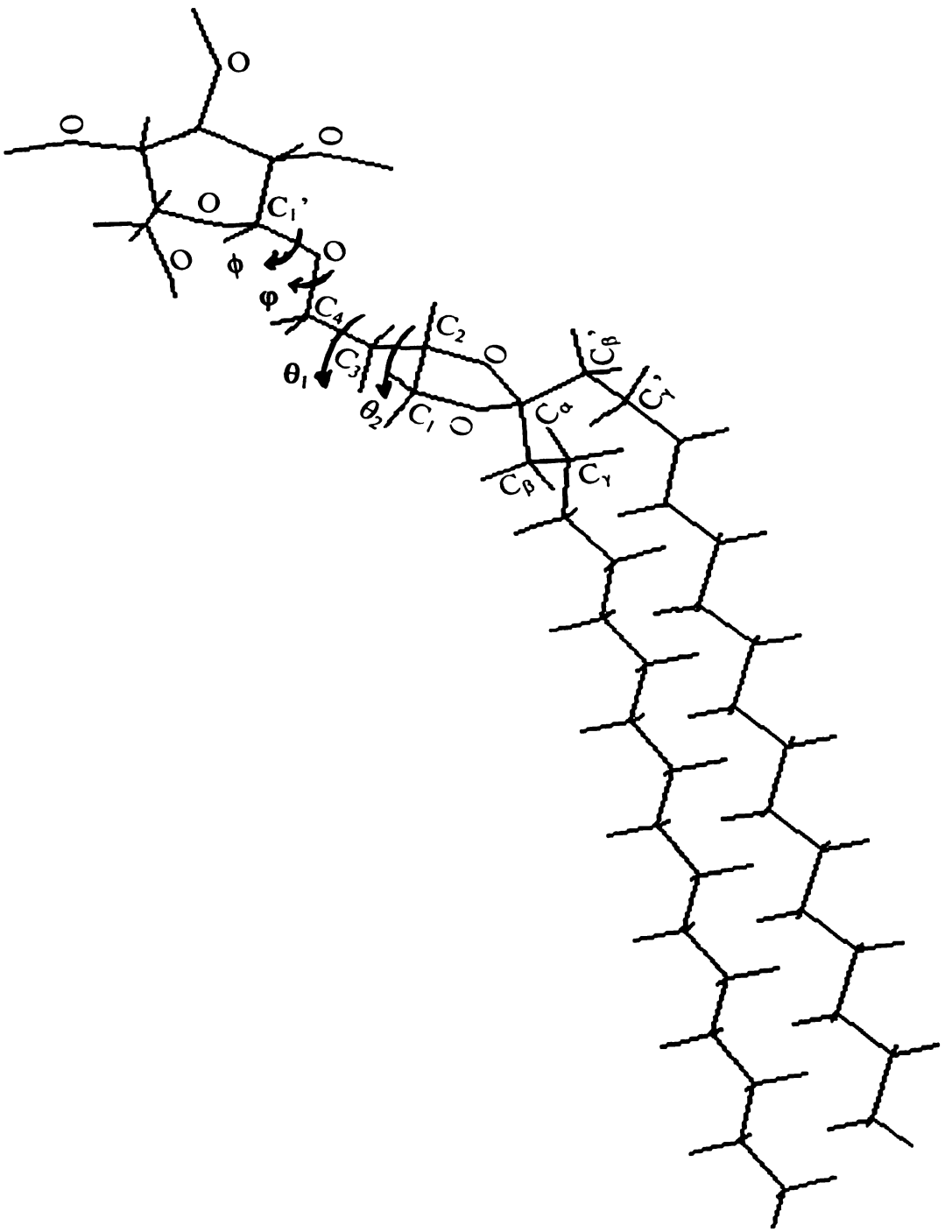


Figure 3. Favorable conformation of compound **2** obtained by molecular mechanics calculation.

nature of molecular motions at the surface of membranes.[20-24] While the results indicated more restricted motions of the carbohydrate ring in some of these systems, the average orientation of the headgroup is similar to those obtained by more simplified studies such as this one, where axial symmetry was assumed.

To gain insight into the relative flexibility about the various dihedrals of the headgroup linker region of molecule **2**, grid searches of dihedral angles ϕ-φ and θ_1-θ_2 were performed. The grid search results are shown in Figure 4. It is clearly shown that there is a relatively restricted conformational preference at the anomeric position whereas the 4-carbon linker employs a large degree of flexibility. In Figure 4A, there is essentially only one major energy minimum and the center of the energy contour matches the measured ϕ and φ of the MM calculated structure. However, in Figure 4B, there are many energy minima, which indicates that these very closely populated conformations all significantly contribute to the averaged solution conformation. Such regional conformational flexibility is consistent with the observations of the glycerol linker flexibility in natural glycerolipids[17,19] and is also expected on adding one more carbon to the glycerol linker.

The flexibility of the linker region was confirmed by an analysis of the coupling constants for the protons in the ^{1}H NMR spectrum of that region. Despite their complex chiral environments, the simple splitting patterns of the 7 spins indicated a mutual conformationally-averaged coupling of 6.6 Hz for all of the vicinal pairs. Protons 1a and 1b appeared as triplets; protons 2, 3a and 3b appeared as quintets; protons 4a and 4b appeared as doublets of doublet. The $^2J_{HH}$ (geminal) coupling constants were $^2J_{1a\text{-}1b}$ = 9 Hz, $^2J_{4a\text{-}4b}$ = 8.4 Hz and $^2J_{3a\text{-}3b}$ =6.6 Hz. Using these values, a spin simulation of this 7-spin

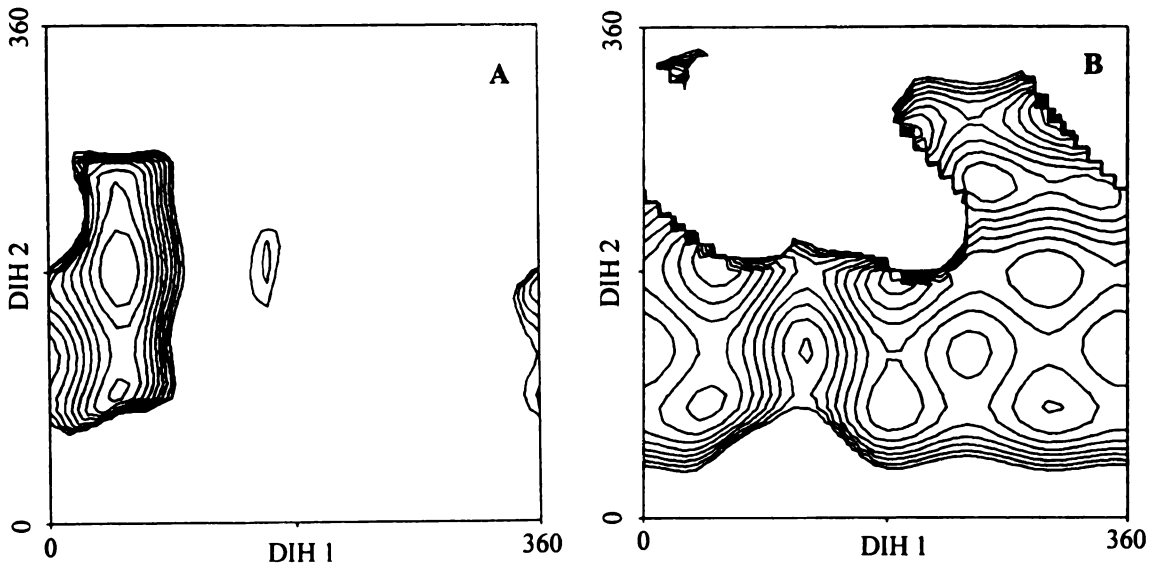


Figure 4. Grid search plots of compound **2**. (A) DIH 1 = ϕ = H_1'-C_1'-O-C_4; DIH 2 = φ = C_1'-O-C_4-C_3; (B) DIH 1 = θ_1 = H_{4a}-C_4-C_3-C_2; DIH 2 = θ_2 = C_4-C_3-C_2-C_1.

system was carried out and the result is shown in Figure 5. The excellent match of the simulated spectrum with the original spectrum proved the assignments of the coupling constants to be correct.

Characterization of Phase Behavior

To further explore the packing and phase behavior of compound **2**, especially in water, differential scanning calorimetry (DSC), x-ray diffraction and optical microscopy data were collected.

A transition at 33.7°C was observed in a DSC scan of compound **2** (in water) from 10°C to 98°C (Fig. 6), which may correspond to a transition from the gel-state to less ordered liquid crystalline state. This is lower than major phase transition temperatures of most natural dialkyl or diacylglycerol phospholipids and glycolipids,[25] but consistent with the reported phase transition temperature of some acetal-based double chain vesicles.[26] One can take advantage of this property using local heating to induce preferential release of entrapped species from liposomes. It was proposed that local hyperthermia could preferentially release drugs from liposomes in the heated area.[27] For this purpose, glycolipid **2** may be mixed with other lipids to construct liposomes exhibiting gel to liquid crystalline phase transitions at a temperature slightly above body temperature and thus attainable by local hyperthermia.

X-ray powder diffraction of the dry sample showed a very strong and sharp low angle reflection corresponding to a d-spacing of 40.16 Å and a medium reflection corresponding to 47.50 Å, which unequivocally revealed the long-range order in this system. They are about 1.4 times the full length of the calculated structure of compound

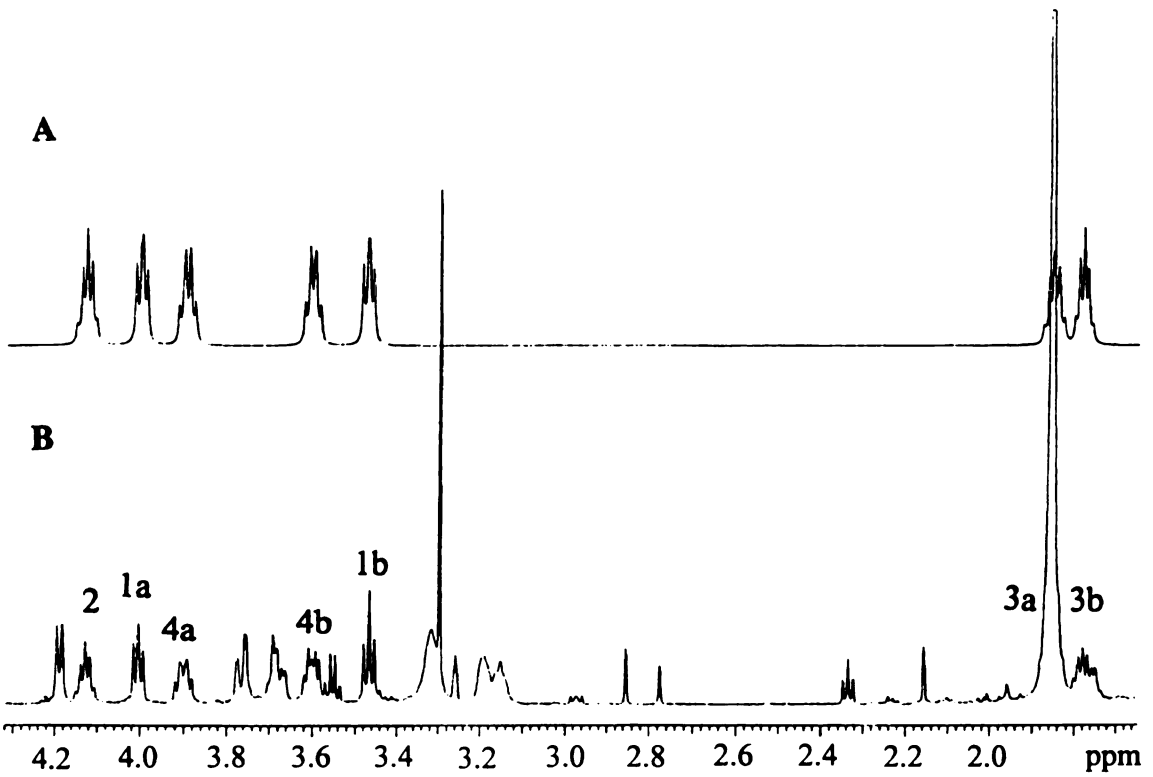


Figure 5. Spin simulation of the chiral linker part of compound **2**. (A) Simulated spectrum; (B) Original spectrum.

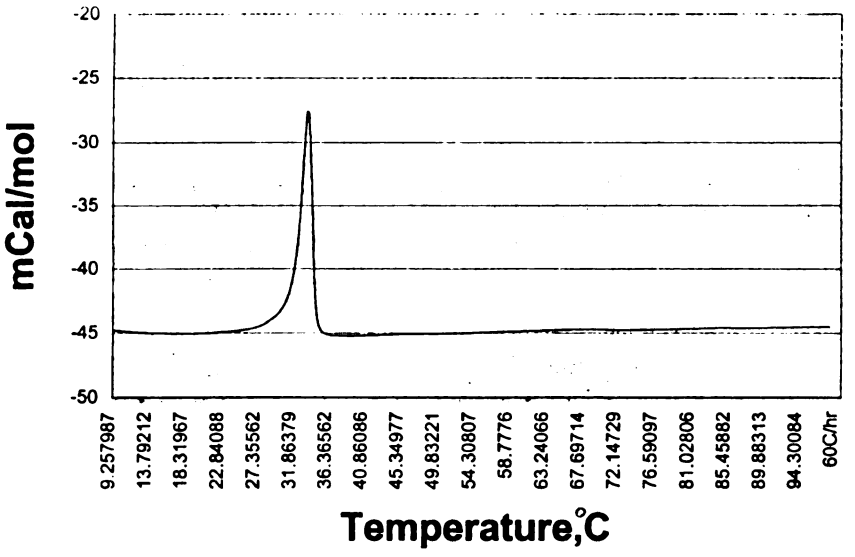


Figure 6. Differential scanning calorimetry thermogram of compound **2**. Scanning rate was 60°C/hr.

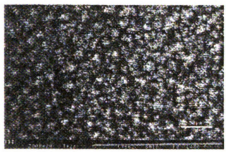
2, which suggests the formation of interdigitated bimolecular layers in the solid phase.[28] The two different values may correspond to (probably slightly) different molecular packing. Reflections at 19.81 Å and 9.78 Å corresponded to the second and third order reflections of the one at 40.16 Å. There was also a peak corresponding to twice the 40.16 Å separation. These all indicated a very high lamellar organization[29]. A wide-angle reflection corresponding to a d-spacing of 4.41 Å, which is characteristic of the hydrocarbon chain separation in smectic mesophases[30], was also observed.

The x-ray diffraction pattern of fully hydrated compound **2** (> 50% water content) in a capillary showed a strong reflection corresponding to a 63.10 Å d-spacing. This is twice the calculated length of one molecule and corresponds to a solvated bilayer structure, considering that the layer spacing would be reduced due to the tilt of the molecule. Its second and third order reflections at 31.00 Å and 15.51 Å were also observed. This again was consistent with the x-ray diffraction pattern of 1-D smectic phase, and distinguished itself from phases such as 2-D hexagonal, 3-D cubic, nematic, etc. [29] Two wide angle reflections were observed, which corresponded to 4.25 Å and 3.31 Å. The shorter distance corresponds to lipid chains that are very tightly packed, and indicates the presence of highly crystalline domains imbedded in a more fluid, but ordered, lamellar matrix.

The concentration range at which compound **2** starts to associate and form vesicles or other supramolecular systems was examined by measuring its light scattering behavior at different concentration levels in water. In the range of 10^{-14} to 10^{-6} mM, a periodic rise and fall was observed when the absorbance was plotted against concentration. Three distinct maxima appeared within this range. The appearance of

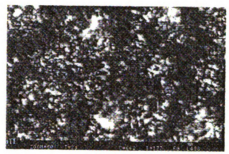
several such sudden changes instead of one is expected, since different types and sized vesicles would be formed in solutions at different concentrations. For instance, as the concentration is increased, at some critical value, the particles might fuse to form a smaller number of units with multiple layers or larger radii. Either one of these will result in a reduction of light scattering.

It is known that amphiphilic carbohydrates form thermotropic and lyotropic liquid crystals if the molecules have an appropriate shape.[31] Smectic A_d phases are well established for amphiphilic carbohydrates with one aliphatic chain.[32] However, the texture of polarized light micrographs of amphiphilic carbohydrates with the complexity similar to molecule **2** have never been reported. Tilted phases of chiral molecules, which possess permanent polarizations and are thus ferroeletric, are of particular interest because of their high potentials in electrooptic applications. One characteristic of chiral smectic C phases is the spiral or concentric circular defects that are observed on the free surface. [33] The tilt of the molecular director away from the surface normal results in the possible motion or placement of the molecule along a cone, the axis of which is the layer normal. It is the offsetting of the alignment of molecules from layer to layer that leads to this spiral defect that is characterized by a winding spiral morphology. Polarized-light micrograph of hydrated compound **2** showed a texture characteristic of smectic C phase (Fig. 7A), which further agreed with the proposed lamellar structure. Phase contrast micrographs (Fig. 7B) and confocal reflection micrographs (Fig. 7C) clearly showed the spiral defects with their fine layered features placed in the characteristic spiral fashion. The presence of these defects again indicated a tightly packed lamellar structure. A lamellar structure is obligatory for producing these effects. 3-D reconstruction of the z-



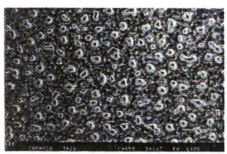
50 μm / 9 mm

A



10 μm / 7 mm

A



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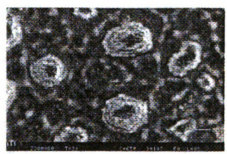
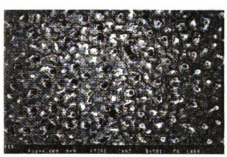
B



10 μm / 7 mm

B



50 μm / 9 mm

C

10 μm / 7 mm

C

Figure 7. The optical texture of compound **2** observed under laser scanning microscopy. (A) Polarized-light micrograghs; (B) Phase contrast micrograghs; (C) Confocal reflection micrographs.

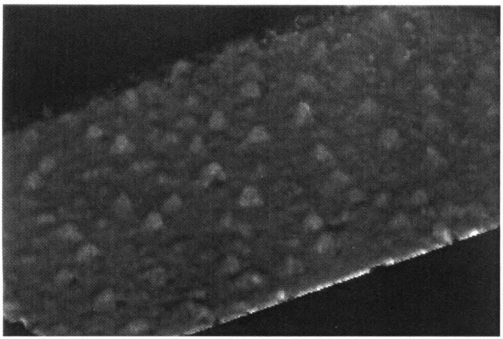


Figure 8. 3-D reconstruction using confocal reflection images from ten sequential laser confocal microscopy scans through compound **2**. The overall depth of the reconstructed image is 5 μm. Note the conical shape of the dislocations and the complimentary concave shapes of their bases (see edges) consistent with an upward dislocation of the layers.

sectioning confocal reflection micrographs using 10 scans along the z-axis with a growth step of 500 nm revealed the conical shape of the dislocations and the complimentary concave shapes of their bases (Fig. 8). This is consistent with an upward dislocation of the layers.

Acid Susceptibility

To get an idea of how sensitive compound **2** is towards acidic environments, ^{1}H-NMR spectroscopy experiments were carried out to monitor the rate of acetal cleavage in different acidic solutions. These experiments were carried out in d^{6}-ethanol since it was the most polar protic solvent in which the solubility was high enough to afford the many measurements to be taken in a reasonable time. As references, compound **2**'s stability in pure ethanol was assessed at temperatures varying from 25°C to 55°C. The spectra (Fig. 9) clearly shows that it is stable under these conditions. Thirty-seven degrees centigrade was chosen as the temperature for all acid susceptibility tests of compound **2** for the consideration of its potential application in pH-controlled drug release systems with the understanding that the rate of solvolysis in ethanol could be scaled to a rate in water. Different concentrations of d^{4}-acetic acid (from 1% to 20%) were added to the NMR sample and the reactions were monitored up to 14 hours. No acetal cleavage was detected even at acid concentration as high as 20%(Fig. 10). The only change observed was the slight broadening of the sugar ring proton signals, which is expected since the increase of solvent polarity upon the addition of acid would force the glycolipids to pack more tightly and therefore the sugar headgroup mobility would decrease. However, when strong acid DCl was used, compound **2** became very sensitive. When 0.01% concentrated DCl (pD slightly lower than 3) was added, the acetal linkage was slowly cleaved with

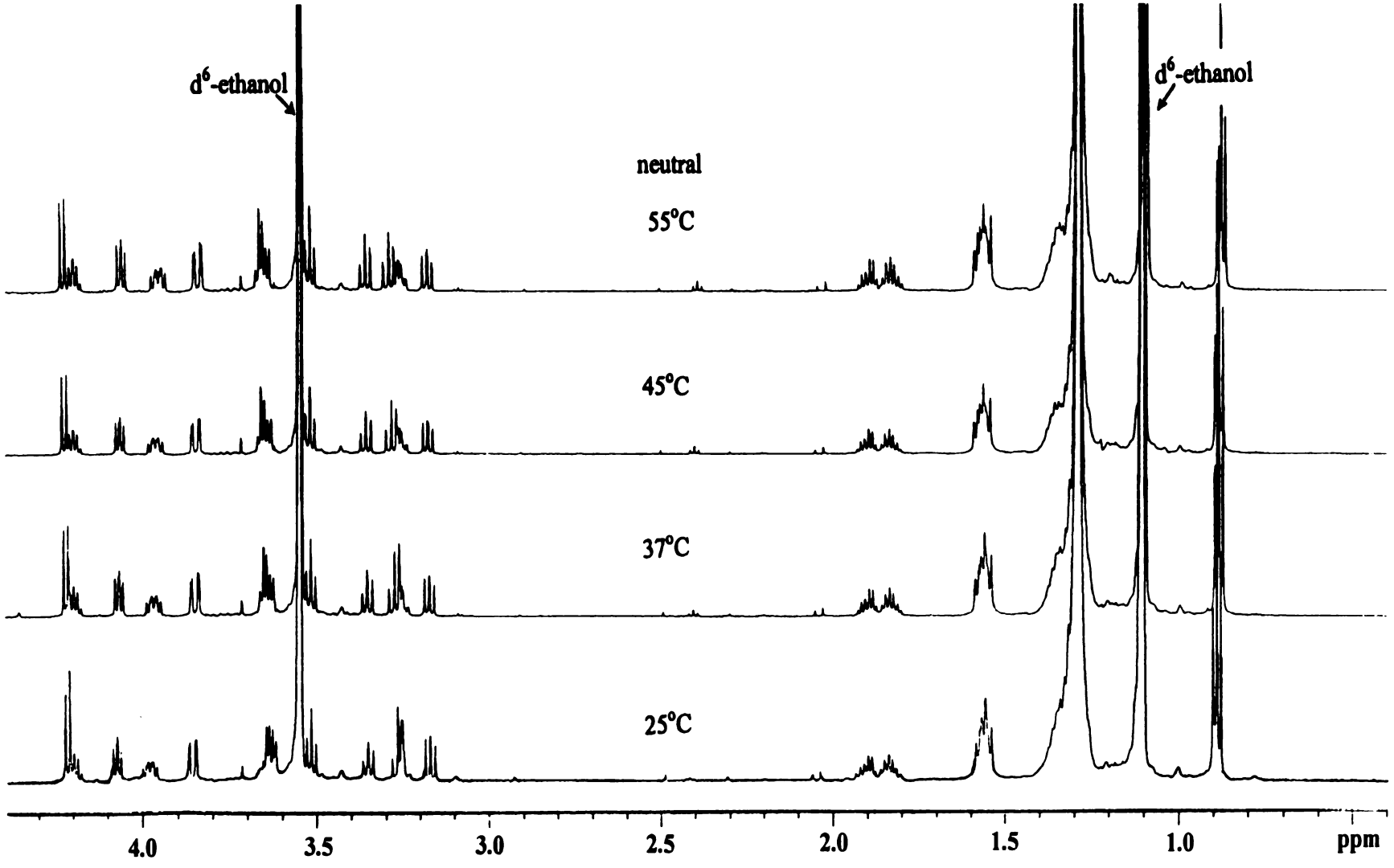


Figure 9. ^{1}H-NMR spectra of compound **2** in pure d^{6}-ethanol with different temperatures.

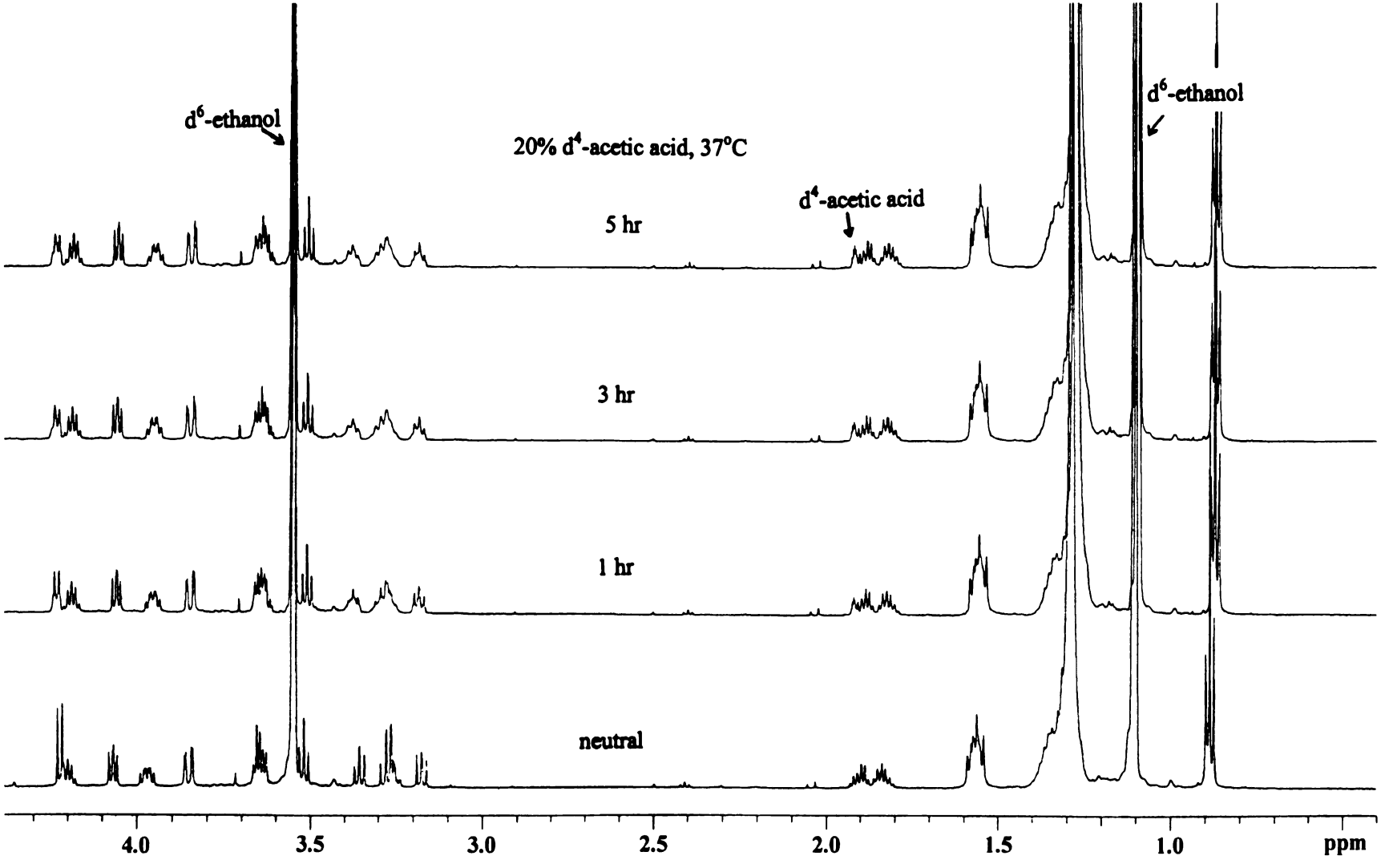


Figure 10. ^{1}H-NMR spectra of compound **2** in d^{6}-ethanol with 20% d^{4}-acetic acid solution over time.

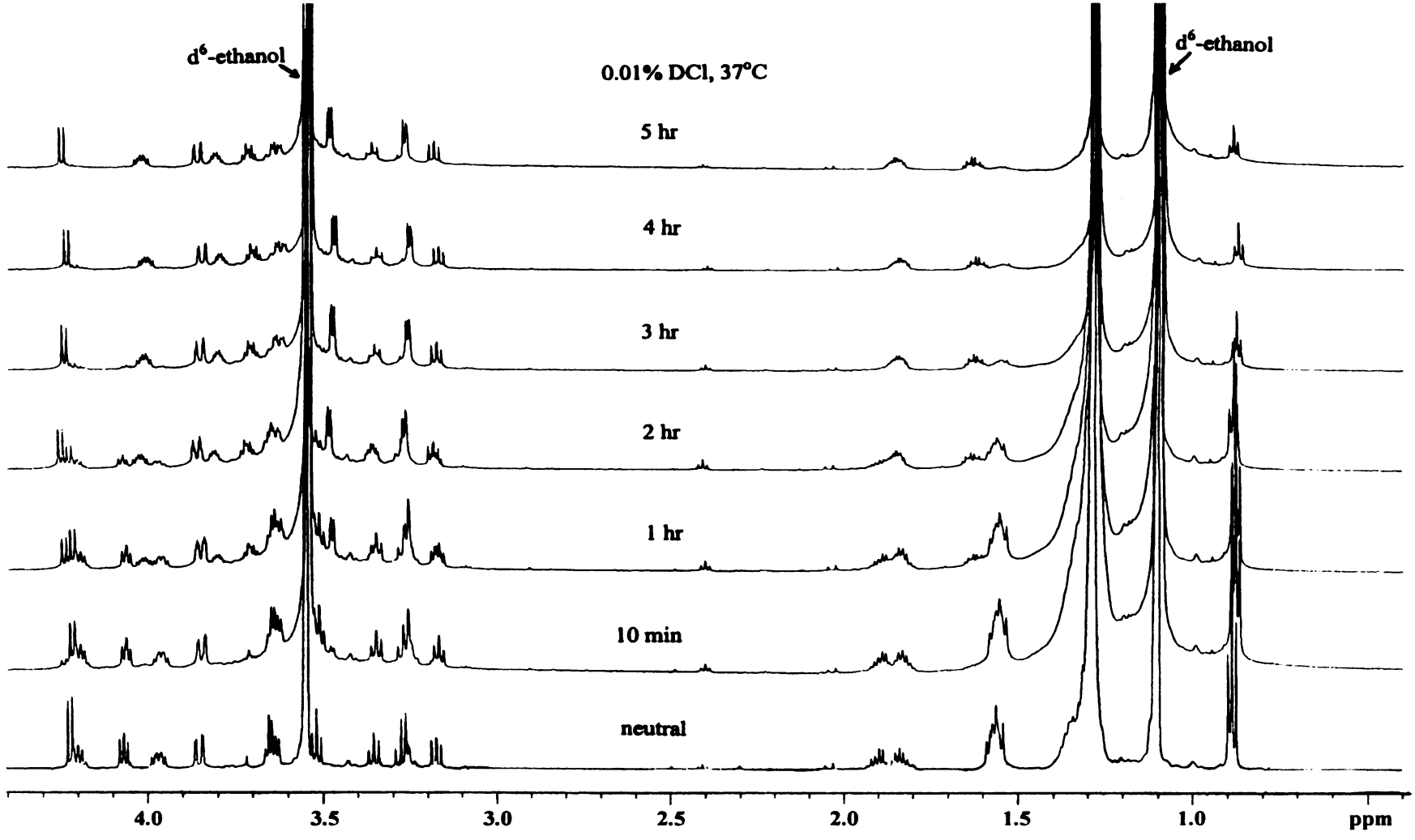


Figure 11. ^{1}H-NMR spectra of compound **2** in d^{6}-ethanol with 0.01% DCl solution over time.

cleavage being completed within 5 hours. The spectra (Fig. 11) clearly showed this process. As the reaction proceeded, the released long chain symmetric ketone precipitated out of the alcohol solution and their signals in the upfield region significantly decreased, while the anomeric proton and the linker proton signals were upfield shifted. When the DCl concentration was increased to 1% (pD around 1), the solution turned cloudy immediately once compound **2** was dissolved, and by the time the first NMR acquisition was recorded the reaction was already completed.

The above results demonstrated that compound **2**'s acid susceptibility allows one to set specific conditions under which the rate and extent of cleavage can be controlled. It is quite stable in weak organic acids such as acetic acid (its Ka is lower in ethanol than in water), while labile in strong inorganic acids such as DCl in a controllable fashion. There is a lot of room for screening appropriate conditions, such as choice of acids, temperature, etc, to control the kinetics of this acetal cleavage for various practical applications. In addition to these, another level of control can be exerted by modifying the structure and/or environment of the carbohydrate group. For instance, the net charge on the carbohydrate group can be altered by preparing the liposomes in borate buffers. Under these conditions carbohydrates form negatively charged cyclic borate esters that buffer the surface of the liposomes. The pH required for cleavage under such circumstances would be appreciably lower. The number of borate groups attached to the carbohydrate ring can be altered by using sugars with different configurations.[34]

Conclusion

An optically pure glycolipid **2** containing a (S)-2,2-di-hexadecyl-1,3-dioxolane ring and designed as a structural surrogate of typical glycolipids such as MGDG was successfully synthesized in a short route with satisfactory yields. Various physical measurements and conformational studies revealed that this molecule forms stable lamellar system and the sugar headgroup orientation, relative to the hydrocarbon chains, is very close to that in MGDG. The 4-carbon linker has a considerable amount of conformational flexibility, which is similar to the naturally occurring glycerol linker in MGDG and is also to be expected on adding one more carbon to the glycerol linker. Hence, this synthetic molecule may serve as a good substitute of the expensive naturally occurring glycolipids in a broad range of studies involving highly ordered 2-dimensional molecular systems. Moreover, the unique structural feature and phase behavior of this molecule opens a large variety of new potential applications. This amphiphile's relatively low phase transition temperature (33.7°C), its glycosidic linkage and its acid-sensitive acetal function make it a good candidate in the construction and engineering of liposomes with triggered release control mediated by physically or chemically induced phase transitions, such as thermal triggering, enzymatic triggering (e.g. by glucosidases) or pH-triggering. These are all important tools in solving drug delivery problems. The fact that the polar headgroup of this amphiphile is glucose and the butane triol linker is a close analog of glycerol also makes this molecule a promising candidate in preparing biocompatible films. The nature of the carbohydrate headgroup can be easily altered without significantly changing the short synthetic route. Therefore, it offers an excellent model for studying the stereo- and regio-chemistry of oligosaccharide synthesis upon

ordered surfaces, using such kind of glycolipids with different carbohydrate headgroups. Such glycolipids serve as both reactants (the carbohydrate headgroups) and ordered templates (the hydrophobic anchors) with versatile chemically and physically tunable properties. This part of the work is currently ongoing and the results will be reported in due course. Finally, this work describes the facile preparation of a molecule that forms chiral smectic C type phases. Such chiral smectics are very important in technology applications of liquid crystals because of their ferroelectric properties. They are, however, not easily accessible.

Experimental

General Techniques

All reagents used were reagent grade. Reaction temperatures were measured externally. Flash chromatography was performed on Aldrich silica gel (60 Å 200-400 mesh). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

NMR spectra were recorded on a Varian 300 MHz, 500 MHz or 600 MHz VXR spectrometer at ambient temperature unless it is otherwise specified. Chemical shifts are reported relative to the residue solvent peak unless it is otherwise specified. Optical rotations were measured using a Perkin polarimeter at 589 nm. High-resolution mass spectra (HRMS) were recorded on a JEOL HX-110-HF spectrometer using Fast Atom Bombardment (FAB) conditions and an N-benzyl alcohol (NBA) matrix. Melting points were measured on a Fisher-Johns melting point apparatus.

Synthesis

Synthesis of Compound 3

A mixture of methyl methylthiomethyl sulfoxide (3.2 ml, 30 mMol), hexadecyl bromide (21.1 ml, 67 mMol), sodium hydride (60% dispersion in oil, 10 g), and freshly distilled tetrahydrofuran (200 ml) was stirred under nitrogen at 50°C for 24 hr. After cooling, the reaction was quenched with methanol-water and extracted with ether. The ether layer was washed with water, then dried over sodium sulfate and concentrated. The residue was stirred at room temperature for 24 hr with tetrahydrofuran (200 ml) and 6 N hydrochloric acid (200 ml). The reaction mixture was then diluted with water and ether. The ether layer was washed with sodium bicarbonate solution and water, then dried over sodium sulfate and concentrated. The residue was crystallized from ethanol to give 11.5 g compound **3** (overall 80%). M.p. 80.0-82.0°C [lit.[35] m.p. 79.7-81.7°C]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (4H, t, J = 7.4 Hz), 1.54 (4H, m), 1.23 (m), 0.86 (6H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.82, 31.93, 29.70, 29.66, 29.62, 29.49, 29.42, 29.37, 29.27, 23.89, 22.69, 14.14 (signal corresponding to carbonyl carbon was not observed); FAB-HRMS (NBA): calcd $C_{33}H_{67}O$ $[M+H]^+$, 479.5192, found 479.5201.

Synthesis of Compound 4

A mixture of compound **3** (4.78 g, 10 mMol), trimethyl orthoformate (3.3 ml, 30 mMol), *p*-toluenesulfonic acid monohydrate (0.1 g), methanol (25 ml) and tetrahydrofuran (50 ml) was refluxed for 12 hr. After being quenched by triethylamine, the reaction mixture was poured into sodium bicarbonate - ice water and extracted with petroleum ether. The ether layer was dried over sodium sulfate and concentrated. The identity of the dimethoxy acetal intermediate was confirmed by the presence of a signal at

δ 3.10 (6H, s, OCH_3) in ^1H NMR (300 MHz, $CDCl_3$ with trace d^5-Py) and the signals at δ 103.91 (quaternary carbon) and δ 47.58 (OCH_3) in ^{13}C NMR (75 MHz, $CDCl_3$ with trace d^5-Py). The residue was dissolved in anhydrous N,N-dimethylformamide (50 ml) and tetrahydrofuran (50 ml) with (S)-1,2,4-butanetriol (1.6 g, 15 mMol). The mixture was refluxed for 24 hr. The reaction was then quenched by triethylamine. The mixture was poured into sodium bicarbonate - ice water and extracted with petroleum ether. The ether layer was dried over sodium sulfate, concentrated and stored with a trace of pyridine added. The yield was 3.9 g (69% from compound **3**). ^1H NMR (300 MHz, $CDCl_3$ with trace d^5-Py): δ 4.19 (1H, m), 4.06 (1H, m), 3.77 (2H, t, J = 5.7 Hz), 3.50 (1H, t, J = 8.0 Hz), 1.78 (2H, m), 1.55 (4H, m), 1.22 (m), 0.84 (6H, t, J = 6.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ with trace d^5-Py): δ 112.46, 75.31, 69.90, 60.56, 37.71, 37.24, 35.45, 31.87, 29.89, 29.65, 29.57, 29.31, 23.93, 23.72, 22.64, 14.08; FAB-HRMS (NBA): calcd $C_{37}H_{75}O_3$ $[M+H]^+$, 567.5716, found 567.5730.

Synthesis of Compound 5

A mixture of compound **4** (1.4 g, 2.5 mMol), acetobromo-α-D-glucose (1.5 g, 3.6 mMol), mercury(II) oxide (0.22 g, 1 mMol), mercury(II) cyanide (1.3 g, 5 mMol), freshly distilled benzene (60 ml) and nitromethane (60 ml) was stirred at 65°C under nitrogen for 12 hr. Solvent was then evaporated and dichloromethane (80 ml) was added. The majority of mercury salts were filtered out on a celite pad. After evaporating dichloromethane, the residue was subjected to column chromatography purification (silica gel, 1.8% methanol in benzene, R_f = 0.50). 1.3 g pure compound **5** was obtained (58%). $[\alpha]_D$: -5.2° (c 0.79, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$ with trace d^5-Py): δ 5.16 (1H, t, J = 9.6 Hz), 5.05 (1H, t, J = 9.6 Hz), 4.94 (1H, dd, J = 9.6, 8.1 Hz), 4.46 (1H, d, J

= 8.1 Hz), 4.24 (1H, dd, J = 12.1, 4.8 Hz), 4.10 (1H, dd, J = 12.1, 2.4 Hz), 4.00 (2H, m), 3.90 (1H, m), 3.66 (1H, m), 3.60 (1H, m), 3.43 (1H, t, J = 7.5 Hz), 2.08 (2H, m), 2.05 (3H, s), 2.01 (3H, s), 1.99 (3H, s), 1.97 (3H, s), 1.52 (4H, m), 1.22 (m), 0.84 (6H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ with trace d^5-Py): δ 170.80, 170.24, 169.35, 169.17, 111.97, 100.58, 73.62, 72.76, 71.74, 71.15, 69.81, 68.28, 66.84, 61.84, 37.71, 37.32, 33.21, 31.87, 29.90, 29.65, 29.31, 23.96, 23.71, 22.64, 20.70, 20.57, 14.08; FAB-HRMS (NBA): calcd $C_{51}H_{93}O_{12}$ $[M+H]^+$, 897.6667, found 897.6644.

Synthesis of Compound 2

Anhydrous potassium carbonate (2 g) was added to a methanol solution (10 ml) of compound **5** (0.67 g, 0.75 mMol). The mixture was stirred at room temperature for 6 hr before it was filtered. The clear filtrate was neutralized with acetic acid and then passed through a celite pad. After removing solvent, compound **2** was obtained in quantitative yield. $[\alpha]_D$: -8.0° (c 1.82, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$:CD_3OD/2:1 with trace d^5-Py, chemical shifts relative to TMS signal): δ 4.19 ($H_{1'}$, d, J = 7.8 Hz), 4.13 (H_2, m), 4.00 (H_{1a}, t, J = 7.8 Hz), 3.90 (H_{4a}, m), 3.76 ($H_{6a'}$, dd, J = 12.2, 2.2 Hz), 3.68 ($H_{6b'}$, dd, J = 12.2, 4.8 Hz), 3.60 (H_{4b}, m), 3.47 (H_{1b}, t, J = 7.8 Hz), 3.32 ($H_{3'}$ & $H_{4'}$, m), 3.19 ($H_{5'}$, m), 3.15 ($H_{2'}$, m), 1.86 (H_{3a}, m), 1.77 (H_{3b}, m), 1.50 ($H_{\beta/\beta'}$, m), 1.18 (CH_2's, m), 0.79 (CH_3's, t, J = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD/2:1 with trace d^5-Py): δ 111.89 (C_α), 102.51 (C_1), 76.01 ($C_{3'}$), 75.80 ($C_{5'}$), 73.42 (C_2), 73.16 ($C_{2'}$), 69.74 ($C_{4'}$), 69.36 (C_1), 66.24 (C_4), 61.12 ($C_{6'}$), 37.16 (C_β), 36.69 ($C_{\beta'}$), 33.20 (C_3), 31.43 ($C_\gamma/C_{\gamma'}$), 29.40, 29.18, 29.09, 28.86, 23.48, 23.22 (C_δ), 23.14 ($C_{\delta'}$), 22.16 ($C_\varepsilon/C_{\varepsilon'}$), 13.38 ($CH_3$'s); FAB-HRMS (NBA): calcd $C_{43}H_{84}O_8K$ $[M+K]^+$, 767.5803, found 767.5804.

Conformational Analysis

Two-dimensional (2-D) NMR Experiments

All NMR spectra of compound **2** were obtained in a solvent system consisting of chloroform/methanol/pyridine in the ratio of 2/1/trace. All 2-D spectra were measured at 600 MHz. Double quantum filtered J-correlated spectroscopy (DQF-COSY), gradient ^{1}H-^{13}C hetero-nuclear multiquantum coherence spectroscopy (^{1}H-^{13}C HMQC) and phase sensitive nuclear Overhauser effect spectroscopy (NOESY) experiments were performed using a total of 256 real data sets, with 32 acquisition transients each and a relaxation delay of 1.8s between transients. Mixing times of 300, 400 and 500 ms were used in the NOESY experiments. Volume integrations of crosspeaks were performed using the standard VARIAN software. A mixing time of 500 ms was used to calculate nOe crosspeak volumes since it gave the best signal-to-noise ratio.

^{1}H NMR Spin Simulation

Protons 1a, 1b, 4a, 4b, 2, 3a and 3b of compound **2** were described as a 7-spin system ABCDEXY. Spin simulation of this spin system was carried out using VARIAN standard spin simulation software.

Molecular Mechanics (MM) Calculations and Grid Search

MM calculations were performed on a Silicon Graphics 4D310 computer using the DREIDING force fields[36] implemented in the BIOGRAF (Molecular simulations Inc., Waltham, MA 02154) program. The default parameters given in this program for the carbohydrate rings were used without modification since they have been validated earlier.[37] The MM calculations were performed in vacuo. Calculated nOe distance

constraints were included as harmonic restraints with a force constant of 10,000 kcal•mol^{-1}•Å^{-2}.

Grid search was performed using sequential search mode in the BIOGRAF program, with a 10° step growth search from 0° to 360° for each defined dihedral angle.

Packing/Phase Behavior Characterization

Differential Scanning Calorimetry (DSC)

DSC measurements were made on a Microcal-2 ultrasensitive scanning calorimeter with a scanning rate of 60°C / hr. Approximately 12 mg of compound **2** was suspended in 1.5 ml water and 5 scans were recorded from 10°C to 98°C.

X-ray Powder Diffraction

The x-ray diffraction spectra were recorded on a one-dimensional scanning detector with a Rigaku rotating anode x-ray generator (CuK_α, 40kV, 100mA). 2.6mg of compound **2** in dichloromethane was deposited on a microscope slide and air-dried. Its x-ray diffraction pattern was obtained in the reflection mode (DS = 1/6°, SS = 1/6°, RS = 0.3mm, RS_m= 0.45mm; scan rate = 0.4°/min; 2θ = 0.5°-45°). Gel form of compound **2** in water (10 mg / 50 µl) was placed into a 0.7 mm glass capillary (Charles Super Co.). The sealed capillary was heated at 70°C for 2 hr to ensure full hydration prior to recording its diffraction pattern in the transmittance mode (DS = 0.05°, SS = 1/6°, RS = 0.3mm, RS_m= 0.45mm; scan rate = 0.3°/min; 2θ = 0.2°-45°). Both diffraction patterns were recorded at room temperature.

Measuring Association by Light Scattering

12.73 mg of compound **2** was uniformly suspended into 2 ml well-stirred distilled water. 200 µl of this solution (8.74 mM) was transferred into one well of an ELISA plate.

100 µl was removed and placed into the adjacent well containing 100 µl of water. The process of serial 50% dilution was continued to give a total of 60 wells. The ELISA plate was equilibrated for 4 hours at room temperature before light scattering measurements were performed by taking absorbance readings on a Bio-Tek Instruments EL-307 ELISA plate reader with a long-wavelength filter.

Laser Scanning Microscopy

Polarized-light microscopy, phase contrast microscopy and confocal reflection images were visualized using a Zeiss LSM210 laser scanning microscope equipped with a 488 nm Ar laser. 40× air objective lens were used. Compound **2** (pre-dissolved in dichloromethane) was deposited on a carefully cleaned microscope slide in a thin film and dried in a vacuum oven. It was then slowly hydrated in a closed chamber of water at 60°C for days. The hydrated sample was then covered by a cover slip and kept under 100% humidity till the microscope observations.

Acid Susceptibility

D^6-ethanol, d^4-acetic acid and d-hydrochloric acid (37% in D_2O) were used as solvent and acids. All spectra were recorded on a 600 MHz VXR spectrometer. Compound **2** was dissolved in various acidic solutions right before NMR experiments and reaction time was recorded since then. Compound **2** in pure ethanol was monitored at 25°C, 37°C, 45°C and 55°C. Compound **2** in 1%, 10% and 20% d^4-acetic acid – d^6-ethanol solutions were monitored at 37°C. Compound **2** in 0.01% and 1% DCl - d^6-ethanol solutions were monitored at 37°C.

Acknowledgements

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§ 2.2 Approaches to the Synthesis of a Close Structural Analog of the Potent Anti-HIV Agent Sulfoquinovosyl Diacylglycerol

Abstract

A close structural analog of potent anti-HIV agent sulfoquinovosyl diacylglycerol (SQDG) was designed replacing (*S*)-glycerol with (*S*)-1,2,4-butanetriol (a one carbon homolog) as the chiral linker between the hydrophilic carbohydrate headgroup and the hydrophobic lipid chains. All other structural features were preserved to maximize the similarity between the analog and the natural glycolipid in an effort to maintain the desired biological activity. As key intermediates of this synthesis, two close structural analogs of monoglucosyl diacylglycerol (MGDG) with (*S*)-1,2,4-trihydroxybutane instead of glycerol were obtained in very high yields in a few steps. The final transformation from MGDG analogs to the targeted SQDG analog was realized with very low yields, presumably due to the steric blocking of the approach of the nucleophile from the appropriate direction within a highly ordered and therefore motionally restricted supramolecular ensemble.

Introduction

The sulfolipid 6-sulfo-α-D-quinovosyl diacylglycerol (SQDG) is found in the photosynthetic membranes of all plants and most photosynthetic bacteria.[1,2] It is also present in some members of the family *Rhizobiaceae*.[3] Since its discovery[4] and structural elucidation[5], different biosynthetic pathways for it have been suggested.[1] To this day, however, there is still no definite answer to this question and therefore it remains a keen target of discussions.[6] Recently, SQDG has attracted more attention because of its potent anti-HIV activity.[7] The sulfolipids tested all contain an α-linked D-sulfoquinovose and a glycerol linker with the *2S* absolute configuration. The fatty acyl chains have different

chain lengths (sixteen or eighteen carbons) and varied degrees of unsaturation (from zero to three separated C=C bonds each chain). At noncytotoxic concentrations, all tested sulfolipids were strikingly active against human immunodeficiency virus (HIV-1), which is implicated as a causative agent of AIDS, in cultured human lymphoblastoid T-cell lines using several different assays. The same study also showed that structurally related acyl glycerols, complex lipids, detergents, and simple sulfonic acid derivatives did not protect against HIV-1 infection. This suggests that acyl chain length and degree of unsaturation in SQDG, at least over the small variation examined, do not critically affect potency[7] but that other structural aspects are the primary determinants of activity. These are the sulfonic acid containing headgroup, the α-anomeric linkage, the *S*-configuration of the chiral linker and the hydrophobicity of acyl chains. This provides useful information for the successful design of SQDG analogs as potential anti-AIDS drugs. The National Cancer Institute has selected the sulfolipid class as a high priority for further preclinical investigation and for evaluation of its feasibility as a candidate for clinical testing.

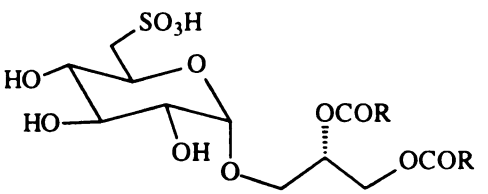
We are interested in designing and synthesizing homogeneous close structural analogs of SQDG as candidates for anti-AIDS drugs. Such design should include important structural features that are important for SQDG's anti-HIV-1 activity as indicated by the previous study.[7] In the meantime, we hope to take the advantage of the fact that some portions of the SQDG structure are not critical for its activity and therefore may be altered to simplify the design and synthesis of the homogeneous analog. The chemical synthesis of such a charged amphiphile itself would be valuable as well, since it provides biologically pertinent self-assembling material for a broad range of studies including biocompatible films, drug delivery vehicles, biosensors, nanotechnology, etc.[8]

Design and Synthesis

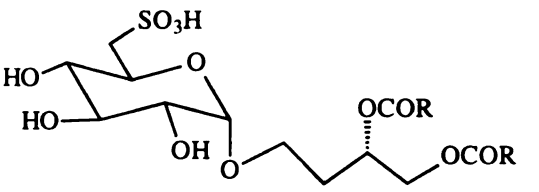
The basic structural feature of SQDG includes a sulfoquinovose headgroup, an α-linked (*S*)-glycerol linker and two long acyl chains connecting to the 2- and 3-hydroxyls of the glycerol linker. The acyl chains are typically sixteen to eighteen carbons' long. SQDG is usually extracted from natural sources. Such products usually have a great deal of acyl chain heterogeneity including acyl chain length distribution, varied degrees of unsaturation, chain branching and other modifications. The purification of homogeneous SQDG sample is quite difficult, requiring multiple step column separations after the already tedious extraction of membrane lipids from plants or bacteria. The yields from such extraction and purification procedure are typically very low.

Currently there is no effective chemical synthesis of SQDG. There are mainly two reasons for this. First of all, the chiral glycerol linker is easily racemized during the synthesis if simple acetal protection is employed.[9] To avoid such problems, stepwise regioselective protection and deprotection of the glycerol linker are required.[10] Secondly, the introduction of charged sulfonic group into such highly ordered self-assembling system is generally difficult and there has not been much effort directed towards this area so far. We tried to design a close structural analog of SQDG with slight modification of the basic structure in whose synthesis the first of the common problems mentioned above could be avoided. In the meantime, we hope to probe the possibility of solving the second general problem associated with the low reactivity of self-assembling systems by using different solvent systems that may disrupt the tight packing of such amphiphilic supramolecular ensembles.

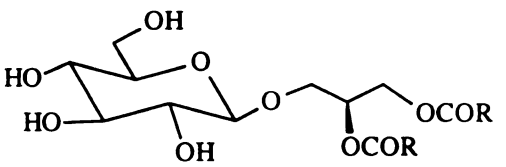
In our design, we used (*S*)-1,2,4-butanetriol instead of (*S*)-glycerol as the chiral linker, thus extending the glycerol unit by one more carbon. Such a design could avoid the racemization problem that is characteristic of glycerol and allow us to use simple acetal protection in the synthesis and therefore significantly simplify the synthetic route. We also chose by convenience two sixteen-carbon saturated fatty acyl chains connecting to the chiral linker since it was suggested earlier that the nature of the lipid chains would not affect the potency of SQDG against HIV-1.[7] Using two identical fatty acyl chains could further simplify the synthesis. In the meantime, we preserved all other critical structural features of SQDG such as the sulfoquinovose headgroup and the α- anomeric configuration.



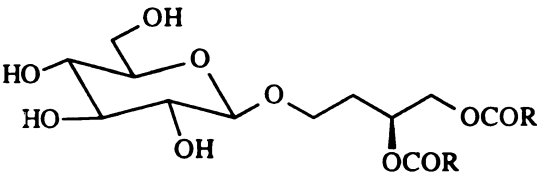
SQDG



SQDG Analog



MGDG

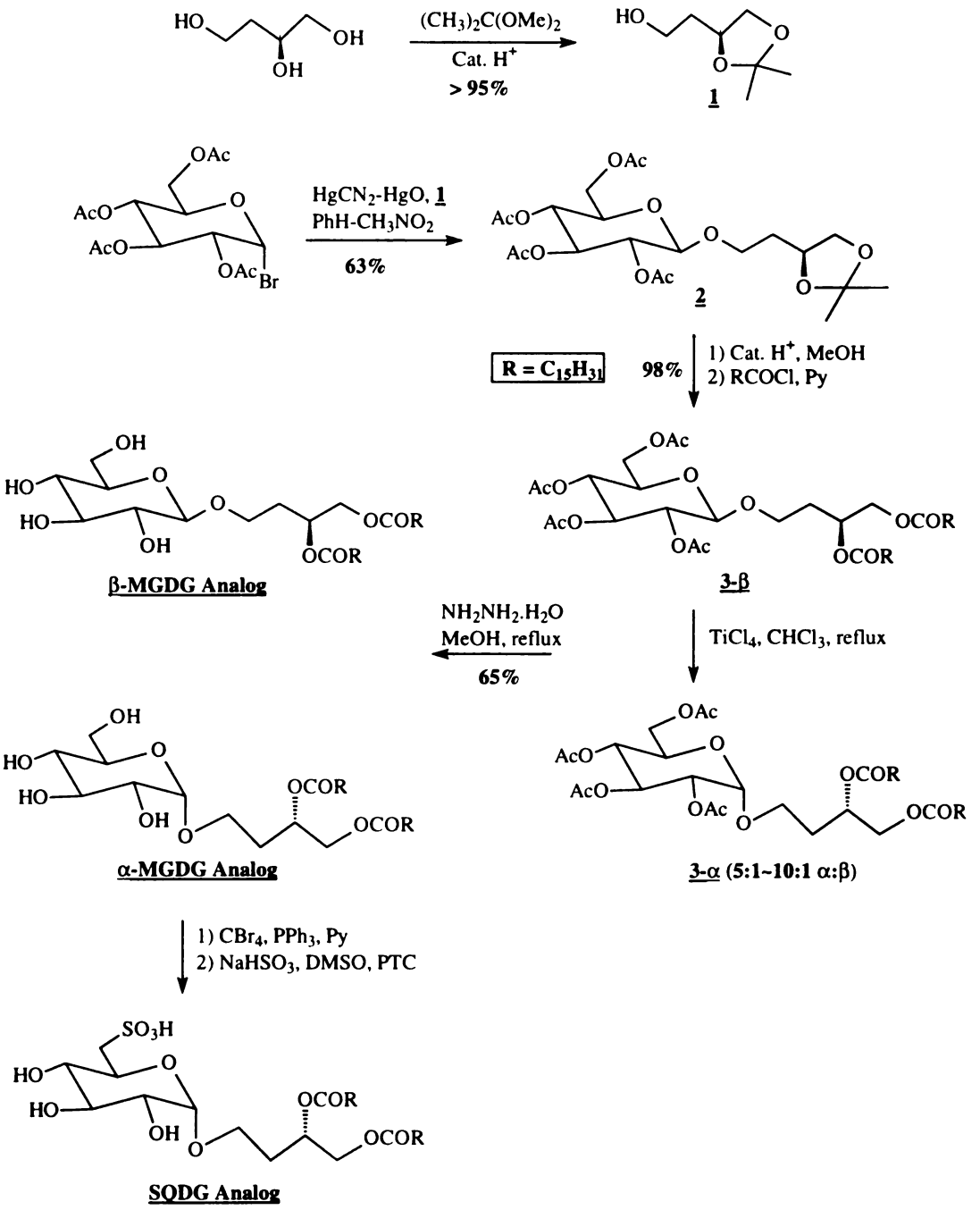


MGDG Analog

In the synthetic route towards this SQDG analog, we chose a close structural analog of monoglucosyl diacylglycerol (MGDG) as the key intermediate. This MGDG analog also has a one-carbon extension at the chiral linker region, again using (*S*)-1,2,4-butanetriol instead of (*S*)-glycerol. MGDG is known to be a very useful glycolipid with a broad range of applications.[8] An easy access to its structural analog is valuable. In our

strategy, the β-MGDG analog could be easily converted to its α-anomer by well established anomerization procedure, from which the targeted α-SQDG analog could be obtained by modifying the 6' position of the glucosyl headgroup. With this synthetic route, four analogs of important natural glycolipids, SQDG and MGDG, with both α- and β-configurations could be quickly accessed.

The synthetic route is summarized in Scheme 1. The (*S*)-1,2,4-butanetriol was first protected by an acetal group. 5-Membered acetal **1** was obtained in high yield. The formation of small amount of dimerized acyclic acetal was occasionally observed. However, the acyclic acetal could be quickly cleaved and converted to the desired, more stable cyclic acetal **1** by mild heating under slightly acidic condition. The final yield of **1** was 95%, with small amount of the less stable 6-membered acetal as the only by-product. The protected chiral linker **1** was then coupled with the peracetylated α-bromoglucoside in a stereoselective fashion to give the β-anomer **2**.[11] The acetal protecting group was then removed and the diol was acetylated with palmitoyl chloride in almost quantitative yield to give the peracetylated glucosyl diacyl lipid **3**-β. The α-anomer was obtained using standard anomerization procedure.[12] The deacetylation of the peracetylated sugar without losing the two fatty acyl chains requires a mild deacetylation reagent. Refluxing with hydrazine in methanol[13] gave both β- and α-MGDG analogs in satisfactory yields. All NMR signals of these two compounds were fully assigned with the help of double quantum filtered J-correlated spectroscopy (DQF-COSY) and gradient ^{1}H-^{13}C hetero-nuclear multiquantum coherence spectroscopy (^{1}H-^{13}C HMQC) experiments (Fig.1 and Fig. 2) performed on a 600 MHz Varian VXR spectrometer.



Scheme 1. Synthesis of close structural analog of SQDG via close structural analogs of MGDG.

Figure 1. DQF-COSY (top) and ^{1}H-^{13}C HMQC spectra of β-MGDG analog.

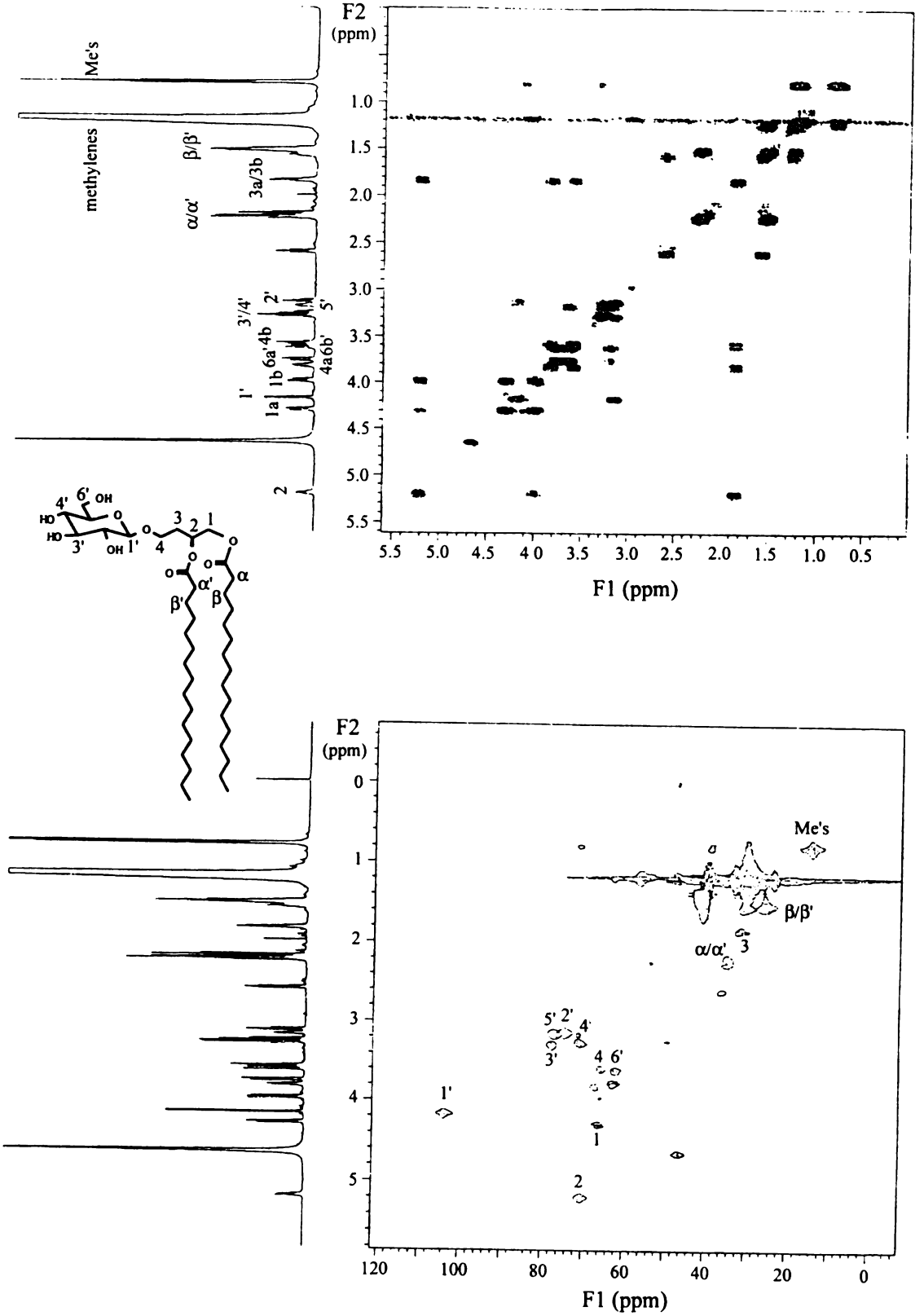
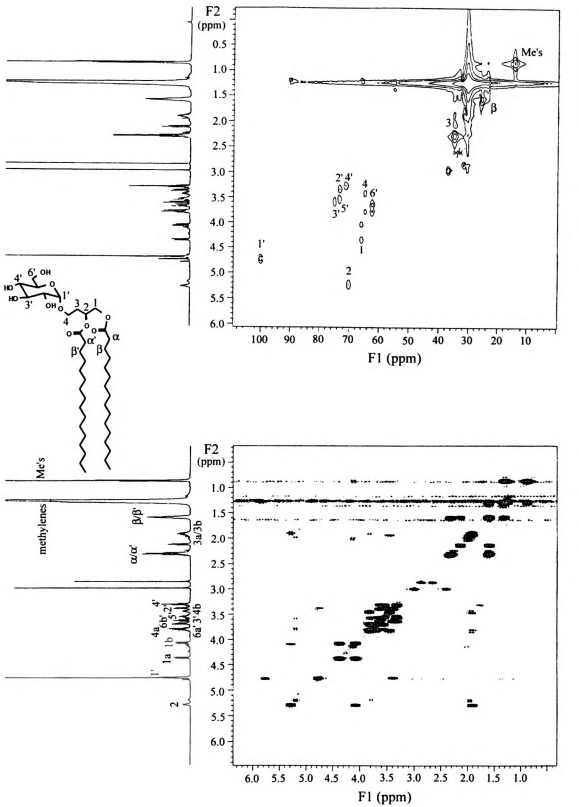

F2
(ppm)
Me's
methylenes
β/β'
α/α'
3a/3b
3'/4'
2'
5'
4a6b'
6a'4b
1b
1a
1'
2
F1 (ppm)
Me's
β/β'
α/α'
3
5' 2'
4'
3'
4 6'
1'
1
2
F1 (ppm)

Figure 2. ^{1}H-^{13}C HMQC (top) and DQF-COSY spectra of α-MGDG analog.


F2
(ppm)
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(ppm)
F1 (ppm)

The final transformation from the MGDG analog to the targeted SQDG analog was via selective bromination of the 6'-position of the sugar headgroup and the subsequent S_N2 substitution of the 6'-bromo by sulfite. Although the formation of both the 6'-bromo intermediate and the final SQDG analog were supported by NMR and high resolution mass spectra, the reactions suffered from extremely low yields even at elevated temperature. (In the ^{13}C NMR spectrum of β-6'-bromo intermediate, the characteristic peak at 62ppm corresponding to the $C_{6'}$-OH disappeared and a new peak at 33.8ppm corresponding to $C_{6'}$-Br appeared. In the case of the SQDG analog, a peak at 55ppm was observed instead, supporting the formation of $C_{6'}$-sulfite. In the ^1H NMR spectrum of the SQDG analog, the new 6' protons also appeared at a higher field around 3ppm. These are both consistent with the data reported for the natural SQDG.[3] We think this low conversion probably is due to the highly ordered packing of MGDG analogs in polar reaction solvents such as pyridine, aqueous methanol solution and dimethyl sulfoxide (DMSO). These may prevent the nucleophiles (bromide or sulfite) approaching the reactive site from appropriate directions and therefore significantly lower the reaction efficiency. Less polar cosolvents such as chloroform were added to the reaction system in order to disrupt the tight packing of the amphiphilic reactants. Unfortunately, no significant improvement was observed. The use of less polar solvent such as chloroform or dichloromethane alone did not help either, because they not only brought the solubility problem of sulfite, but also restricted the upper temperature at which the reaction could be performed. Such less polar solvent alone may also induce inversed micellar or hexagonal packing of the amphiphiles where the polar carbohydrate headgroups are very tightly packed and pointing towards each other. This would further restrict the exposure

of the polar reactive sites and lower the reactivity. As an alternative, one may try to introduce the sulfonic group to the sugar headgroup before attaching the hydrophobic lipid chains. Of course in such an approach, the preparation of protected sulfoquinovose for further coupling may become a relatively difficult problem.

Conclusion

A close structural analog of naturally occurring glycolipid SQDG was designed as a potential anti-AIDS drug. As key intermediates of this synthesis, two close structural analogs of another important natural glycolipid MGDG were obtained in high yield via an efficient synthetic route. The final transformation to the SQDG analog proceeded in low yields. The effort of enhancing the reactivity of the highly ordered self-assembling system by changing reaction solvents was not successful. However, the attempt at synthesizing the SQDG analog brought closer the opportunity of examining such supramolecular systems, their design, synthesis and reactivity.

Experimental

General Techniques

All reagents used were reagent grade. Reaction temperatures were measured externally. Flash chromatography was performed on Aldrich silica gel (60 Å 200-400 mesh). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

NMR spectra were recorded on a Varian 300 MHz or 600 MHz VXR spectrometer at ambient temperature. Chemical shifts are reported relative to the residue

solvent peak, in cases where the mixed solvent of methanol and chloroform were used, methanol was chosen as the reference. Optical rotations were measured using a Perkin polarimeter at 589 nm. High-resolution mass spectra (HRMS) were recorded on a JEOL HX-110-HF spectrometer using Fast Atom Bombardment (FAB) conditions and an N-benzyl alcohol (NBA) or glycerol (Gly) matrix at either positive or negative mode (for the characterization of the SQDG analog).

Synthesis

Synthesis of compound 1.

To 10.6 g of (*S*)-1,2,4-butanetriol were added 25 ml of 2,2-dimethoxypropane, 7.4 ml of absolute acetone and a couple of drops of concentrated sulfuric acid. The reaction mixture was stirred at room temperature overnight. The reaction was then quenched with a few drops of triethylamine and then poured into ice-cold sodium bicarbonate solution. The product was extracted with dichloromethane, dried over anhydrous potassium carbonate and filtered. After the removal of solvent, 14.8 g of colorless liquid was obtained in quantitative yield. The crude product was then dissolved in methanol and stirred with a drop of glacial acetic acid at slightly elevated temperature (~ 35°C) for 2 hours to ensure that any formed dimerized acyclic acetal be converted to the desired product **1**. ^{1}H NMR indicated the purity of the product was higher than 95%, with the 6-membered acetal as the only by-product. Rigorous purification was conducted by either chromatography or distillation at 80° C. $[\alpha]_D$ = -3.0° (C 1.36, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.14 (1H, m), 3.98 (1H, dd, J = 8.1, 6 Hz), 3.64 (2H, m), 3.47 (1H, t, J =

8.1 Hz), 1.70 (2H, m), 1.30 (3H, s), 1.24 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 108.65, 74.18, 69.18, 59.56, 35.60, 26.60, 25.40.

Synthesis of compound 2.

A mixture of 4.14 g of **1**, 3.68 g of mercury oxide and 4.3 g of mercury cyanide was stirred at room temperature in 160 ml of dry nitromethane and 80 ml of dry benzene under N_2 flow. After 30 min, 7 g of acetobromo-α-glucoside was added. The reaction mixture was heated to 70°C in darkness overnight. Filtration through celite pad and evaporation of solvent afforded crude syrup, which was then purified by flash column chromatography (3:2 / hexanes:ethyl acetate as eluant) to give 5.1 g of compound **2** (63% yield). $[\alpha]_D$ = -74.6° (C 0.73, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.20 (1H, dd, J = 9.6, 9.3 Hz), 5.08 (1H, dd, J = 9.9, 9.6 Hz), 4.98 (1H, dd, J = 9.6, 8.1 Hz), 4.50 (1H, d, J = 8.1 Hz), 4.26 (1H, dd, J = 12.3, 4.5 Hz), 4.14 (1H, m), 4.10 (1H, m), 4.03 (1H, dd, J = 7.8, 5.4 Hz), 3.95 (1H, m), 3.67 (1H, m), 3.64 (1H, m), 3.53 (1H, dd, J = 7.8, 7.2 Hz), 2.09 (3H, s), 2.05 (3H, s), 2.02 (3H, s), 2.00 (3H, s), 1.87 (2H, m), 1.40 (3H, s), 1.34 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.99, 170.30, 169.93, 169.25, 108.62, 100.62, 73.50, 72.82, 71.80, 71.22, 69.38, 68.37, 66.74, 61.91, 33.32, 26.88, 25.67, 20.61; FAB-HRMS (NBA): calcd $C_{21}H_{33}O_{12}$ $[M+H]^+$, 477.1972, found 477.1973.

Synthesis of compound 3-β.

To 200 ml of 0.1% HCl-methanol solution, 4 g of compound **2** was added and stirred at room temperature for 3 hours. The freed diol was obtained quantitatively after the removal of solvent. The diol was then stirred with 12 ml of palmitoyl chloride and 100 ml of pyridine at room temperature overnight. After the removal of solvent and the purification by flash column chromatography (7:3 / hexanes:ethyl acetate as eluant), 7.5 g

of **3-β** was obtained (98% for two steps). **Diol intermediate:** $[\alpha]_D$ = -14.2° (C 0.62, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.10 (1H, t, J = 9.3 Hz), 4.96 (1H, dd, J = 9.6, 9.3 Hz), 4.87 (1H, dd, J = 9.3, 8.1 Hz), 4.40 (1H, d, J = 8.1 Hz), 4.10 (2H, m), 3.91 (1H, m), 3.73 (1H, m), 3.60 (1H, m), 3.58 (1H, m), 3.49 (1H, dd, J = 11.4, 3.3 Hz), 3.36 (1H, dd, J = 11.4, 6.6 Hz), 1.98 (3H, s), 1.94 (3H, s), 1.91 (3H, s), 1.89 (3H, s), 1.60 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.80, 170.23, 169.64, 169.40, 100.53, 72.58, 71.81, 71.18, 69.71, 68.30, 67.15, 66.42, 61.72, 32.61, 20.70, 20.64, 20.56; FAB-HRMS (Gly): calcd $C_{18}H_{29}O_{12}$ $[M+H]^+$, 437.1659, found 437.1670. **Compound 3-β:** $[\alpha]_D$ = -5.2° (C 1.24, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.16 (1H, dd, J = 9.6, 9.3 Hz), 5.11 (1H, m), 5.05 (1H, dd, J = 9.6, 9.3 Hz), 4.94 (1H, dd, J = 9.3, 8.1 Hz), 4.47 (1H, d, J = 8.1 Hz), 4.22 (2H, m), 4.10 (1H, dd, J = 12, 3.2 Hz), 4.02 (1H, dd, J = 12, 6.3 Hz), 3.86 (1H, m), 3.65 (1H, m), 3.54 (1H, m), 2.25 (4H, t, J = 7.5 Hz), 2.06 (3H, s), 2.02 (3H, s), 1.99 (3H, s), 1.97 (3H, s), 1.86 (2H, m), 1.56 (4H, m), 1.22 (m), 0.84 (6H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.38, 173.02, 170.64, 170.27, 169.35, 169.31, 100.33, 72.70, 71.73, 71.11, 68.75, 68.59, 65.47, 64.48, 61.80, 34.29, 34.06, 31.89, 30.69, 30.10, 29.65, 29.47, 29.33, 29.10, 24.92, 24.84, 22.65, 20.66, 20.54, 14.07; FAB-HRMS (NBA): calcd $C_{50}H_{89}O_{14}$ $[M+H]^+$, 913.6252, found 913.6265.

Synthesis of compound 3-α.

1.69 g of **3-β** was refluxed with 0.25 ml of titanium tetrachloride in 10 ml of dry chloroform for 24 hours. The reaction mixture was then poured onto ice and extracted by chloroform. The organic layer was washed with sodium bicarbonate solution several times, dried with anhydrous sodium sulfate and concentrated. ^1H NMR of the crude product revealed a 5:1~10:1 α:β anomer ratio. The α-anomer was separated from the

residue β-anomer by flash column chromatography (4:1 / hexanes:ethyl acetate as eluant). $[\alpha]_D$ = +10.6° (C 3.01, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.44 (1H, dd, J = 9.9, 9.6 Hz), 5.20 (1H, m), 5.03 (1H, dd, J = 10.2, 9.3 Hz), 4.96 (1H, d, J = 3.6 Hz), 4.85 (1H, dd, J = 10.2, 3.6 Hz), 4.26 (2H, m), 4.06 (2H, m), 3.96 (1H, m), 3.75 (1H, m), 3.35 (1H, m), 2.28 (4H, m), 2.07 (3H, s), 2.06 (3H, s), 2.00 (3H, s), 1.98 (3H, s), 1.84 (2H, m), 1.58 (4H, m), 1.22 (m), 0.85 (6H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.42, 173.03, 170.66, 170.40, 170.16, 169.60, 96.11, 70.41, 70.10, 68.53, 68.15, 67.24, 64.85, 64.31, 61.85, 34.29, 34.09, 33.88, 31.90, 30.77, 29.69, 29.65, 29.49, 29.42, 29.34, 29.29, 29.23, 29.12, 29.06, 25.00, 24.87, 24.71, 22.68, 20.69, 20.60, 14.11; FAB-HRMS (NBA): calcd $C_{50}H_{89}O_{14}$ $[M+H]^+$, 913.6252, found 913.6281.

Synthesis of β-MGDG analog.

0.56 g of **3-β** was refluxed in 100 ml of methanol with 5 ml of hydrazine hydrate for 1 hour. The reaction was quenched by formic acid. After careful removal of solvent, the residue was subjected to column purification using ethyl acetate as eluant. 0.29 g of pure β-MGDG analog was obtained (63%). $[\alpha]_D$ = -9.1° (C 0.73, $CHCl_3$); ^1H NMR (600 MHz, CD_3OD:$CDCl_3$/1:1): δ 5.21 (1H, m), 4.29 (1H, dd, J = 12, 3 Hz), 4.17 (1H, d, J = 7.8 Hz), 3.99 (1H, dd, J = 12, 6.6 Hz), 3.83 (1H, m), 3.76 (1H, dd, J =12, 3 Hz), 3.62 (1H, dd, J = 12, 5.4 Hz), 3.58 (1H, m), 3.27 (2H, m), 3.18 (1H, m), 3.13 (1H, dd, J = 8.4, 7.8 Hz), 2.22 (4H, m), 1.84 (2H, m), 1.52 (4H, m), 1.18 (m), 0.80 (6H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, CD_3OD:$CDCl_3$/1:1): δ 174.77, 103.43, 77.19, 76.90, 74.21, 70.72, 70.01, 69.85, 65.92, 65.64, 62.19, 34.99, 34.69, 32.52, 31.44, 30.82, 30.25, 29.94, 29.71, 25.58, 25.47, 23.24, 14.36; FAB-HRMS (NBA): calcd $C_{42}H_{80}O_{10}Na$ $[M+Na]^+$, 767.5649, found 767.5635.

Synthesis of α-MGDG analog.

α-MGDG analog was obtained in similar yield starting with **3-α** using same procedure described above. $[\alpha]_D$ = +22.8° (C 0.61, $CHCl_3$:MeOH/1:1); ^1H NMR (600 MHz, CD_3OD:$CDCl_3$/1:1): δ 5.28 (1H, m), 4.75 (1H, d, J = 3 Hz), 4.37 (1H, dd, J = 12, 3 Hz), 4.07 (1H, dd, J = 12, 6.6 Hz), 3.80 (2H, m), 3,68 (1H, dd, J = 12, 5.4 Hz), 3.62 (1H, dd, J = 10.2, 8.4 Hz), 3.55 (1H, m), 3.44 (1H, m), 3.38 (1H, dd, J = 10.2, 3.6 Hz), 3.30 (1H, m), 2.30 (4H, m), 1.91 (2H, m), 1.59 (4H, m), 1.25 (m), 0.87 (6H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, CD_3OD:$CDCl_3$/1:1): δ 174.84, 99.96, 74.73, 73.27, 73.05, 71.17, 70.01, 65.86, 64.64, 62.25, 35.07, 34.83, 32.70, 31.44, 30.45, 30.27, 30.13, 29.87, 25.78, 25.66, 23.40, 14.40; FAB-HRMS (NBA): calcd $C_{42}H_{80}O_{10}Na$ $[M+Na]^+$, 767.5649, found 767.5671.

Synthesis of SQDG analog.

80 mg of β-MGDG analog was stirred with 90 mg of carbon tetrabromide, 70 mg of triphenyl phosphine in 20 ml of pyridine at 50°C for 2 hours. TLC and ^1H NMR indicated the formation of the 6'-bromo intermediate. After the removal of pyridine, 50 mg of sodium sulfite, trace amount of hexadecyltrimethyl ammonium bromide (PTC) and 10 ml of methanol with several drops of water was added. The reaction mixture was refluxed overnight. TLC and ^1H NMR showed that most 6'-bromo compound was still not converted. Prolonged heating resulted in the hydrolysis of the bromo compound. Only very small amount of SQDG analog was isolated upon separation. Reaction conducted in DMSO instead of methanol-water mixture at 140°C gave similar result. The addition of chloroform as cosolvent of DMSO, or the use of pure chloroform or dichloromethane as solvent also failed to improve the yield. **6'-Bromo intermediate:** $[\alpha]_D$ = -3.5° (C 0.39,

$CHCl_3$:MeOH/1:1); ^1H NMR (300 MHz, CD_3OD:$CDCl_3$/1:1): δ 5.24 (1H, m), 4.37 (1H, dd, J = 12, 3 Hz), 4.25 (1H, d, J = 7.8 Hz), 4.05 (1H, dd, J = 12, 6.6 Hz), 3.92 (1H, m), 3.74 (1H, dd, J = 10.8, 1.5 Hz), 3.41 (3H, m), 3.22 (2H, m), 2.30 (4H, q, J = 7.2 Hz), 1.94 (2H, m), 1.60 (4H, m), 1.25 (m), 0.86 (6H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, CD_3OD:$CDCl_3$/1:1): δ 175.22, 175.10, 104.03, 78.79, 76.60, 74.92, 73.64, 70.45, 66.09, 66.07, 35.31, 35.05, 33.80, 33.06, 31.79, 30.79, 30.65, 30.47, 30.18, 26.03, 23.73, 14.50; FAB-HRMS (NBA): calcd $C_{42}H_{79}O_9BrNa$ $[M+Na]^+$, 829.4805, found 829.4810. **β-SQDG analog:** $[\alpha]_D$ = -5.0° (C 0.40, $CHCl_3$:MeOH/1:1); ^1H NMR (300 MHz, CD_3OD:$CDCl_3$/1:1): δ 4.26 (1H, d, J = 7.8 Hz), 4.03 (1H, m), 3.86 (1H, m), 3.78 (1H, m), 3.68 (1H, m), 3.48 (2H, m), 3.25 (2H, m), 3.16 (1H, dd, J = 9, 7.8 Hz), 2.20 (4H, m), 1.85 (2H, m), 1.64 (4H, m), 1.28 (m), 0.89 (6H, t, J = 6.9 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 178.18, 104.04, 78.66, 77.86, 77.05, 71.51, 70.42, 67.50, 67.13, 55.07, 38.44, 34.95, 34.80, 34.22, 32.87, 30.55, 30.37, 30.26, 25.55, 23.54, 14.39; FAB-HRMS (NBA, negative mode): calcd $C_{42}H_{79}O_{12}S$ $[M-H]^-$, 807.5292, found 807.5289.

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Chapter 3

Models for the study of collective effects in ensemble systems: An exploration of the encodement of 1-D linkage and sequence information in the organization of 2-D molecular ensembles

Abstract

The idea that the packing order, symmetry properties, steric and proximity factors of membrane systems or other highly ordered supramolecular ensembles can encode the regio- and stereo-regularities of reactions occurring on their surfaces is being investigated.

A series of glycolipids, which should self-assemble into ordered supramolecular structures, were either purchased or designed and synthesized as model compounds for the study. In these models, the reacting species (carbohydrates' multi-hydroxyl groups and their anomeric linkages) are first pre-organized in a regular array such as a lamellar system or a micelle so that the reactive sites between the units have a regular orientation relative to each other. Ideally only one of the hydroxyl groups might be predisposed to react with an activated anomeric position on a neighboring molecule upon the activation. This would presumably result in the production of new molecular species (oligo- or polysaccharides) with high regio- and stereochemical integrity. These model systems were subjected to different forms of chemical and enzymatic activation to trigger regio- and stereo-selective linkages between the headgroups. We present here the synthesis and characterization of several candidates for testing the idea of encodement of linkage information by order. Preliminary results on the preparation of oligosaccharides using this 2-dimensional linkage encodement strategy and the analysis of their linkage types using enzyme activation are also presented and discussed.

Introduction

Ordered packing of molecules is an important feature of biological systems. Membranes for example consist of a macromolecular aggregate of lipids (and proteins) that retain their association with each other without the benefit of covalent bonds. Governed largely by electrostatic forces, the hydrophobic effect and other weak interactions, membrane lipids can exist in a variety of organized structures, with the bilayer structure as the most common form[1].

As one of our many efforts devoted to the understanding of collective systems and collective effects, we are trying to shed some light on whether and how collective effects due to long range molecular order influence reactions in highly organized biological systems such as biomembranes. By collective systems we mean a large array of similar molecules. By collective effects we mean chemical reactions in which the individual units in a collective system participate to give products with structures that are determined by the configuration or order of the collective system. An immediate benefit of the understanding of collective effects would be to utilize the order within pre-organized systems to achieve regio- or stereo-selective organic synthesis where the reacting species are either self-organized or buried in an ordered templating matrix.

Historically the unique position that membranes have in cell organization has drawn much attention from scientists in various areas. However, the function of membranes is still not fully understood due to the huge varieties of lipid components in membranes, their complexity and the diversity of reactions that occur on them. The relatively common occurrence of the membrane bilayer structure in different kinds of cells suggests to us that such a highly ordered structure not only helps membranes to

function as barriers for the cells, but may also provide important structural templates driving various regio and stereospecific reactions on their surfaces.

It is known that the biosyntheses of various oligosaccharides, polysaccharides and glycolipids occur on the membrane. Some of these syntheses are via activated UDP-glycoside or UDP-mono/disaccharide units and a one-by-one chain growth mechanism with the involvement of specific enzymes[2,3]. Other processes do not appear to involve any specific enzymes and seem to be spontaneous processes on the membrane surface. For instance, all higher plants contain β-glucan synthases, which are responsible for the biosynthesis of β-1,4-glucans (cellulose); however, they seem also synthesize β-1,3-glucans (callose) in response to wounding, physiological stress or infection. It is remarkable that callose synthesis is usually latent in intact cells that are producing at the same time large amount of cellulose. After perturbation of the physiological equilibrium, however, the cellulose synthesis stops while callose formation is initiated. The nature of the enzymes involved in the synthesis of these important polysaccharides and the control of their activities during wall formation are not yet well understood[4]. It was proposed that there may be two different enzymes, cellulose synthase and callose synthase, involved in such processes and one enzyme tended to be active when the other is inactive[5a]. However, it is not known how these two glucan synthase activities (if there indeed are two different ones) are controlled. It was then postulated further that either some unknown effectors activate or suppress alternately each of these enzymes or there exists only one glucosyl transferase, which can be switched, for instance, by conformation changes between the formation of both linkage types depending on the requirements of a cell[6]. It seems reasonable to us that there is another possibility. Such process could be

solely a spontaneous one without necessarily the involvement of any specific enzyme that sets the linkage specificity. We propose that the formation of the stereo- and regio-regular linkage may be a result of the ordered membrane templates in which the glycolipid precursors are imbedded. The specificity of the linkage formation could be set by the proximity of one hydroxyl group on one glycoside to the activated anomeric carbon of the other (upon the participation of enzymes that are responsible for general anomeric bond activation) by virtue of their proximity and relative orientation in the membrane. In the case of the synthesis of callose, it may very well be the conformational change of the membrane templates upon physiological disturbance instead of the conformational change of the enzyme or the involvement of a different enzyme that results in the change of linkage formation from β-1,4 to β-1,3.

A considerable amount of efforts has been expended to achieve *in vitro* synthesis of cellulose using membrane preparations of various degrees of purity from different organisms[5]. Interestingly, almost all attempts at *in vitro* production of cellulose have resulted either in the formation of β-1,3-glucans or only very limited synthesis of β-1,4-glucans, in the latter case, without sufficient proof of the characteristic crystallinity of the product[6]. Such surprises would not be coincidences and could be well explained if our proposed hypothesis is correct in this scenario. When the synthesis is performed *in vitro*, the factors controlling the linkage formation were narrowed down to two: the cellulose synthase used to catalyze the synthesis and the ordered membrane template. The role of cellulose synthase, in this case, may just be a general enzyme that activates the anomeric position to initiate the polymerization. The linkage formations, therefore, are left to be controlled by the order of the membrane template. Since the membrane structure is highly

ordered, the glycolipid precursors incorporated into it have only very limited options to locate themselves. In other words, they can only be inserted into the membrane in a certain direction and therefore the sugar headgroups exposed on the membrane surface will also form highly ordered packing. In this scenario, the formation of stereo-regular new glycosidic linkage could be very well assured since the ordered packing may only allow one of the free hydroxyls on each sugar ring, the most well positioned one, to participate and react. This explanation for callose synthesis means that 1,3-linkages are to be expected from transglycosylation reactions involving highly ordered glycolipids.

To investigate the possibility of our proposed mechanism for the formation of stereo-regular linkages, some models were built to mimic the organized system on membrane surface.

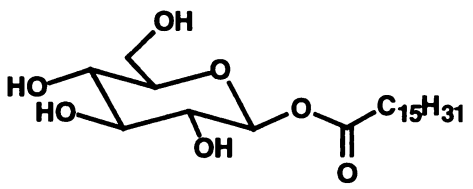
Suitable model systems for this investigation should possess several characteristics. First of all, they should be able to self-assemble into ordered supramolecular structures whose conformation and phase behavior can be predicted or characterized through calculation, spectroscopic or other analytical methods. They should also be easily accessible through synthetic methods to provide homogeneous materials for the investigation in sufficient quantity. A third important feature of such model systems is that they should bear proper functionalities that allow either enzymatic or chemical activation of the system to trigger reactions between the self-assembling units. Finally, such models should bear structural resemblance to important membrane components such as glycolipids, which are excellent targets of this investigation since they are directly involved in the biosynthesis of oligo- or polysaccharides on membrane

surfaces and are responsible for the regio- and stereo-control of the forming glycosidic linkages of resulting saccharides.

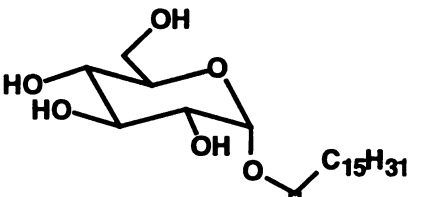
Design and Synthesis

A series of glycolipids were designed and synthesized in stereo-specific or stereo-selective fashion and uniformly good yields with variations in hydrophobic chain-length (either 12 or 16-carbon), number of hydrophobic chains (either single or double-chain), degree of unsaturation in hydrophobic chains (either saturated or 4,5-unsaturated forms), headgroup identities (either glucose or galactose), anomeric configurations, and functionalities connecting the glycon and aglycon parts (varying from ether, ester to acetal linkages). All model compounds are illustrated in Scheme 1.

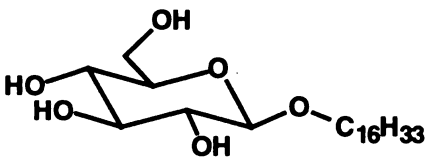
In compound **A-β**, a 16-carbon lipid tail is attached to glucose with the β-configuration. In many organisms, sixteen is statistically the most predominant lipid acyl chain length[7] and the β-configuration is also the most common linkage type. This lipid tail is expected to function as both the packing unit and the potential leaving group in the polymerization process. The synthetic route towards compound **A-β** and its anomer is shown in Scheme 2. In the transformation from tetrabenzylated glucose **A-2** to tetrabenzylated acyl glucoside **A-3**, successful stereo-control of the configuration of the forming glycosidic ester linkage was achieved by butyl lithium's chelation-controlled deprotonation in different solvent systems[8], followed by *in-situ* acylation. When the reaction was carried out in the non-polar (non-participating) solvent benzene, the β-configuration was strongly favored (5:1/β:α) since such a configuration would promote intramolecular chelation between the lithium ion, the deprotonated anomeric hydroxylate



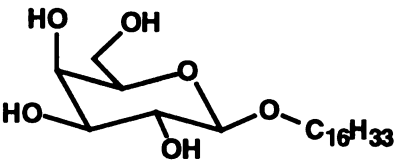
A-β 39% (5:1 β:α)



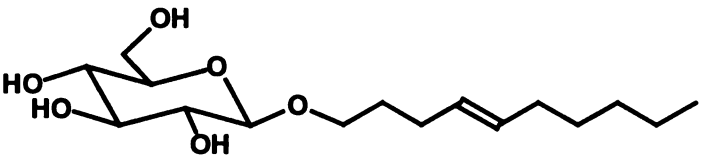
A-α 43% (1:2 β:α)



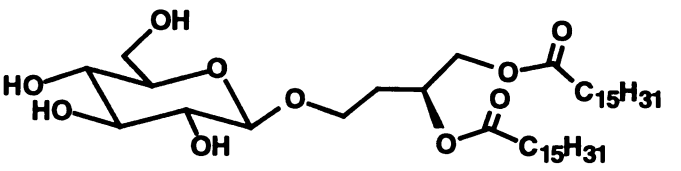
B 69%



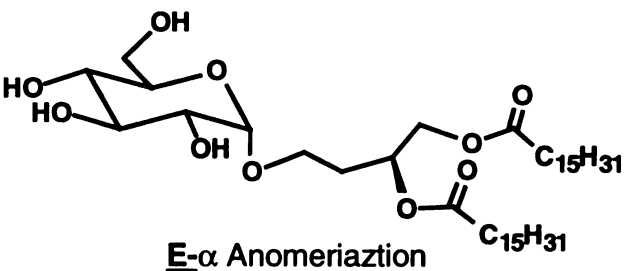
C 78%



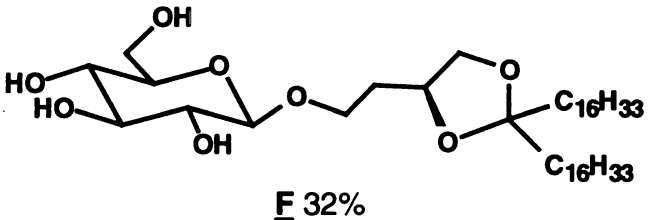
D 86%



E-β 40%

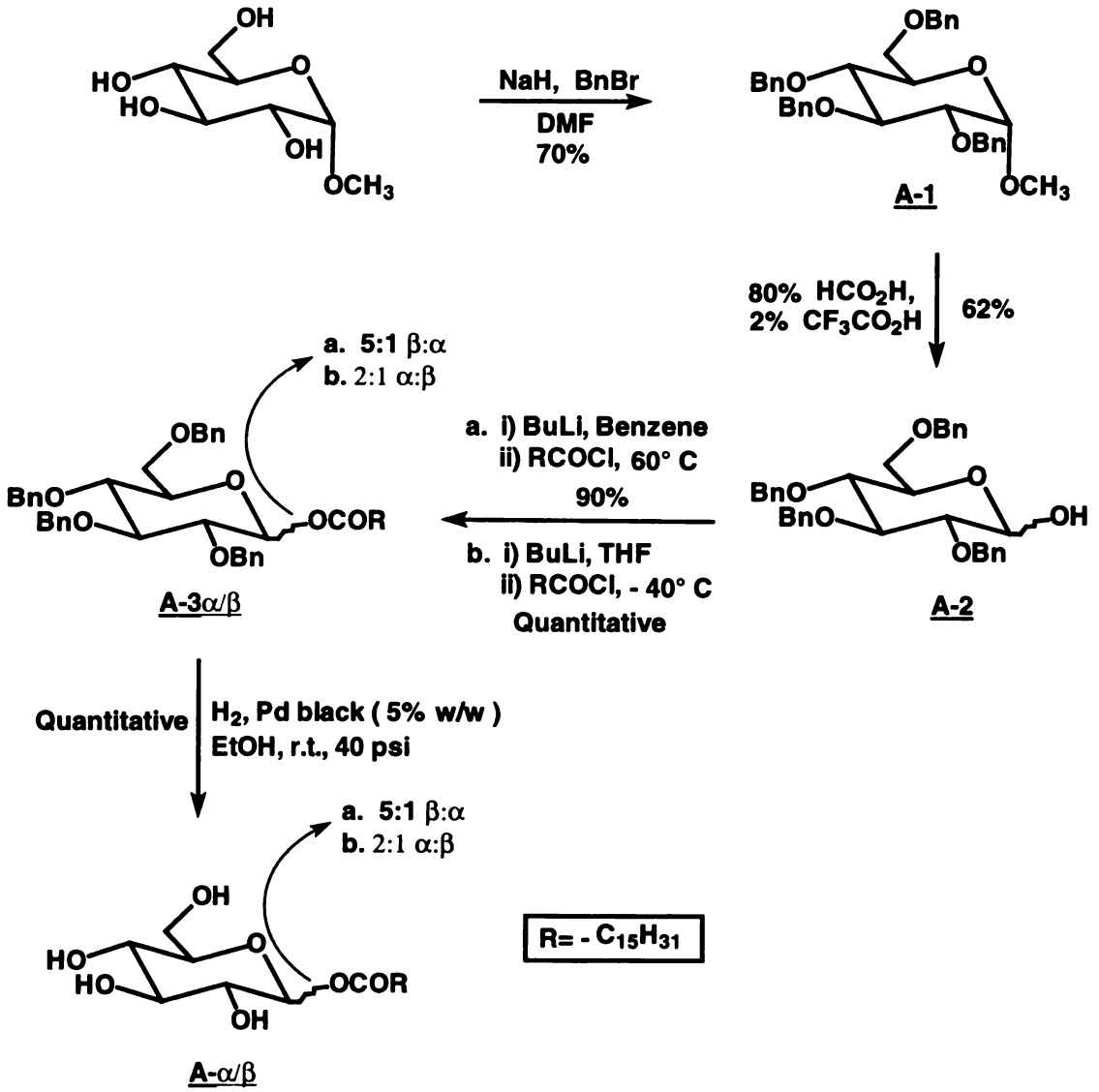


E-α Anomeriaztion
(5:1~10:1 α:β)



F 32%

Scheme 1. Building blocks of self-assembling systems and their overall yields.



Scheme 2. Stereoselective synthesis of compounds **A-α** and **A-β**.

and the ring oxygen. However, when more polar (chelating) solvent such as THF was used, the α-configured intermediate, which possesses a more open geometry for chelation between the lithium ion, the anomeric hydroxylate and the solvent THF, was preferentially formed (2:1/α:β). This chelation effect is illustrated in Figure 1. Apparently the intermolecular chelation with solvent lowers the reaction barrier more effectively than intramolecular chelation. Therefore α-selective reaction should be carried out at low temperature while the β-selective reaction may require elevated temperature. Indeed, the successful stereoselectivity of the two reactions were achieved at -40°C and 60°C, respectively. Excellent overall yields (around 40%) for both anomers were observed.

Compounds **B** and **C** are typical glycolipids with one 16-carbon hydrocarbon chain linked to glucose or galactose through β-glycosidic ether linkages. Unlike the commercially available β-octyl glucoside, these compounds may form major supramolecular structures different from micelles[9]. They offer complementary model systems for the investigation of the influence of the packing configuration of an ensemble on its intrinsic 1-D linkage encodement. The purpose of introducing a galactose headgroup in compound **C** is that when this compound is mixed with other self-assembling glucosides and the system was triggered by β-glucosidase, **C** would only function as a glucosyl acceptor and the axial hydroxyl at its 4-position may then provide information on whether it is the reactivity or the proximity factor that dictate the reaction consequence in such system. These two compounds were synthesized in high yields within two steps, using well-established Konigs-Knorr condition[10] (Scheme 3).

Compound **D** was designed to effect the chemical activation of the anomeric position by Fraser-Reid condition[11], where the double bond of a pent-4-enoyl glycoside

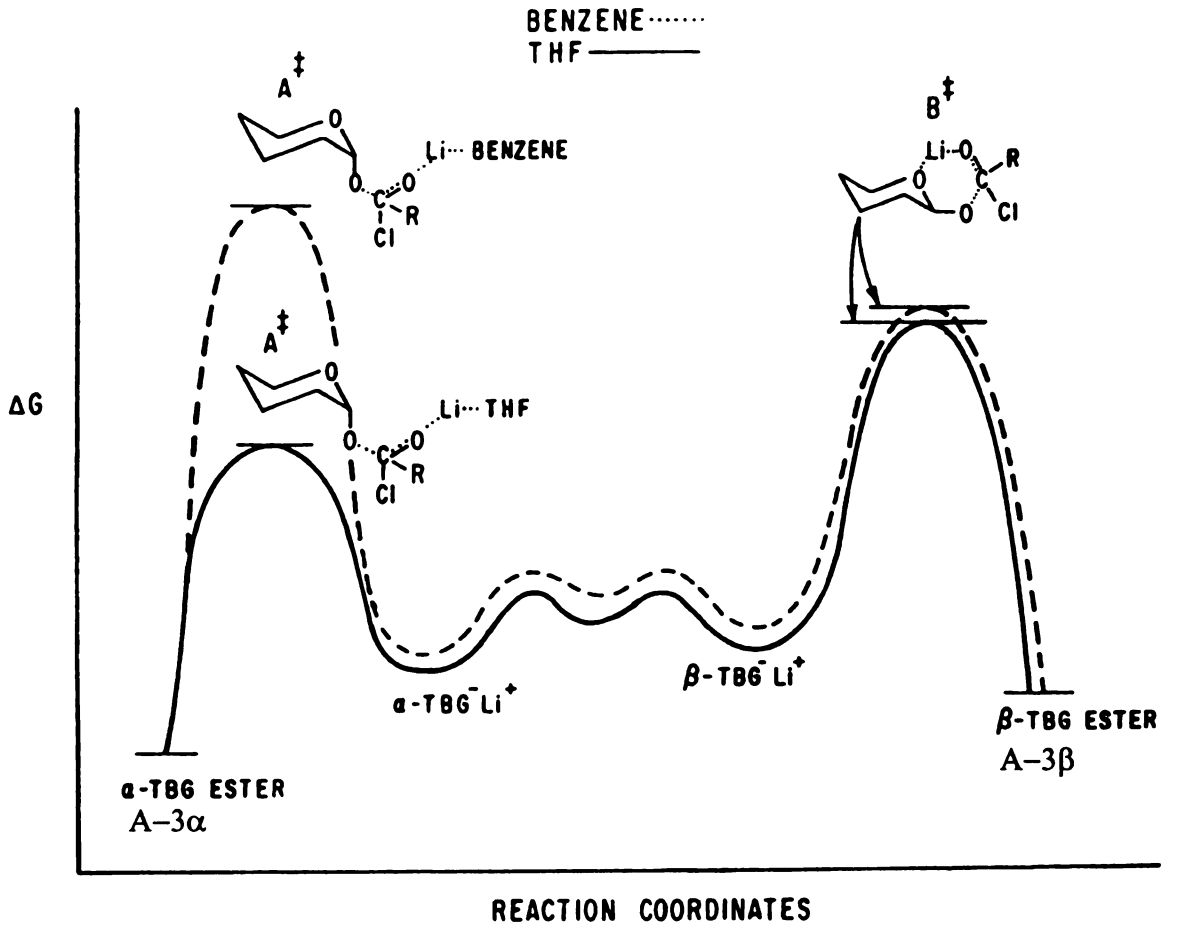
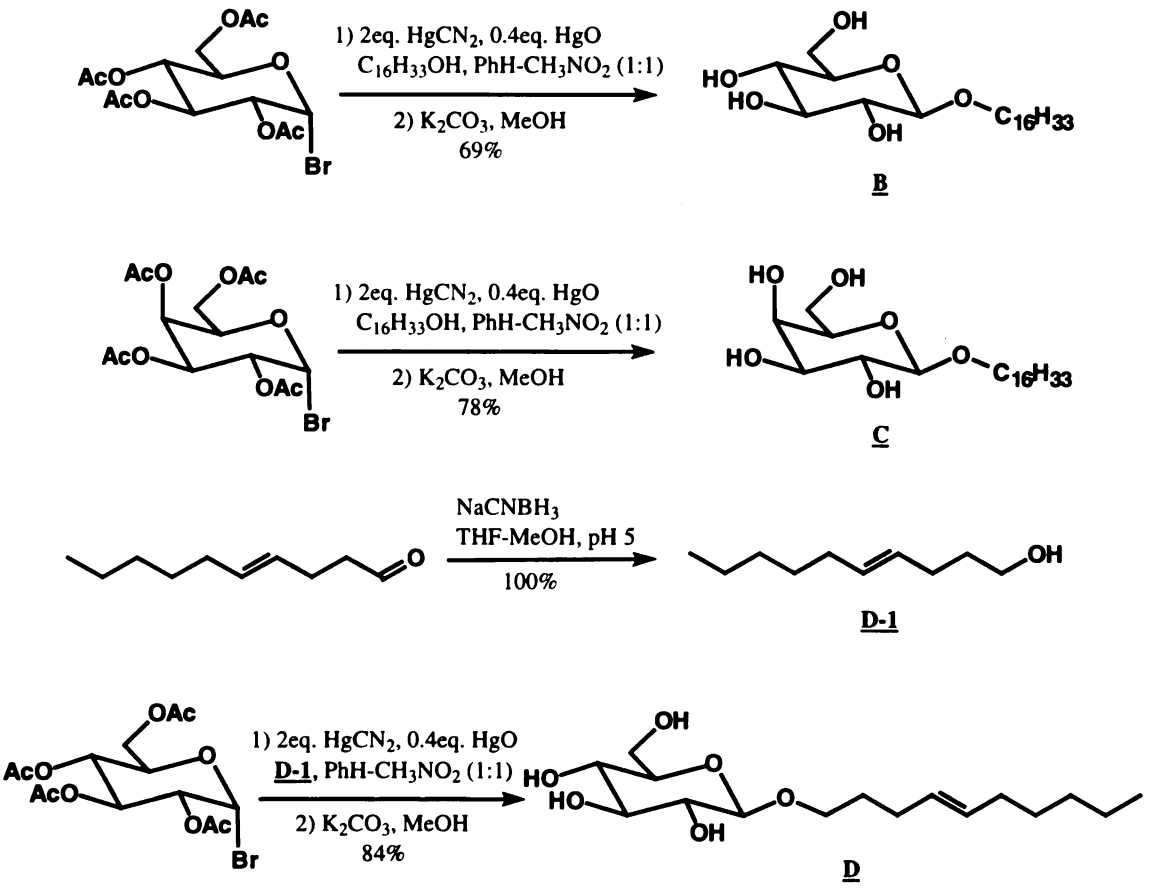


Figure 1. Chelation effects and reaction barriers for stereo-selective acylation in non-participating benzene and chelating THF.



Scheme 3. Stereospecific synthesis of compounds **B**, **C** and **D**.

could be activated by treatment with a halonium ion (e.g. NBS or NIS) or other strong electrophile (e.g. Hg^{2+}) and the subsequent formation of a 5-membered furan ring would result in the cleavage of the glycosidic linkage. We designed a 10-carbon hydrocarbon chain with the unsaturation at the 4,5-position to satisfy the requirements for both the anomeric center activation and the micelle formation. We started with commercially available trans-4-decenal. After reduction, coupling and deprotection, target molecule **D** was obtained in an 84% overall yield (Scheme 3).

Compounds **E-β**, **E-α** and **F** bear double hydrocarbon chains. They are close structural analogs of naturally occurring glycolipid monoglucosyl diacylglycerol (MGDG) and tend to form lamellar structure in water. The strategy of using a chiral butane triol linker instead of a glycerol linker makes these molecules synthetically much more accessible in large quantity (gram scale) than their natural counterpart. They were all obtained in satisfactory overall yields (30-40%). The design, synthesis and characterization of these molecules are presented in detail in Chapter 2. These compounds form supramolecular assembly other than micelles or interdigitated bilayers, and therefore provide further opportunity for future investigation of the influence of packing configuration on 1-D linkage encodement in highly ordered ensemble systems.

Conformational Analysis and Phase Characterization

Membrane lipids and their analogs are invariably polymorphic, that is, they can exist in a variety of different kinds of organized structures[2]. The particular polymorphic form that predominates depends not only on the structure of the lipid molecule itself, but also upon variables such as temperature, pressure, ionic strength, pH and its degree of

hydration. It is impossible to draw any conclusion of this study without any idea of the conformation and packing behavior of the ensemble system formed by specific model compounds, since the intrinsic order in such system is the core of our investigation. Some model compounds are known to enable certain supramolecular structure formation, while others are either not very well studied or completely novel molecules, and therefore require conformational analysis and phase characterization. 2-D NMR experiments, especially NOESY experiment, are very powerful tools in conformational analysis. They are often complemented by computer modeling. X-ray diffraction techniques are usually used to determine the structure of different lipid phases, and Differential Scanning Calorimetry (DSC) is used to study the transition of one lipid phase to another. The combination of X-ray diffraction and DSC has proven extremely valuable in studies of lipid structures in both model and biological membranes. Finally, polarized microscopy pattern could sometimes provide helpful information on specific phase formation as well.

Single-chain Glycolipids with Ether Linkage

Commercially available octyl-β-D-glucoside is known to form different supramolecular structures in water above its critical micelle concentration (CMC) with micelles as the most common form and therefore it serves as a convenient model for this study. Our synthetic homologous compound **D**, which bears a 10-carbon hydrocarbon chain through an anomeric ether linkage should form similar structure in water since it is known empirically that glycolipids with 8 to 12-carbon hydrocarbon chain are good micellar forming molecules.[12]

Model compound **B** bears one 16-carbon hydrocarbon chain and it may predominantly form partially interdigitated bilayer structures instead[9]. Indeed, X-ray

diffraction pattern of compound **B** showed a wide angle reflection at 3.7 Å, which corresponds to the hydrocarbon chain-chain separation, and a sharp and intense low angle reflection at 32 Å (along with its higher order reflections at 16 Å and 8 Å), which corresponds to the layer repeat spacing. These reflections unequivocally revealed the long-range order in this system.[13] The relatively smaller separation of the alkyl chains (3.7 Å) as opposed to the typical separation (4.2-4.6 Å) in well-characterized lamellar systems formed by phospholipids or other membrane lipids[14,15] suggests a partial interdigitation of the bilayer. The fact that the layer spacing falls between 1.2-1.6 times the extended length of the molecule (~ 26 Å, from energy minimization result) also supports a interdigitated bilayer structure.[16,17] There are also less intense reflections at 10 Å, 6 Å and 5 Å observed. They are not higher order reflections of the major peak and could be due to the existence of different molecular packings.

A major phase transition at 47°C was observed in a DSC scan in water.

Single-chain Glycolipid with Ester Linkage

Glycolipids with ester glycosidic linkage are not very commonly seen in nature and their conformation and packing behavior are certainly less studied and rarely documented. To address these questions to the model system formed by compound **A-β**, 2-D NMR experiments, differential scanning calorimetry and X-ray powder diffraction experiments were performed.

It is already known from grid searches using molecular mechanics calculation that for glycolipids there are two major conformers, the one with the lipid tail perpendicular to the sugar ring and the one with the tail fully extended at almost the same plane of the sugar ring. Various X-ray single crystal structure data showed that β-glycosides with long

alkyl chains almost exclusively take the latter conformation.[9] In model compound **A-β**, an acyl chain is glycosidically connected to the glucose instead of the alkyl chain. Unfortunately, there is no crystal structure of the β-linked acyl glycoside available. Although it is reasonable to assume similar packing behavior since both compound **B** and **A-β** adopt a 16-carbon hydrocarbon chain and a β-configuration, it is still necessary to support this assumption by experimental data.

First of all, the conformational question could be easily answered by NOESY experiment. The sugar ring is known to be rigid. If the lipid tail is perpendicular to the sugar ring, we would expect to see a quite intense nOe corresponding to the anomeric proton H_1 and the proton α to the carbonyl group (H_α), comparing to the nOe between the ring protons H_5 and H_1. This is because geometrically the distance of the former two is supposed to be very close to that of the latter two (2.51 Å), which is taken as a reference in this case. If the alternative conformation is taken, then we will not be able to see any nOe corresponding to H_1 and H_α since in this conformation they are too far apart from each other. In real situation, there may be an equilibrium between these two conformers. By measuring the volumes of the NOESY crosspeaks we should be able to tell the ratio of these two conformers. The NOESY data (Figure 2 and Table 1) showed that the volumes of the crosspeaks corresponding to H_1-H_α and H_1-H_β are only about 10% and 5% of the reference volume (H_1-H_5), respectively. This clearly suggests that the conformer with perpendicular lipid tail is the less predominant one in the CD_3OD solution where the NOESY experiment was performed. This is within our expectation since the conformation with the lipid tail extended at the same plane of the sugar ring will

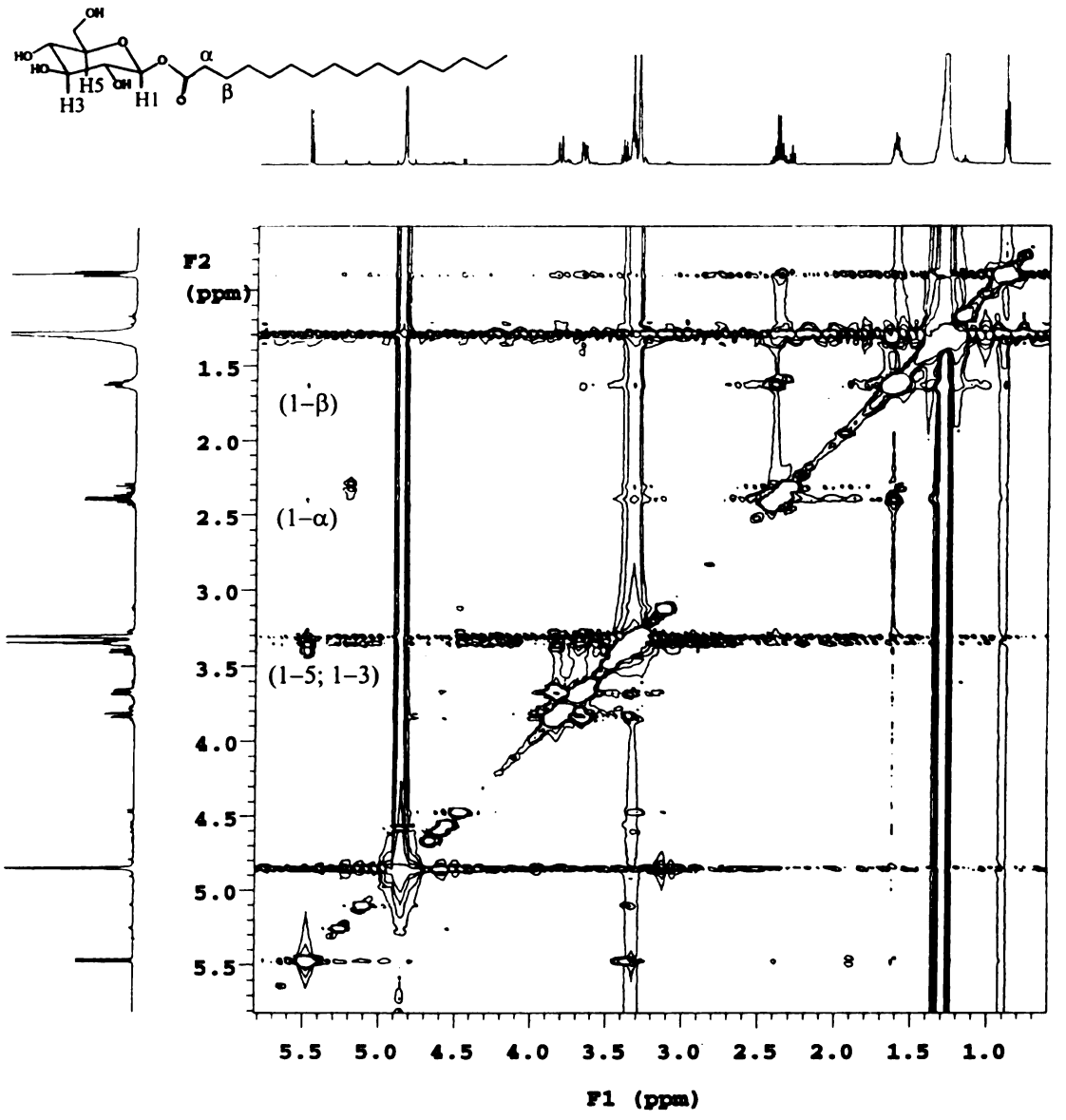


Figure 2. NOESY spectrum of compound **A-β** in CD_3OD, with a mixing time of 400 ms.

Proton Pairs	H_1-H_3	H_1-H_5	H_1-H_α	H_1-H_β
nOe Volumes	0.2979	0.3906	0.0468	0.0214

Table 1. Proton pairs and the nOe volumes of compound **A-β**.

result in a more tightly packed system in polar solvent than the other one, just as in the case of β-linked alkyl glycosides.

The X-ray powder diffraction pattern of compound **A-β** were also very similar to that of compound **B**. At the wide angle region, a 4 Å d-spacing which is typical for lipid bilayer chain-chain separation was observed. And at the low angle region, a sharp reflection at 32 Å corresponding to the layer spacing, along with its higher order reflections at 16 Å and 8 Å, were observed. Considering the same extended molecular length of **A-β** as that of **B** (26 Å) measured from energy minimization result, this suggests a partially interdigitated bilayer structure as well. There were some other less intense reflections (corresponding to 37 Å, 11 Å and 9 Å) observed at the low angle region. They may correspond to the different domain structures caused either by impurities or just by alternative packing arrangements with energies close to that of the bilayer structure. The existence of other minor domain structures apart from the major bilayer structure seem to be supported by the DSC data (Fig.3), which showed a sharp major phase transition around 43°C and some minor phase transitions around 52°C.

With the above evidence, we conclude that the carbonyl group in compound **A-β** does not seem to bring significant changes in its hydrocarbon chain arrangement and packing behavior as compared to its alkyl glycoside counterpart **B**.

Double-chain Glycolipid with Ester Linkage

Model compound **E-β** is the one of the closest structural analogs of naturally occurring double-chain glycolipid monoglucosyl diacylglycerol (MGDG), with one carbon extension at the chiral linker region. It is known that MGDG forms stable lamellar system in water and its glycerol linker has a large degree of flexibility.[18] Therefore it is

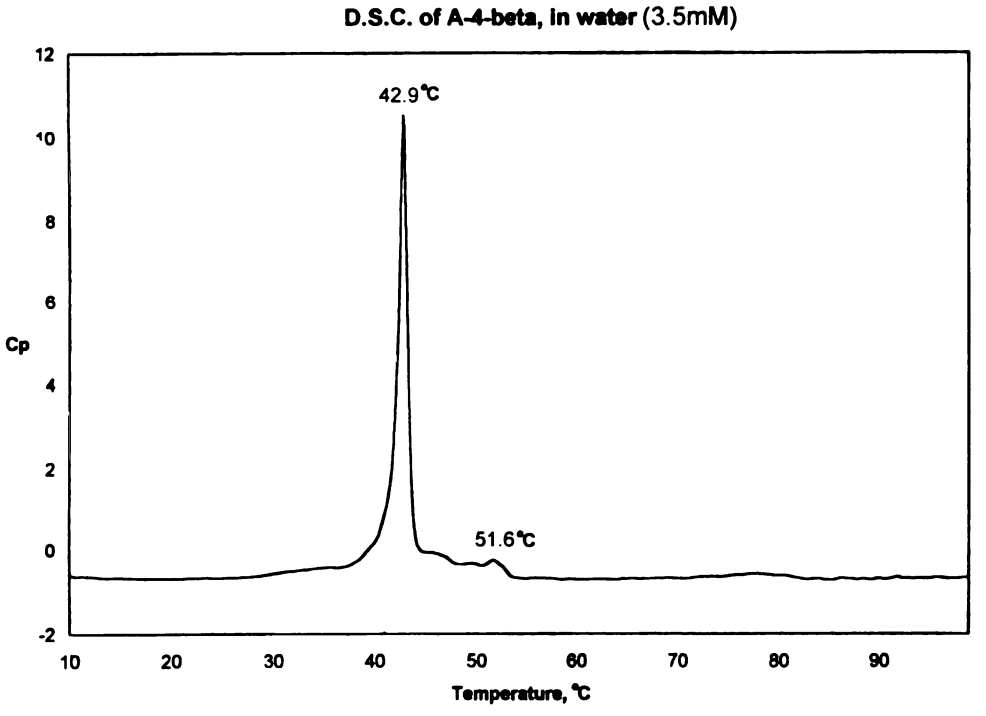


Figure 3. Differential scanning calorimetry thermogram of compound **A-β** in water.

reasonable to speculate a very similar packing behavior for this MGDG analog since the one carbon extension locates at the already flexible linker region.

Novel Double-chain Glycolipid with Acetal Linkage

The novel MGDG analog **F** has a spiral acetal linkage connecting the hydrophobic region to the hydrophilic headgroup through a 4-carbon linker. The conformational analysis and phase behavior characterization was presented in detail in Chapter 2, through the combination of a variety of NMR experiments, computer modeling, DSC, X-ray diffraction and laser scanning microscopy experiments. This model compound forms stable lamellar system in water. And it adopts a lowest energy conformation that is similar to that of MGDG in terms of headgroup orientation, linker flexibility and hydrocarbon chain arrangement.

Activation of Model Systems

When pre-organized molecules are brought together, they form ensemble systems through a variety of non-covalent weak interactions such as H-bonding, van der Waals interaction, etc. If the resulting ensemble system is sufficiently stable, then some long-lived chemical reactions can occur within the well-packed system. In our model systems, highly ordered micellar, partially interdigitated bilayer or lamellar structures are stably formed. Therefore the reactive sites between the units should have a regular orientation relative to each other. In multifunctional reacting species such as, in this case, carbohydrates (which have several hydroxyl groups), ideally only one might be predisposed to react with an activated site on a neighboring molecule. When the reactive sites are very close to each other, which should be the exact case in our model systems,

then not only the intermolecular reactions between the neighboring packing units could happen, but the highly stereoregular linkages may be obtained as well. The expected high regio- and stereo-chemical integrity is based on the assumption that the geometrically most available and the conformationally most matching reacting group will react first.

The key for triggering the formation of new glycosidic linkages (transglycosylation) between carbohydrate headgroups of such glycolipid-based self-assembling system is to activate the anomeric position. This could be achieved by either chemical or enzymatic method depending on the structural nature of the model system.

Enzymatic Activation

It is known that β-glucosidase is highly specific towards the glycon and anomeric linkage identity, namely it only activates glucosides with β-anomeric linkage, while is quite tolerating with variations on the aglycon part.[19] Most of our model systems are β-glucolipids and therefore can be subjected to β-glucosidase's activation.

It is important to control proper reaction time to harvest appreciable amount of transglycosylation products. On the one hand, with infinitely long reaction time, glucosidase would eventually hydrolyze all glucosides including the transglycosylation products. On the other hand, transglycosylation is a relatively slow process and certain period of time is required before the amount of transglycosylation product was accumulated to a level that is useful for further linkage analysis. The concentration of model compounds in such reaction is also crucial. In principle, higher concentration should promote more ordered packing and therefore could lead to higher regio- and stereo-chemical integrity in products. However, in reality this is often restricted by the solubility and mobility limits of both the substrates and the enzyme. At too high a

concentration, not only proper gel formation is impossible, the enzyme's optimal activity cannot be achieved, either.

Octyl-β-D-glucoside (OG) and the control methyl-β-D-glucoside (MG) were subjected to the activation by β-glucosidase at different concentration levels (varying from 1 M, 1.5 M, 2 M, 3 M, 5 M, 7 M up to 10 M). And different time points were taken to trace all reactions (30 min, 1 hr, 2 hrs, 4 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs) by TLC. The formation of glucose, disaccharides and higher oligomers (in both the polyglycolipid form and the free oligosaccharide form with reducing end) were detected in all systems, using glucose and maltose as TLC markers. MALDI-MS detected the formation of up to hexasaccharides in 3 M OG (as opposed to trisaccharides in 3 M MG). However, at very high concentration such as 5 M, 7 M and 10 M, the reaction was extremely slow and there were only very trace amount of oligosaccharides were formed. This is because at such high concentration, it was very hard to hydrate the substrate and the enzyme well. It was also shown that at prolonged reaction time (after 2 days at 37°C), glucose became almost the exclusive product. It was therefore concluded that a concentration less than 3 M and the reaction time less than 12 hrs might be the upper limit of the optimal condition range for harvesting transglycosylation products for further detailed analysis.

Model compounds **B** and **A-β** were subjected to same enzymatic activation at a concentration level higher than their CMC (0.2 M, 200 μl), along with OG and MG at the same concentration. The results for OG and MG were very similar to those obtained at higher concentration level, while for longer chain compounds **B** and **A-β**, the reaction rate was much slower. Even after 2 days' incubation at 37°C, only small amount of glucose were formed and only trace amount of oligosaccharides were detected by TLC

with orcinol spray (very sensitive spray for carbohydrates). This is mainly because of the poor solubility of such substrates in water even at such a relatively low concentration. This brings a potential problem for harvesting enough material for further analysis.

Chemical Activation

Fraser-Reid activation of the 4,5-unsaturated model compound **D** to trigger the formation of the reactive oxonium species was realized by NIS, NBS and Hg^{2+} in acetonitrile. The formation of disaccharides and higher oligomers were detected on TLC in all cases, with Hg^{2+} as the best activating reagent. It needs to be pointed out that this reaction can only be carried out in organic solvent. The packing order of compound **D** in such solvent system would be very different from that in water.

Linkage Analysis of Transglycosylation Products

The linkage analysis of the transglycosylation products, their regio- and stereo-regularity, is not a trivial task. It requires the combination of comprehensive 2-D NMR experiments and GC-MS analyses.

Detailed analyses were performed on products of enzymatic reaction of octyl-β-D-glucoside (OG) and the control methyl-β-D-glucoside (MG). Solutions of OG and MG (1.5 M) incubated with β-glucosidase at 37°C for 1 hr, 4 hrs, 12 hrs, 24 hrs and 48 hrs were desalted, lyophilized, re-dissolved in water and passed through C-18 column. The water fraction should be free of cleaved hydrocarbon chains and most of the unreacted OG. All transglycosylation products should be water-soluble and therefore retained in the water fraction. Overloaded OG and unreacted MG were also expected to be present in the water fraction. In order to simplify the ^1H NMR spectra of this complex mixture,

reduction by sodium borohydride was performed on all samples. This removed the complexity caused by the α/β anomeric protons on the reducing end of the sugars and left signals at the anomeric region only possibly belonging to OG, MG and newly formed transglycosylation linkages (the most interested linkages). 1-D ^{1}H NMR of all reduced fractions revealed that the 4 hrs reaction fractions made the most significant difference between OG and the control MG, in terms of the ratio of transglycosylation products and the completely hydrolyzed and reduced product alditol (glucitol in this case). Therefore they were selected for further detailed analyses. The ^{1}H NMR spectra were taken at 6°C, 25°C and 50°C in D_2O. The shifting of the solvent peak at different temperature assured that there were no anomeric peaks buried underneath the HDO residue line. Equal amount of sodium formate was then added to same volume of NMR sample of OG and MG (the 4 hrs fraction), which contain all transglycosylation products, to quantify the formation of oligosaccharides in these two reactions. The expanded anomeric regions of these two samples are shown in Figure 4. The spectrum vertical scales were adjusted according to the internal calibration by sodium formate. Although the quantity of transglycosylation products formed in OG and MG were comparable, in OG only one type of linkage was predominantly formed (note the doublet at 4.14 ppm in Fig. 4a). In the case of MG there was a much higher diversity of linkage types formed. This was indicated by different anomeric proton signals at comparable intensities (note the peaks at 4.18, 4.17, 4.08 and 4.02 ppm in Fig. 4b). These results suggest that the ordered packing in OG promotes the regularity in linkage formation, while in randomly ordered system like MG, all possible linkage formations occur based on reactivity of different hydroxyl groups.

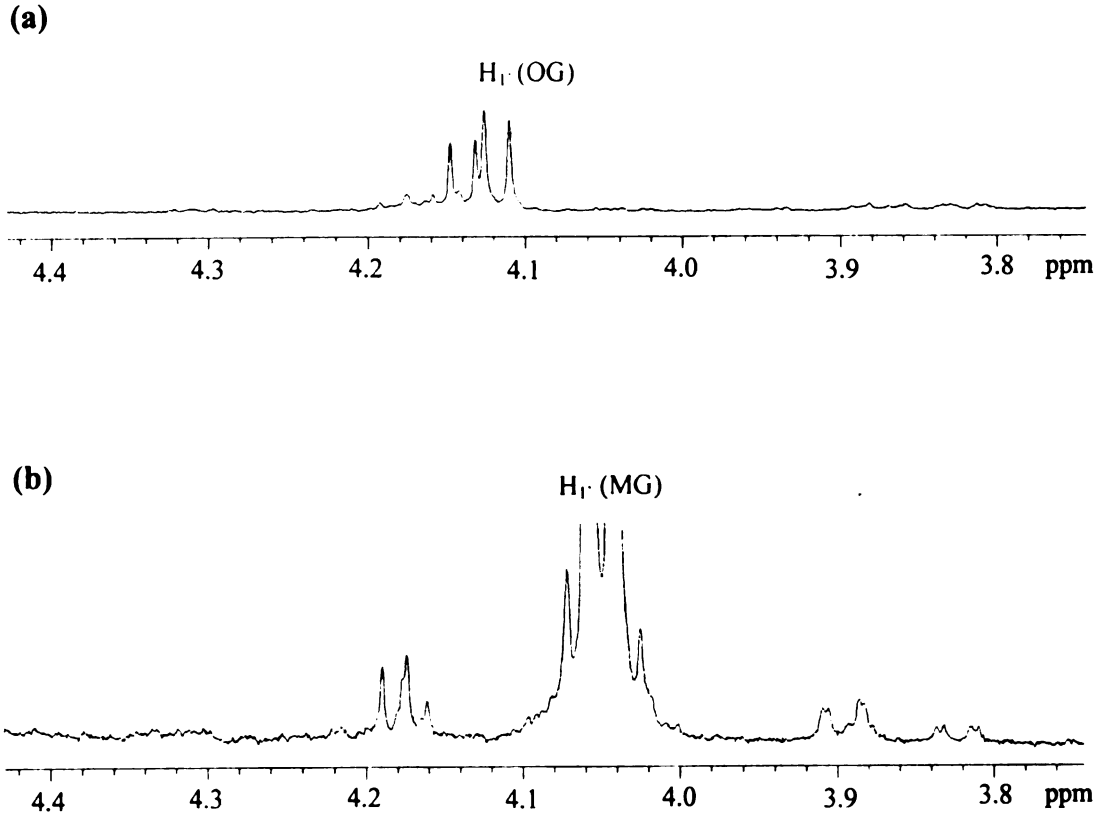


Figure 4. ^{1}H NMR (500 MHz, 6°C, in D_2O) anomeric regions of the reduced transglycosylation reaction mixture of (a) octyl-β-D-glucoside (OG) and (b) methyl-β-D-glucoside (MG). The spectrum vertical scale was adjusted according to the internal calibration standard (sodium formate) so that the intensity shown here reflected the relative quantities of products in these two systems.

The characterization of predominant linkage type in OG was attempted by the combination of double quantum filtered J-correlated spectroscopy (DQF-COSY), gradient ^{1}H-^{13}C hetero-nuclear multiquantum coherence spectroscopy (^{1}H-^{13}C HMQC) and total correlated spectroscopy (TOCSY). The HMQC, TOCST and COSY spectra are shown in Figure 5, Figure 6 and Figure 7. A combined strategy of elimination of possibilities and tracing connections between spectra was used. Unfortunately, the crosspeaks for the H_2' ring protons and anomeric protons of the OG (4.18 ppm, 2.97 ppm) and that for the major transglycosylation product to which glucitol was β-linked (4.22 ppm, 3.02 ppm) were partially overlapped in both TOCSY and COSY spectra. In HMQC, the anomeric crosspeaks for these two compounds were also partially overlapped, with the OG signal at (102.59 ppm, 4.18ppm) and the other at (102.29 ppm, 4.22 ppm). This brought certain level of difficulty in a convincing assignment especially when the transglycosylation product was a minor component comparing to the predominantly formed glucitol in the mixture. In the case of MG, the assignment of the multiple transglycosylation products through 2-D NMR experiments was even more difficult since the proportion of glucitol and MG was overwhelmingly higher due to both the faster hydrolysis rate (to give glucitol) and better solubility of MG in the water fraction.

Methylation analysis is another very powerful tool in characterizing linkage types of oligo- and polysaccharides, especially in detecting minor components due to the high instrument sensitivity.[20] This analysis was performed on both OG and MG transglycosylation products. GC-MS analysis gave diagnostic fragmentations[20] for characterizing all linkage types in both systems. The earliest eluting major peak after the

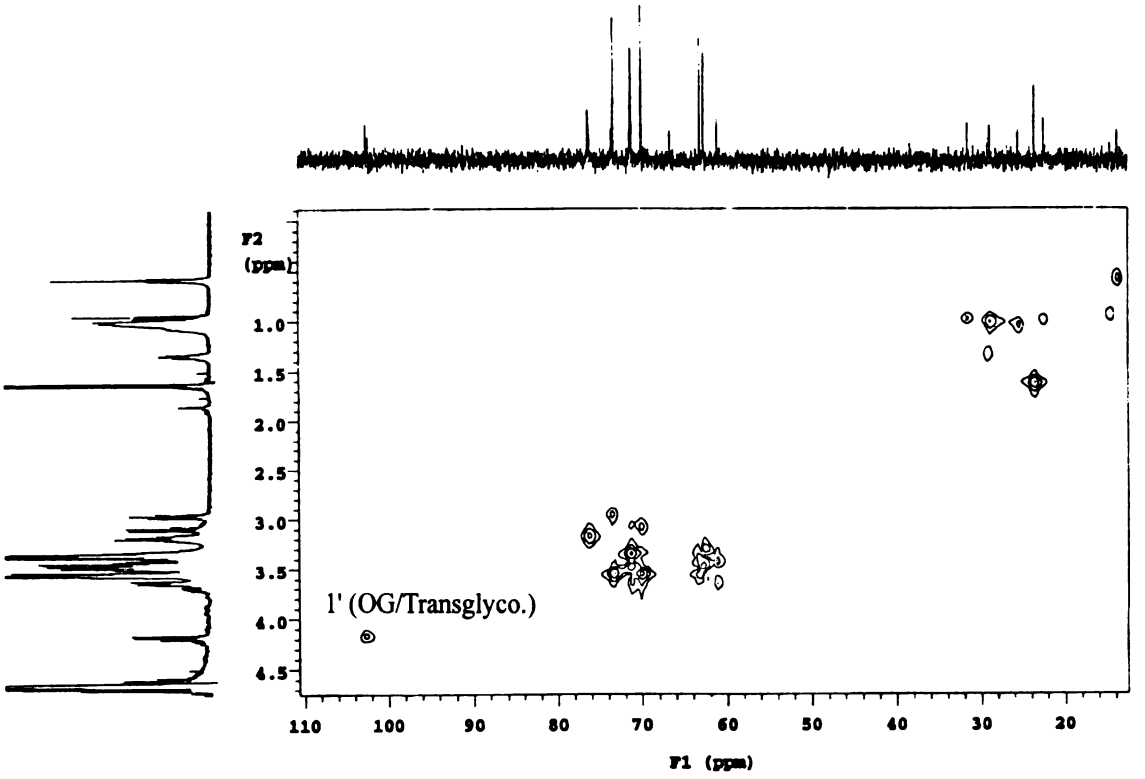


Figure 5. ^{1}H-^{13}C HMQC spectrum (500 MHz, 6°C, in D_2O) of reduced transglycosylation reaction mixture of octyl-β-D-glucoside (OG).

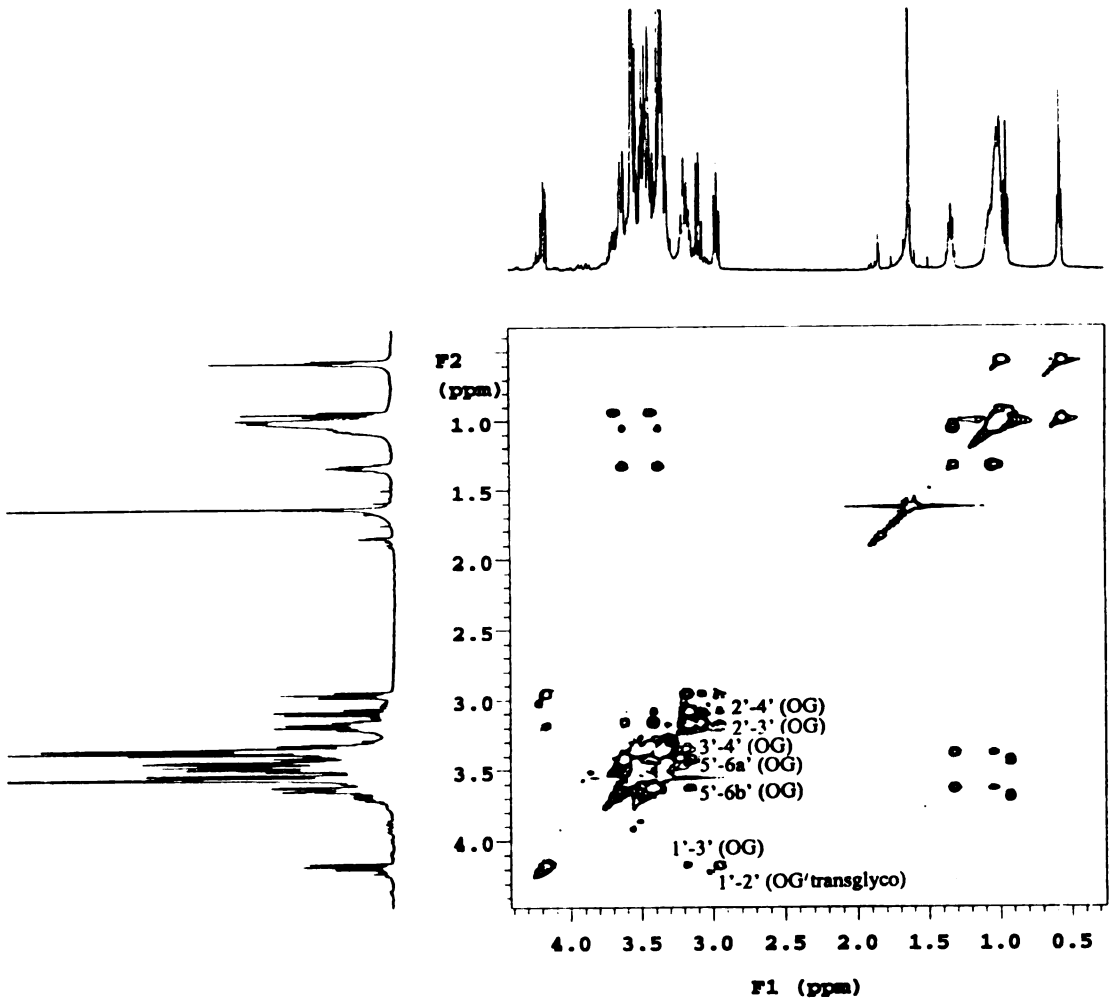


Figure 6. TOCSY spectrum (500 MHz, 6°C, in D_2O) of reduced transglycosylation reaction mixture of octyl-β-D-glucoside (OG).

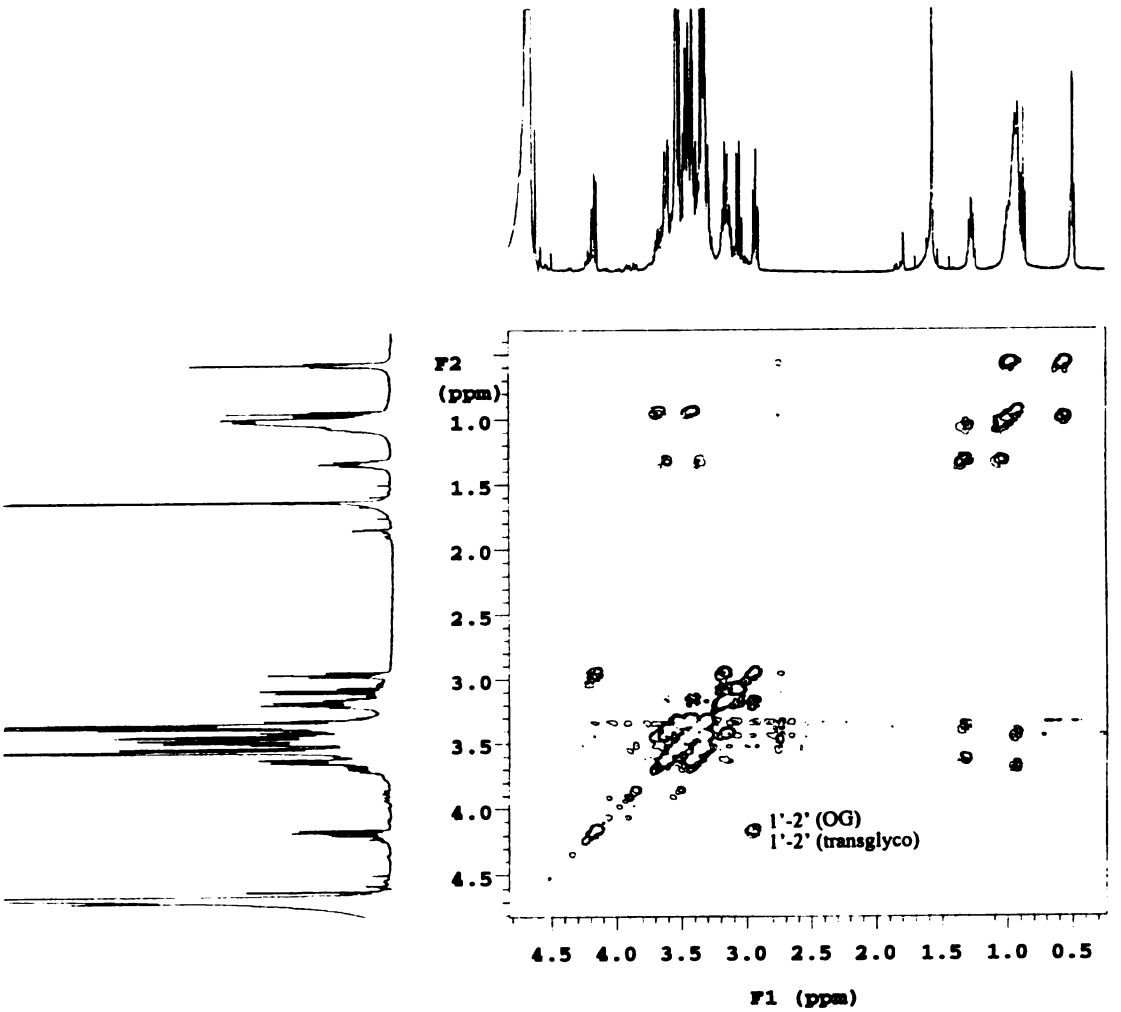


Figure 7. DFQ-COSY spectrum (500 MHz, 6°C, in D_2O) of reduced transglycosylation reaction mixture of octyl-β-D-glucoside (OG).

one for hexamethyl glucitol contained fragments at M/Z 45, 89, 101 and 69 (with a minor peak at 133) clearly suggesting a predominant 1,3-linkage. This linkage pattern was not obtained for the MG sample. The data indicated that a 1,2-linkage was the relatively abundant one in the latter case.

Conclusions and Perspectives

A series of glycolipids were design and synthesized in uniformly good yields. These glycolipids self-assemble into highly ordered supramolecular structures varying from micelles, partially interdigitated bilayers to stable lamellar systems in water. The glycosidic linkages of these glycolipids can be activated either enzymatically or chemically to initiate transglycosylation reactions within the ordered system. They are therefore excellent models for studying templating effects in ordered supramolecular ensemble systems.

Preliminary study revealed that highly ordered self-assembling system (e.g. micelles formed by OG) could not only effect the formation of oligosaccharides upon activation by glycosidase, but the formation of highly selective linkage type (β-1,3) as well. This unequivocally suggested the influence of intrinsic order on the chemical outcome of reactions occurring within such ensemble system. To further reveal how packing configurations, or different supramolecular structures, influence the linkage formation, more detailed analysis need to be carried out on all model systems. One potential difficulty of performing analysis on model systems with longer or double chain glycolipids is the solubility problem. Due to the poor solubility of such amphiphiles in

water, the yield of transglycosylation reaction was generally low. This makes further analyses by 2-D NMR experiments challenging.

By comparing results from self-assembling systems with different conformational and phase behavior, we hope to shed some light on the encodement of 1-D linkage and sequence information in the organization of 2-D molecular ensembles. This will not only be important to further reveal how membrane systems utilize their intrinsic rich structural information to direct or mediate chemical and biological processes on their surfaces, but provide us a foundation to apply this encodement strategy into regio- and stereo-selective organic syntheses. By learning the secrets of nature, we may obtain better controls in utilizing natural law to govern man-made systems.

Experimental

General Techniques

All reagents used were reagent grade. Reaction temperatures were measured externally. Flash chromatography was performed on Aldrich silica gel (60 Å 200-400 mesh). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

NMR spectra were recorded on a Varian 300 MHz or 500 MHz VXR spectrometer at ambient temperature. Chemical shifts are reported relative to the residue solvent peak, in cases where the mixed solvent of methanol and chloroform were used, methanol was chosen as the reference. Optical rotations were measured using a Perkin polarimeter at 589 nm. High-resolution mass spectra (HRMS) were recorded on a JEOL HX-110-HF spectrometer using Fast Atom Bombardment (FAB) conditions and an N-

benzyl alcohol (NBA) or glycerol (Gly) matrix. Low-resolution mass spectra (LRMS) were recorded on a Fisons Platform mass spectrometer using electrospray ionization in positive ion mode (ESI^+).

Synthesis

2,3,4,6-Tetra-O-benzyl-α-methoxy-D-glucopyranoside A-1.

To a solution of 150 ml of anhydrous DMF and 7 g of α-methyl-D-glucoside (dried at 60°C in a vacuum oven for 3 hours prior to use) was added 10.5 g of sodium hydride (60% dispersion in mineral oil, washed with hexanes several times prior to use) in portions under N_2 flow. The reaction mixture was stirred at room temperature for one hour before it was cooled to 0°C. 21.5 ml of benzyl bromide was introduced slowly within an hour and the reaction was kept at 0°C for another hour before it was allowed to warm to room temperature. After 18 hours, with occasional sonication, the reaction was quenched with 3 ml of methanol and 1 ml of water. The mixture was then diluted with 150 ml of toluene, washed by saturated sodium bicarbonate solution and water several times. The organic layer was dried over anhydrous sodium sulfate. After the removal of solvent, 24 g of light brown syrup was obtained. Flash column chromatography (using an eluent of 9:10:1/chloroform:hexanes:ethyl acetate) gave 14 g pure **A-1** in 70% yield. ^1H NMR (300 MHz, $CDCl_3$): δ 7.31 (m), 7.14 (m), 4.75 (m), 4.01 (1H, t, J = 9 Hz), 3.76 (2H, m), 3.67 (2H, m), 3.59 (1H, dd, J = 9.6, 3.4 Hz), 3.40 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.67, 138.12, 138.04, 137.78, 129.18, 128.38, 128.35, 128.34, 128.32, 128.31, 128.29, 128.24, 128.15, 128.03, 127.92, 127.87, 127.85, 127.80, 127.77, 127.75,

127.72, 127.58, 127.56, 127.48, 98.09, 82.02, 79.69, 77.52, 75.65, 74.93, 73.36, 73.29, 69.91, 68.33, 55.06; ESI$^+$-MS: calcd $C_{35}H_{38}O_6Na$ $[M+Na]^+$, 577.6777, found 577.3.

2,3,4,6-Tetra-O-benzyl -D-glucopyranoside A-2.

A mixture of 1 g of **A-1** in 40 ml of 88% formic acid solution containing 2% trifluoroacetic acid was stirred at 100°C for 3 hours before it was diluted with water and extracted with toluene. The organic layer was washed with saturated sodium bicarbonate solution and water twice and then concentrated. Flash column chromatography (3:1/hexanes:ethyl acetate) yielded 0.6 g of **A-2** (62% yield). ^{1}H NMR (300 MHz, $CDCl_3$, listed signals correspond to the predominant α-anomer; minor amount of the β-anomer was also observed): δ 7.34-7.12 (m), 5.23 (1H, d, J = 3.6 Hz), 4.96-4.46 (8H, m), 4.01 (1H, m), 3.96 (1H, t, J = 9.3 Hz), 3.73-3.55 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$, reported signals correspond to the predominant α-anomer; minor amount of the β-anomer was also observed): δ 137.79, 128.51, 128.40, 128.05, 127.96, 127.86, 127.70, 91.35, 81.72, 79.93, 75.72, 75.02, 74.78, 73.48, 73.30, 70.32, 68.47; FAB-HRMS (NBA): calcd $C_{34}H_{37}O_6$ $[M+H]^+$, 541.2590, found 541.2594.

2,3,4,6-Tetra-O-benzyl-α-hexadecanoyl-D-glucopyranoside A-3α.

The solution of 50 mg of **A-2** in 10 ml of anhydrous THF was cooled to -40°C under N_2 flow. 50 μl of 2 M butyl lithium–pentane solution was added and 2 min later 34 μl of palmitoyl chloride was introduced. TLC revealed a complete conversion in 20 min. After concentration, excess reagent was removed by flash column chromatography (8:11:1/chloroform:hexanes:ethyl acetate as eluent) and gave pure **A-3** in quantitative yield with the α-anomer as the main product. ^{1}H NMR (300 MHz, $CDCl_3$, listed signals correspond to the predominant α-anomer; the α to β ratio is 2:1 according to the

integration of corresponding anomeric protons.): δ 7.31-7.09 (m), 6.38 (1H, d, J = 3.6 Hz), 4.95-4.44 (8H, m), 3.91 (1H, t, J = 9.3 Hz), 3.84 (1H, m), 3.72 (4H, m), 2.36 (2H, m), 1.60(2H, m), 1.22 (m), 0.86 (3H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, reported signals correspond to the predominant β-anomer): δ 172.28, 138.61, 137.99, 137.79, 137.62, 128.39, 128.08, 128.00, 127.94, 127.87, 127.73, 89.70, 81.67, 78.92, 77.21, 75.67, 75.30, 73.52, 73.07, 72.80, 68.04, 34.38, 31.91, 29.69, 29.48, 29.35, 29.30, 29.01, 24.91, 22.68, 14.12.

2,3,4,6-Tetra-O-benzyl-β-hexadecanoyl-D-glucopyranoside A-3β.

Heat a mixture of 110 mg of **A-2** in 15 ml of dry benzene at 60°C and maintain this temperature throughout the reaction. Under N_2 flow, 0.11 ml of 2 M butyl lithium-pentane solution was added. After 2 min, 75 μl of palmitoyl chloride was introduced. TLC revealed a complete conversion in 30 min. After concentration, excess reagent was removed by flash column chromatography (6:1/hexanes:ethyl acetate as eluent) and gave 0.14 g of pure **A-3** (90% yield) with the β-anomer as the main product. ^1H NMR (300 MHz, $CDCl_3$, listed signals correspond to the predominant β-anomer; the β to α ratio is 5:1 according to the integration of corresponding anomeric protons.): δ 7.34-7.12 (m), 5.62 (1H, d, J = 8.1 Hz), 4.97-4.45 (8H, m), 3.73 (4H, m), 3.59 (2H, m), 2.32 (2H, m), 1.61(2H, m), 1.25 (m), 0.88 (3H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, reported signals correspond to the predominant β-anomer): δ 172.13, 138.37, 138.08, 138.01, 137.91, 128.38, 128.07, 127.92, 127.86, 127.72, 127.66, 93.93, 84.79, 81.04, 77.22, 76.58, 75.69, 75.48, 74.95, 73.48, 68.05, 34.26, 31.91, 29.68, 29.43, 29.35, 29.25, 29.06, 24.53, 22.68, 14.11.

Hexadecanoyl-β-D-glucopyranoside A-β.

40 mg of **A-3** (β:α/5:1) was dissolved in 10 ml of ethanol containing a few drops of glacial acetic acid and 2 mg of palladium-black (5% w/w). Hydrogenation was performed at 40 psi overnight. After filtration and concentration, compound **A** was obtained quantitatively. Pure **A-β** was separated from the α-isomer via flash column chromatography (9:1/chloroform:methanol as eluent). $[\alpha]_D$ = -26° (C 1.78, MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 5.47 (1H, d, J = 8.1 Hz), 3.83 (1H, m), 3.67 (1H, m), 3.36 (4H, m), 2.39 (2H, m), 1.63 (2H, m), 1.29 (m), 0.90 (3H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.11, 95.54, 78.81, 78.01, 73.96, 71.06, 62.31, 34.89, 33.09, 30.81, 30.79, 30.78, 30.74, 30.61, 30.50, 30.45, 30.14, 25.64, 23.75, 14.45; FAB-HRMS (NBA): calcd $C_{22}H_{43}O_7$ $[M+H]^+$, 419.3009, found 419.3008..

Hexadecyl-β-D-glucopyranoside B.

A mixture of pre-dried 2.5 g of acetobromo-α-D-glucose (dried in vacuum oven at 40°C for 2 hours prior to use), 7.7 g of hexadecanol, 3 g. of mercury cyanide and 0.5 g of mercury oxide (all dried over phosphorus pentoxide in a vacuum desiccator) was stirred at 50°C in 220 ml of 1:1 anhydrous benzene and nitromethane under nitrogen overnight. The reaction solvent was then evaporated under reduced pressure. 100 ml of chloroform was then added and most of the mercury salts were removed by filtration. The filtrate was then concentrated and subjected to deacetylation directly without purification. The syrup was dissolved in 200ml of methanol and stirred with 15g of granular potassium carbonate at room temperature for 3 hours. TLC indicated that the deacetylation was completed. After filtration and concentration, the resulting syrup was subjected to flash column chromatography purification (9:1/chloroform:methanol as eluent) and 1.7 g of compound **B** was obtained (69% overall yield). $[\alpha]_D$ = -47° (C 1, MeOH); ^1H NMR

(300MHz, CD_3OD): δ 4.24 (1H, d, J = 7.8 Hz), 3.89 (2H, m), 3.66 (1H, dd, J = 12, 5.4 Hz), 3.53 (1H, m), 3.29 (2H, m), 3.16 (2H, dd, J =8.7, 8.1 Hz), 1.62 (2H, m), 1.29 (m), 0.90 (3H, t, J = 6.9 Hz); ^{13}C NMR (75MHz, CD_3OD): δ 104.35, 78.11, 77.90, 75.11, 71.64, 70.90, 62.75, 33.09, 30.81, 30.78, 30.66, 30.49, 27.14, 23.75, 14.47; FAB-HRMS (Gly): calcd $C_{22}H_{45}O_6$ $[M+H]^+$, 405.3216, found 405.3228.

Hexadecyl-β-D-galactopyranoside C.

A mixture of pre-dried 4 g of acetobromo-α-D-glucose (dried in vacuum oven at 40°C for 2 hours prior to use), 7.5 g of hexadecanol, 4.9 g. of mercury cyanide and 0.8 g of mercury oxide (all dried over phosphorus pentoxide in a vacuum desiccator) was stirred at 55°C in 250 ml of 1:1 anhydrous benzene and nitromethane under nitrogen for 24 hours. The reaction was worked up as describe above and the crude syrup was dissolved in 200ml of methanol and stirred with 20g of granular potassium carbonate at room temperature for 2 hours. After filtration, concentration and flash column chromatography purification (9:1/chloroform:methanol as eluent), and 3 g of compound **C** was obtained (78% overall yield). $[\alpha]_D$ = -323° (C 0.38, MeOH); ^1H NMR (300MHz, CD_3OD): δ 4.20 (1H, d, J = 7.5 Hz), 3.89 (1H, m), 3.82 (1H, dd, J = 3, 0.9 Hz), 3.73 (2H, m), 3.55 (1H, m), 3.48 (3H, m), 1.62 (2H, m), 1.29 (m), 0.90 (3H, t, J = 6.9 Hz); ^{13}C NMR (75MHz, CD_3OD): δ 104.92, 76.50, 75.01, 72.54, 70.83, 70.24, 62.40, 33.61, 33.00, 30.79, 30.71, 30.56, 30.39, 27.07, 26.89, 23.66, 14.40; FAB-HRMS (Gly): calcd $C_{22}H_{45}O_6$ $[M+H]^+$, 405.3216, found 405.3225.

***Trans*-4-decenol D-1.**

4 ml of 95% trans-4-decenal was reduced with 3 g of sodium cyanoborohydride in 20 ml of THF and 20 ml of pH 3 hydrochloric acid-methanol solution at room

temperature. The acidity of the reaction media was maintained constantly around pH4 by additional hydrochloric acid. After 4 hours, the reaction was quenched with more hydrochloric acid and then neutralized by sodium bicarbonate solution. The product was extracted by dichloromethane, washed by water twice and dried over anhydrous sodium sulfate. After concentration, 3.3 g of **D-1** as colorless liquid was obtained quantitatively. ^{1}H NMR (300MHz, CD_3Cl_3): δ 5.41 (2H, m), 3.63 (2H, t, J = 6.6 Hz), 2.83 (2H, m), 1.97 (2H, m), 1.75 (1H, b), 1.61 (2H, m), 1.28 (6H, m), 0.86 (3H, t, J = 6.9 Hz); ^{13}C NMR (75MHz, $CDCl_3$): δ 131.31, 129.31, 62.61, 32.52, 32.42, 31.37, 29.22, 28.91, 22.52, 14.06.

2',3',4',6'-Tetra-O-acetyl-β-1-(*trans*-4-decenyl)-D-glucopyranoside D-2.

A mixture of 1 g of **D-1**, 5.26 g of acetobromo-α-D-glucose (dried in vacuum oven at 40°C for 2 hours prior to use), 3.23 g. of mercury cyanide and 0.55 g of mercury oxide (both dried over phosphorus pentoxide in a vacuum desiccator) was stirred at 60°C in 80 ml of 1:1 anhydrous benzene and nitromethane under nitrogen overnight. After the same workup as described in the preparation of hexadecyl-β-D-glucopyranoside, the crude product was subjected to flash column chromatography purification (3:2/hexanes:ethyl acetate as eluent) to yield 2.7 g of **D-2** (86% yield). $[\alpha]_D$ = -11.7° (C 2.27, $CHCl_3$); ^{1}H NMR (300MHz, $CDCl_3$): δ 5.33 (2H, m), 5.16 (1H, t, J = 9.6 Hz), 5.04 (1H, t, J = 9.6 Hz), 4.95 (1H, dd, J = 9.6, 7.8 Hz), 4.44 (1H, d, J = 7.8 Hz), 4.23 (1H, dd, J = 12, 4.8 Hz), 4.09 (1H, dd, J = 12, 2.4 Hz), 3.83 (1H, m), 3.65 (1H, m), 3.44 (1H, m), 2.04 (3H, s), 2.00 (3H, s), 1.98 (3H, s), 1.96 (3H, s), 1.93 (4H, m), 1.59 (2H, m), 1.23 (6H, m), 0.84 (3H, t, J = 6.9 Hz); ^{13}C NMR (75MHz, $CDCl_3$): δ 170.74, 170.34, 169.41, 169.31, 131.35, 128.81, 100.77, 72.78, 71.62, 71.27, 69.46, 68.35, 61.91, 32.45, 31.31,

29.18, 28.52, 22.45, 20.70, 20.62, 20.58, 20.56, 14.03; FAB-HRMS (NBA): calcd $C_{24}H_{38}O_{10}Na$ $[M+Na]^+$, 509.2363, found 509.2357.

1-(*Trans*-4-decenyl)-β-D-glucopyranoside D.

2.5 g of **D-2** was stirred with 5 g of granular potassium carbonate in 50 ml of methanol at room temperature for 5 hours. After filtration, neutralization and concentration, the product was passed through a short celite pad and washed off by 4:1/ethyl acetate:methanol. 1.7 g of compound **D** was obtained in quantitative yield. $[\alpha]_D$ = -12.8° (C 2.28, MeOH); ^1H NMR (300MHz, CD_3OD): δ 5.42 (2H, m), 4.24 (1H, d, J = 7.5 Hz), 3.88 (2H, m), 3.67 (1H, m), 3.53 (1H, m), 3.31 (3H, m), 3.18 (1H, dd, J = 9, 7.8 Hz), 2.08 (2H, m), 1.98 (2H, m), 1.66 (2H, m), 1.30 (6H, m), 0.89 (3H, t, J = 6.9 Hz); ^{13}C NMR (75MHz, CD_3OD): δ 131.97, 130.73, 104.31, 77.99, 77.78, 75.03, 71.52, 70.26, 62.65, 33.57, 32.48, 30.73, 30 39, 29.95, 23.55, 14.43; FAB-HRMS (Gly): calcd $C_{16}H_{31}O_6$ $[M+H]^+$, 319.2121, found 319.2105.

Conformational Analysis

Two-dimensional (2-D) NMR Experiments

All 2-D NMR spectra of compound **A-β** and the transglycosylation products of methyl-β-D-glucoside and octyl-β-D-glucoside were measured at 500 MHz. Double quantum filtered J-correlated spectroscopy (DQF-COSY), gradient ^1H-^{13}C hetero-nuclear multiquantum coherence spectroscopy (^1H-^{13}C HMQC) and total correlated spectroscopy (TOCSY) of the transglycosylation products were performed at 6°C using a total of 512 real data sets, with 32 acquisition transients each and a relaxation delay of 2 s between transients. The phase sensitive nuclear Overhauser effect spectroscopy (NOESY)

experiment of compound **A**-β was performed at 25°C using a total of 256 real data sets, with 32 acquisition transients each and a relaxation delay of 2s between transients. Mixing time of 400 ms was used in the NOESY experiment. Volume integrations of crosspeaks were performed using the standard VARIAN software.

Molecular Mechanics (MM) Calculations

MM calculations were performed on a Silicon Graphics 4D310 computer using the DREIDING force fields[21] implemented in the BIOGRAF (Molecular simulations Inc., Waltham, MA 02154) program. The default parameters given in this program for the carbohydrate rings were used without modification since they have been validated earlier.[22] The energy minimization were performed in vacuo.

Packing/Phase Behavior Characterization

Differential Scanning Calorimetry (DSC)

DSC measurements were made on a Microcal-2 ultrasensitive scanning calorimeter with a scanning rate of 60°C / hr. Approximately 1.5 ml 3.5 mM suspension of compound **A**-β in water was injected into the cell and 5 scans were recorded from 10°C to 98°C. A DSC scan for compound **B** was performed in the same fashion (8.76 mg of **B** was suspended in 2 ml water).

X-ray Powder Diffraction

The x-ray diffraction spectra were recorded on a one-dimensional scanning detector with a Rigaku rotating anode x-ray generator (CuK_α, 45 kV, 100 mA) at room temperature. Compound **B** and **A**-β (2-3 mg each) in dichloromethane-methanol solution (1:2/v:v) were deposited separately on microscope slides and air-dried. Their x-ray

diffraction patterns were obtained in the reflection mode (DS = 1/6°, SS = 1/6°, RS = 0.15 mm, RS_m= 0.45 mm; scan rate = 0.4°/min; 2θ = 0.75°-45°).

Activation of Model Systems

Enzymatic Activation

β-Glucosidase (EC 3.2.1.21) extracted from almonds were purchased from Sigma in lyophilized powder form (chromatographically purified, essentially salt-free). 1.36 mg of the enzyme (40.8 units) was dissolved in 81.6 µl of double distilled (d.d.) deionized water to make a 0.5 unit/µl solution and stored at 4°C. 1 M Sodium acetate buffer (pH 5.0, 10× buffer) was prepared according to Sigma's protocol.

To a suspension of 17.64 mg OG (or 11.69 mg MG) in 32 µl of d.d. water was added 4 µl of enzyme solution (2 units) and 4 µl of 10× buffer to give a 1.5 M reaction mixture. Reaction mixtures at 1 M, 2 M, 3M, 5 M, 7 M and 10 M concentration were prepared in the same way. Reaction mixture of model compounds **B** and **A-β** at 0.2 M were prepared in the same manner but into a total 200 µl volume. The Ampendorf tubes containing these reaction mixtures were vortexed several times before sent into a 37°C water bath for incubation. Six identical reaction mixtures were made for each sample at a specific concentration level for taking different time points to trace the reactions. At 30 min, 1 hr, 4 hrs, 12 hrs, 24 hrs and 48 hrs, one of each different reaction mixtures were taken out of the 37°C incubation bath, lyophilized, redissolved in 1 ml water, desalted by mixed ion resin, passed through a C18 (octadecylsilyl silica-bound (Waters-Millipore)) reverse phase cartridge and lyophilized again. The product was then dissolved in 0.5 ml methanol with 5 mg of sodium borohydride to reduce the aldoses. Reaction stood at room

temperature for 3 hrs. before the excess borohydride was destroyed by adding a couple of drops of acetic acid. Methanol (1 ml) was then added and removed under nitrogen at 45°C. Four other 1 ml volumes of methanol were added and removed in the same fashion. Such reduced alditol mixtures were then dissolved in equal amount of D_2O with mixed-ion exchange resin to remove final trace of salts before NMR experiments were performed.

For calibration purpose, 25 µl of 1.35 mM sodium formate solution (in D_2O) were added to each NMR tube containing same volume of alditol mixtures and sonicated.

Chemical Activation

Compound **D** (40 mg) was dissolved in 4 ml acetonitrile with 2 eq. NBS, NIS and in-situ prepared mercury trifluoroacetate, respectively. Reactions were allowed to stand at room temperature for 24 hours and traced by TLC at 1hr, 2 hrs, 4 hrs, 12 hrs and 24hrs.

Mercury trifluoroacetate were prepared from mercury oxide in trifluoroacetic acid solution at room temperature overnight. After removal of excess trifluoroacetic acid, the obtained mercury trifluoroacetate was dissolved in acetonitrile for immediate use.

Methylation Analysis of Transglycosylation Product Mixtures

Transglycosylation product mixture (~ 0.5 mg) was dissolved in dry dimethyl sulfoxide (50 µl), and 30 µL of a 4M solution of sodium dimesylate in dry dimethyl sulfoxide was added. The mixture was stirred at room temperature for 8 hours with sonication and vortexing every hour. Sodium iodide (30 µl) was then added and the mixture kept at room temperature for 2 hours with sonication and vortexing every 15 minutes. The excess methyl iodide was removed under a stream of nitrogen and the

reaction mixture diluted with 5 ml of water. The aqueous solutions were passed through a C18 (octadecylsilyl silica-bound (Waters-Millipore)) reverse phase cartridge. The adsorbed material was released by eluting with methanol (5 ml) and then with a 4:1 mixture of methanol and chloroform (5 ml). The two organic fractions were concentrated to dryness under a stream of nitrogen. The methylated products thus obtained were then hydrolyzed to yield free sugars by treating with 75% acetic acid in water at 100°C for 1.5 hours. The acetic acid and water were removed under a stream of nitrogen and the hydrolysate was taken up in methanol (100 µl) to which water (20 µl) was added. Sodium borohydride (~ 5mg) was then added to reduce the free aldoses to alditols. Reduction was conducted at room temperature for 2 hours and then the excess borohydride was destroyed by adding acetic acid (20 µl). Methanol (1 ml) was then added and removed under nitrogen. Four other 1 ml volumes of methanol were added and removed. The partially methylated alditols so formed were peracetylated by treatment with pyridine (60 µl) and acetic anhydride (40 µl) with dichloromethane (60 µl) as solvent. The mixture was allowed to stand at room temperature overnight. Chloroform (1 ml) was then added and the solution was freed of a white precipitate of sodium acetate by filtration through a plug of cotton that was pre-washed with chloroform. It was then concentrated to dryness under a stream of nitrogen with a bath temperature not greater than 35°C. The residue was taken up in chloroform, freed of a further precipitate of sodium acetate in the manner previously described, concentrated to dryness and subjected to GC-MS analysis.

GC-MS Analysis of Methylation Products

GC-MS analysis was performed on a JEOL JMS-AX505H mass spectrometer and a Hewlett Packard 5890J GC on both methanol and chloroform fractions of OG and MG methylation products, using a 30 m DB-5MS column (0.32 mm ID, 0.25 μm film). 100 mA ionization current and 3 KV acceleration voltage was applied. GC started at 120°C, held for 10 min; then temperature was gradually increased to 220°C at a rate of 3°C/min, and held for 10 min. M/Z was scanned from 44 to 400. Stack ion chromatograms were plotted.

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Chapter 4

Development of Concise and General Routes towards Optically Pure Five- and Six-carbon β-Hydroxy Acids, γ-Hydroxy Acids, and γ-Amino Acids from Naturally Occurring Carbohydrates

Abstract

General and concise routes towards chiral alkenyl-containing β-hydroxy acids and γ-hydroxy acids from naturally occurring carbohydrates were developed. The first part of this chapter (§ 4.1) describes a convenient approach to the synthesis of chiral β-hydroxy acid using 2-deoxyribose as chiral synthon. Two important pharmaceutical intermediates (*S*)-β-hydroxy-4-pentenoic acid and (*S*)-4-pentene-1,3-diol were synthesized in high yield and e.e. via an efficient 3-step sequence. The second part of this chapter (§ 4.2) describes an approach to both enantiomers of chiral 4-hydroxy-5-hexenoic acids using inexpensive δ-gluconolactone and *L*-mannonic-γ-lactone as chiral synthons. Such γ-hydroxy acid intermediates are logical precursors of many important chiral γ-amino acids. As a demonstration of one of its many potential applications, the increasingly important pharmaceutical target Vigabatrin® (γ-vinyl-γ-amino-butyric acid) was synthesized in a short synthetic route from its chiral γ-hydroxy acid precursor. The highly functionalizable alkenyl group in these chiral building blocks brings extra handles for these molecules to be attached to more complex molecular architectures and therefore should have valuable applications in various natural compound syntheses.

§ 4.1 A Concise Route towards (*S*)-β-Hydroxy-4-pentenoic acid and (*S*)-4-Pentene-1,3-diol, Important Chiral Intermediates of Diverse Pharmaceuticals

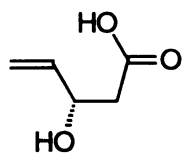
Abstract

Important pharmaceutical intermediates (*S*)-β-hydroxy-4-pentenoic acid and (*S*)-4-pentene-1,3-diol were synthesized in high yield and e.e. via an efficient and concise route from 2-deoxyribose. This synthesis provides a general route towards chiral β-hydroxy acids.

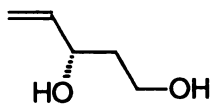
Introduction

(*S*)-3-Hydroxy-4-pentenoic acid **1a** and (*S*)-4-pentene-1,3-diol **2** and their chiral counterparts are important building blocks of many natural compounds and pharmaceutical targets. Optically pure 3-hydroxy-4-pentenoic acids have been used in the total syntheses of (-)-Slaframine[1], the lactone moiety of Mevinic acids[2], some polyether antibiotics[3] and Spirovetivane Phytoalexin (±)-Lubiminol[4]. They are also excellent acyclic substrates for studying stereoselective iodocyclizations.[5-8] Another important application is the diastereoselective *anti* alkylation at the α-position of the chiral 3-hydroxy esters using Seebach's procedure[9]. Such anti-addition provides *anti*-3-hydroxy-2-alkyl esters, another extremely important class of natural product fragments. They have been widely used, for instance, in the syntheses of (+)-Cassiol[10] (a potent antiulcerogenic compound) and a variety of beetle pheromones[11-13]. The *anti* relationship could also be altered to *syn* by tautomerization and therefore the usage could be further broadened, for instance, into the study of Pentalene Synthase biosynthetic pathway, where the *syn*-isomer is a key intermediate. 3-Hydroxy acids are also logical precursors of various β-amino acids and β-lactams, which are very important in the design and synthesis of a variety of enzyme inhibitors and β-peptides. Finally, (*S*)-3-Hydroxy-4-pentenoic acid **1a**

was recently found to be an effective mitochondria glutathione depletion agent to potentiate oxidative cell death. It has found use in the study of mitochondria glutathione homeostasis and the role of mitochondria glutathione in cellular protection.[14-17] One unique application of chiral 4-pentene-1,3-diol **2** is its potential as a chiral auxiliary. It is well known that chiral 1,3-diols serve as excellent chiral auxiliaries by forming 6-membered complex with their substrates.[18-21] They are usually very expensive. In this case, the vinyl group would serve as one of the steric differentiating factors that dictate the attack of incoming reagent from only one side. And the π-electrons of the vinyl group may even increase the facial differentiation by enforcing the chelation to Lewis acids together with the two ring oxygens, for instance, in the case of metal hydride reduction.



1a



2

Due to their high synthetic value, many procedures have been developed towards the synthesis of these two compounds. The most widely used method is the addition of lithium enolate of ethyl acetate to acrolein at -78°C to produce the racemic 3-hydroxy-4-pentenoic acid ethyl ester in high yields.[4,22-25] However, an efficient and simple synthesis of optically pure 3-hydroxy-4-pentenoic acid has not been achieved. For a long time, the chiral version of this molecule had been obtained through traditional resolution of racemic compounds by diastereomer crystallization using chiral resolving agents.[26,27] This not only requires extra steps to recover the desired isomer, but sometimes could lead to the waste of half of the product due to the decomposition of the other isomer during

the process. The first real asymmetric synthesis of 3-hydroxy-4-pentenoic acid was not reported until 1993.[28] The (*R*)-isomer was obtained by an aldol addition of doubly deprotonated (*R*)-2-hydroxy-1,2,2-triphenylethyl acetate to acrolein with a reported enantiomeric excess of 83%. This method requires the use of a chiral reagent which is quite heavy in molecule weight. This poses productivity and cost problems especially when the synthesis needs to be scaled up. Also, to enhance its moderate e.e., resolution by diastereomer crystallization using chiral resolving agents had to be used, which required two more steps.

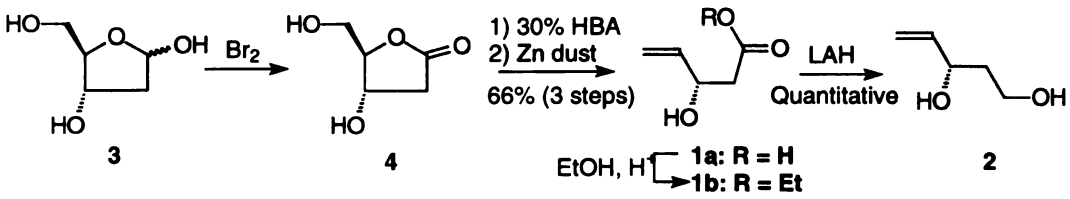
In the case of chiral 4-pentene-1,3-diol, the only reported method in a long time was to use glucose as chiral auxiliary to carry out a stereoselective water-promoted Claisen rearrangement of either α- or β-glycosylated diene.[29,30] Apart from the inconvenience of carrying out glycosylation to put on the substrate and the subsequent hydrolysis to release the product from the chiral template, this method suffered most from its low enantiomeric excess. In both cases, a 3:2 ratio of both isomers were formed, which by no means could be regarded as a satisfactory result. Recently, a chemoenzymatic synthesis of pure 4-pentene-1,3-diol derivatives was reported.[31] The racemic diol had to be acylated before it was subjected to *Pseudomonas* lipase's selective hydrolysis of one enantiomer to realize the separation.

The aforementioned endeavors of obtaining the two chiral compounds involve either asymmetric synthesis using chiral reagents[28], chiral auxiliaries[29,30] or traditional resolution of racemic products by diastereomer crystallization using chiral resolving agents[26-28]. They all contributed towards the development of chiral synthesis of these two important pharmaceutical intermediates. However, due to their individual disadvantages,

a simpler and more efficient route is still highly desired. Here, we report a very concise route towards both title compounds **1a** and **2** utilizing carbohydrate as the chiral source. An unoptimized 66% overall yield for this 3-step reaction sequence was obtained, with very high (>99%) enantiomeric excess.

Design and Synthesis

We chose 2-deoxyribose as the starting material for a couple of reasons. First, as a pentose sugar, the transformation from 2-deoxyribose to the 5-carbon title compounds **1a** and **2** does not have the common problem of wasting carbon sources that a lot of asymmetric syntheses using chiral auxiliaries suffer. Secondly, the correct C-3 stereochemistry would remain intact throughout the reaction sequence and therefore ensure the (*S*)-configuration be inherited by the final product, which guarantees a high enantiomeric purity.



Scheme 1. Synthesis of Compound **1a** and **2**.

The reactions are summarized in Scheme 1. 2-Deoxyribose was first quantitatively oxidized to lactone by bromine at 0°C overnight using standard procedure.[30] It was then subjected to 30% hydrogen bromide in acetic acid (HBA) at room temperature to open the lactone ring to give the 5-bromo-5-deoxy-3,4-diacetyl

pentanoic acid, which upon treatment with Zn dust and acetic acid[32] gave the 4,5-unsaturated product **1a** with the 3-hydroxyl group partially acetylated. The subsequent basic workup homogenized the C-3 position and gave either **1a** or **1b** according to different workup procedures. Reduction of ester **1b** by lithium aluminum hydride (LAH) at room temperature gave diol **2** quantitatively. This reaction sequence involved only three steps using very mild reaction conditions and no protecting and deprotecting manipulation associated with most carbohydrate syntheses was involved. It provided an overall yield of 66%. And up to compounds **1a** and **1b**, all reactions were carried out in one pot. Chiral GC analysis of diol **2** revealed a >99% enantiomeric excess. There is no expensive or exotic reagent used in this reaction sequence. The only relative costy part is the starting material. However, with various well-documented methods to make 2-deoxy ribose from inexpensive ribose availabe[33], this should not be a big issue anymore. Indeed, the price of commercial 2-deoxyribose has kept dropping over the years. Some companies like Czech Moravian & Slovak Chemicals in England are now selling it in very competitive prices for large-scale orders. Finally, using the same method, one should be able to make the enantiomers of **1a** and **2** from xylose.

Conclusion

In conclusion, a concise and efficient route has been developed towards two common chiral intermediates of diverse pharmaceuticals. It gives the highest e.e. reported so far and has the great potential to be applied into large-scale synthesis due to the short reaction sequence, mild reaction conditions used and the continuing dropping price of the starting material 2-deoxy sugars.

Experimental

General Techniques

All reagents used were reagent grade. Reaction temperatures were measured externally. Flash chromatography was performed on Aldrich silica gel (60 Å 200-400 mesh). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

NMR spectra were recorded on a Varian 300 MHz VXR spectrometer at ambient temperature. Chemical shifts are reported relative to the residue solvent peak. Optical rotations were measured at 20°C using a Perkin polarimeter at 589 nm . High-resolution mass spectra (HRMS) were recorded on a JEOL HX-110-HF spectrometer using Fast Atom Bombardment (FAB) conditions and an N-benzyl alcohol (NBA) matrix. Chiral gas chromatography (GC) were performed on a Betadex cyclodextrin phase column (Supelco, Bellefonte, PA).

Synthesis

2-Deoxy-D-ribonolactone 3.

25 g of 2-deoxy-D-ribose **1** was stirred with 29 ml of bromine in 1.5 liters of water at 0°C in darkness for 16 hours. The product was concentrated under vacuum with mild heating to give light yellow syrup. NMR analysis indicated a clean conversion to 2-deoxyribonolactone. ^{1}H NMR (300 MHz, D_2O): δ 4.34 (2H, m), 3.66 (1H, dd, J=12.9, 3 Hz), 3.55 (1H, dd, J=12.9, 4.2 Hz), 2.84 (1H, dd, J=18.6, 6.9 Hz), 2.36 (1H, dd, J=18.6, 3.0 Hz); ^{13}C NMR (75 MHz, D_2O): δ 174.72, 84.14, 63.49, 56.19, 32.94; FAB-HRMS (Gly): calcd $C_5H_9O_4$ $[M+H]^+$, 133.0457, found 133.0501.

(*S*)-3-Hydroxy-4-pentenoic acid (1a) and the ethyl ester 1b.

1 g of lactone **3** was stirred with 5 ml of 30% HBr in acetic acid (HBA) at room temperature overnight. Residue HBr and acetic acid was evaporated and the resulting syrup was dissolved in 10 ml of 50% acetic acid in water. 3 g of zinc dust was added in portions and stirred at room temperature for 3 hours. The mixture was then filtered and the filtrate was concentrated. To remove the zinc salt and deacetylate the 3-OH position, the residue was dissolved in water and the pH was brought up to 10 by KOH. White zinc hydroxide precipitate was removed and the filtrate was concentrated again. In ethanol, the residue was acidified by concentrated HCl to around 3 where the 3-hydroxy carboxylate get fully protonated and became soluble in the solution. And the KCl salt was removed by filtration. Removal of ethanol gave 0.7 g of crude compound **1a**. ^{1}H NMR (300 MHz, D_2O): δ 5.72 (1H, m), 5.09 (1H, m), 4.98 (1H, m), 4.31 (1H, m), 2.33 (2H, m); ^{13}C NMR (75 MHz, D_2O): δ 175.20, 134.16, 110.90, 65.05, 38.51; FAB-HRMS (Gly): calcd $C_5H_9O_3$ $[M+H]^+$, 117.0508, found 117.0552.

The reaction was scaled up starting with 20 g of lactone **3**. In the final workup, the corresponding ethyl ester **1b** was made by stirring with ethanol under acidic condition at elevated temperature. And the product was purified by flash column (chloroform as eluant) with an overall yield of 66% for 3 steps. $[\alpha]_D$ -5° (C 0.98, $CHCl_3$); ^{1}H NMR (300 MHz, D_2O): δ 5.85 (1H, m), 5.27 (1H, m), 5.11 (1H, m), 4.50 (1H, m), 4.14 (2H, q, J=7.2 Hz), 2.52 (2H, m), 1.23 (3H, t, J=7.2 Hz); ^{13}C NMR (75 MHz, D_2O): δ 172.22, 138.76, 115.33, 68.89, 60.74, 41.11, 14.11 (both ^{1}H and ^{13}C NMR data agree with literature data[4,22]); FAB-HRMS (NBA): calcd $C_7H_{13}O_3$ $[M+H]^+$, 145.0865, found 145.0849.

(*S*)-4-Pentene-1,3-diol 2.

0.5 g ester **1b** was dissolved in 15 ml of THF and stirred with 0.17 g of LAH at room temperature for a few hours. The reaction was then quenched and dumped into acidic ice-water and extracted by ethyl acetate. Removal of solvent yielded 1,3-diol **2** as colorless liquid quantitatively. $[\alpha]_D$ +11° (C 1, MeOH) [lit.[34] $[\alpha]_D$ +11° (C 1, MeOH)]; ^1H NMR (300 MHz, $CDCl_3$): δ 5.87 (1H, m), 5.24 (1H, m), 5.09 (1H, m), 4.34 (1H, m), 3.80 (2H, m), 3.04 (2H, b), 1.74 (2H, m) (^1H NMR data agree with literature data[35]); ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.54, 114.60, 72.47, 60.84, 38.05; e.e. >99% by chiral GC; FAB-HRMS (Gly): calcd $C_5H_{11}O_2$ $[M+H]^+$, 103.0759, found 103.0741.

Acknowledgements

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§ 4.2 Chiral 4-Hydroxy-5-hexenoic Acids: An Easy Access from Carbohydrate Chiral Pool and an Application in the Synthesis of Optically Pure Vigabatrin ®

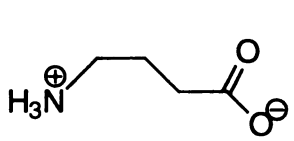
Abstract

The increasingly important pharmaceutical target Vigabatrin was successfully synthesized in a protected form via a short synthetic route using inexpensive gluconolactone as chiral synthon. This synthetic pathway also provides an efficient general route towards other chiral γ-hydroxy acids or γ-amino acids from carbohydrates.

Introduction

γ-Aminobutyric acid (GABA) **1** is an important neurotransmitter in mammalian systems. When the concentration of GABA in the brain decreases below a threshold level, seizures and a variety of other neurological disorders occur[1]. GABA concentrations are mainly regulated by two pyridoxyl 5'-phosphate dependent enzymes *L*-glutamic acid decarboxylase (GAD), which catalyzes the conversion of *L*-glutamate to GABA, and GABA-aminotransferase (GABA-T), which degrades GABA to succinic semialdehyde[2]. Peripheral administration of synthetic GABA as an anticonvulsant agent is limited by GABA's inability to effectively cross the blood-brain-barrier (BBB) presumably due to its low lipophilicity[3]. An attractive alternative is to use more lipophilic GABA derivatives or mimics that can cross the BBB and selectively bind to GABA-T to irreversibly inactivate the degradation of GABA by GABA-T. 4-Amino-5-hexenoic acid (γ-vinyl GABA or Vigabatrin) **2** was found to be such a highly selective irreversible inhibitor of GABA-T[4]. Marketed in Europe to treat epilepsy, Vigabatrin has also been found to be useful or potentially useful for treating other disorders associated with depletion of GABA levels in the central nervous system (CNS) such as Parkinson's disease[5], Huntington's disease[6], tardive dyskinesia, schizophrenia[7] and West's syndrome[8]. Most

recently, vigabatrin was found to be very promising in treating cocaine[9] and nicotine[10] addictions. Like many psychostimulant drugs, cocaine and nicotine elevate extracellular and synaptic dopamine (DA) concentrations in the nucleus accumbens. Their addictive liability has been linked to their pharmacological actions on DA reinforcement/reward pathways in the CNS. Dopaminergic transmission within these pathways is modulated by GABA[9]. Vigabatrin cancels nicotine and cocaine's DA-producing effect by curbing breakdown of GABA. It is striking that the doses of vigabatrin needed to block nicotine are one-quarter those for cocaine and about one-tenth to one-twentieth the dose used to treat epilepsy[9,10]. Researchers are now investigating whether vigabatrin could potentially treat addictions to amphetamine, methamphetamine, alcohol, heroin and mophine[11].



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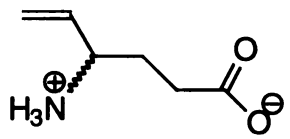


2

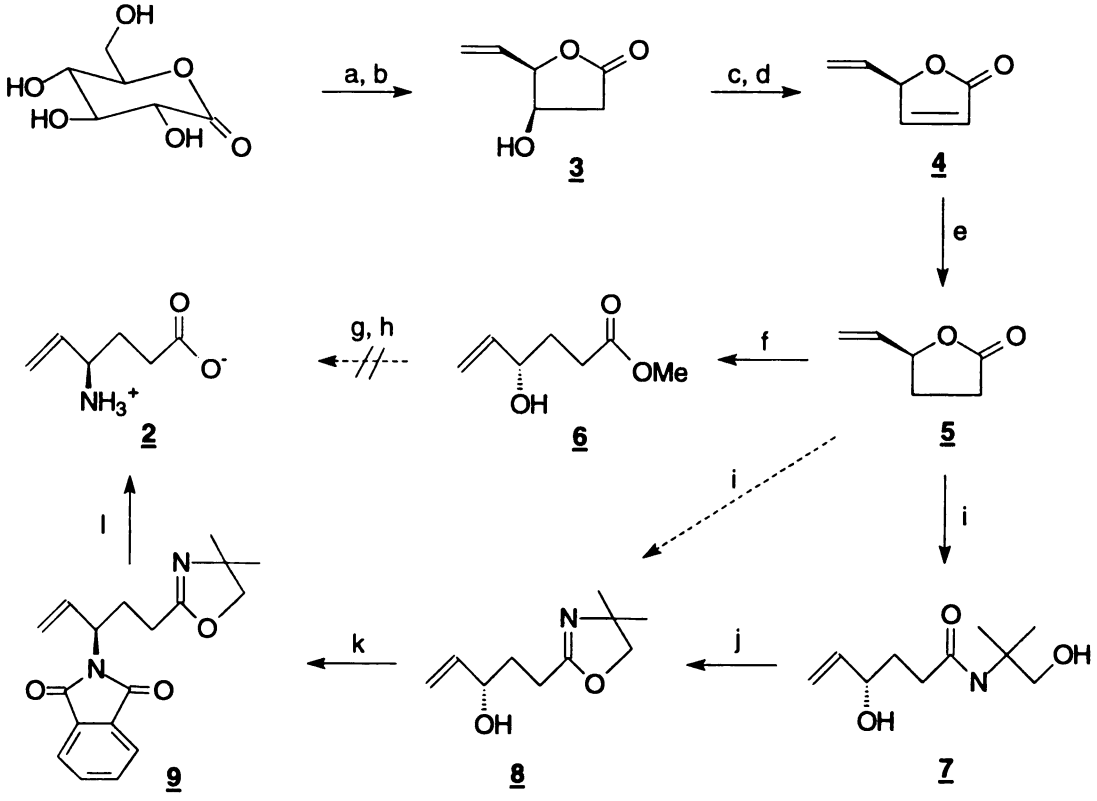
Because of vigabatrin's extreme importance, the development of effective synthesis of this drug has been a constant pursuit by researchers form both academia and industry. A variety of syntheses of racemic vigabatrin were reported over the years. They were based on either a Michael addition of propargylic anions followed by catalytic hydrogenation[12], or biscondensation of malonate anion on 1,4-dichloro-2-butene and aminolysis[13]. More recently, a couple of syntheses based on allylic amination via silicon induced [2,3]sigmatropic rearrangement[14] or thermal rearrangements (an Overman rearrangement to introduce the allylamine function and a Claisen rearrangement to introduce the carboxylic function)[15] were reported. Synthesis of optically pure vigabatrin was realized by either enzyme catalyzed resolution of racemic compounds[16], asymmetric

synthesis (e.g. Sharpless asymmetric epoxidation, aminohydroxylation, etc.) using transition metal catalysts[17], or using natural α-amino acids or their derivatives as chiral pool[18]. Some of these methods involve lengthy synthetic procedures and/or suffer from mild enantiomeric excess. Another common problem for these procedures[17b, 17c, 18b-d] is that they all relied on Wittig reaction to introduce the vinyl group and involved various oxidation procedures, which are undesirable in industrial scale-ups. Finally, those transition metal catalyzed processes[17] pose not only the cost issue, but more importantly the environmental issue as well. Therefore, a cost-efficient, environmental benign and concise synthesis towards optically pure vigabatrin is still highly desirable.

Design and Synthesis

We now report a synthesis of (*R*)-vigabatrin **2** using easily accessible and inexpensive δ-gluconolactone as chiral synthon. We used Zn-induced elimination instead of Wittig reaction to introduce the vinyl group. An important chiral intermediate (*S*)-4-hydroxy-5-hexenoic acid was generated in only a few steps. An amino group was then introduced to the γ-position to give the protected form of (*R*)-vigabatrin. A whole spectrum of functionalities could be added to the key intermediate to give other chiral γ-hydroxy acids and amino acids. Another advantage of choosing carbohydrates as chiral synthon, apart from its cost and environmental advantages, is the richness of the available choice of substrates with all sorts of desired stereochemistry. One should be able to expand this chemistry to generate the opposite isomers of desired γ-hydroxy acids and amino acids by using carbohydrates or lactones with opposite stereochemistry to glucose

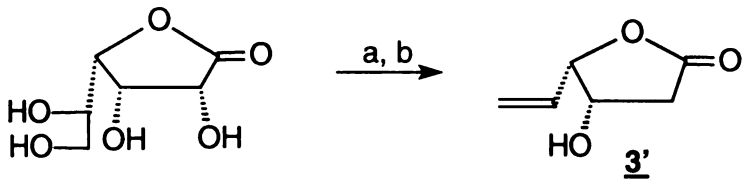
Scheme 1. Synthesis of (*R*)-Vigabatrin **2.** *Reagents, conditions and yields:* (a) 30% HBr in HOAc, 60°C for 1 hr and r.t. overnight; (b) Zn dust, 50% HOAc in water, r.t. for 2 hrs and reflux for 1 hr, 58% (2 steps); (c) 1.2 eq. MsCl, 1.1 eq. Et_3N, CH_2Cl_2, -40°C, 2 hrs; (d) 0.8 eq. Et_3N, CH_2Cl_2, -78°C, 30 min -1 hr, 70% (2 steps, based on converted 3-mesylated intermediate); (e) 2 eq. CuI, 5 eq. LiCl, 4 eq. TMSCl, 3.6 eq. $n$$Bu_3SnH$, anhydrous THF, -70°C, 30-45 min, 80%; (f) 1.1 eq. NaOMe, MeOH, 0°C, 3 hrs, quantitative; (g) 1.2 eq. PPh_3, 1.2 eq. DEAD, 2.5 eq. NaN_3, DMF, 110°C, 8 hrs, 94% (intramolecular cyclization product); (h) $SnCl_2$ reduction of azide (not performed); (i) 2 eq. $H_2N(CH_3)_2CCH_2OH$, toluene, reflux, 12 hrs, 96% (based on 72% conversion); or 10 eq. $H_2N(CH_3)_2CCH_2OH$, no solvent, 120°C, 5hrs, quantitative; (j) 1.2 eq. PPh_3, 1.2 eq. DEAD, 1.3eq. phthalimide (not necessary), THF, r.t. 12 hrs, 80%; (k) 2 eq. PPh_3, 2 eq. DEAD, 1.5eq. phthalimide (not necessary), THF, r.t. 12 hrs, 71%; (l) 6N HCl, 100°C. MsCl = methanesulfonyl chloride; TMSCl = chlorotrimethylsilane; DEAD = diethyl azodicarboxylate.


OH
HO
HO
OH
O
a, b
HO
3
c, d
4
e
5
f
OMe
OH
6
g, h
NH3+
2
i
i
7
j
8
k
9
l

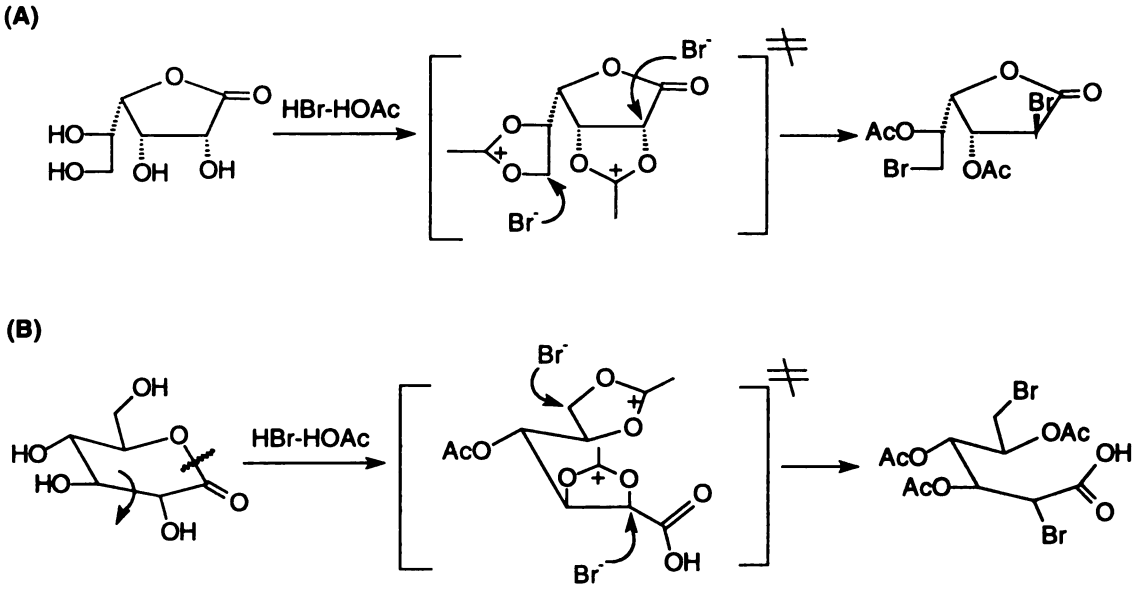
at the C-4 position. Indeed, we demonstrated the possibility of using *L*-mannonic-γ-lactone as starting material to get the other enantiomer.

The synthesis is summarized in Scheme 1. Inexpensive starting material δ-gluconolactone was subjected to 30% hydrogen bromide in acetic acid (HBA) followed by Zn-dust to open the lactone ring and introduce the 5,6-vinyl function via Zn-facilitated elimination[19]. This reaction took advantage of the high reactivity of the primary C-6 hydroxy and C-α hydroxy groups to regioselectively bring in bromo groups without the usual protection-deprotection manipulations associated with carbohydrate chemistry. An effective 2-debromination by Zinc occurred subsequently, presumably via an enol intermediate. This one-pot process quickly led to the first key intermediate (3*R*, 4*R*)-3-hydroxy-4-vinyl-4-butyric lactone **3** in 58% yield. When *L*-mannonic-γ-lactone was used instead of δ-gluconolactone, the enantiomer (3*S*, 4*S*)-3-hydroxy-4-vinyl-4-butyric lactone **3'** was obtained in comparable yield (Scheme 2). Clean ^{1}H NMR spectrum indicated that there was no scrambling of stereochemistry during this process.

Despite the similar reaction outcome, the dibromination of δ-gluconolactone and *L*-mannonic-γ-lactone may go through slightly different mechanisms. It was well known that in strongly acidic medium, bromination of aldonolactones happens at either the α-position or the primary position, or at both positions. Lactones having the hydroxy groups *cis* within the lactone ring tend to form an acetoxonium ion, while *trans* oriented hydroxy groups do not[20]. Opening of a 2,3-acetoxonium ion has been found to take place exclusively at C-2 with inversion of the stereochemistry. This probably is the case for the dibromination of *L*-mannonic-γ-lactone (Scheme 3A). In the case of δ-gluconolactone, despite the *trans* relation of the 2,3-hydroxy groups, the dibromination was still observed



Scheme 2. Synthesis of compound **3'** from *L*-mannonic-γ-lactone. ***Reagents, conditions and yields:*** (a) 30% HBr in HOAc, 60°C for 1 hr and r.t. overnight; (b) Zn dust, 50% HOAc in water, r.t. for 2 hrs and reflux for 1 hr, 50% (2 steps).

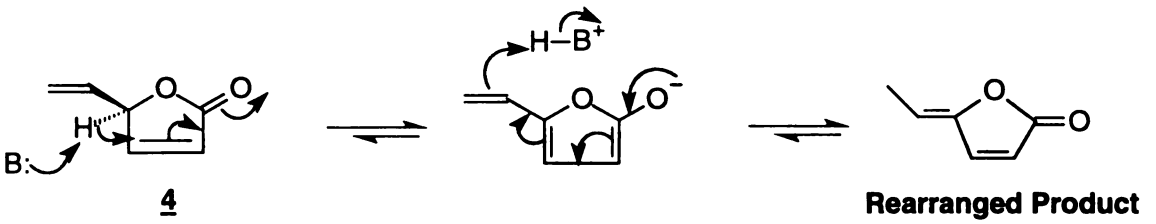


Scheme 3. Proposed mechanism for dibromination of (A) *L*-mannonic-γ-lactone and (B) δ-gluconolactone.

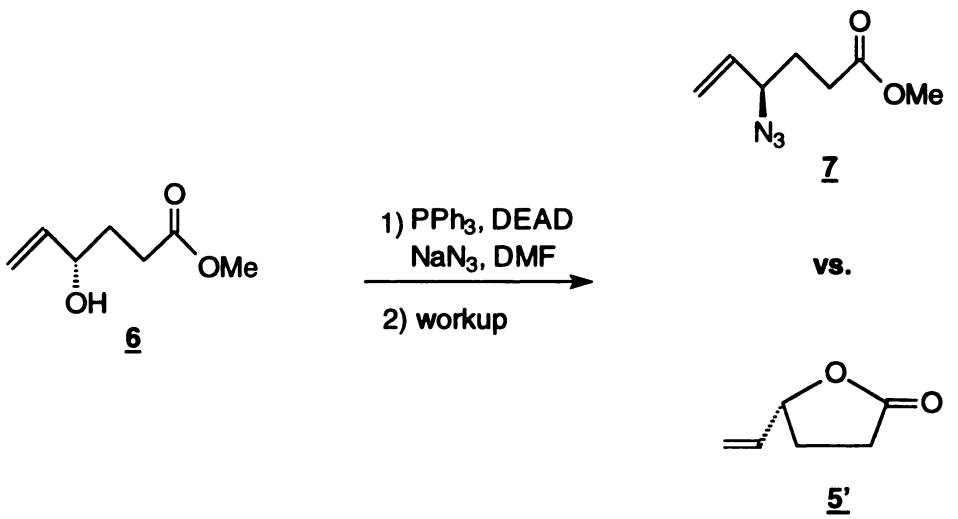
under prolonged reaction time as opposed to the usually employed 3-4 hours[19]. This can probably be explained by the ring opening of the 6-membered lactone ring and the consequent free rotation of C_2-C_3 bond in the resulting acyclic structure, which allows the formation of acetoxonium ion and the subsequent 2-bromination (Scheme 3B). However, it should be pointed out that there are many other possible scenarios and competing processes in such reactions and the overall reaction outcome is quite sensitive to specific neighboring group environment and ring size of the substrates. One should be very careful to draw any general conclusions from just a few examples. Indeed, we have found that the 2-bromination of the γ-galactonolactone under similar condition did not proceed with significant yield.

After the elimination of 3-hydroxy group, a 1,4-reduction[21] of the resulting α,β-unsaturated lactone **4** and the ring opening of lactone **5** gave the second key intermediate (*S*)-4-Hydroxy-5-hexenoic acid methylester **6** in satisfactory yields. Due to the acidity of the doubly allylic C-4 proton of lactone **4**, a rearrangement of compound **4** tended to occur under basic conditions to give an achiral conjugated by-product (Scheme 4), if cautions were not applied during the elimination. This problem could be alleviated by careful control of the amount of base used in the elimination of the 3-mesylated intermediate. Indeed, the occurring of the rearrangement was significantly reduced at the cost of an incomplete conversion of the 3-mesylated intermediate to lactone **4**. The unconverted 3-mesylate was recovered and used in a second round reaction.

Mitsunobu reaction[22] on the methyl ester **6** to effect an allylic substitution by azide was complicated by a competition between intramolecular cyclization assisted by the carbonyl oxygen and the desired intermolecular S_N2 substitution (Scheme 5). The

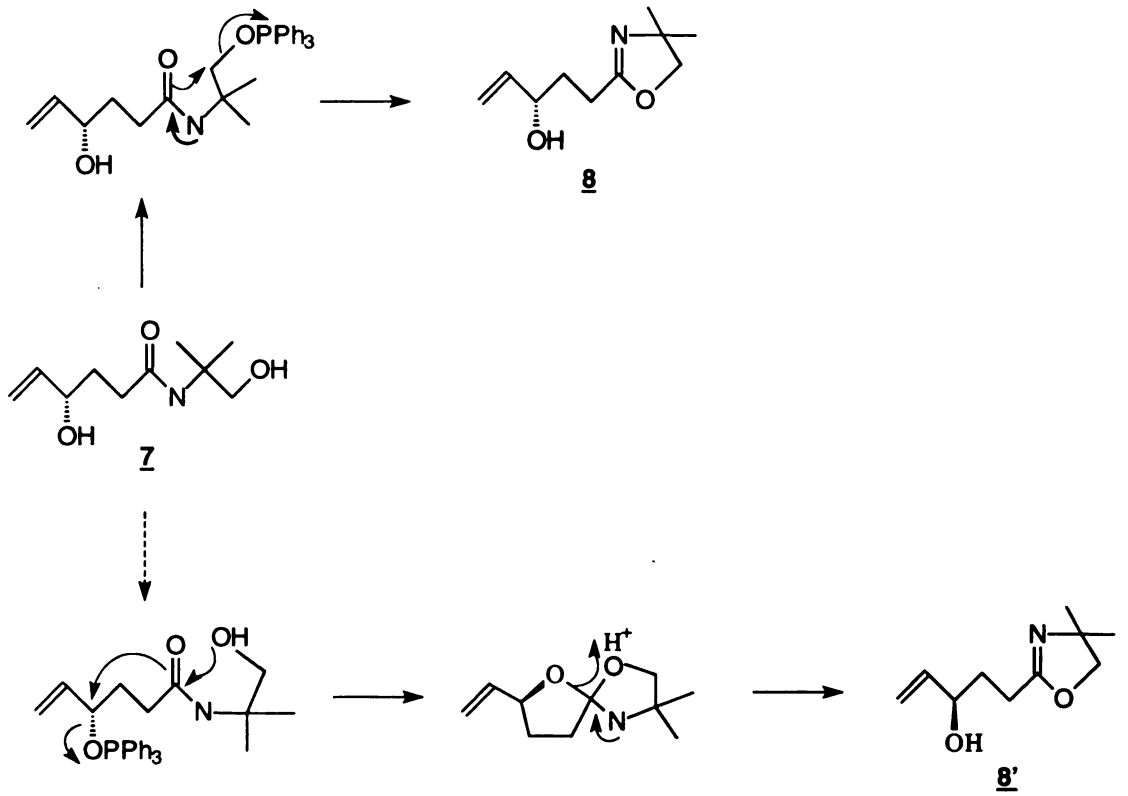


Scheme 4. The rearrangement and loss of chirality of compound **4** under basic conditions.



Scheme 5. A competition between intramolecular cyclization assisted by carbonyl oxygen (to give product **7**) and intermolecular nucleophilic substitution by azide (to give product **5'**).

intramolecular cyclization was strongly favored in this case with the cyclized 5-membered lactone **5'**, the enantiomer of lactone **5**, as the exclusive product. No azide derivative via either S_N2' or intermolecular S_N2 pathway was isolated. In order to avoid the intramolecular cyclization, the carbonyl group has to be masked by a non-participating functionality. Oxazoline is an ideal choice for this purpose. Lactone **5** was refluxed with 2-amino-2-methyl-1-propanol in toluene. Surprisingly, instead of getting the oxazoline **8** by this standard procedure[25], amide **7** was obtained. Even heating neat amide **7** at 160°C did not effect the formation of the oxazoline ring. The dehydration of **7** apparently requires more stringent conditions. However, under Mitsunobu condition amide **7** cyclized to give oxazoline **8**, which then proceed to react with nitrogen-containing nucleophile phthalimide to give allylic amino product. This is because the primary hydroxy group in amide **7** is more reactive than the allylic hydroxy group towards bulky activating reagent triphenylphosphine (Scheme 6). With the activated leaving group at the primary position and the participation of the amide carbonyl oxygen, intramolecular cyclization happened to give oxazoline **8** when only one equivalent of Mitsunobu reagents was employed. If the allylic hydroxyl competed in this process, one would expect the formation of enantiomeric oxazoline **8'**, which could lead to racemization of the product. The optical rotation value of the product clear indicates that the primary hydroxyl group was more reactive and the product was predominantly the *S*-isomer **8**. When a second equivalent of Mitsunobu reagents and the nucleophile phthalimide were introduced, the protected form γ-amino acid **9** was obtained in satisfactory yield. Deprotection under acidic condition[25b,c] should be able to free the amino and carboxyl groups simultaneously to give the final target (*R*)-vigabatrin[17a].



Scheme 6. Intramolecular Mitsunobu reactions: primary hydroxy group vs. allylic hydroxy group.

With (3*S*, 4*S*)-3-hydroxy-4-vinyl-4-butyric lactone **3'** available, (*S*)-vigabatrin should be obtained in comparable yields since identical reactivities are expected for enantiomers.

Conclusion

In conclusion, an efficient synthesis of optically pure vigabatrin (in its protected form) was developed using inexpensive carbohydrates as the chiral synthon. This synthesis also provided an important chiral γ-hydroxy-5-hexenoic acid intermediate. This

synthetic strategy could be applied to the synthesis of isomers with the opposite stereochemistry and therefore serves as a quite general route towards vinyl-containing 6-carbon chiral γ-hydroxy acids and amino acids.

Experimental

General Techniques

All reagents used were reagent grade. Reaction temperatures were measured externally. Flash chromatography was performed on Aldrich silica gel (60 Å 200-400 mesh). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

NMR spectra were recorded on a Varian 300 MHz VXR spectrometer at ambient temperature. Chemical shifts are reported relative to the residue solvent peak. Optical rotations were measured at 20°C using a Perkin polarimeter at 589 nm . High-resolution mass spectra (HRMS) were recorded on a JEOL HX-110-HF spectrometer using Fast Atom Bombardment (FAB) conditions and an N-benzyl alcohol (NBA) matrix. Chiral gas chromatography (GC) was performed on a Betadex cyclodextrin phase column (Supelco, Bellefonte, PA).

Synthesis:

(3*R*, 4*R*)-3-Hydroxy-4-vinyl-4-butyric lactone 3.

25 g of δ-gluconolactone was stirred in 80 ml of 30% hydrogen bromide in acetic acid at 60°C for 1 hr and then at room temperature overnight. Excess of hydrogen bromide and acetic acid was removed under reduced pressure. The resulting syrup was

dissolved in 200 ml of 50% acetic acid in water. 50 g of zinc dust was added sequentially and stirred for 2 hrs before refluxing for an additional hour. After filtration, the filtrate was concentrated under reduced pressure. The resulting syrup was dissolved in 100 ml water and potassium hydroxide was added to precipitate the remaining zinc salt and homogenize the C-3 position by deacetylating 3-acetylated product. After filtration, the basic filtrate was acidified to pH 5 by concentrated hydrochloric acid. Water was evaporated under reduced pressure and the product was dissolved in ethanol and potassium chloride was filtered out. The product was purified by flash column chromatography using ethyl acetate / hexanes (1:1/v:v) as eluent. 10.5 g of pure compound **3** was obtained (58% overall yield for 2 steps). $[\alpha]_D$: +43° (C 1.15); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.93 (1H, m), 5.49 (2H, m), 4.87 (1H, m), 4.51 (1H, m), 2.77 (1H, dd, J = 17.7, 5.4 Hz), 2.58 (1H, dd, J = 17.7, 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.74, 130.12, 120.77, 84.65, 69.42, 38.60; FAB-HRMS (NBA): $[M+H]^+$ $C_6H_9O_3$ calcd 129.0552, found 129.0553.

(3*S*, 4*S*)-3-Hydroxy-4-vinyl-4-butyric lactone 3'.

25 g of *L*-mannonic-γ-lactone was subjected to the one-pot reaction sequence described above. After the flash column chromatography purification, 9.1 g of pure compound **3'** was obtained (50% overall yield). $[\alpha]_D$: -44° (C 1.56, $CHCl_3$); ^{1}H and ^{13}C NMR (300 MHz, $CDCl_3$): identical with those of compound **3**; FAB-HRMS (NBA): $[M+H]^+$ $C_6H_9O_3$ calcd 129.0552, found 129.0551.

(*R*)-4-Vinyl-2,3-butenyric lactone 4.

1.5 g Compound **3** was dissolved in 25 ml of dichloromethane and the solution was cooled to -40°C. 1.1 ml of methanesulfonyl chloride was added, followed by the

slow addition of 1.8 ml triethylamine (diluted with 10 ml of dichloromethane) over 30 min. After stirring for 2 hrs at -40°C, compound **3** was all converted to 3-mesylated compound judging by TLC. The reaction temperature was then reduced to -78°C and 1.3 ml of triethylamine (diluted with 5 ml of dichloromethane) was slowly introduced over 30 min. Reaction was traced by TLC and stopped when the fast-moving UV-active spot (rearrangement product) started to appear while part of the 3-mesylated compound still not converted to the elimination product. Reaction mixture was dumped into 40 ml of ice cold dilute hydrochloric acid solution and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate and then concentrated. Flash column chromatography (chloroform as eluent) gave 0.64 g compound **4** (50% yield) as pale yellow liquid. 0.7 g (29%) of unconverted 3-methylated product was also recovered.

(3*R*, 4*R*)-3-O-Mesyl-4-vinyl-4-butyric lactone: ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.88 (1H, m), 5.49 (2H, m), 5.36 (1H, m), 5.00 (1H, m), 3.01 (3H, s), 2.96 (1H, dd, J = 18.3, 5.7 Hz), 2.82 (1H, dd, J = 18.3, 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.75, 129.01, 121.49, 82.49, 76.80, 38.52, 36.68. **Rearrangement product**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (1H, d, J = 5.1 Hz), 6.09 (1H, d, J = 5.1 Hz), 5.32 (1H, q, J = 7.5 Hz), 1.92 (3H, d, J = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.04, 150.30, 143.51, 118.70, 112.28, 11.88. **Compound 4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (1H, dd, J = 5.7, 1.5 Hz), 6.12 (1H, dd, J = 5.7, 2.1 Hz), 5.70 (1H, m), 5.46 (1H, m), 5.41 (1H, m), 5.34 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.67, 154.69, 131.62, 121.45, 119.82, 83.63; HRMS-EI(+): M^+ $C_6H_6O_2$ calcd 110.0368, found 110.0352.

(*S*)-4-Vinyl-4-butyric lactone 5.

0.21g Lithium chloride and 0.38 g copper (I) iodide were dissolved in 10 ml anhydrous THF under nitrogen flow. After stirring for 20 min at room temperature, the mixture was cooled to -70°C. 0.11 g Compound **4** was then added, followed by 0.54 ml chlorotrimethylsilane. After stirring for 15 min, 1 ml of tributyltin hydride was slowly added over 5 min. The reaction mixture was then allowed to warm up to 0°C during the next hour. Reaction was quenched with 5 ml of 10% potassium fluoride solution and stirred for 30 min. The mixture was filtered through a celite pad and the filtrate was extracted with THF. The organic layer was concentrated and the residue was stirred with 10 ml of 10% potassium fluoride solution for 15 min. 10 ml Ether was then added and the stirring was continued for another 15 min. The mixture was passed through a celite pad and the filtrate was extracted with ether. Ether layer was washed with brine, dried over anhydrous sodium sulfate and then concentrated. Flash column chromatography gave 90 mg pure compound **5** as colorless liquid (80% yield). $[\alpha]_D$: +28° (C 1.48, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.85 (1H, m), 5.34 (1H, m), 5.23 (1H, m), 4.92 (1H, m), 2.51 (1H, m), 2.39 (1H, m), 1.98 (1H, m) [Lit. ^1H NMR (60 MHz)[24]: δ 4.78-6.20 (4H, m), 1.78-3.15 (4H, m); ^1H NMR (60 MHz, CD_3CN)[25]: δ 4.7-6.3 (4H, m), 1.6-2.7 (4H, m)]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.94, 135.49, 117.44, 80.48, 31.54, 28.24 [Lit. ^{13}C NMR (d^5-pyridine)[25]: δ 178.4, 137.8, 118.1, 81.9, 29.3 (2)]; HRMS-EI(+): M^+ $C_6H_8O_2$ calcd 112.0524, found 112.0541.

(*S*)-4-Hydroxy-5-hexenoic acid methylester 6.

50 mg of compound **5** was stirred with 26 mg of sodium methoxide in 5 ml of absolute methanol at 0°C for 3 hrs before it was quenched and neutralized by

concentrated hydrochloric acid. The reaction mixture was concentrated and the product was extracted by chloroform. Compound **6** was obtained quantitatively. $[\alpha]_D$: +1.6° (C 1.33, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.84 (1H, m), 5.24 (1H, m), 5.12 (1H, m), 4.16 (1H, m), 3.68 (3H, s), 2.43 (2H, t, J = 7.2 Hz), 1.85 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.35, 140.33, 115.13, 72.13, 51.68, 31.58, 29.93; FAB-HRMS (NBA): $[M+H]^+$ $C_7H_{13}O_3$ calcd 145.0865, found 145.0866.

(*R*)-4-Vinyl-4-butyric lactone 5'.

To a solution of 22 mg of compound **6**, 49 mg of triphenyl phosphine in 5 ml of dry DMF was added 25 mg of sodium azide and 0.05 ml of DEAD. Reaction mixture was stirred at 110°C for 8 hours. TLC revealed a complete conversion of the starting material into a faster moving species (chloroform as solvent). After the removal of solvent (using xylene as cosolvent to evaporate trace DMF), flash column chromatography gave 16 mg of product identified as the enantiomer of lactone **5** (94%). $[\alpha]_D$: -28° (C 0.8, $CHCl_3$); ^1H NMR and ^{13}C NMR data identical with those of lactone **5**.

N-2-(1-hydroxy-2-methylpropanyl) (*S*)-4-hydroxy-5-hexenoic acid amide 7.

0.17 g lactone **5** was refluxed with 0.31 ml 2-amino-2-methyl-1-propanol in toluene overnight. After the removal of solvent, the product was subjected to flash column chromatography purification (chloroform:methanol/19:1). 48 mg (28%) of starting material **5** and 0.21 g of compound **7** (69%, or 96% based on 72% conversion) were obtained. When the reaction was carried out in 10 eq. of 2-amino-2-methyl-1-propanol at 120°C for 5 hrs without the addition of any solvent, the conversion was nearly quantitative. $[\alpha]_D$: +3.0° (C 0.62, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.84 (1H, m), 5.78 (1H, b), 5.18 (2H, m), 4.17 (1H, m), 3.56 (2H, s), 2.30 (2H, s), 1.91 (1H,

m), 1.78 (1H, m), 1.26 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.31, 140.42, 114.86, 72.12, 70.11, 56.14, 33.17, 32.11, 24.61; FAB-HRMS (NBA): calcd $C_{10}H_{20}O_3N$ $[M+H]^+$, 202.1443, found 202.1437.

2-[(*S*)-3'-hydroxy-4'-pentenyl]-4,4-dimethyl oxazoline 8.

To a solution of 50 mg compound **7** and 80 mg triphenyl phosphine in 5 ml dry THF was added 50 mg phthalimide and 0.05 ml DEAD. The reaction mixture was stirred at room temperature overnight. Flash column chromatography purification (chloroform:methanol/19:1) yielded 36 mg of compound **8** (80%). $[\alpha]_D$: +4.2° (C 0.33, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 6.46 (1H, b), 5.85 (1H, m), 5.18 (2H, m), 4.19 (1H, m), 3.92 (2H, s), 2.38 (2H, m), 1.86 (2H, m), 1.25 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.05, 140.64, 114.40, 79.35, 72.09, 66.86, 32.32, 28.32, 24.70; FAB-HRMS (NBA): calcd $C_{10}H_{18}O_2N$ $[M+H]^+$, 184.1338, found 184.1329.

2-[(*R*)-3'-phthaloyamino-4'-pentenyl]-4,4-dimethyl oxazoline 9.

To a solution of 30 mg compound **8** and 90 mg triphenyl phosphine in 5 ml dry THF was added 38 mg phthalimide and 0.06 ml DEAD. The reaction mixture was stirred at room temperature overnight. The product was isolated by preparative TLC (chloroform:methanol/19:1) in 36 mg (71%). $[\alpha]_D$: -4° (C 0.36, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.80 (2H, dd, J = 5.5, 3 Hz), 7.68 (2H, dd, J = 5.5, 3 Hz), 6.18 (1H, m), 5.22 (2H, m), 4.76 (1H, m), 3.86 (2H, s), 2.38 (1H, m), 2.29 (1H, m), 1.97 (1H, m), 1.87 (1H, m), 1.23 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.65, 170.63, 167.90, 134.94, 133.94, 123.21, 118.11, 79.07, 68.70, 53.32, 30.83, 28.31, 25.16; FAB-HRMS (NBA): calcd $C_{18}H_{21}O_3N_2$ $[M+H]^+$, 313.1552, found 313.1556.

Acknowledgements

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