# Synthesis, Conformational Analysis, and Phase Characterization of a Versatile Self-Assembling Monoglucosyl Diacylglycerol Analog

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**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 homonuclear and heteronuclear 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 trialkoxybutane 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.

## 1. 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 stereochemistry 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

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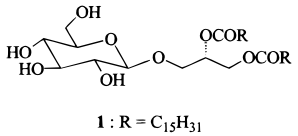
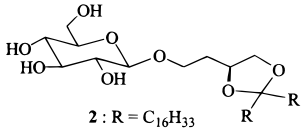
would be very expensive especially if large quantities are needed. The chemical synthesis is therefore the solution to large-scale production of homogeneous products. However, this is not trivial, either. This is especially due to the inevitable problem of the enantioselective protection and deprotection 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 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 = $C_{15}H_{31}$

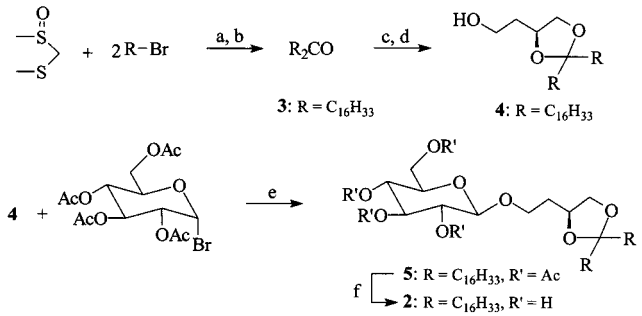



**2** : R = $C_{16}H_{33}$

## 2. Results and Discussion

**Design and Synthesis of Glycolipid 2.** 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 aglycon. 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-dihexadecyl-4-hydroxyethyl-1,3-dioxolane was then $\beta$-linked to a glucose moiety to give glycolipid **2**.

**Scheme 1.** Synthesis of Compound **2**[a]




[a] Reagents, conditions and yields: (a) NaH, THF, 50 °C, 24 h; (b) 6 N HCl, THF, r.t., 24 h, 80% (for 2 steps); (c) 3 equiv of TMOF, MeOH−THF (1:2, v/v), Cat. *p*TSA, Reflux, 12 h; (d) 1.5 equiv of (S)-1,2,4-butanetriol, DMF-THF (1:1, v/v), Reflux, 24 h, 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, 12 h, 58%; (f) $K_2CO_3$ (s), MeOH, r.t., 6 h, Quantitative. TMOF = Trimethyl Orthoformate; *p*TSA = *p*-toluenesulfonic acid.

The synthetic route is summarized in Scheme 1. A 33-carbon symmetric ketone **3** was prepared by using methyl methylthiomethyl sulfoxide (MMTS) as the carbonyl donor.[12] MMTS was dialkylated with hexadecyl bromide, and direct treatment of the crude dialkylated intermediate with hydrochloric acid in tetrahydrofuran gave symmetric ketone **3** in 80% overall yield. Ketone **3** was converted to dimethoxy acetal and then transacetalized 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 $\beta$-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 compounds **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 turning into clear liquid.

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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 the 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).

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 heteronuclear multiquantum coherence spectroscopy ($^1$H−$^{13}$C HMQC) experiments (Figure 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 $\beta/\beta'$ were also observed (Figure 2), although the volumes could not be accurately measured because of the overlapping of the four $\beta/\beta'$ protons. Semiquantitative 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 $\beta/\beta'$ protons and $\gamma/\gamma'$ protons on the downfield edge of the large methylene peak envelope. Although this information cannot be 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 with use of 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 angles $\phi$ and $\varphi$ ($\phi = H_1'-C_1'-O-C_4$; $\varphi = C_1'-O-C_4-C_3$), 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 ($\phi = 47°$; $\varphi = 169°$).[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 $\theta_1$ and $\theta_2$ ($\theta_1 = H_{4a}-C_4-C_3-C_2$; $\theta_2 = C_4-C_3-C_2-C_1$), 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 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 $\phi-\varphi$ and $\theta_1-\theta_2$ 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 $\phi$ and $\varphi$ 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 $^2J_{1a-1b} = 9$ Hz, $^2J_{4a-4b} = 8.4$ Hz, and $^2J_{3a-3b} = 6.6$ Hz. Using these values, a spin simulation of this 7-spin 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.

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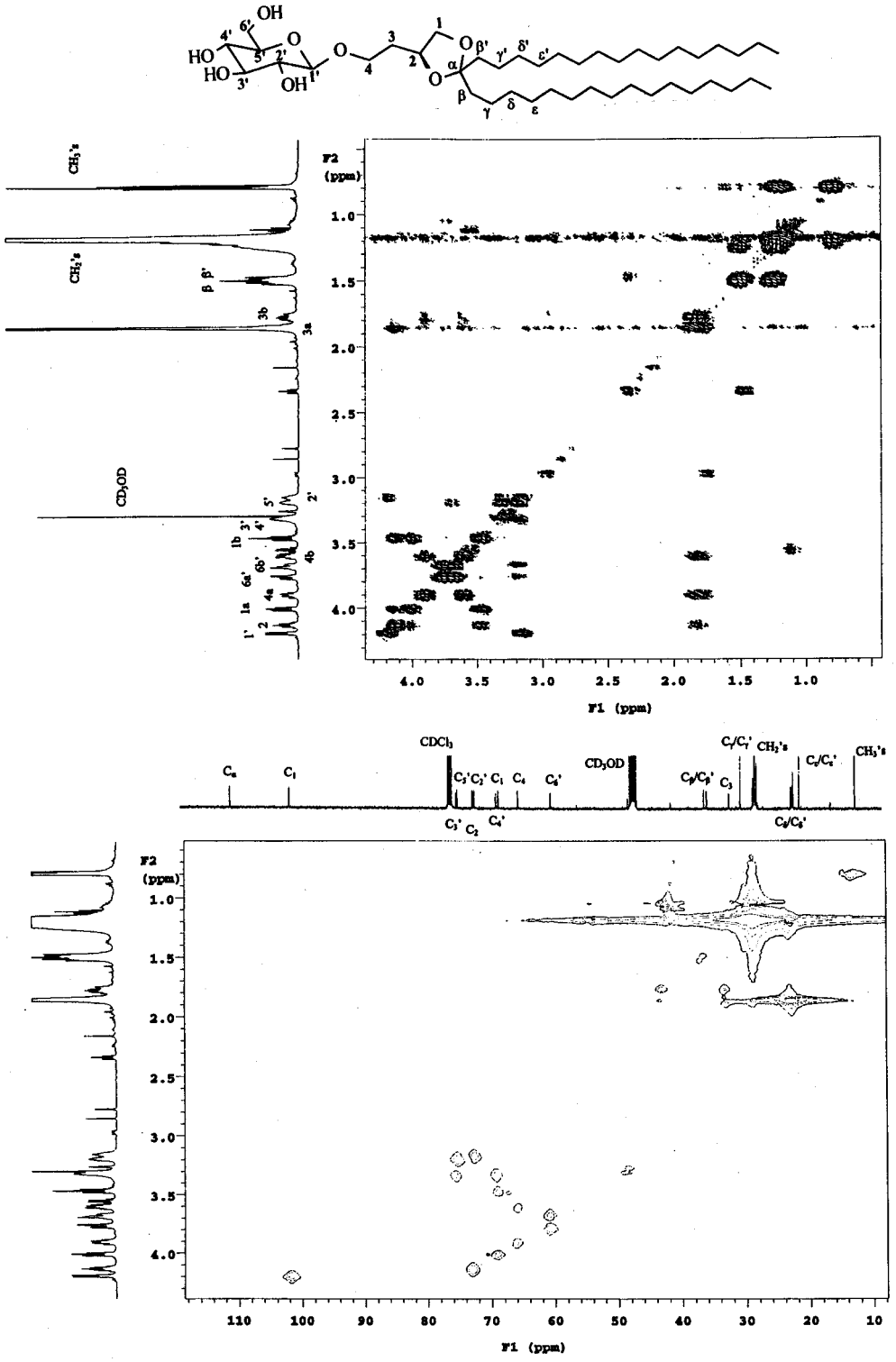


**Figure 1.** DQF-COSY (top) and $^{1}H-^{13}C$ HMQC spectra of compound **2**.

**Table 1.** Distances Calculated from NOESY Experiment for Compound 2

| 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 |

A transition at 33.7 °C was observed in a DSC scan of compound **2** (in water) from 10 to 98 °C (Figure 6), which may correspond to a transition from the gel-state to the 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

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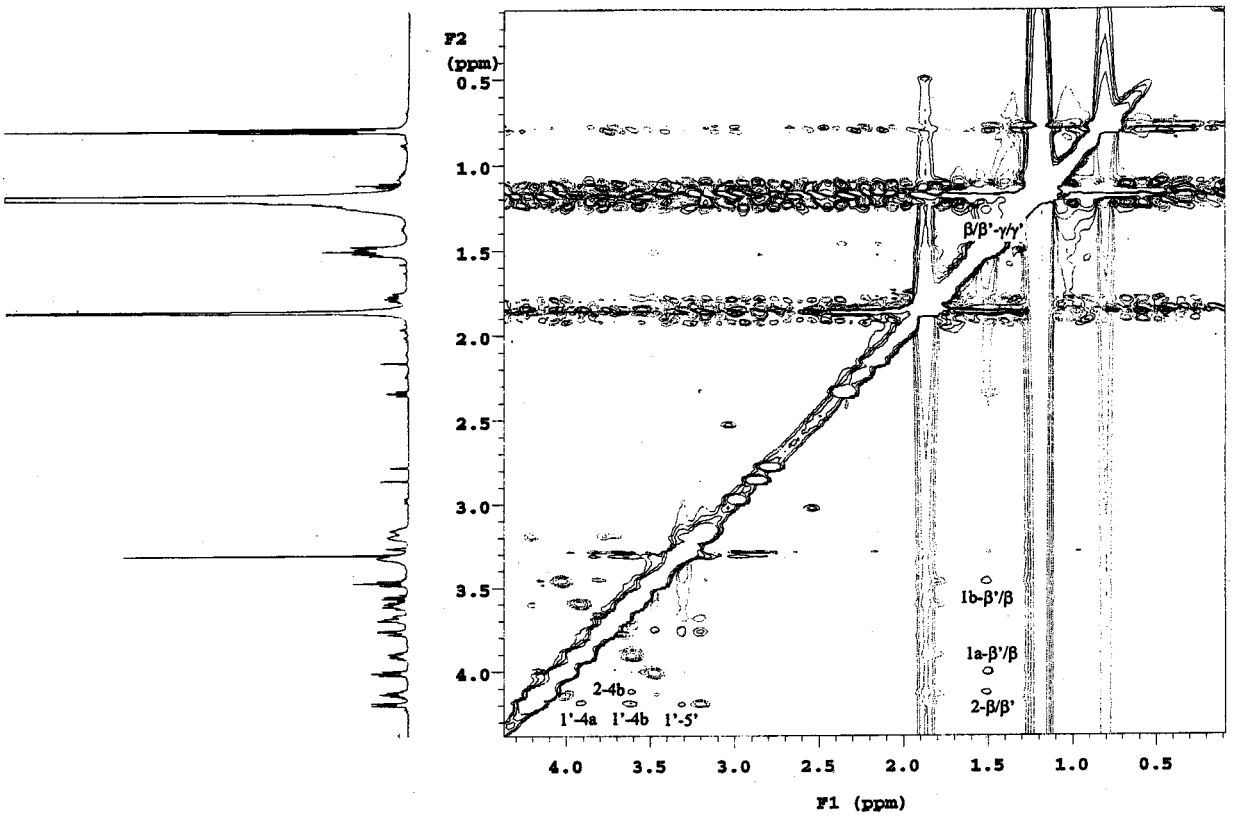


**Figure 2.** NOESY spectrum of compound **2**. A mixing time of 500 ms was used.

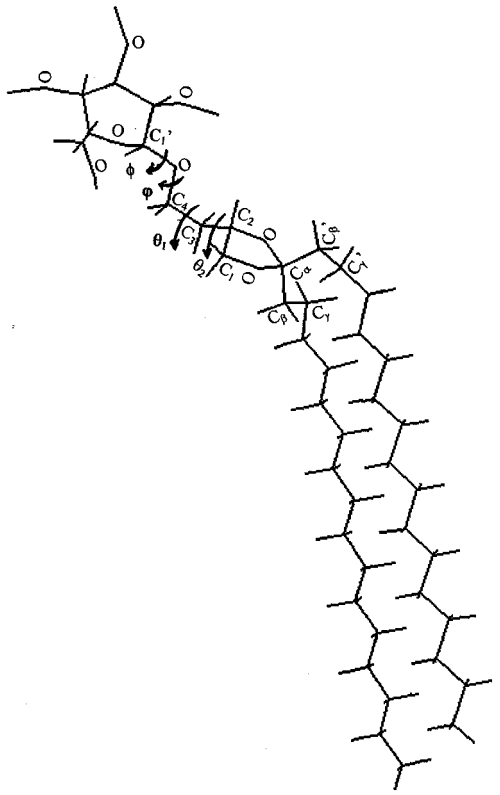

**Figure 3.** Favorable conformation of compound **2** obtained by molecular mechanics calculation.

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 **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 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 and 15.51 Å were also observed. This again was consistent with the X-ray diffraction pattern of the 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 and 3.31

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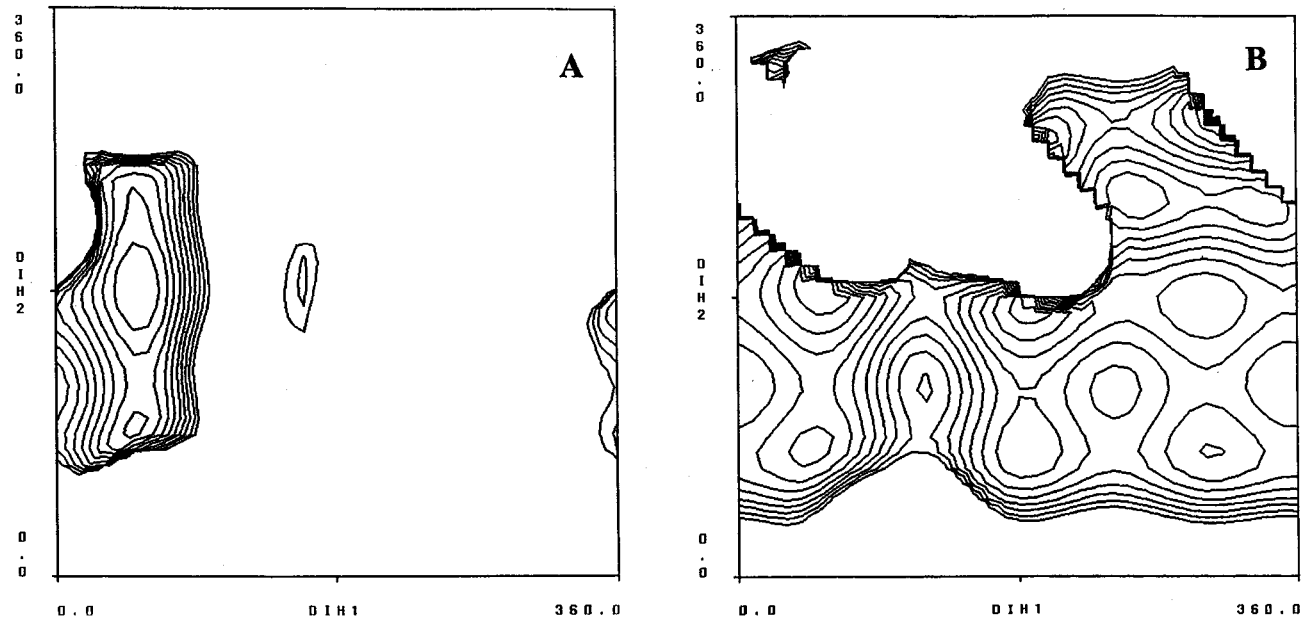


**Figure 4.** Grid search plots of compound **2**. (A) DIH 1 = $\phi = H_1'-C_1'-O-C_4$; DIH 2 = $\varphi = C_1'-O-C_4-C_3$; (B) DIH 1 = $\theta_1 = H_{4a}-C_4-C_3-C_2$; DIH 2 = $\theta_2 = C_4-C_3-C_2-C_1$.

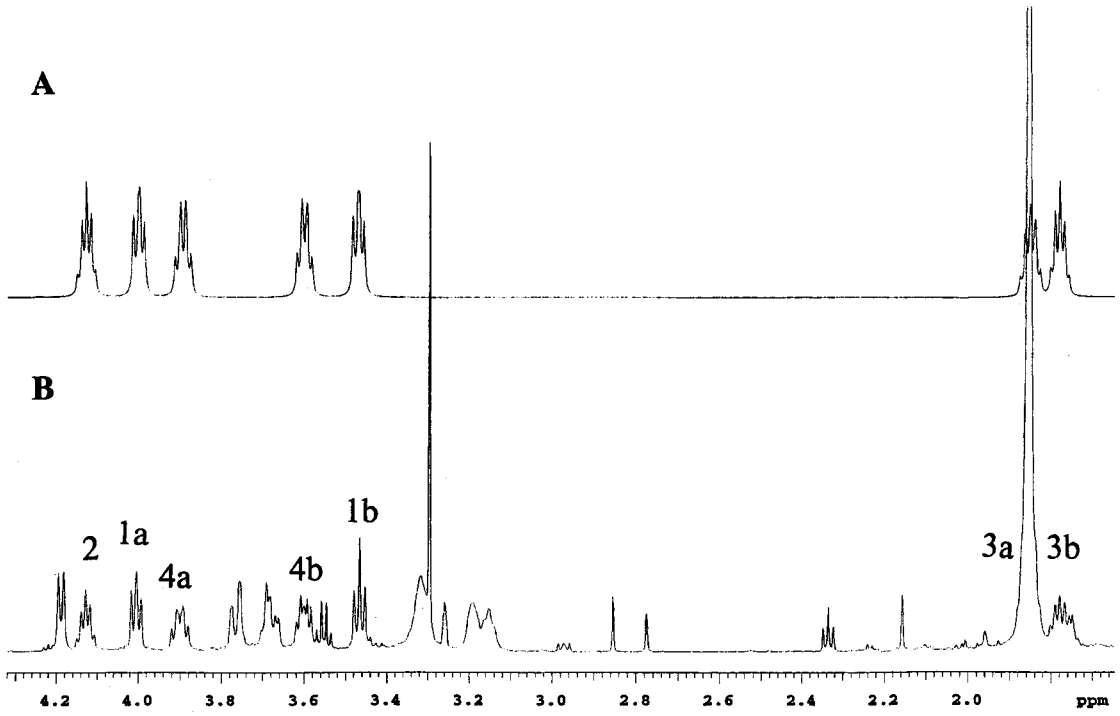


**Figure 5.** Spin simulation of the chiral linker part of compound **2**: (A) Simulated spectrum and (B) original spectrum.

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

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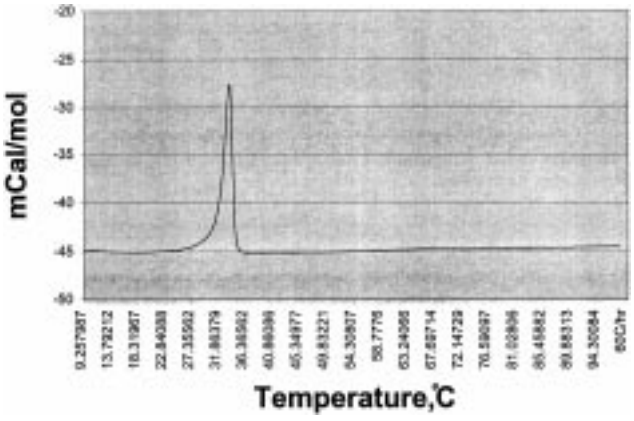


**Figure 6.** Differential scanning calorimetry thermogram of compound **2**. Scanning rate was 60 °C/h.

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. The polarized-light micrograph of hydrated compound **2** showed a texture characteristic of smectic C phase (Figure 7A), which further agreed with the proposed lamellar structure. Phase contrast micrographs (Figure 7B) and confocal reflection micrographs (Figure 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*-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 complementary concave shapes of their bases (Figure 8). This is consistent with an upward dislocation of the layers.

**Acid Susceptibility.** To get an idea of how sensitive compound **2** is toward 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 to 55 °C. The spectra (Figure 9A) 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 h. No acetal cleavage was detected even at acid concentration as high as 20% (Figure 9B). 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 cleavage being completed within 5 h. The spectra (Figure 9C) 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 $K_a$ 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]

## 3. Conclusion

An optically pure glycolipid **2** containing a (*S*)-2,2-dihexadecyl-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 the 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 for 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 analogue 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 regiochemistry of oligosaccharide synthesis upon ordered

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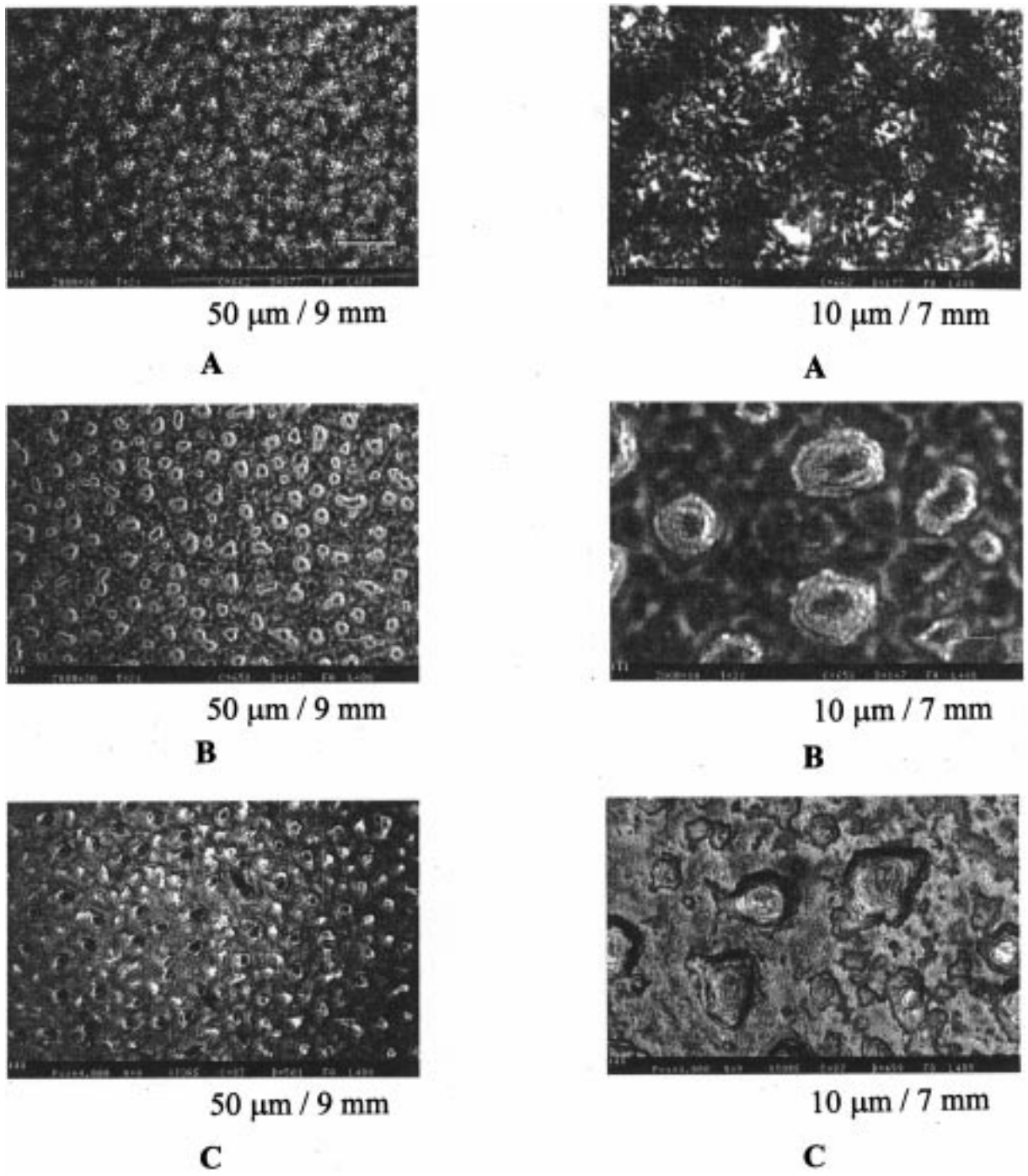


**Figure 7.** The optical texture of compound **2** observed under laser scanning microscopy: (A) polarized-light micrograghs; (B) phase contrast micrograghs; and (C) confocal reflection micrographs.

surfaces, using such 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.

## 4. Experimental Section

**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, 500, or 600 MHz VXR spectrometer at ambient temperature unless otherwise specified. Chemical shifts are reported relative to the residue solvent peak unless otherwise specified. Optical rotations were measured with 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: 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

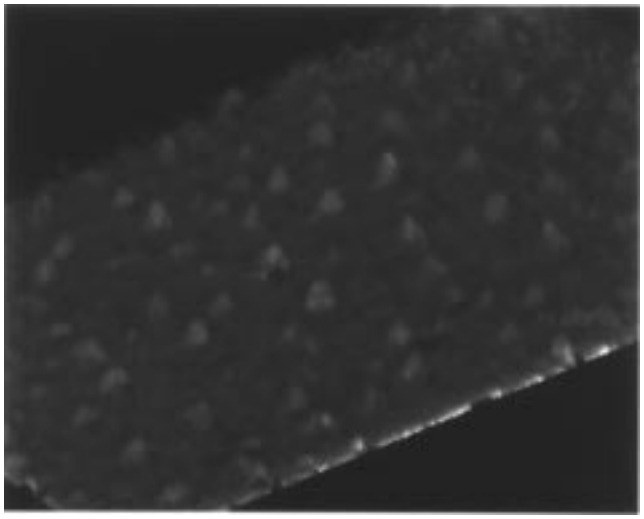

**Figure 8.** 3-D reconstruction with use of confocal reflection images from 10 sequential laser confocal microscopy scans through compound **2**. The overall depth of the reconstructed image is 5 $\mu$m. Note the conical shape of the dislocations and the complimentary concave shapes of their bases (see edges) consistent with an upward dislocation of the layers.

tetrahydrofuran (200 mL) was stirred under nitrogen at 50 °C for 24 h. After the mixture was cooled, 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 h 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 of **3** (overall 80%). Mp 80.0−82.0 °C [lit.[35] mp 79.7−81.7 °C]; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 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, $CDCl_3$) $\delta$ 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.

**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 h. After the reaction was 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 and concentrated. The identity of the dimethoxy acetal intermediate was confirmed by the presence of a signal at $\delta$ 3.10 (6H, s, $OCH_3$) in $^1$H NMR (300 MHz, $CDCl_3$ with trace $d^5$-Py) and the signals at $\delta$ 103.91 (quaternary carbon) and $\delta$ 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 h. 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**). $^1$H NMR (300 MHz, $CDCl_3$ with trace $d^5$-Py) $\delta$ 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) $\delta$ 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.

**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 h. Solvent was then evaporated and dichloromethane (80 mL) was added. The majority of mercury salts were filtered out on a Celite pad. After dichloromethane was evaporated, the residue was subjected to column chromatography purification (silica gel, 1.8% methanol in benzene, $R_f$ = 0.50). Pure compound **5** (1.3 g) was obtained (58%). $[\alpha]_D$ −5.2° (*c* 0.79, $CH_2Cl_2$); $^1$H NMR (300 MHz, $CDCl_3$ with trace $d^5$-Py) $\delta$ 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) $\delta$ 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.

**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 h 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, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$:$CD_3OD$ 2:1 with trace $d^5$-Py, chemical shifts relative to TMS signal) $\delta$ 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 ($H_{3'}$ and $H_{4'}$, 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) $\delta$ 111.89 ($C_\alpha$), 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 ($C_\beta$), 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 ($C_\delta$), 23.14 ($C_{\delta'}$), 22.16 ($C_\epsilon$/$C_{\epsilon'}$), 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 heteronuclear multiquantum coherence spectroscopy ($^1$H−$^{13}$C HMQC), and phase sensitive nuclear Overhauser effect spectroscopy (NOESY) experiments were performed by using a total of 256 real data sets, with 32 acquisition transients each and a relaxation delay of 1.8 s between transients. Mixing times of 300, 400, and 500 ms were used in the NOESY experiments. Volume integrations of cross-peaks were performed with the standard VARIAN software. A mixing time of 500 ms was used to calculate nOe cross-peak 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 with 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 had 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 by using the 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

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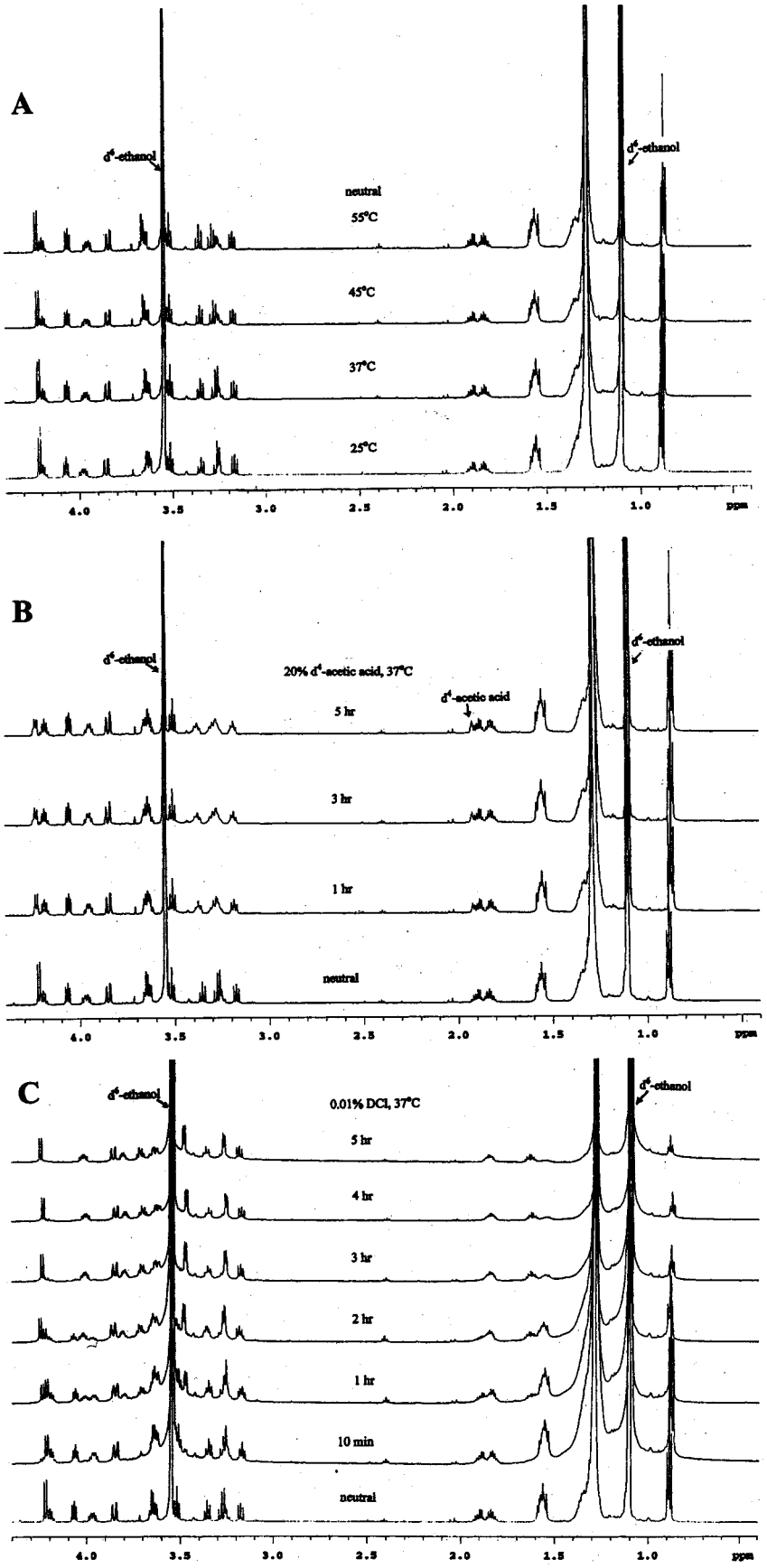


**Figure 9.** $^1$H NMR spectra of compound **2** in $d^6$-ethanol with different acidities: (A) spectra obtained in pure $d^6$-ethanol with different temperatures; (B) spectra obtained in 20% $d^4$-acetic acid solution over time; and (C) spectra obtained in 0.01% DCl solution over time.

ultrasensitive scanning calorimeter with a scanning rate of 60 °C/h. Approximately 12 mg of compound **2** was suspended in 1.5 mL of water and 5 scans were recorded from 10 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 (Cu Kα, 40 kV, 100 mA). Compound **2** (2.6

mg) 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 = 0.3 mm, $RS_m$ = 0.45 mm; scan rate = 0.4°/min; $2\theta$ = 0.5−45°). The gel form of compound **2** in water (10 mg/50 $\mu$L) was placed into a 0.7 mm glass capillary (Charles Super Co.). The sealed capillary was heated at 70 °C for 2 h to ensure full hydration prior to recording its diffraction pattern in the transmittance mode (DS = 0.05°, SS = 1/6°, RS = 0.3 mm, $RS_m$ = 0.45 mm; scan rate = 0.3°/min; $2\theta$ = 0.2−45°). Both diffraction patterns were recorded at room temperature.

**Measuring Association by Light Scattering.** Compound **2** (12.73 mg) was uniformly suspended into 2 mL of well-stirred distilled water. A 200 $\mu$L sample of this solution (8.74 mM) was transferred into one well of an ELISA plate, then 100 $\mu$L was removed and placed into the adjacent well containing 100 $\mu$L of water. The process of serial 50% dilution was continued to give a total of 60 wells. The ELISA plate was equilibrated for 4 h 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 by using a Zeiss LSM210 laser scanning microscope equipped with a 488 nm Ar laser. 40× air objective lens were used. Compound **2** (predissolved 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, 37, 45, and 55 °C. Compound **2** in 1%, 10%, and 20% $d^4$-acetic acid−$d^6$-ethanol solutions was monitored at 37 °C. Compound **2** in 0.01% and 1% DCl−$d^6$-ethanol solutions was monitored at 37 °C.

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