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Synthesis of new glycolipids and study their liquid crystalline phases for potential drug delivery systems.

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Abstract— In this study, new glycolipids derivatives have been synthesized by modification of the head group. The glycosylation reaction was perform using monosaccharide pentacetates with n-dodecanol in presence of borontriflouride. After deacetylation under basic medium, the benzylidene protecting on 4 and 6 hydroxyls on sugar follow by introducing of two propargylic group on remaining hydroxyls on 2 and 3 positions. The click reaction of sodium azide provide two triazole rings on the final structures of glycolipids **6** and **7**. The liquid crystalline phases of the materials were investigated extensively by optical polarized macroscope (POM), and differential scan calorimetry (DSC). The thermotropic behavior of these glycolipids reveal the formation of lamellar phase at the range of 60-70 oC. While the lyotropic under penetration of water reveal a cubic phase for **6** and bi-continues cubic phase for **7**. All the compounds was characterized by nuclear magnetic resonance spectroscopy (1H and 13CNMR), Fourier transform infrared spectroscopy (FTIR) and mass spectrometry (MS).

Keywords: Glycolipids, Liquid crystals, Thermotropic, Lyotropic, drug delivery

1. Introduction

Glycolipids are involving two parts a sugar head-groups attached covently to a long chain fatty alcohols or acids.[1] The highly diversity of their structures are characterized glycolipids from other bioactive materials in the living cells. They have the capability to decrease both the surface tension and the interface.[2] Furthermore, they can destabilize the membrane by forming a pores, which allows them to use in medicine against bactria, fungi and hemolytic. In addition, glycolipids can be applied as anti-adhesion agents due to their ability to prevent the bio-adhesive process in bacteria.[3] Also, the glycolipids are found applications in industrial process like detergents, cosmetics and pharmaceutical preparations. In last few decades, the glycolipids have found uses as nonionic bio-surfactant due to their amphiphilic structures. These amphiphile interestingly form a liquid crystalline phases under variety of environments by heat or under penetration of liquids.[4]
Liquid crystals (LCs) is the case that the materials are organized in special arrangement that nor solid neither liquid states. The solid state will be rigid and the molecules are arranged in strong intermolecular forces, while in liquid state the molecules move randomly. There are two types of liquid crystalline materials, a thermotropic LCs is produced when the materials are heated in a rate that allow them to melt from their solid state to more flexible phases which verified depending upon the type of them. In other hand, thermotropic LCs is formed when temperature raised slightly

above melting point (Tm) and the solid crystal convert to liquid crystals.[5] The nematic, smectic, chiral nematic, discotic and conic are among famous examples of thermotropic liquid crystalline phases. The second type of LCs is Lyotropic LCs which is alternative class of liquid crystal in which the LC phase forms when mixing the amphiphile molecules with a certain solvent. However, the lyotropic LCs are chiefly categorized into lamellar, cubic and hexagonal phases depending on their variety of internal constructions. Among them the cubic and hexagonal phases are of interest and have received more consideration in research through their highly organized structure. Which provide a potentially low rate release of the active drug ingredient with various dimension and polarities.[6]

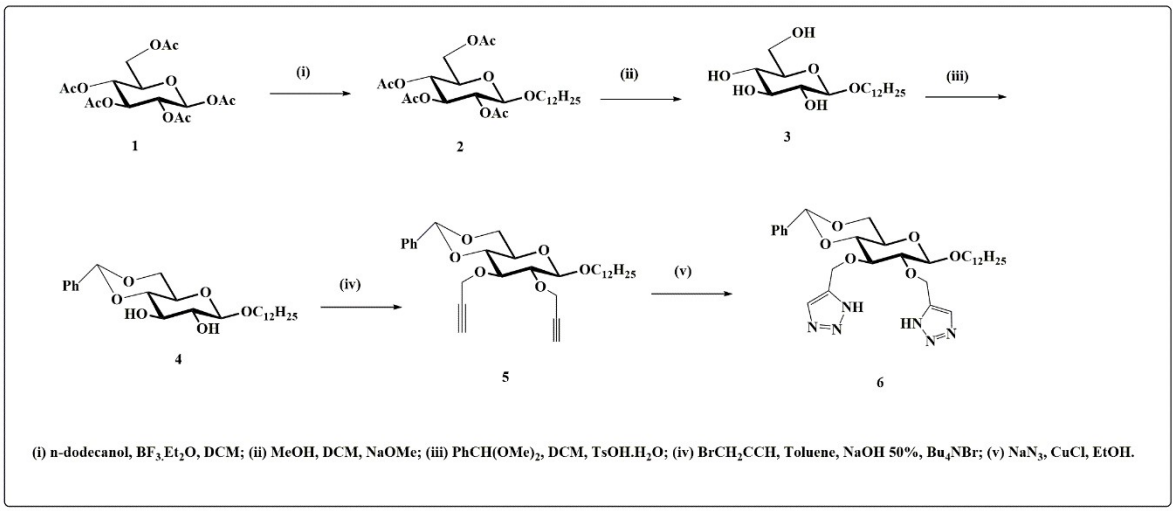
The delivery of certain drugs in an effective and improved way could be a big challenge. Over last few decads, extensive work have been done for trials to develop and improve drug delivery systems. New systems of delivery should enhance the therapy and compliance of the patient. Pioneering study shows special deliver drug to cancer cells could be accomplished by lictins in the surface of target cell. However, a variety of sugar terminal of glycolipids could be recognized only by particular lectin produced to target delivery.[7]

In these bases, we synthesized new glycolipids containing two triazole rings in the sugar head group for potential use as drug delivery systems based on their liquid crystalline behaviors upon expose to aqueous environments. Therefore, our expectation could be proved when the lamellar phase of normal alkyl glycoside could be moved to either cubic or hexagonal phases based on changing the hydrophilic-lipophilic balance with respect to polarity of triazole rings.[8]

2. Result and Discussion

2.1 Synthesis

The synthesis of glycolipid **3** has been achieved from commercially available glycose pentacetate in two steps as shown in scheme 1. After glycosylation with n-dodecyl alcohol in presence of Lewis acid , i.e. $BF_3.Et_2O$, the de-protection of acetate have been performed in the basic condition applying sodium methoxide in methanol via Zemplen reaction.



Scheme (1): synthesis of di-triazole **6** on glucose.

The benzlidination of free glycolipid **3** will allow protecting the hydroxyl groups on **4** and **6** sites. The treatment of **3** with benzaldehyde dimethyl acetal in acidic medium would form the protecting glycolipid 4 in acceptable yield in the form of β-anomer after recrystallization from n-hexane. The synthesis going straightforward to build up the new glycolipids in two more steps. First, the propargyl groups has been introduced in direct treatment of glycolipid 4 with propargyl bromide under phase transfer catalyst technique in toluene/ aqueous sodium hydroxide solvents. The product **5** is pure enough to process to the final products and simple work-up was applied. The ^{1}H-NMR spectrum of compound **5**, showed the all-characteristic peaks, start with the multiplet at 2.44 ppm for the propargylic protons on both 2 and 3 positions of the pyranoside ring. In the same way the ^{13}C-NMR of compound **5**, exhibit the signals at 80.24 and 80.34 ppm for the quaternary carbons of triple bonds (2 **C**≡CH). In similar, the signals at 74.74 and 74.36 ppm are assigned to the carbons of CH in triple bonds.

The compound **5** was treated with sodium azide to produce derivative **6** in presence of sodium ascorbate and cupper (I) chloride in a mixture of ethanolic chloroform.

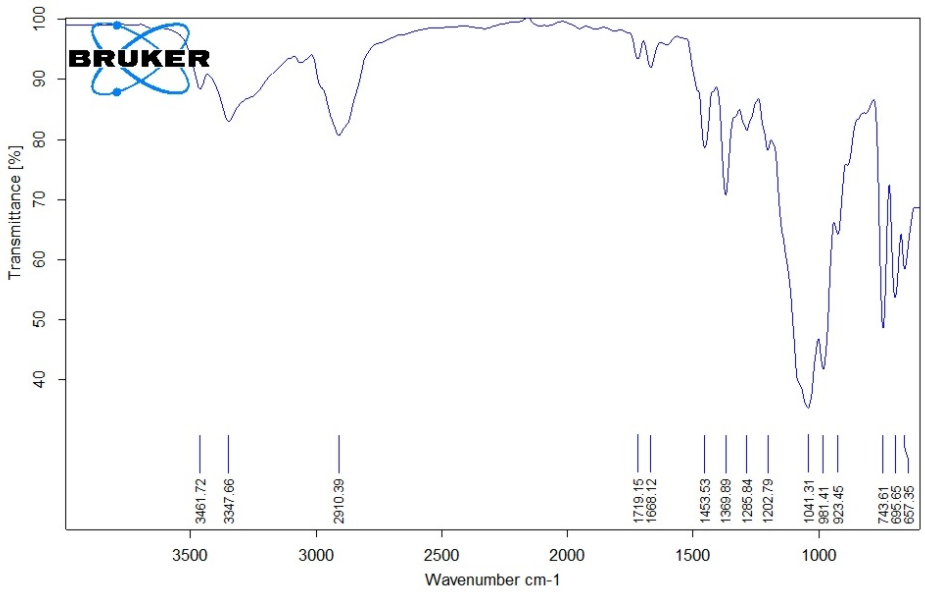


Figure (1): The FTIR spectrum of ditriazole **6**.

The compounds **6** was confirmed by the FT-IR figure (1) which are shown that absence of the stretching vibration at 3203 cm^{-1} for the terminal alkyne, beside the stretching vibration of triple C-C bonds at 2120 cm^{-1}. The spectrum is present as well some difference in the finger print region of compound **6**.

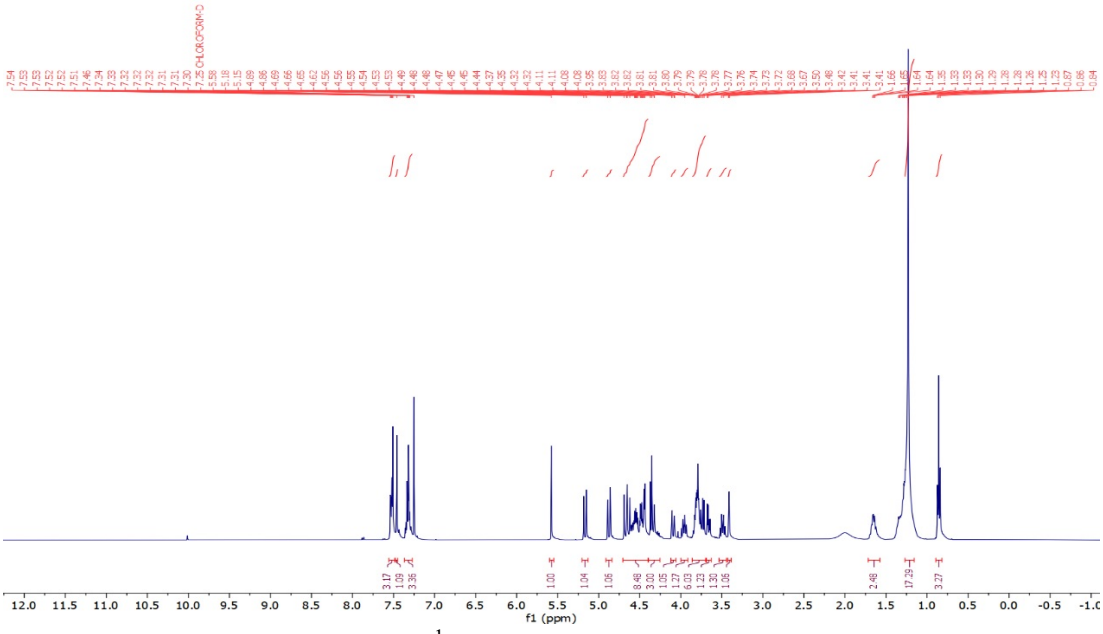


Figure (2): The ^{1}H-NMR spectrum of ditriazole **6**.

Moreover, the proton NMR spectrum of triazole **6** is indicate all the characteristic signals of compound **6** exactly Fig (2). The disappearance of terminal proton of alkynes at 2.43 ppm is good indication that the reaction occurance. Furthermore, the two signals at the 7-8 ppm region was prove that the triazole formation based on the protons of two triazole C-H which is slightly near with the protons of the benzylidene group. Furthermore, the ^{13}CNMR spectrum of compound **6** Fig (3), indicated the following peaks that confirm the characteristic peaks of product, as well the disappearance of some other peaks at 80.19 and 80.15 ppm of the quaternary carbon of carbon-carbon triple bond. In addition, there is absence of the peaks at 74.80 and 74.05 ppm for terminal carbons of triple bonds. The new aromatic carbons at 150.10 and 140.29 are assigned to the two carbons of triazole ring (C-triazole).

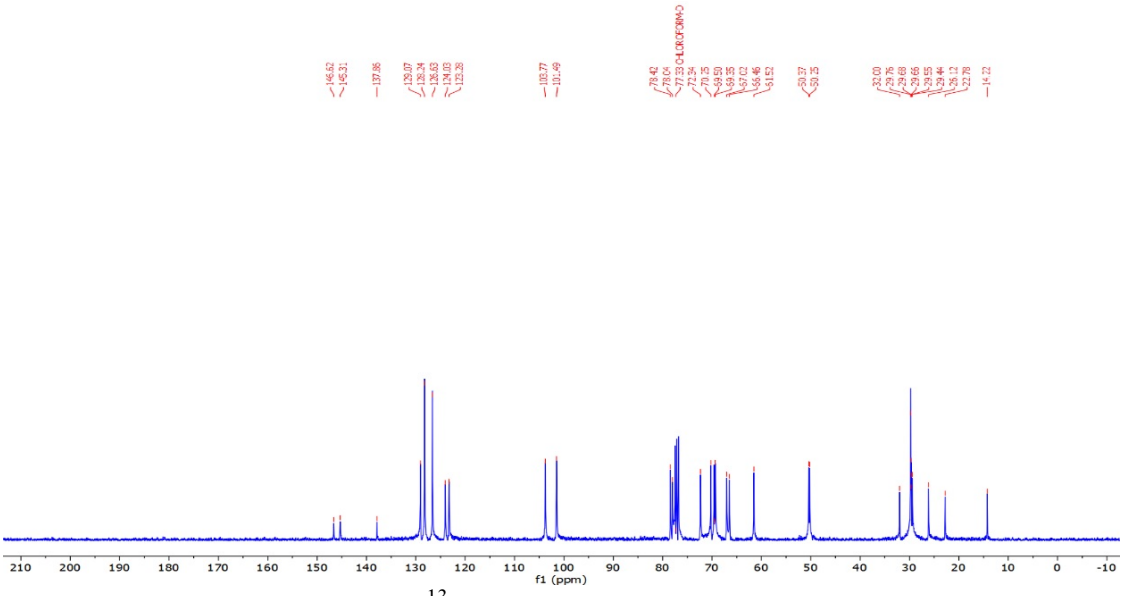
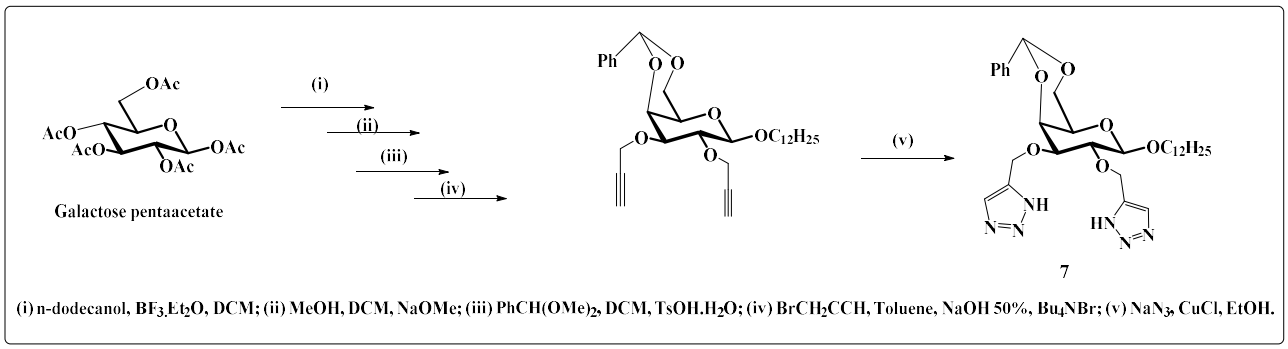


Figure (3): The ^{13}C- NMR spectrum of di=triazole **6**.

According to the same strategy, the compound **7** was synthesis in the same reaction consequences of compound **6** starting from β-galactose pentaacetate scheme 2. The chemical characterization are compatible with the structure and subjected directly to the physical investigation of liquid crystalline behaviours.



Scheme (2): synthesis of di-triazole **7** on galactose.

2. Study of the liquid Crystalline Behaviors

The two selected compounds (**6** and **7**) are studied extensively under the more powerful instruments for characterization of their liquid crystalline behaviors of such material which classified as amphiphiles due to the two parts in their structure hydrophilic (head group) and hydrophobic (hydrocarbon long chain). The details of their liquid crystalline phases as below:

2.1. n-dodecyl 4,6-O-benzylidene-2,3-ditriazole-β-D-Glucopyranoside 6.

2.1.1 Optical Polarized Microscope (OPM): -

2.1.1.1 Thermotropic study study of compound 6:

The result of ditriazole compound **6** identifies the phase transition at 65 °C upon heating as a melting manner from a firm crystal to an isotropic phase. If the cooling is slowdown rate was employed, the transition of the liquid crystal phase is shown as a texture of circle polarized light that indicating that the compound exhibitions a monotropic transition [9] (Figure 4). This observed texture, can be normally as a chiral nematic [10] which appear at 38 °C when a cooling rate is slowly decrease due to the phase can be rearrangement in slow mode and that requires slightly long time for the species to reorganize again in texture [11].

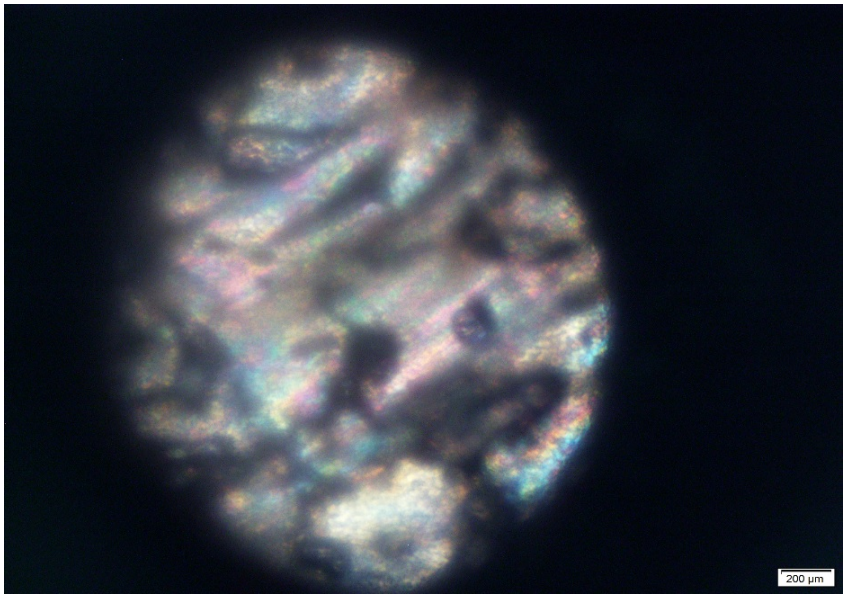


Figure 4: Texture of compound **6** at zoom (X10)

2.1.1.2. Lyotropic phase behavior of ditriazole 6:

OPM pictures for lyotropic behavior of the compound at room temperature demonstrated a birefringent line at the edge of the sample, which normally characteristic of the lamellar phase (Lα) exactly at the water-rich side. Furthermore, beside the producing of the lamellar phase, the compound was form a cubic phase at side when water-poor as shown in (Figures 5).

The modified glycolipid with ditriazole rings, which somewhat increases the head-group size with reducing of the hydrophilicity, can promote the formation of the non-lamellar phase.[12] However, The results are noticeably proves the effect of water making a hydrogen bond network with the monosaccharides head-groups [13].

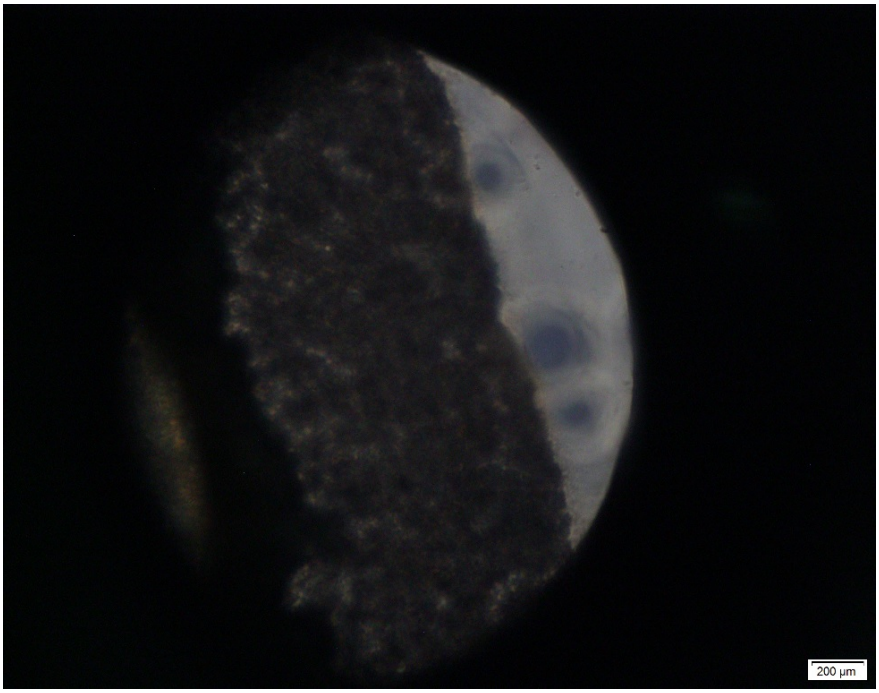


Figure 5: Texture of compound **6** water penetrate at zoom (X3)

2.1.2. Differential Scanning Calorimeter (DSC) of compound 6: -

The transition temperature of phase was obtained calorimetrically during the DSC second heating round of the sample to remove the thermal history and minimize the kinetic effect. Figure (6) shows that the compound exhibit recrystallization in the reheating process which is followed by a melting transition at 48°C (ΔH= 17.56 J/g) upon further heating A similar observation has been reported by monoalkylated glucosides [52] [11]. The OPM texture confirms this assignment. The liquid crystal phase transition is only observed on cooling , indicating that this compound displays a monotropic transition behavior [9].

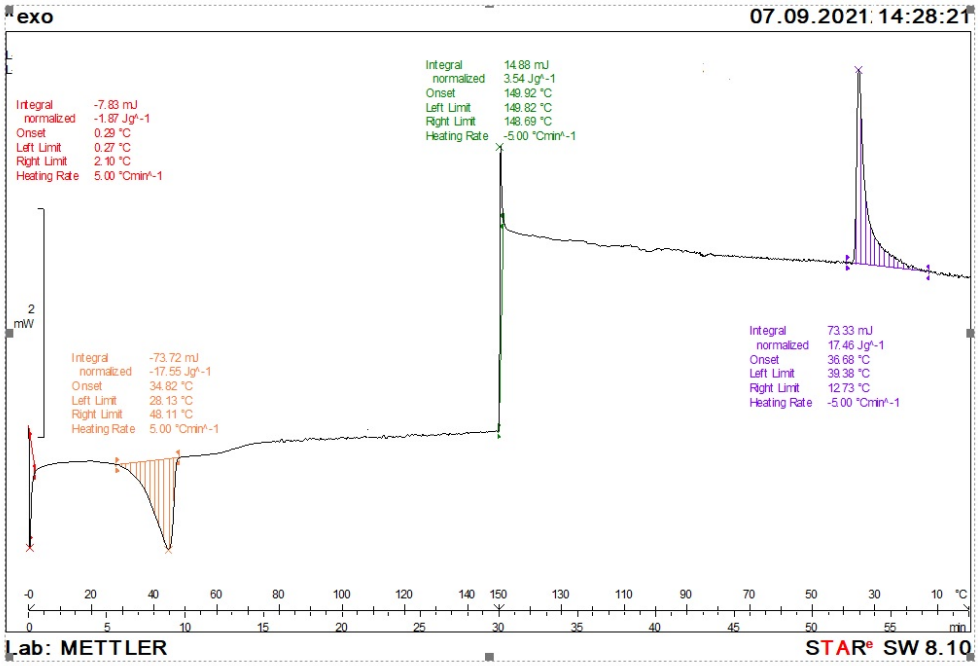


Figure 6: DSC of compound **6**.

2.2.2. n-dodecyl -2,3-ditrizolyl-β-D-Glucopyronoside 7

2.2.2.1. Optical Polarizing Microscopy

Thermotropic study of compound 7:

On heating for compound **7** under OPM, a contrast birefringent texture was appeared (Figures 7) suggesting a lamellar phase and the compound clears at 49°C. The change in morphology in OPM texture is not observed below transition of glass temperature (Tg). However, if the liquid crystal phase experience glass transition, new texture keep the same phase for liquid crystal, which of course relax into more order texture[14], [15]

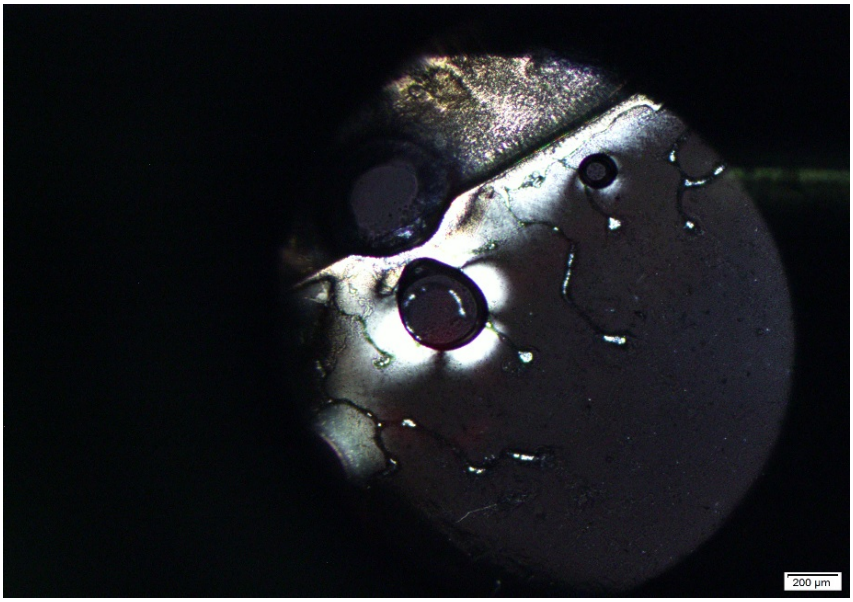


Figure 7: Texture of compound **7** at (RT) at zoom (X20)

2.2.2.2. Lyotropic study of compound 7:

The lyotropic investigation of compound **7** showed the following phases under high water concentrations (figure 8). This phase was followed by lamellar phase when the water is penetrated gradually reduced. The cubic phase is the more stable phase under increasing the water penetration.

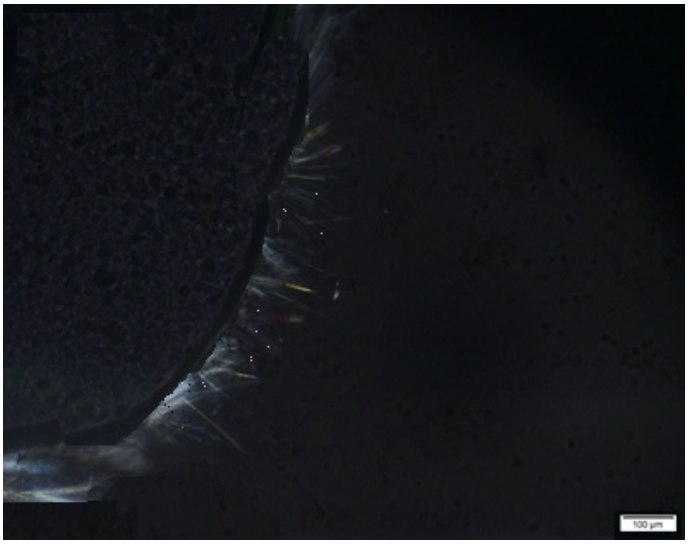


Figure 8: Texture of compound **7** at (RT) at zoom (X20)

2.2.3 Differential scanning calorimetry

The phase transition temperature for the sample was obtained calorimetrically during the DSC second heating cycle to remove the thermal history and minimize the kinetic effect. Figure (9) shows the typical endothermic clearing transition into the isotropic phase at 52°C (ΔH= 40 mJ/g), but no melting was observed; instead, step-shaped peak associated with glass transition was discernible.

The compound was found to have high clearing temperature and thus, it exhibits relatively a stable mesophase for a wide range of temperature [16].

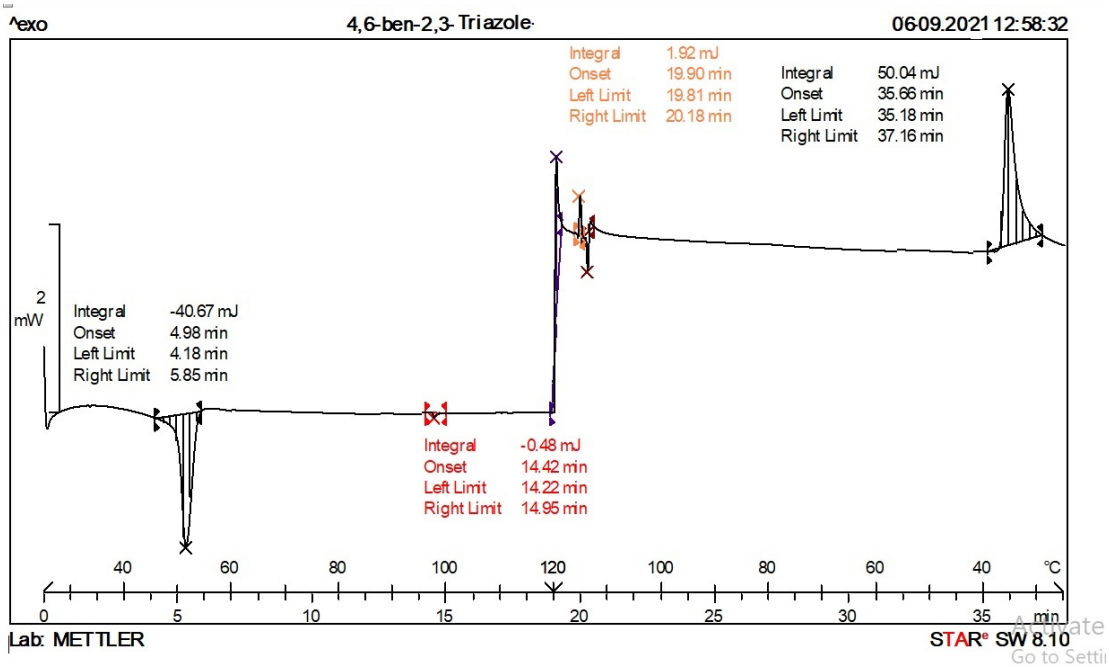


Figure 9: DSC of compound 7.

3 Conclusion:

In summary, two di-traizoles derived from glucose and galactose based glycolipids are successfully synthesized in good yields. The structures of compounds have been confirmed by ^{1}HNMR, ^{13}CNMR and FT-IR spectrometry. The liquid crystalline behavior of final compounds are studied extensively and confirm that the cubic phase could be obtain upon penetration of water because of the polarity of di-triazole moieties. This cubic liquid crystalline phase is important in drug delivery systems as well as crystalline of important macromolecules.

4. Experimental Part:

4.1 General methods:

All reagents were obtained from commercial sources and used without further purification. Flash column chromatography was carried out on silica gel 60 (230–400 mesh, E. Merck). TLC was performed on pre-coated aluminum plates of Silica Gel 60 F254 (0.25 mm, E. Merck. NMR spectra were recorded on Bruker Avance and Joel ECA spectrometers at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. Chemical shifts were in ppm from Me_4Si, calibrated using the residual proton and carbon of the deuterated solvent. Proton peak assignments were performed with the aid of 2D NMR techniques (HMQC).

4.2. General Procedure for alkylation with propargyl

The glycolipid **4** (2 mmol) was dissolved in a mixture of toluene (50 mL), 50% sodium hydroxide solution (25 mL) and tetrabutylammonium hydrogen sulfate (1 mmol) and stirred at 10 ^{o}C for 30 minutes. Propargyl bromide (4 mmol) was added dropwise. The mixture was stirred for 12 hours. Hexane (50 mL) was added, and the organic layer was separated, filtered through pad of celite,

dried over magnesium sulfate, and the solvents were evaporated. The crude material was purified by column chromatography.

4.3 The general procedure of ditriazoles glycolipid 6

Dipropargyl compounds **5** (1 mmol) were dissolved in 100 mL of a mixture of ethanol: water (85:15). The sodium azide, sodium ascorbate (6 .0 mmol %) and Cu salt (2.0 mmol %), were added and stirred at 60 ^{o}C for 24 hrs. The mixture was cooled to room temperature, extracted with water (3* 50 mL), dried over magnesium sulfate and evaporated the organic solvent under vacuum. The desired product was obtained after column chromatography.

5. References

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