Iraqi National Journal of Chemistry

Journal homepage:
http://iqnjc.com/Default.aspx

Synthesis and antibacterial study of new thiadiaza-crown ethers on mannose based glycolipid and its analogues.

Karem Jaber Sabah

Chemistry Department, Faculty of Science, University of Kufa, 54001 An-Najaf Al-Ashraf, Iraq.

kareemj.alabidi@uokufa.edu.iq

Abstract:

Two new thiadiaza- and triaza-crown ethers on n-dodecyl mannopyranoside have been synthesized and characterized by proton, carbon NMR, high resolution mass spectrometry CHN analysis and FTIR. The antibacterial activities of synthesized macrocycles and their analogues was performed against two *gram*-negative and two *gram*-positive bacteria in two cultural media.

Keywords: macrocycles, mannose, glycolipids, antibacterial, crown ethers.

Introduction:

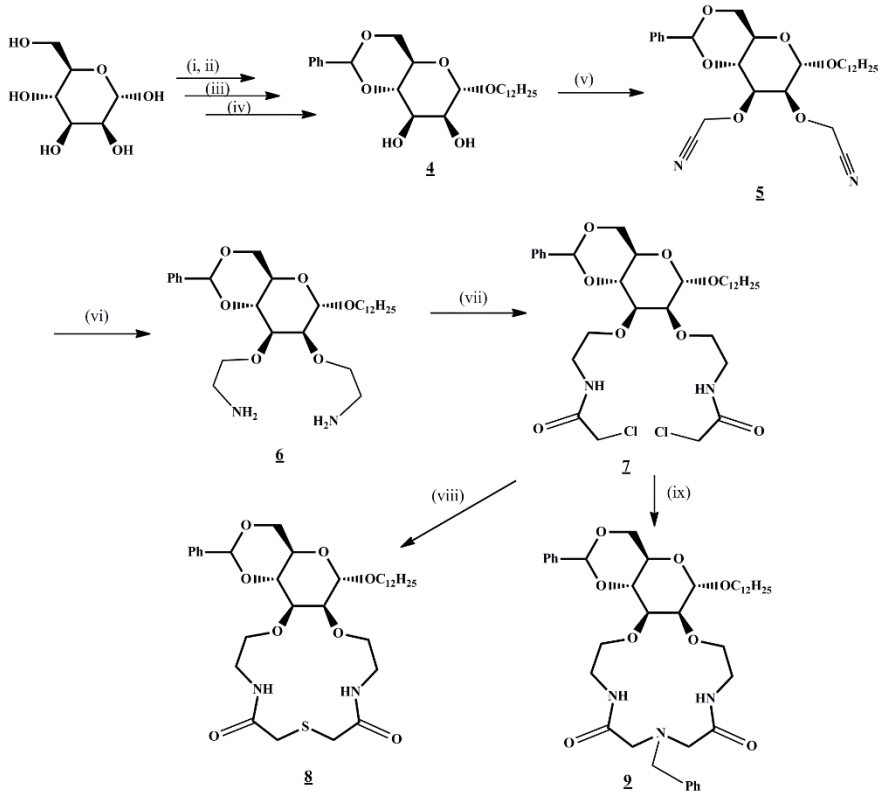
Macrocycles, such as crown ethers, catenane and cryptands are among the most widely investigated molecules in chemistry during the past five decades [1-2]. Although, many of macrocycles have been prepared earlier, the formal beginning of crown ethers started with the appearance of Pedersen's seminal paper of dibenzo-crown ethers.[3] Beside Pedersen's work, Donald Cram[4] and Jean- Lehn[5] are established the supramolecular chemistry based on their talent hard working. In spite of the high toxicity of most crown ethers[6], their unique properties led to verify the sources of building blocks.[7] Among the all naturally occurring building blocks, carbohydrate[8] are used extensively due to they are available, inexpensive, non-toxic and their verity of

chiral centers.[9] The carbohydrates with macrocycles was applied in several fields including the extraction of alkali metals[10] and organic cations, phase transfer catalysts [11], molecular recognition,[12] and asymmetric synthesis[13]. In addition, the sugar units of the glycolipids are acting as an antenna in the phospholipids cell membrane.[14] Glycolipids involving macrocycles, as a new class of amphiphilic molecules, play significant role in the self-assembly of the parent glycolipids.[15] As a continues of our ongoing project of synthesis a new macrocycles based glycolipids[15-16], we report here the synthesis of new mannopyranoside macrocycles and evaluated the toxicity of some others towards four types of bacteria, two gram-negative and two gram-positive bacteria in tow cultural media.

Result and Discussion:

1-Synthesis of Macrocycles:

To synthesis the target macrocycles **8** and **9**, protected glycolipid **3** was required (scheme 1), which is synthesis according to the methodology was described in literature .[17]



(i) acetic anhydride, NaOAc, (ii) n-dodecanol, BF_3, DCM, (iii) MeOH, NaOMe, (iv) benzaldehyde dimethyl acetal, TsOH, DCM, (v) chloroacetonitrile, NH_4HSO_4,(vi) $LiAlH_4$, THF, (vii) chloroacetic anhydride, triethyl amine, (viii) $Na_2S.9H_2O$, ethanol, (ix) benzyl amine, Na_2CO_3

Scheme 1: The synthetic route of n-dodecyl mannose involving crown ether

The slightly low yield of protecting of 4,6- hydroxyl groups by benzylidene group selectively can be attributed to the difficulty to obtained the single isomer on 4,6-hydroxyls due to the competition of 2,3-hydroxyl groups to benzylidenation, as shown in scheme **2**.[18] Fortunately, the 4,6-benzylidene isomer **4** could be crystallized by mixture of hexane:petroleum ether to remove the other undesired by-products. The ^{1}HNMR spectrum was shown a multiplate at 7.3-7.5 ppm for aromatic protons, while the singlet at 5.5 ppm was assigned to CH proton of benzylidene group. The cynomethylation of compound **4** by electrophilic substitution of chloroacetonitrile on the 2,3-hydroxyls of compound **4** by phase transfer catalyst approach furnished the cyno-derivative **5** in high yield (83%). The 1HNMR spectrum of **5** was shown the following signals, a multiplate at 4.55 ppm for the C$\underline{H}_2$ group attached directly to cyano group, with disappear of proton of both hydroxyl group. In addition the ^{13}CNMR spectrum indicate the signal at 57.78 and 57.73 ppm for (2 $\underline{C}H_2C\equiv N$) while the two signals at 116.16 and 116.00s ppm for $\underline{C}H_2$ and $\underline{C}N$ respectively. The HRMS was support the formation of both cyno group as indicate in exact molecular mass.

The derivative **5** was treated with lithium aluminum hydride in THF at 0^{o}C to obtain free amines in almost quantitive yield **6**, as seen in the TLC which was showed the disappear of starting materials. In other words, the losing of some product might be a result of recovering the product after working-up. Furthermore, the derivative **7** has been synthesized from compound **6** by simple amidation process using chloroacetic anhydride as a reagent in chloroform and triethyl amine as a scavenger. Although, the compound **7** can be obtained as well from the reaction of **6** with chloroacetyl chloride, the resulting by products was tricky to workup and was reduced the yield of product. The ^{1}HNMR spectrum was showed a two triplet signals at 7.05 and 7.02 ppm which was attributed to the two N$\underline{H}$ amides, while the doublet at 4.08 ppm could be assigned for C$\underline{H}_2$Cl group (Fig 1). Moreover, the ^{13}CNMR was indicated the signals at 167.51 and 167.45 ppm for $\underline{C}O$ carbonyl groups, and the signals at 44.27 and 44.07 ppm were assigned to the carbon of $\underline{C}H_2$Cl groups (Fig 2). The HRMS was showed the exact molecular mass of compound **7**.

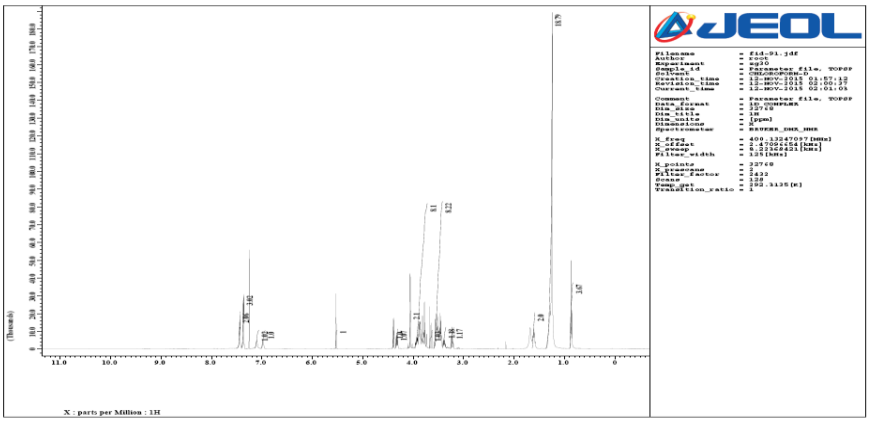


Fig 1: ^{1}HNMR spectrum of compound **7**

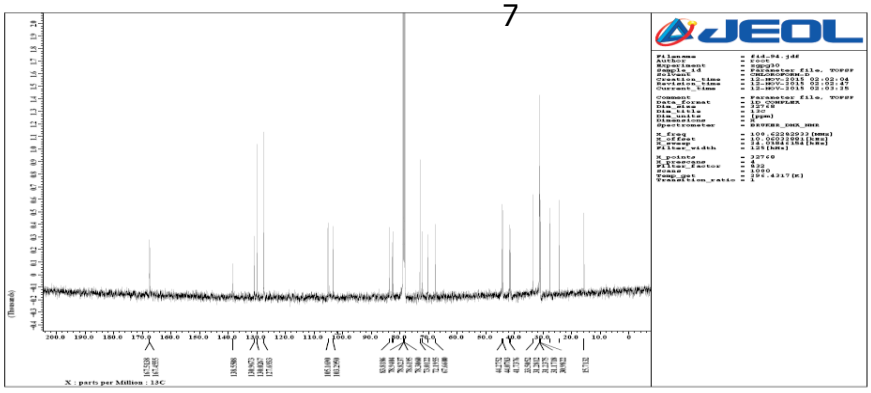


Fig 2: ^{13}CNMR spectrum of compound **7**

The cyclization process is the crucial step in this strategy, therefore, some tricks have been taken to get excellent results. The macrocycle **8** was obtained from the treatment of **7** with sodium sulfide nonahydrates in ethanol at room temperature, while the reflux could be accelerated the reaction significantly. Finally, macrocycle **9** was prepared by the direct reaction on compound **7** with benzyl amine in acetonitrile and sodium carbonate as a base in considerable yield (65%).

The ^{1}HNMR spectrum was showed a two triplet signals at 6.92 and 6.87 ppm which was attributed to the two N<u>H</u> amides, while the doublet at 4.05 ppm was disappeared and the two multipletes at 2.92 and 2.83 ppm was assigned to C<u>H</u>$_2$S in macrocycle **8** (Fig 3). In the same manner, macrocycle **9** was showed one mulitplete at 3.21 ppm which assigned to the <u>C</u>H$_2$NBn groups (Fig 5). The ^{13}CNMR was indicated the signals at 167.51 and 167.45 ppm for <u>C</u>O carbonyl groups, and the signals at 36.61 and 36.54 were assigned to the carbon of both <u>C</u>H$_2$S groups for macrocycle **8** (Fig 4). On the other hands, the

macrocycle **9** showd signals at aromatic region aromatic region 150-120 ppm for the benzyl ring, and two signals at 39.74 and 39.55 ppm which assigned to the $\underline{C}H_2NBn$ groups (Fig 6) . The HRMS was showed the exact molecular mass of compounds **8** and **9** (Fig 7) and (Fig 8) respectively.

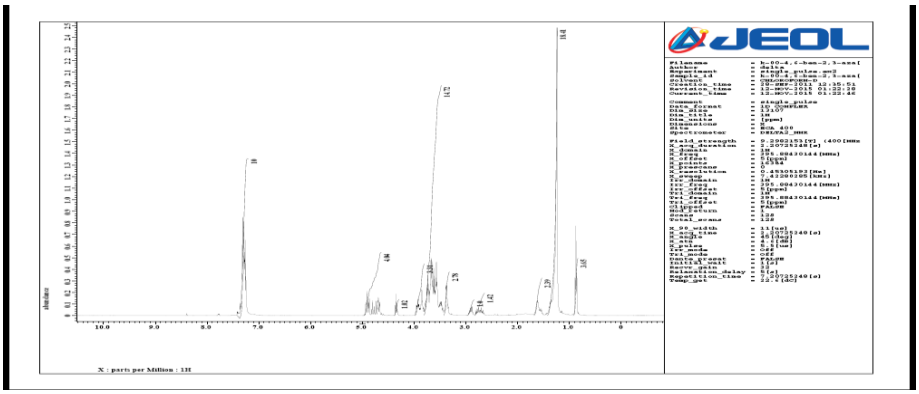


Fig 3: ^{1}HNMR spectrum of compound **8**

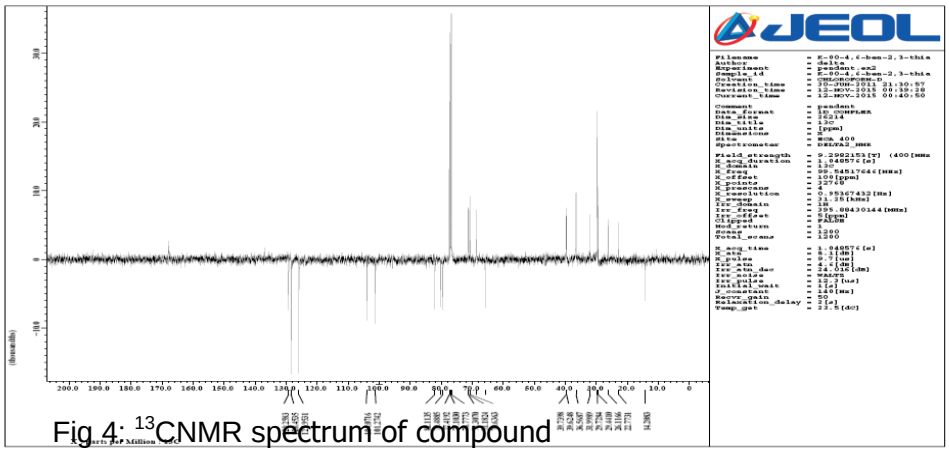


Fig 4: ^{13}CNMR spectrum of compound **8**

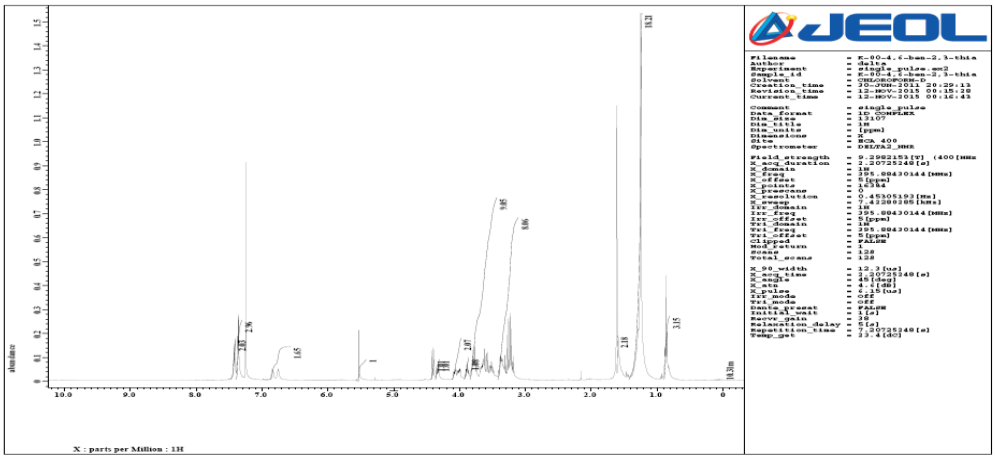


Fig 5: ^{1}HNMR spectrum of compound **9**

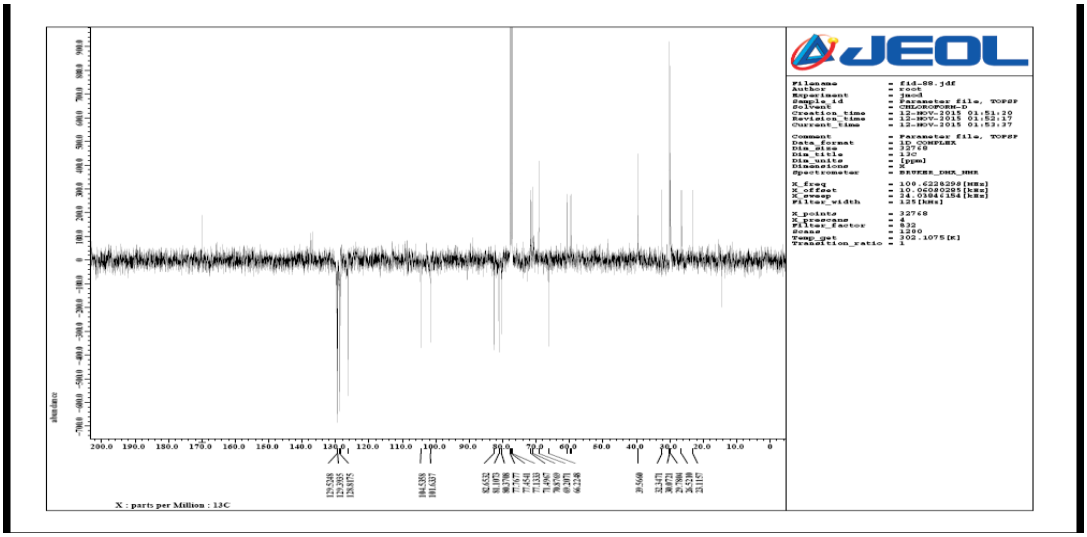


Fig 6: ^{13}CNMR spectrum of compound **9**

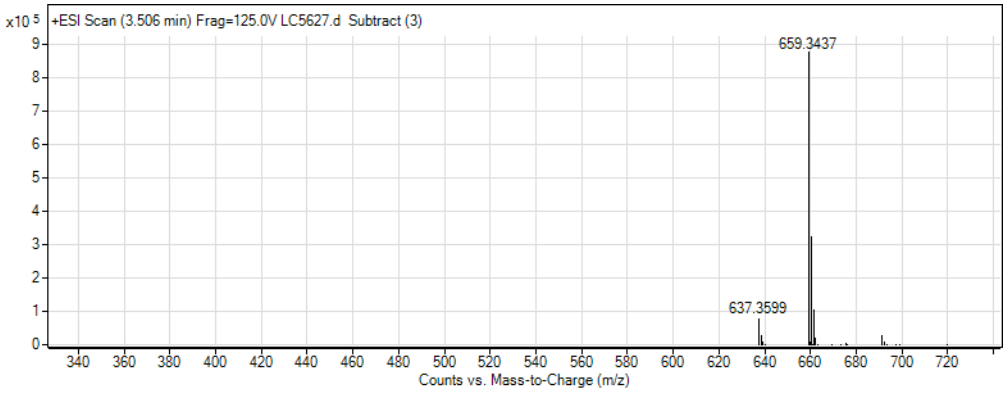


Fig 7: HR-mass spectrum of compound **8**

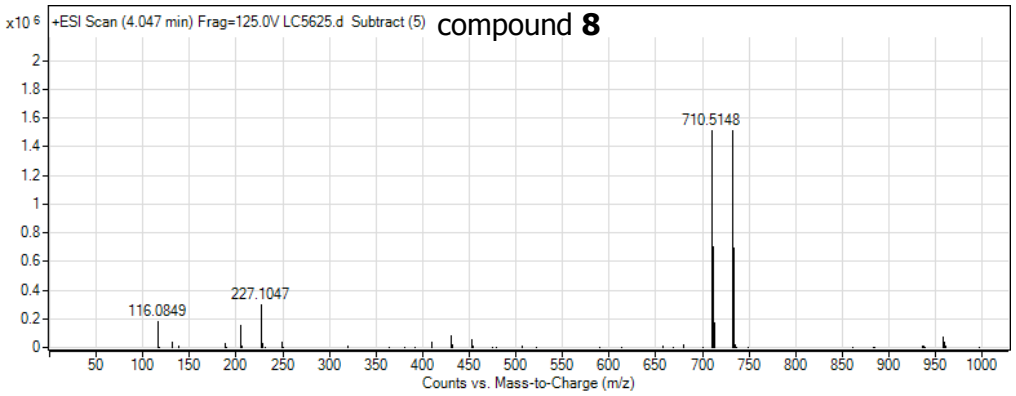


Fig 8: HR-mass spectrum of compound **9**

2- Anti-bacterial study:

In the present study, the evaluation of antibacterial activity of macrocycles based glycolipids **8**,**9**, **10**, **11**, **12** and **13** and their non-sugar analogues **14** and **15** against two gram-negative and two gram-positive bacteria in tow cultural media

were studied using agar well diffusion method.[19] The data pertaining to the antibacterial potential were presented in Table **1**.

Table **1** The antibactrial activity of crown ethers with various bactria in different caltural media.

	E.coli		*Klebsiella* spp		*Pseudomonas spp*		*staph aureus*	
Compound	MHA	BA	MHA	BA	MHA	BA	MHA	BA
8	++	++	-	-	-	-	-	-
9	++	++	-	-	-	-	-	-
10	++	++	-	-	-	-	-	-
11	+	+	-	-	-	-	-	-
12	++	++	-	-	-	-	-	-
13	++	++	-	-	-	-	-	-
14	++	++	++	++	++	++	++	++
15	++	++	++	++	++	++	++	++

Note : MHA = Mueller-Hinton agar

BA = Blood Agar

++ = Sensitive , - = Resistant

The results showed variability in the inhibitory of each macrocycles except macrocycles **14** and **15** against bacteria. The diameters of growth inhibition zone were in the range maximum of 10± 0.5 mm and minimum of 12 ± 0.5 mm (Table 1). The glycolipids crown ether **8**, **9, 10, 11, 12** and **13** showed antibacterial activity against *E. coli* only expect crown ether **11**. The highest inhibition zone was observed on macrocycle **8** against *E. coli* (10±0.5 mm). It is clear that there is no biological activity was demonstrated by these macrocyles with other types of bactria in different caltural media. In opposite, the non-glycolipid macrocycles analogues **14** and **15** was showed highly inhibition for all type of bacteria under examinations. It is known that most crown ethers are toxic [20], so compounds **14** and **15** like them. The slightly toxicity of all sugar based glycolipids under study, may be attributed to the presence of glycolipid in their structures which reduce the toxicity of macrocyles as these moieties are naturally available.

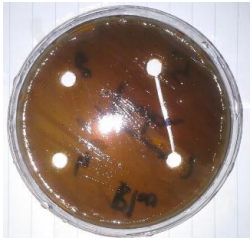
Compound **8** (MHA media) 	Compound **9** (BA media) 	Compound **10** (MHA media) 
Compound **11** (BA media) 	Compound **12** (BA media) 	Compound **13** (MHA media) 

Fig 9: The antibacterial activity of some prepared compounds aginst *E.coli* in MHA and BA media .

Experimental:

All reagents and solvents were received from commercial sources and were used without further purification. Thin layer chromatography was performed on silica gel (Merck GF_{254}) coated on aluminum plates. The detection applied UV light and dipping of the plates into diluted sulfuric acid H_2SO_4:H_2O: EtOH (2:8:90) solution followed by heating. Compounds were purified by flush column chromatography on silica gel 0.035-0.070 µm using either hexane/ethyl acetate or toluene/isopropanol mixture in varying ratios. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and CD_3OD on JEOL JNM-LA 400, JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. Internal calibration of the spectra used the solvent signals, i.e. 7.26 ppm for ^{1}H-NMR and 77.0 ppm for ^{13}C-NMR in $CDCl_3$ and 3.30 ppm for ^{1}H-NMR and 49.0 ppm for ^{13}C-NMR in CD_3OD. 2D COSY, 2D Homo J-resolved, HMQC, and HSQC experiments were applied to assist the NMR assignments. High resolution mass-spectra were recorded on an Agilent 6530 Q-TOF mass spectrometer. Optical rotations were determined on a Jasco P-1020 digital polarimeter using a 10 cm cell.

Synthesis of n-dodecyl 2,3,4,6-tetra acetyl β-D-mannopyranoside 2.

To a stirred solution of β-peracetylated mannose (7.8 g, 20 mmol) and n-dodecanol (4.65 g, 25 mmol) in dichloromethane (120 mL), boron trifluoride diethyl etherate (4.3 g, 30 mmol) was added using a syringe. The solution was stirred for five hours at room temperature after which it was quenched with saturated sodium hydrogen carbonate solution. The aqueous phase was extracted twice with dichloromethane and the combined organic extracts were washed with water, dried over magnesium sulphate, filtered and evaporated to give the crude product. The crude material was then extracted using acetonitrile/hexane in order to remove the excess of alcohol. The evaporated acetonitrile solution provided the crude product (7.3 g, 81% yield).

Synthesis of n-dodecyl β-D-mannopyranoside 3.

The compound **2** (7.0 g, 36 mmol) was dissolved in anhydrous methanol (100 mL). A catalytic amount of sodium methoxide was added to the solution to maintain a basic medium. The reaction was stirred for two hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the methanol was evaporated, and the absence of ester groups was confirmed by IR spectroscopy. The crude material was extracted using 1-butanol/water in order to remove the unreacted sugar. The evaporated 1-butanol solution provided the glycoside in α/β-mixture form (4.2 g, 90% yield)

Synthesis of 4,6-O-benzylidene- β-D-mannopyranoside 4.

Compound **3** (2 g , 6 mmol) and benzyldehyde dimethyl acetal (1.35 g, 9 mmol) were mixed in Dichloromethane (100 mL). *p*-Toluenesulfonic acid (100 mg) was added , and the starting suspension became a clear solution after a short period of time (15-20 min.). The mixture was stirred until the TLC indicated absence of starting material. Water (100 mL) was added, and the organic phase was extracted with 20% sodium bicarbonate solution and then with water, dried over magnesium sulfate, and concentrated. The residue was stirred with hexane and a gel was formed. The gel was filtrated and washed successfully with hexane (5 x 5mL), to provide the pure β-anomer (1.7 g, 68 % yield) as a white solid.

$[\alpha]_D$ = - 39 (c = 0.2, $CHCl_3$). ^{1}H-NMR (400MHz, $CDCl_3$) δ = 7.51-7.28 (m, 5H; Ph), 5.54 (s; Ph-CH), 4.40 (d; H-1), 4.35 (dd; H-6eq), 3.92-3.77 (m,3 H; H-2, H-3 ,H-6ax), 3.60-a3.44 (m, 4 H, H-4, H-5,α-CH_2), 2.78 (d, 1 H, OH-3), 2.60 (d, 1 H, OH-

2),1.67-1.60 (m, 2H; β-CH_2), 1.32-1.23 (m, 18 H; bulk-CH_2), 0.88 (t, 3 H; CH_3); $^3J_{1,2}$ = 4.0, $^3J_{2,OH}$ = 2.4, $^3J_{3,OH}$ = 2.5, $^3J_{5,6eq}$ = 5.0, $^2J_6$ = 10.5 Hz.

Synthesis of 4,6-O-benzylidene-2,3-bis(cynomethyl)-β-D-mannopyranoside 5.

The compound **4** (2.6g, 6 mmol) was dissolved in a mixture of toluene (30 mL), 50% sodium hydroxide solution (20 mL) and tetrabutyl ammonium hydrogen sulfate (1.1 g, 3 mmol) and stirred at 10 ^oC for 30 minutes. Bromoacetonitrile (4 mmol) was added dropwise. The mixture was stirred for 2 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 could be crystallized or purified by column chromatography to give (2.5 g, 83% yield). $[\alpha]_D$ = - 28.0 (c = 1.0, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.51-7.28 (m, 5 H; Ph), 5.54 (s, 1H; Ph-CH), 4.55 (m_c, 4 H; CH_2CN), 4.47 (d, 1H; H-1), 4.37 (dd, 1H; H-6eq), 3.91 (dt, 1H; α-CH_2a), 3.81 (t, 1H; H-6ax), 3.74-3.64 (m, 2 H; H-3, H-4), 3.59 (dt, 1H, α-CH_{2b}), 3.43 (ddd, 1H; H-5) 3.37 (dd~t, 1H; H-2), 1.66 (m_c, 2H; β-CH_2), 1.39-1.24 (m, 18 H; bulk-CH_2), 0.88 (t, 3 H; CH_3).

$^3J_{1,2}$ = 7.5 Hz, $^3J_{2,3}$ = 9.5, $^3J_{3,4}$ = 9.1, $^3J_{4,5}$ = 9.5, $^3J_{5,6ax}$ = 10.0, $^3J_{5,6eq}$ = 5.0, $^2J_{6eq,6ax}$ =10.8 Hz.

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 136.63 (Ph-C), 129.35 / 128.41/ 126.08 (Ph-CH), 116.16/ 116.00 (2 C≡N), 102.76 (C-1), 101.51 (PhCH), 82.01 (C-2), 80.77 / 80.56 (C-3, C-4), 70.69 (α-CH_2), 68.53 (C-6), 65.67 (C-5), 57.78/ 57.73 (2 CH_2C≡N), 31.94 (β), 29.66 / 29.63 / 29.58 / 29.38 (bulk-CH_2), 25.97 (γ), 22.72 (ω-1), 14.18 (ω). HRMS: [M+Na] calcd. for $C_{29}H_{42}N_2O_6Na$: 537.2941,; found: 537.2934.

Synthesis of 4,6-O-benzylidene-2,3-bis(aminoethyl)-β-D-mannopyranoside 6.

Compound **5** (2.4 g, 4.6 mmol) was dissolved in tetrahydrofuran (50 mL) and cooled to 0^oC. Lithium aluminum hydride (0.74 g, 20 mmol) was added in small portions carefully in a 30 minute period. The mixture was stirred for 1 hour. Thin layer chromatography indicated complete conversion. Ethyl acetate (10 mL) was added and stirring was continued for 15 minutes to destroy excess $LiAlH_4$. The solid was

filtered and the solvent was evaporated to give the crude amine (2.2 g, 91 % yield), which was pure enough and used without further purification.

Synthesis of 4,6-O-benzylidene-2,3-bis(2-chloroacetylamidoethyl)-β-D-mannopyranoside 7.

Compound **6** (2 g, 3.8 mmol) was dissolved in dichloromethane (100 mL) and cooled to 0°C. Chloroacetic anhydride (1.7 g, 10 mmol) was added in small portions. The mixture was stirred for 2 hours at 0 °C and at room temperature overnight. Thin layer chromatography indicated complete consumption of starting materials. The mixture was successfully washed with saturated sodium bicarbonate solution followed by water. The organic layer was dried over magnesium sulfate, and evaporated to give the reasonable pure diamide (2.4 g, 93% yield), which was either used or purified by flash chromatography. $[\alpha]_D$ = +3.0 (c = 0.2, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.51-7.28 (m, 5 H; Ph), 7.05 (t , 1H , CONH), 7.02 (t , 1H , CONH), 5.25 (s, Ph-CH), 4.39 (d ; H-1), 4.32 (dd; 1H, H-6_{eq}), 4.05 (d, 2H, 2 $CH_{2a}Cl$), 3.95-3.73 (m, 7H, α-CH_{2a}, CH_2O, H-6_{ax}, $CH_{2b}Cl$), 3.65 (d, 1H, $CH_{2b}Cl$), 3.59-3.42 (m, 7H, H-3, H-4, α-CH_{2b}, CH_2N), 3.37(ddd, 1H, H-5), 3.21 (dd~t, 1H, H-2), 1.61 (m_c, 2H; β-CH_2), 1.34-1.22 (m, 18 H; bulk-CH_2), 0.88 (t, 3 H; CH_3).

$^3J_{1,2}$ = 7.8, $^2J_{6eq,6ax}$ =10.5 , $^3J_{6eq,5}$ = 10, $^3J_{6ax,5}$ = 5 , $^2J_{CH2aCl,\ CH2aCl}$ =1.8 ,15.0.

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 167.51, 167.45 (C=O), 137.1 (Ph-C), 129.44 / 128.54/ 126.21 (Ph-CH), 103.64 (C-1), 101.76 (Ph-CH), 82.27 (C-2), 81.19/ 80.90 (C-3, C-4), 71.50 (2 CH_2O), 70.63 (α-CH_2), 68.92 (C-6), 66.02 (C-5), 44.27/ 44.07 (2 CH_2Cl), 40.21/ 39.96 (2 CH_2N), 31.99 (β), 29.75 / 29.72 / 29.69 / 29.46/ 29.43 (bulk-CH_2), 26.05 (γ), 22.77 (ω-1), 14.21 (ω). HRMS: [M+Na] calcd. for $C_{33}H_{52}Cl_2N_2O_8Na$: 697.2998; found: 697.2989.

Synthesis of 4,6-O-benzylidene-2,3-thia(15-crown-5)-β-D-mannopyranoside 8.

Compound **7** (1.0 g, 1.4 mmol) was dissolved in ethanol (120 ml) and stirred at room temperature for 15 min. sodium sulfide nonahydrate (0.44 g, 2 mmol) was added and the mixture was heated gently to reflux for 1 hour followed by (0.11 g, 0.5 mmol) and continued refluxed for additional 3 hours. Thin layer chromatography showed there was no starting materials leftover, evaporate the ethanol. The remaining was dissolved in dichloromethane (50 mL) and washed with water, dried

over magnesium sulfate; after the solvent was evaporated, and flash chromatography was applied to get (0.8 g, 85%) as white semi solid.

$[\alpha]_D$ = -38.0 (c = 0.2, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 7.49-7.37 (m, 5 H; Ph), 6.88 (t , 1H , CONH), 6.79 (t , 1H , CONH), 5.56 (s, Ph-CH), 4.44 (d ; H-1), 4.37 (dd; 1H, H-6_{eq}), 4.11 (m_c, 1H, $CH_{2a}O$), 4.05 (m_c, 1H, $CH_{2a}O$), 3.92 (ddd, 1H, α-CH_{2a}), 3.87-3.77 (m, 3H, 2 $CH_{2b}O$, H-6_{ax}), 3.72-3.63 (m, 3H, H-4, 2 $CH_{2a}NH$), 3.61 (dd~t, 1H, H-3), 3.55 (ddd, 1H, α-CH_{2b}), 3.45-3.36 (m, 3H, H-5, 2 $CH_{2b}NH$), 3.35-3.21(m, 5H, H-2, CH_2S), 1.64 (m_c, 2H; β-CH_2), 1.33-1.24 (m, 18 H; bulk-CH_2), 0.90 (t, 3 H; CH_3).

$^3J_{1,2}$ = 7.8, $^2J_{6eq,6ax}$ =10.5 , $^3J_{6eq,5}$ = 10, $^3J_{6ax,5}$ = 5, $^2J_{\alpha\text{-}CH2a,b}$= 9.5, $^3J_{\alpha\text{-}CH2,CH2}$ = 6.5 , 2.5, $^3J_{H2,H3}$= 9.0, $^3J_{H3,H4}$= 9.5.

^{13}C-NMR (150 MHz, $CDCl_3$) δ = 167.85, 167.80 (C=O), 136.97 (Ph-C), 129.21 / 128.41/ 125.90 (Ph-CH), 104.01 (C-1), 101.20 (Ph-CH), 82.03 (C-4), 80.18 (C-2), 79.41 (C-3), 71.24/71.12 (2 CH_2O), 70.58 (α-CH_2), 68.69 (C-6), 65.81 (C-5), 39.66/ 39.54 (2 CH_2NH), 36.52/ 36.49 (2 CH_2S), 31.92 (β), 29.71 / 29.65 / 29.62 / 29.58/ 29.36 (bulk-CH_2), 26.07 (γ), 22.73 (ω-1), 14.16 (ω). HRMS: [M+Na] calcd. for $C_{33}H_{52}N_2O_8SNa$: 659.3342,; found: 659.3337.

Synthesis of 4,6-O-benzylidene-2,3-thia(15-crown-5)-β-D-mannopyranoside 9.

Compound **7** (1.0 g, 1.4 mmol) was dissolved in suspension of acetonitrile (100 ml) and sodium carbonate (1.05 g, 10 mmol). Benzyl amine (0.16 g, 1.5 mmol) was added and the mixture was heated gently to reflux for 12 hours. Thin layer chromatography showed there was no starting materials remained, the mixture was cooled down to room temperature, filtered and the solvent was evaporated under reduced pressure. The remaining was dissolved in dichloromethane (50 mL) and washed with water, dried over magnesium sulfate; after the solvent was evaporated flash chromatography was applied to get (0.68 g, 65%) as a yellow syrup. $[\alpha]_D$ = -47.0 (c = 0.2, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.45-7.25 (m, 10 H; Ph), 7.21 (br. , 1H , CONH), 7.16 (br., 1H , CONH), 5.55 (s, Ph-CH), 4.45 (d ; H-1), 4.36 (dd; 1H, H-6_{eq}), 4.21-4.11 (m, 2H, $CH_{2a}O$), 3.91 (ddd, 1H, α-CH_{2a}), 3.80 (dd~t, 1H, H-6_{ax}), 3.72-3.56 (m, 8H, H-3, H-4, 2 $CH_{2b}O$, Ph-CH_2N, 2 $CH_{2a}NCO$), 3.54 (ddd, 1H, α-

CH_{2b}), 3.42 (ddd, 1H, H-5), 3.27 (dd~t, 1H, H-2), 324-3.13 (m, 6H, 2 $CH_{2b}NHCO$, CH_2N), 1.62 (m_c, 2H; β-CH_2), 1.37-1.15 (m, 18 H; bulk-CH_2), 0.88 (t, 3 H; CH_3).

$^3J_{1,2}$ = 7.8, $^2J_{6eq,6ax}$ =10.5 , $^3J_{6eq,5}$ = 10, $^3J_{6ax,5}$ = 5, $^2J_{\alpha\text{-}CH2a,b}$= 9.5, $^3J_{\alpha\text{-}CH2,CH2}$ = 6.5 , 2.5.

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 169.65 (2 C=O), 137.01/ 136.55 (2 Ph-C), 129.24 / 129.13/ 129.02/ 128.46/ 128.24/ 125.95 (Ph-CH), 104.11 (C-1), 101.20 (Ph-CH), 82.22 (C-4), 80.70 (C-2), 79.98 (C-3), 71.32/71.13 (2 CH_2O), 70.53 (α-CH_2), 68.81 (C-6), 65.80 (C-5), 60.40 ($PhCH_2N$), 59.28/ 59.10 (2 CH_2CONH), 39.20/ 39.18 (2 CH_2NH), 31.98 (β), 29.72 / 29.66 / 29.45 / 29.35 (bulk-CH_2), 26.15 (γ), 22.77 (ω-1), 14.21 (ω). HRMS: [M+Na] calcd. for $C_{40}H_{59}N_3O_8Na$: 732.4200; found: 732.4194.

2- Antibacterial assay (Zone of inhibition)

Antibacterial activity was assayed by disc diffusion method described by Nongponga et al [16]. The concentration of a certain macrocycle was prepared by diluted with DMSO (Dimethylsulfoxide), and then with sterile distilled water. For each of the calture media exact procedure was applied to produce fresh bectria in this study. The organisms were spread on caltural media plates by cotton swab, wells of 6 mm diameter were pouched into the agar medium filled with 50µl of different macrocycle solutions. The plates were incubated for 24 h at 37°C and antibacterial activity was evaluated by measuring the inhibition zone diameter against the test organism.

Conclusion:

In summary, we synthesis new mix-heteroatoms macrocycles on mannopyranoside glycolipids. The overall yield was reasonable. The non-sugar analogues macrocycles were showed a high toxicity towards bacteria, while the antibacterial activity was low for most of the macrocycles on glycolipids due to the biocompatibility of these compounds with the living organisms.

Acknowledgement:

The author thank the staff of NMR / mass spectra labs at university of Malaya for helping to record NMR and MS spectra.

References:

1. Lehn, J. M. Cryptates: inclusion complexes of macropolycyclic receptor molecules. *Pure Appl. Chem.* **50,** 871–892 (1978).
2. Fraser, J. Chiral Crowns Incorporating Pyridyl Units and Tertiary Amine Functions. 979–980 (1976).
3. Pedersen, C. J. Cyclic polyethers and their complexes with metal salts. *J. Am. Chem. Soc.* **89,** 7017–7036 (1967).
4. Planar, H. Chiral Recognition with Crown Ethers. **32,** 8–9 (2003).
5. Lehn, J. M. Cryptates: the chemistry of macropolycyclic inclusion complexes. *Acc. Chem. Res.* **11,** 49–57 (1978).
6. Smithrud, D. B., Wang, X., Tarapore, P. & Ho, S.-M. Crown Ether Host-Rotaxanes as Cytotoxic Agents. *ACS Med. Chem. Lett.* **4,** 27–31 (2013).
7. Supek, F. *et al.* Could LogP be a principal determinant of biological activity in 18-crown-6 ethers? Synthesis of biologically active adamantane-substituted diaza-crowns. *Eur. J. Med. Chem.* **46,** 3444–54 (2011).
8. Bakó, P. & Tőke, L. Novel monoaza-and diazacrown ethers incorporating sugar units and their extraction ability towards picrate salts. *J. Inclus. Phenom. Mol.* **23,** 195–201 (1995).
9. Coxon, A. & Stoddart, J. F. Synthesis of a [2]-cryptand with carbon bridgeheads. *Carbohydr. Res.* **44,** 1–4 (1975).
10. Bakó, P. *et al.* Alkai Metal- and Ammonium Picrate Extraction and Complex Forming Capabilities of d-Glucose and d-Mannose-based Lariat Ethers. *J. Incl. Phenom. Macrocycl. Chem.* **55,** 295–302 (2006).
11. Bako, P. *et al.* Synthesis, extraction ability and application in an asymmetric synthesis of aza-crown ethers derived from D-mannitol. *J. Incl. Phenom. Macrocycl. Chem.* **39,** 247–251 (2001).
12. Julian, R. R. & Beauchamp, J. L. The unusually high proton affinity of aza-18-crown-6 ether: implications for the molecular recognition of lysine in peptides by lariat crown ethers. *J. Am. Soc. Mass Spectrom.* **13,** 493–8 (2002).
13. Bakó, T. *et al.* Enantioselective Michael addition of 2-nitropropane to chalcone analogues catalyzed by chiral azacrown ethers based on α-d-glucose and d-mannitol. *Tetrahedron: Asymmetry* **14,** 1917–1923 (2003).
14. Podder, S. K., Chakraborti, A., Vijayalakshmi, K. & Singh, P. L. K. Reactivity of glycoconjugate in membrane system II: Can a neutral glycolipid function as lectin receptor in the presence of gangliosides in plasma membrane? *J. Biosci.* **11,** 495–502 (1987).
15. Sabah, K., Heidelberg, T. & Hashim, R. Novel crown ethers on glucose based glycolipids. *Carbohydr. Res.* **346,** 891–6 (2011).
16. Sabah, K. J. & Hashim, R. Novel mixed-heteroatom macrocycles via templating: A new protocol. *Tetrahedron Lett.* **54,** 1534–1537 (2013).
17. Poláková, M., Belánová, M., Petrus, L. & Mikusová, K. Synthesis of alkyl and cycloalkyl alpha-d-mannopyranosides and derivatives thereof and their evaluation in the mycobacterial mannosyltransferase assay. *Carbohydr. Res.* **345,** 1339–47 (2010).
18. Sharma, I., Bohé, L. & Crich, D. Influence of protecting groups on the anomeric equilibrium; case of the 4,6-O-benzylidene acetal in the mannopyranose series. *Carbohydr. Res.* **357,** 126–31 (2012).
19. Höistad, M., Chen, K. C., Nicholson, C., Fuxe, K. & Kehr, J. Quantitative dual-probe microdialysis: evaluation of [3H]mannitol diffusion in agar and rat striatum. *J. Neurochem.* **81,** 80–93 (2002).
20. Atkins, J. L., Patel, M. B., Cusumano, Z. & Gokel, G. W. Enhancement of antimicrobial activity by synthetic ion channel synergy. *Chem. Commun. (Camb).* **46,** 8166–7 (2010).

تحضير ايثرات تاجية محتوية على الكبريت والناتروجين مشتقة من السكريات الدهنية للمانوز ونظيراته ودراسة فعاليتها كمضادات بكتيرية

كريم جابر صباح

قسم الكيمياء- كلية العلوم – جامعة الكوفة -٥٤٠٠١ النجف الاشرف – العراق

دكتوراه ، مدرس

الخلاصة:

حضرت اثنتان من الايثرات التاجية المحتوية على ذرات كبريت وناتروجين مشتقة من السكريات الدهنية للمانوز، وتم تشخيصها بواسطة اطياف الرنين النووي المغناطيسي(البروتون والكاربون ١٣)، طيف الكتلة عالي الدقة واطياف الاشعة تحت الحمراء. تمت دراسة الفعالية ضد اربعة من البكتريا للمركبات المحضرة، اثنتين كرام موجب واثنتين كرام سالب في وسطين زرعيين.

الكلمات المفتاحية : الحلقات الكبيرة ، مانوز، سكريات دهنية، مضادات بكتيرية، ايثرات تاجية.