Synthesis of some new 1,2,3-triazoles derivatives of D-mannose via click chemistry and evaluation of their effect on urease enzyme

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***Abstract*—In this research some new 1, 2, 3-triazole derivatives of mannose were synthesized by click chemistry .The synthesis was started by the reaction of protected mannofuranose with propargyl bromide to form propargylic derivative of that sugar. The second step of synthesis was performed by preparation of aryl azide derivatives from diazoniume salts by treating them with sodium azide . Finally reaction of propargylic ether of mannose with aryl azide derivatives in click condition was gave triazole derivatives containing carbohydrate moiety. The biological activity of synthesized compounds were tested against urease activity. Some of these compounds (Tr2, Tr3) show high inhibition action on the enzyme activity, while (Tr1) do not show any effect on the activity.**

Keywords*— *Triazoles , D-mannose ,click chemistry , urease.

I. INTRODUCTION

Carbohydrates are one of the most important classes of biomolecules along with nucleic acids, proteins and lipids [1]. Synthesis of some new heterocyclic containing a carbohydrate moiety has great interest because of the possibility to obtain nucleosides and their analogues, which have, in some cases, therapeutic importance [2]. Mannose is a monosaccharide of the aldohexose series of carbohydrates, it is a C-2 epimer of glucose [3]. It's particularly present in fruits and vegetables such as blueberries, apples and in green beans, [4-6]. "Click Chemistry" is a term which was described by K. B. Sharpless of the Scripps Research Institute in 2001 to the reactions that afford products in high yields and in excellent selectivities by carbon-hetero atom bond formation reactions [7]. The term "Click" means joining molecular pieces as easily as clicking together the two pieces of a seat belt buckle [8]. Click chemistry is widely used in biosciences [9-11], drug discovery [12] and material science [13]. Triazole is an aromatic five-membered heterocyclic system consisting of two carbon atoms and three nitrogen atoms including one pair of isomeric chemical compounds: 1,2,3-triazole and 1,2,4-triazole [14] . Some of triazoles derivatives act as therapeutic agent used as Antifungal [15], Anti-bacterial [16], Anti-inflammatory [17], and Anti-cancer [18] and as Analgesic [19].

II. EXPERIMENTAL PART

(A) Materials

- Chemical reagents and starting materials were obtained from Ajax and Sigma-Aldrich Chemical.
- Instrumentations
- FT-IR spectra were recorded by using Fourier transformation infrared Shimadzu FT-IR-8400S infrared spectrophotometer by KBr disc, Faculty of Pharmacy University of Kufa.
- 1H NMR, 13C NMR were recorded by Bruker spectrometer, operating at (400MHZ) with (DMSO-d6). Measurements were made at Faculty of Science, Osmania University, India.
- TLC plates were used with an aluminum backing (0.2 mm, 60 F254).

(B) Synthesis of aryl azide derivatives (general procedure) (A1-A3) [20]

An aniline derivative (1 eq) was dissolved in 10 mL of dilute HCl in a Round Bottomed flask. Reaction mass was cooled to (-10°C to -5°C). Sodium nitrite (1.2 eq) was added in small portions (4 portions) to the reaction mass by maintaining the temperature at -10°C to 0°C and maintained the reaction for 10 min. A solution of sodium azide (1.2 eq) was added in a drop wise manner to the reaction mixture at 0°C. The Reaction mixture was stirred for 10 min. at 0°C. The product was extracted by using chloroform followed by washing with water up to neutral pH. Organic layer was dried with anhydrous sodium sulfate and then the solvent removed to yield aryl azide derivatives. The material was used without further purification.

(C) Synthesis of 1-O-propargyl-2, 3:5, 6-Di-O-isopropylidene α-D-mannofuranoside (A) [8]

2,3:5,6-Di-O-isopropylidene-α-D-manno furanose (1) (5.205 g, 20 mmol) was dissolved in DMF (30 mL) in a dry flask and to this solution was added crushed NaOH (1.6 g, 40 mmol). The contents stirred for (10 min) before propargyl bromide (2.85, 24mmol) was added dropwise. The reaction mixture was then allowed to stir for a further (24h.), gradually warming to rt. The reaction mixture was quenched with distilled water (50 mL) and extracted with diethyl ether (3×100 mL). The combined organic layer was washed with sat. NH4Cl (3×30 mL), distilled water, dried over Na2SO4, filtered and the solvent was evaporated to dryness under reduced pressure to yield a pale yellow oil (36) (85%), Rf = (0.67), (2:1) (hexane: EtOAc).

(D) Synthesis of 1, 2, 3-triazole derivatives of sugar via click chemistry (general procedure) (Tr1-Tr3) [22]

A solution of propargyl ether (1.0 eq) in DMF (5mL) was added to the suspension of sodium ascorbate (1.2 eq) and CuSO4.5H2O (1.2 eq) in DMF (4mL).The mixture was stirred for (10 min) and to this was added an aryl azides derivatives (1.2 eq). The mixture was heated to 50°C with stirring for (10-48 h.). The reaction mixture was diluted with distilled water (30 mL), extracted with EtOAc (3×30 mL), the combined organic layers were washed with sat. NaCl (2× 20 mL), dried over Na2SO4, and evaporated to dryness under reduced pressure. The residue was flash chromatographed (silica gel, n-Hexane: Et2O) to yield the desired compounds as a yellow syrup (Z1 – Z3). (60-80) %.

(E) studying the biological activity of synthesized compounds [23]

(1) Reagents for Urease activity:

The following reagents which supported form—biomerieux ®,, urea kit.

(a) Phosphate buffer PH=8 with concentration 50mM, sodium salicylate (26mM), sodium nitroprosside (3.35mM), EDTA (1mM)

(b) Sodium hydroxide (0.5mM) and sodium hypochloride (24.8mM).

(2) Phosphate Buffer (PH 7)

0.5L of 1M K2HPO4 at 174.18 g mol-1 = 87.09g.
0.5L of 1M KH2PO4 at 136.09 g mol-1 = 68.045g.
Preparation of 0.1 M potassium phosphate buffer at 25°C.

(3) Estimation of Urease Activity:

Urease activity is estimated by using end point method for the formation of ammonia per minutes. Urease catalyzed degradation of urea results in the formation of ammonia, which is determined by the Berthelot method (according to method of urea kit). The assay is simple, sensitive, stable and high-throughput adaptable the steps of the method are as follows:

(a) The blank of reaction consist of five hundred microliters of 1.65mg/ml of free or immobilize or dispersion jack bean urease (dispersion is solution from urease at concentration 1.65 was mixed with 2mg/ml of synthesized compounds) were mixed with a of solution which contain phosphate buffer PH 8 with concentration of 50 mM, sodium salicylate 26mM, sodium nitroprusside 3.35mM, EDTA 1mM).

(b) Twenty microliters of one concentration of urea (50 mg/dl) were mixed with 1 ml of blank, (enzyme and buffer) and then incubated for 5 minutes at room temperature.

(c) Two hundred microliters from alkaline reagent (consist of sodium hydroxide 0.5mM, sodium hypochloride 24.8mM) were added and the mixture incubated for 5 minutes, the absorbance was measured at 580 nm by spectrophotometer.

(d) Calibration curve was obtained from the absorbance of different concentration of ammonium sulfate

(e) Urease activity was determined through measurement released ammonia per minute at room temperature and PH 7.

III. RESULTS AND DISCUSSION

(A) Synthesis of aryl azide derivatives

Compounds (A1-A3) were synthesized by treatment of an aromatic amines derivatives with hydrochloric acid and sodium nitrite to from diazoniume salts at (0-5)^{0}C, followed by reaction of diazoniume salt with sodium azide at the same temperature.

FT-IR spectra of compounds (A1-A3), figures (8-14) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr) summarized in table (1).

TABLE I. FT-IR AND PERCENTAGE YIELD OF COMPOUNDS (A1-A3)

compounds	FT-IR bands / cm $^{-1}$	Yield %
Z1	3032 (υ C-H aromatic),2135,2100(υ N=N=N of two azide groups) , 1602(υ C=C aromatic)	60 %
Z2	3375(υ O-H of phenol), 3040 (υ C-H aromatic),2115 (υ N=N=N of two azide groups) , 1600(υ C=C aromatic),1301(υ C-O of phenol).	55%
Z3	3525(υ O-H of phenol), 3059 (υ C-H aromatic),2114 (υ N=N=N of two azide groups) , 1599(υ C=C aromatic),1303(υ C-O of phenol).	

(B) 3-2- Synthesis of 1-O-propargyl-2,3:5,6-Di-O-isopropyli-dene α-D-mannofuranoside (A)

Williamson etherification of 2, 3:5, 6-Di-O-isopropyli-dene α-D-mannofuranoside with propargyl bromide in DMF as solvent and the presence of basic media (NaOH) produced the terminal alkyne (1) in very good yield (78%)

FT-IR spectrum of compound (A) fig (1) showed the following bands at $\bar{\upsilon}$ cm^{-1}(KBr): 3267 ($\upsilon_{C-H,}$ **alkyne**), 2987($\upsilon_{C-H,}$ **CH$_3$**), 2941($\upsilon_{C-H,}$ **CH$_2$**), 2893($\upsilon_{C-H,}$ **CH**), 2121(**υ** $_{C\equiv C}$), 1452($\delta_{as.C-H,}$ **CH$_3$**), 1377($\delta_{s.C-H,}$ **CH$_3$**), 1216,1261($\upsilon_{C-O,}$ **C–O–C**), 1078($\upsilon_{C-O,}$ **C–O-H**).

FT-IR spectrum is a good evidence that the reaction happened successfully by disappearing the band at 3435 cm^{-1} and appearing sharp bands at (3253, 2115) cm^{-1} attributed to the terminal alkyne (C–H and C≡C) respectively.

^{1}H NMR spectrum,(2) (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.24, 1.26, 1.33, 1.35 (s, 12H, 4C***H*$_3$ isopropylidene**), 3.47 (t, J 2.4 Hz of C≡C **-H**),3.81 (dd, J7.2, 3.6 Hz, 1H, of C4-H), 3.88 (dd, *J 8.4*, 5.2 Hz, 2H, of C**H**$_2$-C≡C), 3.99 (d, *J* 6.4 Hz, 2H, 2 of C6-H), 4.22 (t, *J* 3.3 Hz, 1H, of C3-H), 4.43 (t, *J* 4.0 1H, of C2-H), 4.73 (dd, *J* 5.6,3.2 1H, of C2-H), 5. 70 (1H, of C1-H),

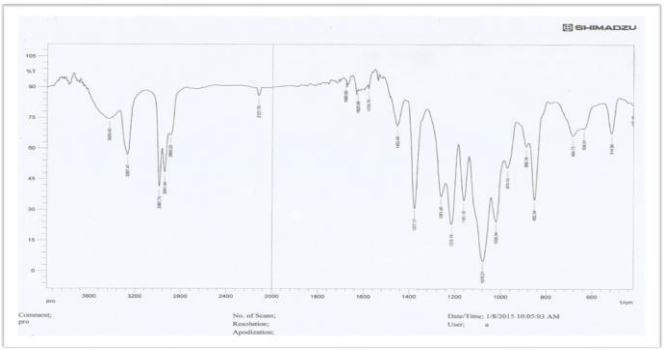


Fig. 1. FT-IR spectrum of compound (A)

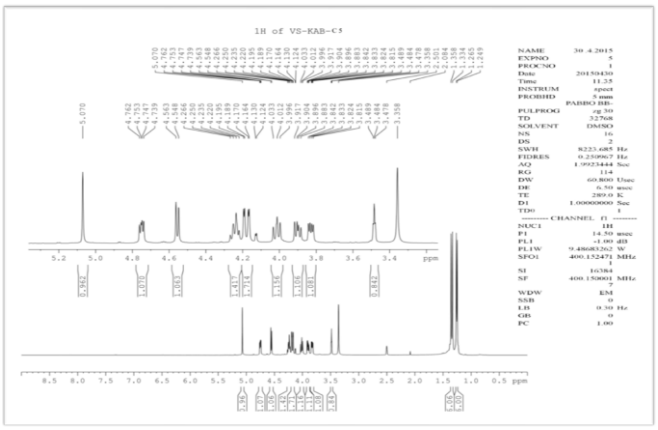


Fig. 2. ^{1}H NMR spectrum of compound (A)

(C) Synthesis of 1-O{(4,4-biphenyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3:5,6-Di-O-isopropylidene α-D-mannofuranoside (Tr1)

Compound (Tr1) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether D-mannofuranoside (A) and 4,4'-diazido-1,1'-biphenyl (A1) to produce a very good yield

FT-IR spectrum fig. (3) of compound (Tr1) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr): 3183 ($\upsilon_{C-H,}$ **triazole**), 3097 ($\upsilon_{\mathbf{C-H\ of\ benzene}}$) 2987($\upsilon_{C-H,}$ **CH_3**), 2937($\upsilon_{C-H,}$ **CH_2**), 1641($\upsilon_{C=C}$, **aromatic**), 1410($\delta_{as.C-H,}$ **CH_3**), 1377 ($\delta_{s.C-H,}$ **CH_3**), 1261($\delta_{C-H\ aromatic,}$), 1211, ($\upsilon_{C-O,}$ **C–O–C**), 1161 (υ_{C-N}), 1089 (**υ_{C-O}**).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the bands around υ (2100, 2135,2121, 3267) cm^{-1} which is attributed to (-N3 ,**C≡C and** $\upsilon_{C-H,}$ **alkyne**) respectively .

^{1}H NMR spectrum fig. (4), (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.24, 1.26, 1.32, 1.36 (s, 12H, 4C***H_3*** **isopropylidene**), 3.81 (m, 1H, of C4-H), 3.90 (d, *J* 6.4 Hz, 2H, 2 of C6-H), 3.99 (m, 1H, of C3-H), 4.24 (m, 1H, of C2-H), 4.52 (m 1H, of C2-H),4.66(m,2H of O-CH_2 of triazole) 5.17 (1H, of C1-H),7.99 -8.93(m , of aromatic and triazoles) .

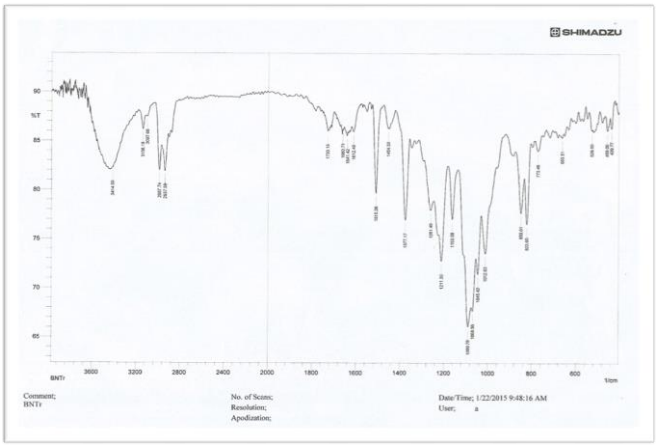


Fig. 3. FT-IR spectrum of compound (Tr1)

^{13}C NMR spectrum fig. (5), (100 MHz, DMSO-d_6) showed the following signals at δ (ppm): 24.19, 25.21[(2 × C(***C***H_3)$_2$], 62.36 (1C of C6), 64.67 (1C of C5), 76.17 (1C of C3),78.26(1C of OCH2C=C of triazole) 79.09 (1C of C4), 83.67 (1C of C2), 104.79 (1C of C1), 112.25,115.54[(2 × C(CH3)2],116.43,122.63,130.36,130.77,137.96,138.49,146.61 (m C of aromatic carbon of benzene and triazole ring).

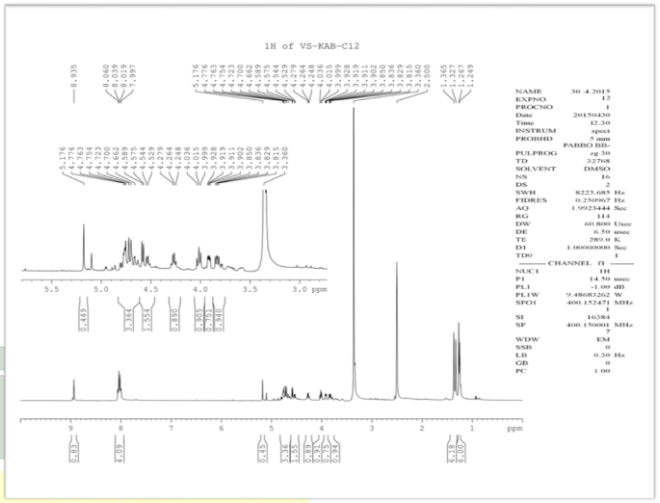


Fig. 4. ^{1}H NMR spectrum of compound (Tr1)

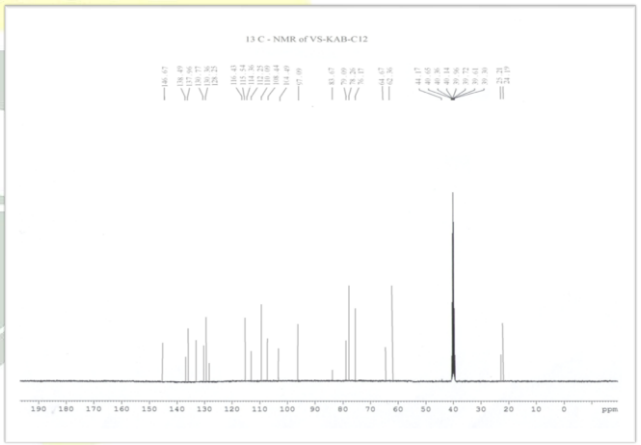


Fig. 5. ^{13}C NMR spectrum of compound (Tr1)

(D) Synthesis of 1-O{(4-hydroxyphenyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3:5,6-Di-O-isopropylidene α-D-mannofuranoside (Tr2)

Compound (Tr2) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether D-mannofuranoside (A) and 4-azidophenol (A2) to produce a very good yield

FT-IR spectrum fig. (6) of compound (Tr2) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr):3415($\upsilon_{\mathbf{O-H}}$ of phenol)3151 ($\upsilon_{C-H,}$ **triazole**), 3097 ($\upsilon_{\mathbf{C-H\ of\ benzene}}$) 2985($\upsilon_{C-H,}$ **CH_3**), 2941($\upsilon_{C-H,}$ **CH_2**), 1606($\upsilon_{C=C}$, **aromatic**), 1521($\delta_{as.C-H,}$ **CH_3**), 1379 ($\delta_{s.C-H,}$ **CH_3**), 1253($\delta_{C-H\ aromatic,}$), 1213, ($\upsilon_{C-O,}$ **C–O–C**), 1159 (υ_{C-N}), 1066 (**υ_{C-O}**).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the

bands around υ (2115, 2121, 3267) cm^{-1} which is attributed to (-N3 ,**C≡C and** $\upsilon_{C-H,}$ **alkyne**) respectively .

^{1}H NMR spectrum fig. (7), (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.22,1.23,1.24, 1.25, 1.31, ,1.35 (4C***H*****$_3$ isopropylidene**) 3.48 (m, 1H, of C4-H), 3.81 (m, 2H, 2 of C6-H),4.12(m ,1H of O-H phenol), 4.16 (m, 1H, of C3-H), 4.22 (m, 1H, of C2-H), 4.54 (m 1H, of C2-H),4.73(m,2H of O-CH_2 of triazole) 4.76 (1H, of C1-H),6.73 -7.29(m , of aromatic and triazoles).

^{13}C NMR spectrum fig. (8), (100 MHz, DMSO-d_6) showed the following signals at δ (ppm):12.16(C of ethyl group) 24.11, 25.29[(2 × C(***C***H_3)$_2$], 62.36 (1C of C6), 64.64 (1C of C5), 76.19(1C of C3),78.26(1C of OCH2C=C of triazole) 79.01 (1C of C4), 83.61 (1C of C2), 108.24 (1C of C1), 114.36,115.64[(2 × C(CH3)2],130.36,130.46,130.36, 148.61, 157.11 (m C of aromatic carbon of benzene and triazole ring).

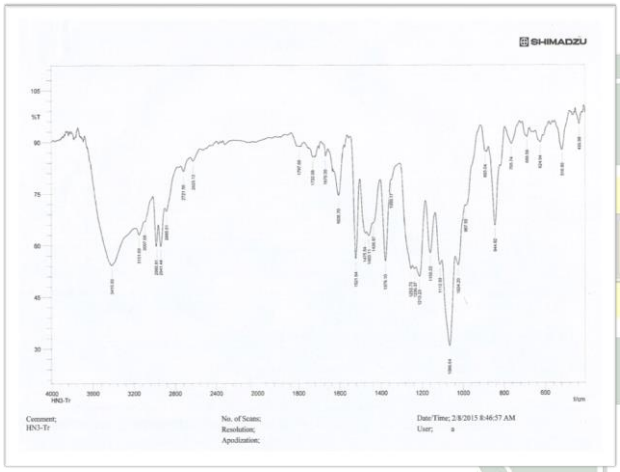


Fig. 6. FT-IR spectrum of compound (Tr2)

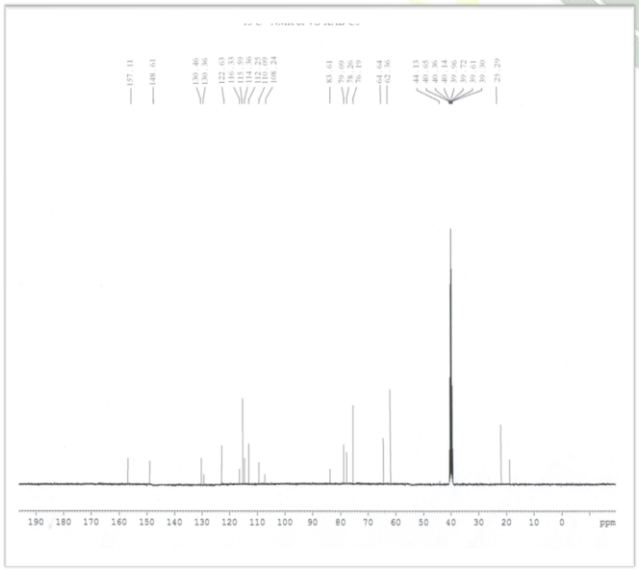


Fig. 7. 1H NMR spectrum of compound (Tr2)

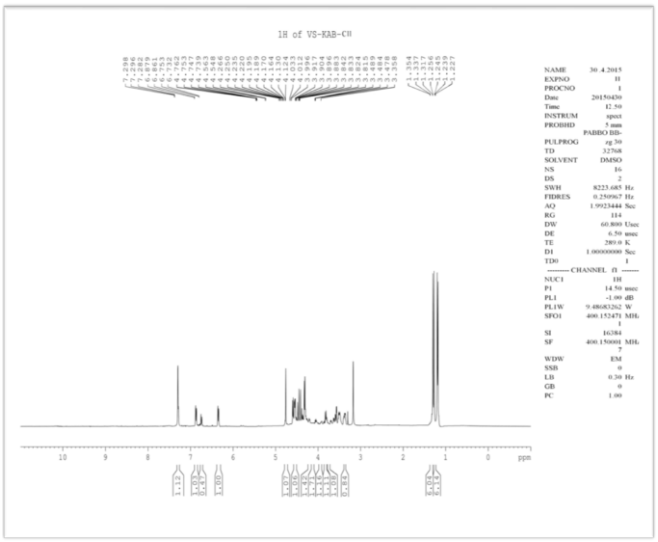


Fig. 8. 13C NMR spectrum of compound (Tr2)

(E) Synthesis of 1-O{(3-hydroxyphenyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3:5,6-Di-O-isopropylidene α-D-mannofuranoside(Tr3)

Compound (Tr3) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether D-mannofuranoside (A) and 23-azidophenol (A3) to produce a very good yield

FT-IR spectrum fig. (9) of compound (Tr3) showed the following bands at cm-1 (KBr):3444(υ O-H of phenol)3136 (υC–H, triazole), 3095 (υC–H of benzene) 2987(υC–H, CH3), 2935(υC–H, CH2), 1612(υ , aromatic), 1508(δas.C–H, CH3), 1375 (δs.C–H, CH3), 1257(δ C–H aromatic,), 1215, (υC–O, C–O–C), 1163 (υC–N), 1080 (υC–O).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the bands around υ (2114, 2121, 3267) cm-1 which is attributed to (-N3 ,C≡C and υC–H, alkyne) respectively .

1H NMR spectrum fig. (10), (400 MHz, DMSO-d6) for the compound showed the following signals at d (ppm): 1.22,1.23,1.24, 1.25, 1.31, ,1.35 (4CH3 isopropylidene) 3.47 (m, 1H, of C4-H), 3.81 (m, 2H, 2 of C6-H),4.12(m ,1H of O-H phenol), 4.16 (m, 1H, of C3-H), 4.22 (m, 1H, of C2-H), 4.54 (m 1H, of C2-H),4.73(m,2H of O-CH2 of triazole) 4.76 (1H, of C1-H),6.73 -7.29(m , of aromatic and triazoles).

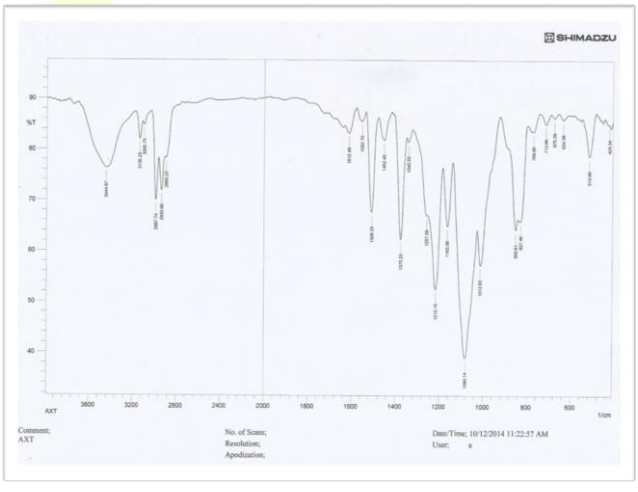


Fig. 9. FT-IR spectrum of compound (Tr3)

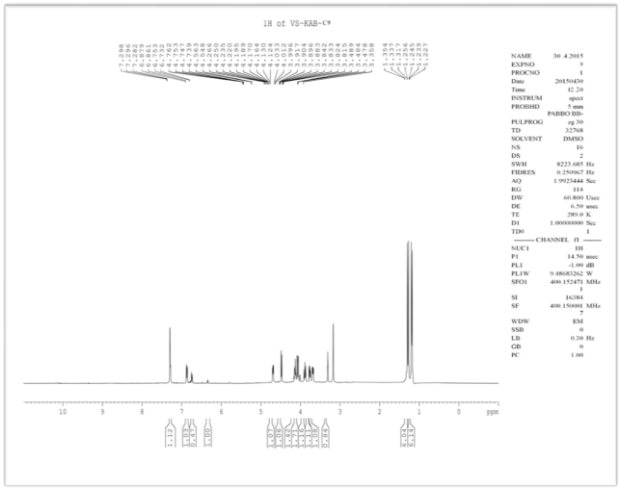
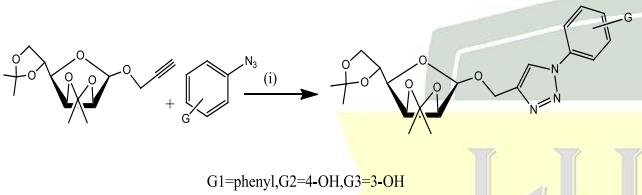


Fig. 10. ^{1}H NMR spectrum of compound (Tr3)



G1=phenyl,G2=4-OH,G3=3-OH

(i) Na Ascorbate , $CuSO_4$.$5H_2O$, DMSO , 5 ^{0}C ,(10 - 48) hr

Scheme (1) Synthesis of 1,2,3-triazole derivatives of sugars

(F) Studying the biological activity of synthesized compounds

The biological activity of the synthesized compounds were tested against the activity of urease enzyme (which is catalyze urea to ammonia and carbon dioxide). The enzyme was immobilize on each one of synthesized compound by taking one concentration of each compound with enzyme

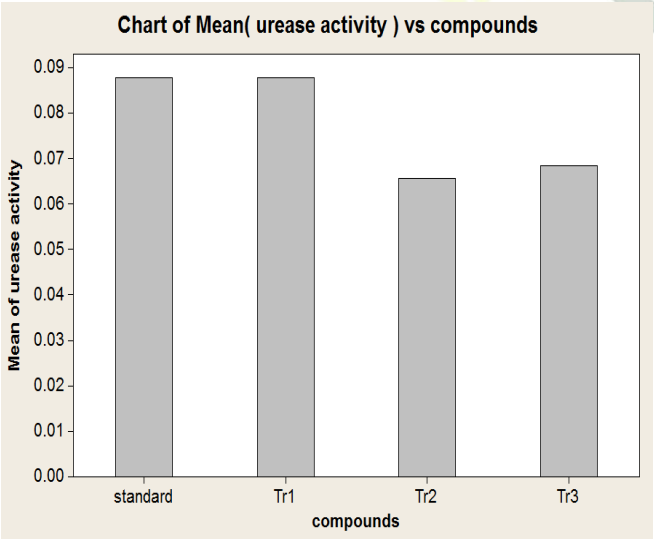


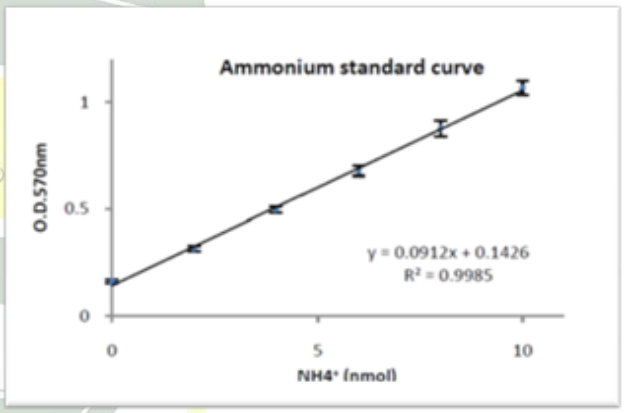
Fig. 11. The effect of synthesized compounds on urease activity

and stirring the mixture for 10 min. The triazoles derivatives show different inhibition action toward the enzyme shown in figure (11)

TABLE II SHOW THE AMOUNT OF LIBERATED AMMONIA AND ENZYME ACTIVITIES

	Name of compound	Absorbance	Ammonia concentration	Urease activity
1	standard	0.180	0.439	0.0878
2	Tr1	0.180	0.439	0.0878
3	Tr2	0.170	0.329	0.0658
4	Tr3	0.172	0.340	0.0680

* The amount of liberated ammonia were calculate from relationship
(ammonia concentration = Absorbance-0.41/0.091) which is obtain from calibration curve figure (12) .
*The enzyme activity were calculate by relationship
(Urease activity = amount of liberated ammonia /time) .
The time was 5 min



*

Fig. 12. calibration curve for librated ammon

Some of these compounds (Tr2,Tr3) show high inhibition action on the enzyme , while (Tr1)do not show any effect on the activity .

The activities of enzyme with different compound were calculated from the amount of liberated ammonia per 5 min as shown in table [2].

TABLE III. SHOW THE AMOUNT OF LIBERATED AMMONIA AND ENZYME ACTIVITIES

	Name of compound	Absorbance	Ammonia concentration	Urease activity
1	standard	0.180	0.439	0.0878
2	Tr1	0.180	0.439	0.0878
3	Tr2	0.170	0.329	0.0658
4	Tr3	0.172	0.340	0.0680

- The amount of liberated ammonia were calculate from relationship
  (ammonia concentration = Absorbance-0.41/0.091) which is obtain from calibration curve figure (12) .
- The enzyme activity were calculate by relationship
  (Urease activity = amount of liberated ammonia /time) .
- The time was 5 min

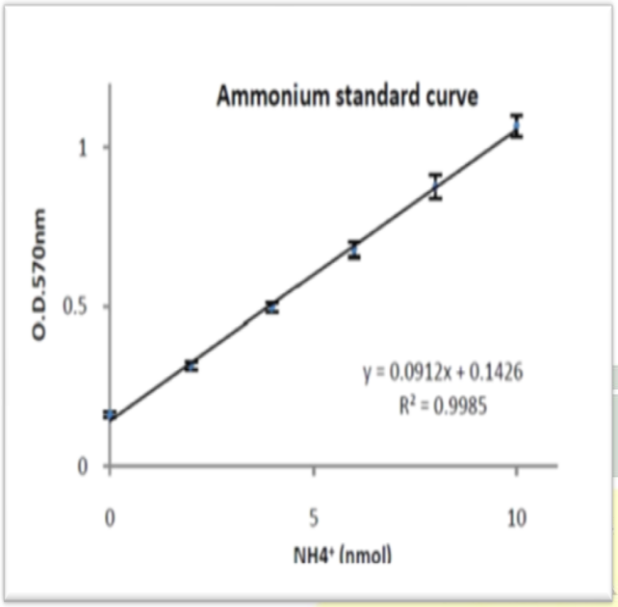


Fig. 13. Calibration curve for liberated ammonia

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