

Synthesis of Some Streoregular Polydiacetylenes and the Slow Release of Drug Using Some Linear Polymers

*Mohammad S. Al-Ajely, A. Y. Sharif and A. A. Qasim
Chemistry Dept, College of Education, Mosul University, Mosul-Iraq
Biology Dept, College of Science, Mosul University, Mosul-Iraq
Email: mohamadalajelee@yahoo.com

ABSTRACT

Some polydiacetylenes have been synthesized using UV light in topochemical polymerization technique. Some diacetylene compounds were reacted with benzocaine and procaine in an attempt to be used as biosensors in topochemical polymerization process (In vivo). The linear (Amorphous) polymer was allowed to react with some commercial antibiotics and were studied as slow drug release polymers. Externally treatment of the drug containing polymers to groups of infected rabbits revealed that these polymers are effective against certain types of micro organisms.

Keywords: Polydiacetylenes, Slow release of drug, Linear polymers

1. INTRODUCTION

It was found that the benzoxazane containing diacetylene monomers can be polymerize at low temperature giving thermally stable ploy diacetylenes¹⁻² has succeed in preparing ploy diacetylenes using jell method in preparing organojell in presence of zinc ion. The resulted spiral fibers were found to change their color according to temperature change in various solvents

Stanish and his coworkers³ used versatile methods to prepare ploy diacetylenes containing lipid moieties. These polymers were found stable in buffer solution and were used as biosensors. Usually the ploy diacetylene components in order to be used as biosensors must have hydrophilic groups like lipid, enzyme or antibiotic as a terminal group to the diacetylene monomer which can be form bilayer upon polymerization similar to the blood cell while the hydrophobic group represent the alkyl backbone of the polymer⁴. Some amino acid containing polymers were prepared from the reaction of stearic acid with 10,12-pentacosadioic acid⁵. Jung and his coworkers⁶ have prepared some ploydiacetylene from the reaction of biotin with 10, 12-pentacosadioic acid for Chlamydia diagnosis of many diseases. Condensation polymers themselves were found to have versatile biological applications⁷⁻⁸. Donaruma and his coworkers⁹ have succeeded in preparing some linear polymers from trobolon and formaldehyde. These polymers were found to have antibacterial activity. Some poly acrylic, methacrylic phenoxarsnes were found to have remarkable activity against several types of bacteria¹⁰. Some maleic anhydride, N-vinylpyrrolidone and vinyl butyl ether were found to have antimicrobial activities¹¹⁻¹².

Other researchers¹³ studied the physical bounding between linear polymer and some additive materials like starch to hold the antibiotics with the polymer backbone. There is another way of bounding with the polymer backbone which is nowadays used by many researchers¹⁴⁻¹⁶ based on the formation of sigma bound between the polymer and the drug and sometime these polymers were allowed to polymerize into a network using some cross linking agents like N,N-methylene bis aryl amide.

2. EXPERIMENTAL

Melting points were measured using HS591, TC92 Controller apparatus. The molecular weight of the synthesized compounds is measured by Elmer Torbo mass spectrometer, PE-5Ms capillary column, Mean average M.wt was measured using gell permeation chromatography. IR, ¹HNMR and ¹³CNMR of the synthesized compounds were measured using FT-IR, 205, ATR Diamond Perkin Elmer and Bruker AMX 400MHz respectively at Sheffield university UK Acrylic-Itaconic copolymer was prepared according to the published procedure¹⁷. Compounds 1, 2, and 3 were also prepared according to the published procedure¹⁸. EDC Compound was prepared following the well known procedure¹⁹.

2.1 Synthesis of 1, 4-Bis (N-alkyl pyridinum) – buta -1,3-diyne dibromide and diiodide²⁰ (4-7)

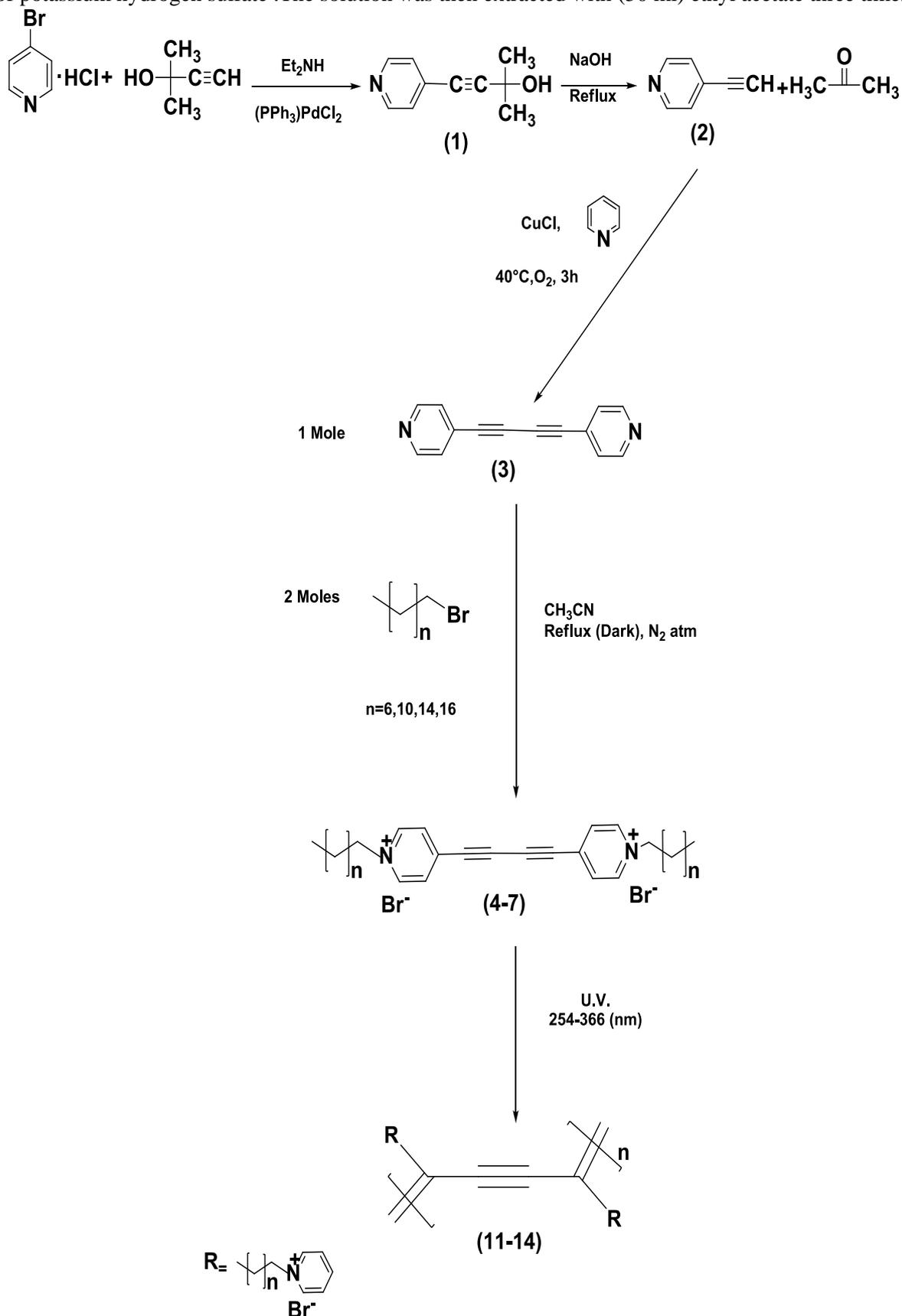
1,4- Di(4-pyridyl)-1,3-butadiyne (0.5g,0,00245 mol.) and alkyl halide (0.005 mol.), 50 ml of actonitilre were mixed together in dark place and under nitrogen atmosphere. The mixture was refluxed for 96 hour, Cooled and the solvent was removed under reduced pressure. The product was recrystallized from ethyl acetate/petroleum ether 1:3 respectively, the physical properties of the above compounds were illustrated in Table-1.

2.2 Synthesis of N-Succinimidyl -10, 12-pentacosadiynoate (13)

This compound was prepared following Murry procedure²¹. The synthesized compound showed the same m.p (64-66 °C) and identical IR spectrum.

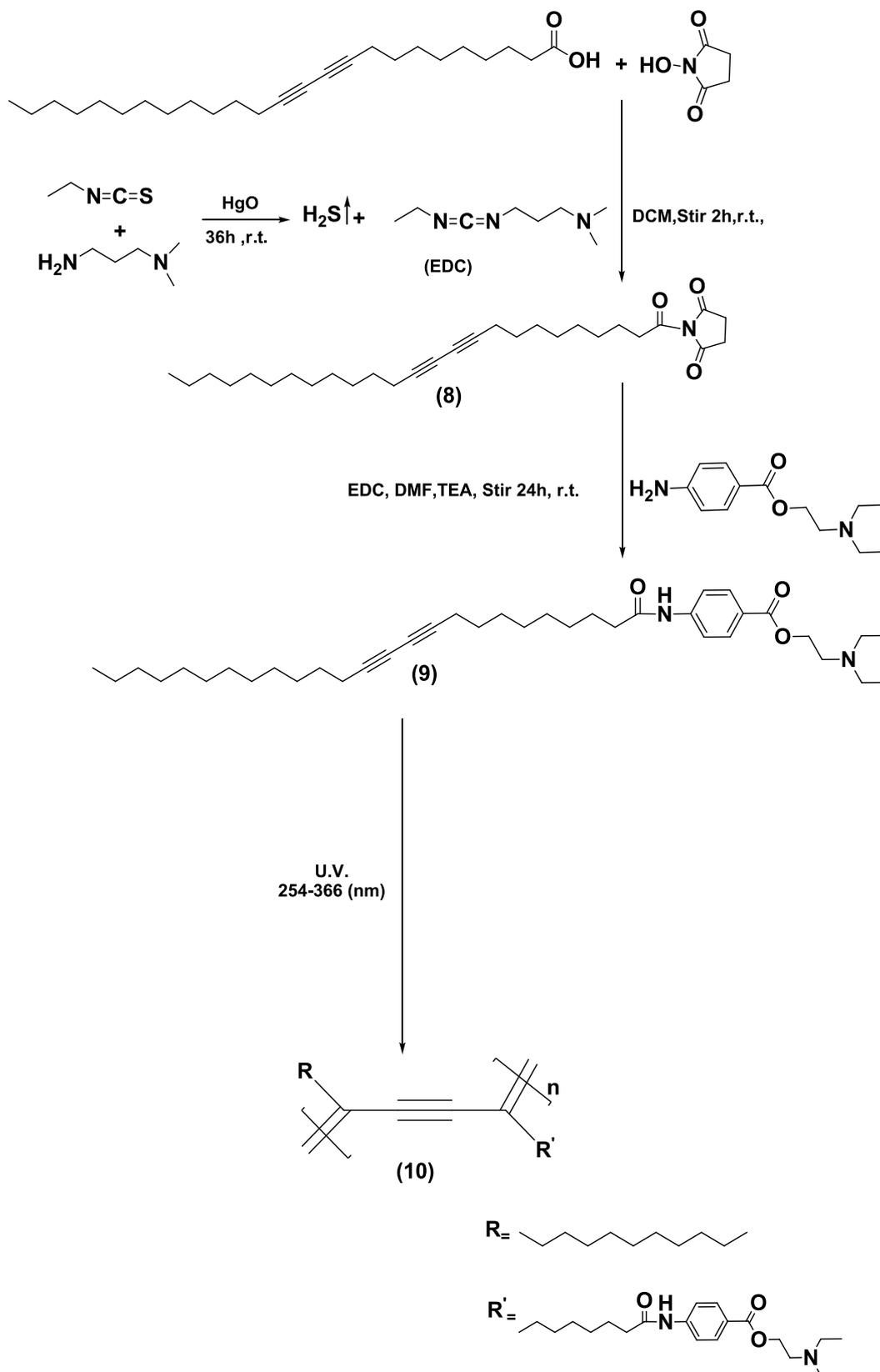
2.3 Synthesis of 1-(*N*- diethylamino) ethyl-4- pentacosa -10, 12-diyne amido benzoate²² (9)

Procaine (0.529, 0.0022 mol.), Triethyl amine (0.726g, 0.007 mol.) and DMF (5 ml) were mixed together. This solution was added to a solution of compound (8, 49 1.0 g, 0.00212 mol.) in DMF (5 ml). The final solution was stirred for 24 hour at r.t The solvent was removed under reduced pressure. The solid product was treated with 1 Molar solution of potassium hydrogen sulfate .The solution was then extracted with (50 ml) ethyl acetate three times. The

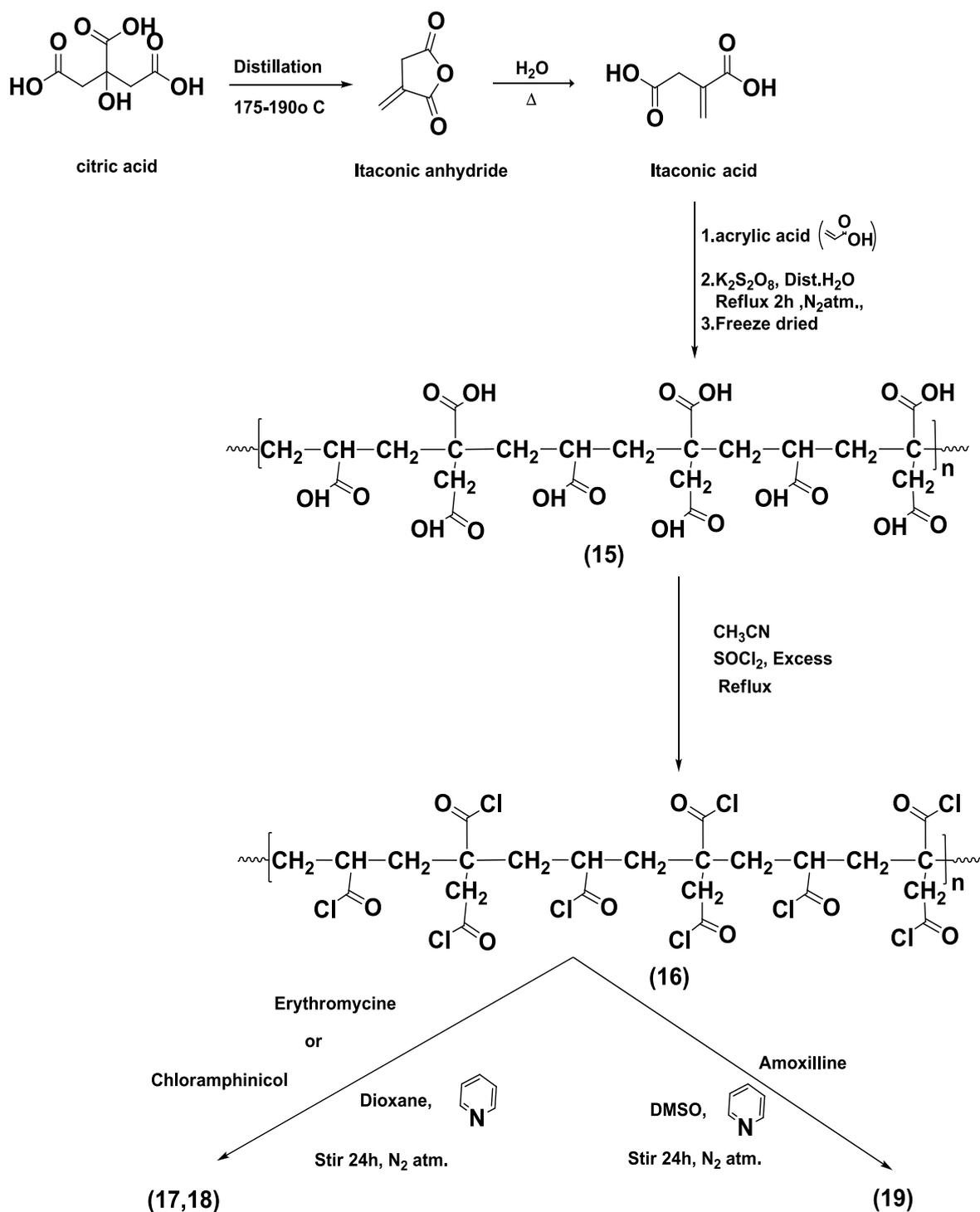


Scheme (1): Synthetic steps of polymers (11-14)

pH of the solution was adjusted at 8.5 using solid sodium hydrogen carbonate and extracted with (50 ml) of CH_2Cl_2 three times. The combined extract was dried on MgSO_4 anhydrous, filtered off. The solvent was removed under reduced pressure using rotary evaporator. The Crude product was recrystallized from ethyl acetate/petroleum ether (40-60), 85/15 giving the above compound as brown crystals m.p 61-62 $^\circ\text{C}$ with 64% yield.



Scheme (2): Synthetic steps of compound (10)



Scheme (3): synthetic steps of compounds (17-19)

2.4 Synthesis of ploy 1-(N-2-Diethylamino) ethyl-4-pentacosa -10, 12-Diyne amido benzoate²³ (10)

Compound (9, 0.012g, 0.02mol.) was dissolved in 1.0 ml. of DMSO .Deionizer water 19 ml. was then added to the mixture. The final solution was sonicated at 80°C for 25 min. The solution was flittered off using $0.45\ \mu\text{m}$ Wattman flitter paper .The filtrate was then cooled and kept at 4°C for 24 h. The final solution was then allowed to stand for 1h.to reach r.t and was irradiated by UV light at wavelength 254 nm for 5 minutes. The monomer solution color was changed from colorless into yellow after this irradiation.

2.5 Synthesis of diacetylene polymers (8-11) General procedure

The diacetylene monomer (0.01 mol.) was placed in watch glass and was irradiated with UV light operating at wavelength between 254-366 nm. The irradiation was stopped 1h. interval for each 3h. irradiation. The final product was washed with chloroform to remove the non polymerized monomer, flittered, The physical constant of the products were illustrated in Table-1.

Table-1: physical constants of compounds(4-7)

Comp.No	n	x	Color	Yield (%)	M.P (°C)	Molecular weight		
						Mol Form	Calc	Meas
4	6	I	Light red	90	107-110	C ₃₀ H ₄₂ N ₂ I ₂	684.514	684.0
5	10	Br	Light red	83	92-94	C ₃₈ H ₅₈ N ₂ Br ₂	702.72	704.0
6	14	Br	Light red	78	77-79	C ₄₆ H ₇₄ N ₂ Br ₂	814.92	815.0
7	16	Br	Light red	77	59-60	C ₅₀ H ₈₂ N ₂ Br ₂	871.04	871.0

2.6 Synthesis of copoly Acryloyl-Itaconyl Chloride¹⁷ (16)

Acrylic-Itaconic copolymer (2.0g, 0.0064 mol.) was dissolved in 30ml. of dry acetonitrile and (15 ml,0.2mol.) of thionyl chloride was then added. The mixture was stirred for 6h.at 60 °C under N₂ gas .The excess of thionyl chloride was removed under reduced pressure .The yellow solid product m.p (117-120 °C) was used as it is in further step.

3. GRAFTING OF ACRYLIC-ITACONIC COPOLYMER WITH COMMERCIAL ANTIBIOTICS

3.1 General procedure²⁴

Compound (16) 1.4g, 0.005 mol. was dissolved in 20ml. of dry dioxin. To this solution was drop-wise added a solution of 0.001 mol. Antibiotic in 10 ml of dioxane . Pyridine 0.5ml, 0.006 mol. Was drop-wise added to the above solution under N₂ atmosphere. After complete addition, the final solution was stirred at r.t for 24h. The solvent was removed under reduced pressure. The solid product was washed with distilled water and dried at r.t . The grfted copolymers 17, 18, 19 showed the following softening points; 61-63, 83-85, 104-105 °C respectively and an average grafting percent of about 50-60%.

3.2 The biological studies

Compounds (17-19) were studied against the following types of bacteria *E.coli*, *Psedu. aeruginosa*, *Staph. aureus*,*Ercherichia.cloi* invivo .Six groups of libratory animals (rabbits) were used for this investigation. These animals (Albino type) weighted between 750-1500 gm and aged between (4-6 months) were supplied by Mosul university research center.

3.2.1 The Fist Group

In this group of animals an infection was created with *Staph. aureus* and this infection was then treated with the commercial erythromycin antibiotic .

3.2.2 The Second Group

Creation of an infection with *Staph aureus* and treated with compound 17.

3.2.3 The Third Group

Creation of infection with *Psedu.aeruginosa* and treated with chloramphinicol.

3.2.4 The Forth Group

Creation of infection with *Psedu aeruginosa* and treated with compound 18.

3.2.5 The Fifth Group

Creation of infection with *Ercherichia.cloi* and treated with Amoxicillin.

3.2.6 The Sixth Group

Creation of infection with *Ercherichia.cloi* and treated with 19.

3.3 Preparation of the laboratory animal for antibacterial study²⁵

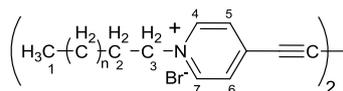
The legs of the studied animal were cleaned with 70% ethanol after removal of the hair. Injury within the legs of these animals (about 1-2 cm) was created. This injury was then treated with a microbial suspension²⁵ containing 10 microorganisms/cm³. The injury was then surgically closed. The 1st, 3nd and 5th groups of animals were treated with the commercial antibiotics while the 2nd, 4th and the 6th groups were treated with 1 g of compounds 17-19 mixed with 10g of Vaseline.

3.4 The bacteriological study

A cotton swab was taken from the infection area at different time intervals (1, 3, 5, 7, days) and growth on nutrition agar (Mannitol / salt agar) and the samples were incubated at 37 °C for 24 h. and the colonies growth were measured after 1, 3 and 7 days.

Table-2: IR spectral data for compounds (1, 4-7)

Comp. No.	IR cm^{-1}					
	+ N-R (Str.)	C—H (Str.)		C≡C (Str.)	C=C, C=C)Str,(Other)Str,(
		Aliphatic	Aromatic			
1	---	3060	2978	2233	1599-1574	C-O 1164
4	3140	3102	2979	2232	1614-163-	---
5	3312	3071	2921	2232	1635-1607	---
6	3278	3086	2920	2225	1635-1607	---
7	3268	3064	2917	2232	1636-1613	---

Table-3: ^1H NMR spectral data for compounds (4-7)

Comp. No.	n	^1H NMR, δ (ppm) $\text{CHCl}_3\text{-d}_1$
4	5	0.85(t,3H) $\text{C}_1\text{-H}$, 1.28(m,2H) $\text{C}_n\text{-H}$, 2.04(m,2H) $\text{C}_2\text{-H}$, 4.82(t,2H) $\text{C}_3\text{-H}$, 8.05(d,1H) $\text{C}_{5,6}\text{-H}$, 9.25(d,1H) $\text{C}_{4,7}\text{-H}$
5	9	0.85(t,3H) $\text{C}_1\text{-H}$, 1.29(m,2H) $\text{C}_n\text{-H}$, 1.98(m,2H) $\text{C}_2\text{-H}$, 4.85(t,2H) $\text{C}_3\text{-H}$, 8.01(d,1H) $\text{C}_{5,6}\text{-H}$, 9.31(d,1H) $\text{C}_{4,7}\text{-H}$
6	13	0.88(t,3H) $\text{C}_1\text{-H}$, 1.33(m,2H) $\text{C}_n\text{-H}$, 2.0(m,2H) $\text{C}_2\text{-H}$, 4.88(t,2H) $\text{C}_3\text{-H}$, 7.99(d,1H) $\text{C}_{5,6}\text{-H}$, 9.30 (d,1H) $\text{C}_{4,7}\text{-H}$
7	15	0.88(t,3H) $\text{C}_1\text{-H}$, 1.26(m,2H) $\text{C}_n\text{-H}$, 1.99(m,2H) $\text{C}_2\text{-H}$, 4.89(t,2H) $\text{C}_3\text{-H}$, 8.0 (d,1H) $\text{C}_{5,6}\text{-H}$, 9.28(d,1H) $\text{C}_{4,7}\text{-H}$

4. RESULTS AND DISCUSSION

The 1,4-Bis(4-pyridyl) buta-1,3-diyne compound(3) was prepared according to the published procedure¹⁸. The m.p of this compound was found 202-204 $^{\circ}\text{C}$ with a yield of 55%. The ^1H NMR spectrum showed a doublet signal at 7.55 ppm related to two pyridyl protons near the acetelynic linkage and doublet signal resonated at 8.87 ppm for the two protons near the nitrogen atom. Compound 4-7, scheme (1) showed identical M. wt of the calculated and the measured values. IR spectra indicate the existence of $\text{C} \equiv \text{C}$ absorbed between 2232-2225 Cm^{-1} for all the above compound.

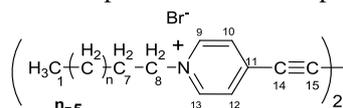
^1H NMR spectra were indicated in Table (3). ^{13}C NMR spectra, Table-4 showed the corresponding signals as appeared in this table. These signals were assigned for different types of carbons related to these compounds. Compound (8) was prepared following the same procedure²¹. It has the same physical properties and identical IR spectra. This compound has melting point(63-64 $^{\circ}\text{C}$, while in Murry thesis it was 64-66 $^{\circ}\text{C}$) 93% yield. The IR spectrum gave the following absorption bands 2053, 1670, 1684 $\text{C} \equiv \text{C}$, $\text{C}=\text{O}$ amide and for succinimide respectively. Compound(9) was prepared as mentioned in the experimental part and as shown in scheme(2). The IR spectrum of this compound showed the following absorption bands: 3289, 2065 Cm^{-1} belong to NH, $\text{C} \equiv \text{C}$ stretching vibration, 1732, 1651, 1556 Cm^{-1} for $\text{C}=\text{O}$, $\text{C}=\text{C}$ Aromatic and NH deformation. Polymer (10) was prepared as a solution polymer following the procedure indicated in the experimental part. The yellow color solution after irradiation indicate the occurrence of the polymerization process in this compound and accordingly it could be used as biosensor which is our next study.

Copoly acrylic-itaconic acid was prepared as mentioned in the experimental part and could be shown in scheme (3). The structure of this polymer was elucidated by combination of spectroscopic methods IR, GPC. The IR spectrum showed the following absorption bands: 2995, 1700, 3430 Cm^{-1} for CH , $\text{C}=\text{O}$, OH carboxylic. The GPC measurements revealed that its M. wt is 3629 g/mol. Using DMF as solvent.

Copolymers (17-19, scheme 3) were prepared by grafting acrylic-itaconic copolymer with erythromycin, chloramphenicol and Amoxicillin as mentioned in the experimental part. The formation of N-C or O-C linkage between the antibiotics and the polymer backbone was followed by the brooding change of the OH group to minimum values and the appearance of the ester, amide linkage as presented in Table-5.

4.1 The biological study⁽²⁵⁾

The studied compound were used as antibacterial agent against three types of bacteria such as *staph. aureus*, *E.col* and *pseudo.arug*. The study reveals that both the tested compounds and the control gave +ve results after 1st, 3rd and 5th days. Compound (17) showed highest activity especially for 3rd and 5th days, while after the 7th day there is negligible growth for the two groups.

Table-4: ^{13}C NMR spectral data for compounds (4-7)

Comp. No.	n	^{13}C NMR, δ (ppm) $\text{CHCl}_3\text{-d}_1$
4	5	14.07 C ₁ , 22.56 C ₂ , 25.99 C ₆ , 28.64 C _{4,5} , 28.98 C ₇ , 30.89 C ₃ , 61.91 C ₁₄ , 61.45 C ₈ , 65.32 C ₁₅ , 127.58 C _{10,12} , 129.99 C ₁₁ , 144.42 C _{9,13}
5	9	14.11 C ₁ , 22.56 C ₂ , 26.07 C ₁₀ , 29.07 C _{4,9} , 29.49 C _{5,8} , 29.9 C ₁₁ , 30.82 C ₃ , 31.64 C ₁₂ , 61.74 C ₁₈ , 65.11 C ₁₉ , 129.98 C _{14,16} , 140.78 C ₁₅ , 144.61 C _{13,17}
6	13	14.07C ₁ , 22.9 C ₂ , 26.5 C ₁₄ , 27.7 C _{4,13} , 29.2 C _{5,12} , 29.4 C ₁₅ , 30.08 C ₃ , 31.9 C ₁₆ , 61.8 C ₂₂ , 65.09 C ₂₃ , 129.99 C _{18,20} , 140.08 C ₁₉ , 145.0 C _{17,21}
7	15	14.12 C ₁ , 22.68 C ₂ , 26.09 C ₁₆ , 29.53 C _{4,15} , 29.71 C _{5,14} , 30.80 C ₁₇ , 31.77 C ₃ , 53.82 C ₁₈ , 62.01 C ₂₄ , 65.18 C ₂₅ , 129.97 C _{20,22} , 140.08 C ₂₁ , 144.57 C _{19,23}

Table (5) IR spectral data for compounds (17-19)

Comp. No.	IR, $\bar{\nu}$ (cm ⁻¹)							
	O—H (Str.)	C—H (Str.)		C=O (Str.)		C=C, C—C (Str.)	N—H Def. (Str.)	Others (Str.)
		Aromatic	Aliphatic	Ester	Amide			
17	3426-2740	---	2979	1726	---	---	---	1172-1039(C-O)
18	3446-2677	3080	2976	1734	1648	1636-1596	1558	1541,1364(N-O) 1171-1069(C-O)
19	3326-2622	3040	2931	1717	1653	1623-1585	1558	1174-1011(C-O)

Compound (18) was tested against *E.coli* it also gave +ve results for the 1st, 3rd and 5th days but the growth was found to extend after 3rd and 5th days for animals treated with this compound. The study also revealed that the growth is slightly higher for this compound than the control.

Compound (19) was used against *pseudo.arug*. The result of growth also +ve for the 1st, 3rd and 5th days for both. The study also revealed that animals treated with this compound shows slightly more growth than the control especially after 3 and 5 days.

5. CONCLUSION

In conclusion of the above study the prepared stereoregular polymers which are all new compounds were structurally elucidated and has been confirmed. It is also clear that the three polymer containing the drug showed release capability of drug for 7 days against the studied bacteria while the commercial antibiotics (treated three times a day) gave nearly the same results and some times the polymers results are higher. These observations gave evidences to use these polymers successfully as drug release polymers.

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