

Synthesis of new Nucleoside & Nucleotide Analogues

Muqdad Irhaem Kadhim* , Yousif Ali Al-fatahi**

Department of chemistry , college of science , Al-Qadisiyah University*

Department of chemistry , college of science , Baghdad University**

Abstract:-

The designed multistep synthetic route to these nucleoside start with diacetone glucose (1) prepared in one step from D-glucose [Scheme.A].

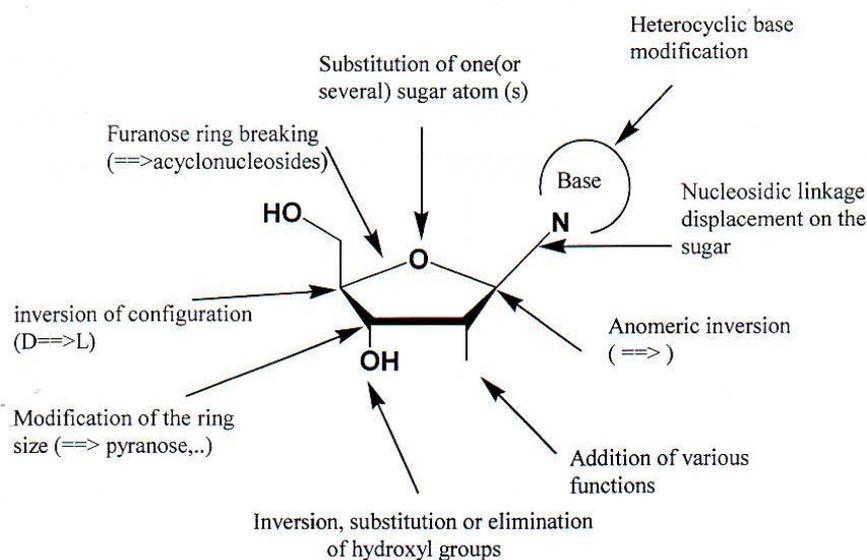
Oxidation of the diacetone glucose (1) with dimethyl sulphoxide and acetic anhydride of the 3-hydroxyl group gave the corresponding ulose derivative (2).Condensation of (2) with nitromethane under PTC condition yielded the nitromethyl derivative (3), which was converted to the derivative (4). Upon treatment with nitromethane in presence of sodium ethoxide. To obtain the first type of nucleoside analogues (18) , (19) , (20) and (21); isopropylidene acetal at -5,6-position was removed with acetic acid followed by periodate oxidation and borohydride reduction to give the derivative (9). The 5-hydroxyl group was protected with benzoyl group using benzoyl chloride to give the 5-benzoate derivative (8). Treatment with trifluoro acetic anhydride in the presence of acetic acid gave the 1,2-di-O-trifluoro acetylated derivative (9). When (9) was allowed to react with mercuric theophylline salt ,mercuric indole salt , silylated uracil and silylated cytosine, the four nucleoside analogues (14),(15), (16) and (17) were obtained.The free nucleoside (18),(19), (20) and (21) were obtained when (14),(15), (16) and (17) were allowed to react with sodium methoxide in methanol.The second type [Scheme. B] of nucleotide analogues was obtained from the condensation of ethylcanoacetate derivative with (2) to (give the derivative (22), followed by addition potassium cyanide in basic media gave (23)).Deprotection of the isopropylidene derivative at 5,6-position followed by periodate oxidation and borohydride reduction in situ gave the ribo derivative (25) .Benzoylation of the ribo derivative (25) with benzoyl chloride protected the 5-hydroxyl group to give (26) [Scheme. B]. Treatment of (26) with acetic acid and trifluoro acetic anhydride gave the 1,2-di-O-trifluoro acetylated derivative (27).The condensation of (27)with the mercuric theophylline salt ,mercuric indole salt , silylated uracil and silylated cytosine yielded the nucleotide analogue (28),(29),(30) and (31) respectively .Hydrolysis under basic condition yield the free nucleotide analogue (32), (33) , (34) and (35)).The synthesized derivatives were characterized by thin layer Chromatography, infrared spectroscopy ,¹³CNMR ,¹HNMR (nuclear magnetic resonance) .It is hoped that the new synthesized nucleoside and nucleotide analogues may possess antiviral , anticancer and antibacterial activity.

Introduction:-

Nucleosides, both of natural and synthetic origin have at least some biological activity. A much smaller, but nevertheless significant number of nucleosides are either in use as or have the potential based upon extensive biological evaluation to be employed as chemotherapeutic agents⁽¹⁾. Such as potential anti-viral ⁽²⁾, fungicidal, and anti cancer agents ^(3,4). More recently, they have been incorporated into oligonucleotides for application in the "antisense" field, where oligonucleotides

complementary to mRNA are sought as inhibitors of gene expression. So the major purpose for the syntheses of nucleosides is, of course, the development of new compounds of chemotherapeutic interest. Chemical modifications of naturally occurring nucleosides have been of interest for over 50 years and numerous nucleoside analogues were synthesized in order to selectively interfere with DNA and RNA. These structural modifications involve either the heterocyclic ring or the sugar moiety (Fig.1) resulting in analogues that act as anti-metabolites through the induction of one of the following effects⁽⁵⁾:

- (i) Inhibition of certain enzymes which are important for nucleic acids biosyntheses.
- (ii) Incorporation of analogues metabolites into nucleic acids which later block their biosyntheses. In many cases the nucleoside is not the actual active agent, but rather the nucleoside is metabolized in cells to a mono-, di-, or triphosphate derivatives before it is able to manifest its effect. These phosphate derivatives may be active themselves, or they may be further metabolized. The inability of phosphate derivatives of nucleosides to penetrate cells in significant amounts has routinely led to the use of the nucleosides themselves as agents, thus requiring intracellular activation⁽²⁾. Nucleoside analogues show importance in several established chemotherapies (anticancer, antiviral and antibacterial) and other attractive fields like immunomodulation or regulation of gene expression which could constitute new therapeutic



approaches⁽⁶⁾.

Fig. (1) : main structure modifications susceptible to transform a natural nucleoside (R = H or R = OH) in to one of its analogues .

Impetus has been provided for the synthesis of branched chain nucleosides by the reports that 2' and 3'-C-methyl adenosine and 3'-C-methyl cytidine are effective antiviral agent in mice and also that two 2'-C-nitromethylhexopyranosylpurines are active against KB tumour cells . There are two main methods for preparing such nucleosides : first by synthesis of a suitable carbohydrate precursor followed by a conventional nucleoside condensation reaction , and secondly by the attack of nucleophiles on 2' and 3'-Keto-nucleosides. Thus , most of the chemistry involved is standard carbohydrate chemistry

and , as such , is covered in references^(7,8) .It will suffice , therefore , to give just a few examples here .

Apparatus & Chemicals

- Melting points were recorded using Gallenkamp electro-thermal melting point apparatus and were uncorrected.
- Infrared spectra were recorded on SHIMADZU FT-IR-8400s spectrophotometer, as KBr disc or thin films.
- ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker, Ultra Shield, 300 MHZ, Switzerland, Tetramethyl silane was used as an internal reference and CDC1₃, MeOD or DMSO, as a solvent.
- TLC was performed on aluminum sheets precoated with silica-gel F₂₅₄ supplied by Merck. Column chromatography was carried out with silica gel 60 (Fluka). Spots were detected with iodine vapor.
 - All chemicals used were supplied from Merck, Fluka and BDH chemicals.
 - Solvents were dried according to the procedures mentioned in literatures. Solvents were removed under reduced pressure using Buchi rotary evaporator type 120.

Material Synthesis:-

1, 2:5, 6- Di-O- isopropylidene - α -D-glucofuranose⁽⁹⁾ 1 To an efficiently stirred suspension of anhydrous glucose (15 g ,83.3 mmol.) in acetone (100 ml) ,anhydrous pulverized zinc chloride (12g ,88.1 mmol) was added followed by 85% phosphoric acid (0.75 g) . This mixture was stirred at room temperature for 30 hours and the undissolved glucose (6.18 g) was filtered and washed with a little acetone. the filtrate and washing were cooled and made slightly alkaline with 40% sodium hydroxide solution, the insoluble inorganic material was removed by filtration and washed with acetone. The almost colorless filtrate and washing were concentrated and the residue was diluted with water (15 ml) and extracted three times with chloroform (30*3 ml) the combined chloroform extract were washed with a little water ,dried over anhydrous sodium sulphate and the solvent was removed to give a white crystalline residue of (1) (11.6 g 91% yield based on the glucose consumed) m.p 95 – 101 .one crystallization from chloroform : n-hexane (1: 10) raised the m.p to 105 -109 °C ,(R_f= 0.84)(chloroform : ethanol) (10 : 1) ; FTIR (KBr disc) (ν_{max} cm⁻¹) , 3446 (OH) , 2875-2985 (C-H)aliphatic ,1373 (C-H) bending .

1,2:5,6-Di-O-isopropylidene- α -D-ribo-hexofuranose-3-ulose ⁽¹⁰⁾ 2

1, 2:5, 6 - Di- O- isopropylidene - α -D-glucofuranose (1) (5g) was dissolved in a mixture of dimethyl sulphoxide (25 mL) and acetic anhydride (15 mL) in stoppered conical flask . After stirring for 24 hours at room temperature, TLC (chloroform –ether 10:1) showed that the reaction mixture consist mainly of the required product with trace of side products.the reaction mixture then diluted with ice water (100 mL) and the resultant yellow syrup was washed with ice water (3*30 mL) followed by extraction with chloroform (3*20 mL).

The combined chloroform extracts were dried over anhydrous sodium sulphate and the solvent was removed to afford a syrup residue of the 3-keto derivative (2) (3 g , 60.2 %)as syrup . R_f (0.67) ;FTIR (film) ($\nu_{max} \text{ cm}^{-1}$) 2935-2987 (C-H)aliphatic ,1749 (C=O).

• 1,2:5,6 -Di-O-isopropylidene-3-C-nitromethyl- α -D-allo- furanose (3) ⁽¹¹⁾

1,2:5,6 -Di-O-isopropylidene- α -D-ribo-hexofuranose-3-ulose (2) (6 g ,23.3 mmol) was dissolved in benzene (60 mL) and treated with nitomethane (10 mL ,190 mmol) for 24 h with constant stirring at room temperature in the presense of 0.2 M sodium hydroxide (10 ml) and tetrabutyl ammonium bromide (0.6 g , 1.9 mmol) TLC (benzene : ethyl acetate ,10:1) showed that the reaction was complete .

The aqueous phase was separated and extracted twice with benzene (10 ml) . The combined organic layers were dried over anhydrous sodium sulphate and the solvent was removed to afford asyrup product (3) of (5.1 g ,70 %) R_f (0.57) ;FTIR (film)($\nu_{max} \text{ cm}^{-1}$) 1560 and 1380 (N-O)3300-3450 (O-H) .

• 3-deoxy -1,2:5,6 -Di-O-isopropylidene-3-C-nitromethyl- α -D-allofuranose (4) ⁽¹¹⁾

To a solution of (3) (5 g , 15.5 mmol) in dimethyl sulphoxide (25 mL) was added (12.5 ml) acetic anhydride and the mixture was stirred for 24 h at 20 . TLC (n-hexane : ethyl acetate ,8:4) showed that the reaction was complete . After the addition of iced water (100 mL) , asyrup residue was separated . The aqueous Layer was decanted and remaining syrup was washed with (3*100 mL) . The combined chloroform extracts were dried over anhydrous sodium sulphate and the solvent was removed to give the 3-nitromethylene derivative (4) as a syrup (4.3 g ,90.9 %) ;(R_f =0.55) FTIR (film) ($\nu_{max} \text{ cm}^{-1}$) 1551 and 1380 (N-O) , 1675 (C=C)

• 3-C-cyano-3-deoxy-1,2:5,6-di-O-isopropylidene-3-C-nitromethyl- α -D-glucohexofuranose (5) ⁽¹²⁾

A mixture of (4) (3 g) and potassium cyanide (1 g) was stirred in benzene (70 ml) &0.2 M sodium hydroxide (7 ml) ,in the presence of tetrabutylammonium bromide (0.3 g) ,for 3 h at room temperature .

TLC (Benzene : ethyl acetate ,10:1) showed that the reaction was complete. the aqueous phase was then separated and extracted twice with benzene (10 ml) .

The combined benzene layers were dried (magnesium sulfate) and evaporated to afford a syrup which crystallized from 2-propanol-petroleum ether to afford (5) (2.2 g ,73 %), FTIR (film) (ν_{\max} cm^{-1}) 1555 and 1380 (N-O) , 2105 weak ($\text{C}\equiv\text{N}$), 2935-2987 (C-H) aliphatic.

3-C-cyano-3-deoxy-1,2-O-isopropylidene-3-C-nitromethyl- α -D-glucohexofuranose (6)

Compound 5 (2.5 g , 9.5 mmol) was dissolved in (60 %) acetic acid (30 ml) and stirred for 48 hours at room temperature . TLC (chloroform : ether,10:1) showed that the reaction was complete. the solution was evaporated under reduced pressure and the resulting residue was coevaporated with toluene to give crystals of 6 (1.9 g , 91 %)

recrystallization from methanol : petroleum ether (1: 1) , FTIR (film) (ν_{\max} cm^{-1}) 1596 & 1373 (N-O) , 2207 ($\text{C}\equiv\text{N}$). 3463 (O-H) .

• 3-C-cyano-3-deoxy-1,2-O-isopropylidene-3-C-nitromethyl- α -D-ribofuranose (7)

To a well – stirred solution of 6 (1.8 g , 6.2 mmol) in ethanol (40 ml) was added a saturated solution of sodium hydrogen carbonate (2 ml) followed by a solution of sodium metaperiodate (1.32 g , 6.2 mmol) in 70 ml water .the resulting reaction mixture was stirred for 3 hours after which the excess sodium metaperiodate was destroyed by adding few drops of ethylene glycol. TLC (chloroform : ether,10:1)

The resulting aldehyde sugar was immediately reduced with sodium borohydride (0.12 g) . after the reaction mixture was kept with stirring for 4 hours , acetone (0.5 ml) was added and the mixture was further stirred for 30 minutes . the solid residue was removed by filtration and the filtrate was extracted with methylene chloride (4* 100 ml) dried over anhydrous sodium sulphate and the solvent was removed to a syrup (7) (1.39 g ,76.8 %) (R_f =0.44) , FTIR (film) (ν_{\max} cm^{-1}) 1540 (N-O) , 2100 ($\text{C}=\text{N}$) , 3500 (O-H) .

5-O-benzoyl-3-C-cyano-3-deoxy-1,2-O-isopropylidene-3-C-nitromethyl- α -D-ribofuranose (8)

To ice cooled solution of 107 (2.12 g , 8.2 mmol) in anhydrous pyridine (6.7 ml , 8.3 mmol) was added (0.96 mL , 8.3 mmol) of benzoyl chloride . after the reaction was kept at room temperature for 24 h , a mixture of ice and water was added . the resulting syrup was extracted with petroleum ether (b.p 40-60) (4*100mL) , then dried with anhydrous sodium sulphate , filtered and concentrated under reduced pressure . Traces of pyridine were removed by coevaporation with dry toluene (3* 10 mL) . The benzoate derivative (8) was obtained as a syrup (1.6 g , 50 %) .) TLC (Benzene : ethyl acetate , 10:1) (R_f =0.67) IR (film) (ν_{\max} cm^{-1}) 2244 ($\text{C}\equiv\text{N}$) , 1595 (N-O) , 1695 ($\text{C}=\text{O}$) benzoate .

1,2-Di-O-trifluoroacetyl-5-O-benzoyl-3-C-cyano-3-deoxy-3-C-nitromethyl- α -D-ribofuranose (9)

A solution of (8) (1.06 g , 2.75 mmol) and 99% trifluoroacetic acid (6 mL , 0.16 mol) was stirred at room temperature . TLC (petroleum ether – diethyl ether 9:1) showed that deacetalation completed after 25 minutes .the reaction mixture was then neutralized with solid hydrogen carbonate and extracted with methylene chloride (2* 50 mL) . the combined extracts were dried over anhydrous sodium sulphate and the solvent was removed to give a syrup (0.71 g , 72%) .this syrup was immediately treated with acetic anhydride (3 mL) in pyridine (7 mL) with stirring for 18 h at which time the acetalation was completed (TLC) .

To the reaction mixture iced water (100 mL) was added with stirring and the resulting syrup was extracted with chloroform (4*100 mL) . the combined chloroform extracts

were dried over anhydrous sodium sulphate and the solvent was removed to give (9) as asyrup (1.343 g , 90%) FTIR (film) (ν_{\max} cm^{-1}) 1556 (N-O) , 1751 (C=O) ester , 1699 (benzoate) .

2,4 – Bis (tri methyl silyl) Uracil 10 to a mixture of uracil (1 g , 8.9 mmol) , ammonium sulphate (60 mg) and 1,1,1,3,3,3-hexamethyldisilazane HMDS (40 mL) was refluxed over night , a clear solution was obtained , cooled to room temperature and the solvent removed under reduced pressure by co distillation with xylene (2*20mL) to give silylated uracil as white powder (1.3 g , 61 %) m.p 330°C. This method was also

used to prepare the reagent 2,4 – Bis (tri methyl silyl) cytosine (11) using cytosine under the same conditions

2,4 – Bis (tri methyl silyl) cytosine 11

This compound was prepared under similar conditions for uracil (10) to give (1.6 g , 64. % yield) m.p 317 °C.

Bis (theophylline -7 -yl) mercury 12

To a solution of theophylline hydrate (2.5 g , 4.5mmol) in hot water (25 mL) was added sodium hydroxide (1 g) .To the vigorously stirred solution was added a hot solution of mercuric chloride (1.75 g) in ethanol (25 mL) , causing an immediate separation of white precipitate .the colorless suspension was cooled and the product was filtered off and washed with distilled water until the filtrate become neutral to give (2.7 ,60 %) m.p 374 °C.The salt was stored in vacuum desecator over calcium chloride .

Bis (Indole-1-yl) mercury (II) 13

This compound was prepared under similar conditions for (12)to give (2.2 g , 59.55 % yield) with respect (1g ,8.53 mmol) of indole ,as a fine white solid m.p 305 °C.

1 (2'-O-tri fluoroacetyl-5'-O-benzoyl-3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) uracil 14

To a mixture of 9 (0.6 gm, 0.78mmol) and silylated uracil (0.2 gm, 1 mmol) in anhydrous 1,2-dichloromethane (10 ml) was added anhydrous stannic chloride (0.08ml) and a few pellets of molecular sieve 4A. The reaction mixture was stirred at 25°C for 15 hours at, which time, TLC (Benzene: ethylacetate, 10:1) showed that the reaction was complete. The reaction mixture was poured into excess sodium bicarbonate solution and extracted with methylene chloride (3x20 ml). The organic layer was dried over anhydrous magnesium sulphate and the solvent was removed to give 14 (0.45 g ,62 % yield). The product was purified on a silica gel column using (chloroform: acetone 10:1) as eluent. Two different fractions were isolated, the first one (0.1 gm) and the second (0.01 gm) as syrup. R_f (0.5), FTIR (film) (ν_{\max} cm^{-1}), 1700-1710 (CO), 1320 (C-N_{tert}) , 2100 (C \equiv N).

1 (2'-O-trifluoroacetyl-5'-O-benzoyl-3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) cytosine 15

To a mixture of 109 (0.53 gm, 0.78mmol) and silylated cytosine (0.12 gm, 1 mmol) in anhydrous 1,2-dichloromethane (10 ml) was added anhydrous stannic chloride (0.08ml) and a few pellets of molecular sieve 4A. The reaction mixture was stirred at 23°C for 15 hours at, which time, TLC (Benzene: ethylacetate, 10:1) showed that the reaction was complete. The reaction mixture was poured into excess sodium bicarbonate solution and extracted with methylene chloride (3x20 ml). The organic layer was dried over anhydrous magnesium sulphate and the solvent was removed to give (15) (0.43 g ,61 % yield). The product was purified on a silica gel column

using Chloroform : acetone 10:1) as eluent. Two different fractions were isolated, the first one (0.2 gm) and the second (0.01 gm) as syrup. R_f (0.5), IR (film)

(ν_{\max} cm⁻¹ 1700-1720 (CO); 1310 (C-N_{tert}))

7(2'-O-trifluoroacetyl-5'-O-benzoyl-3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) theophylline 16

The theophylline mercury salt (0.35 gm, 0.62 mmol), was finely powdered, suspended in (20 ml) sodium-dried xylene and the solvent was partially distilled of to

remove traces of water when the temperature of the mixture was raised to 137°C, the residual suspension was allowed to cool (below 50°C). The acetylated sugar (113) (0.35 gm) in xylene (20 ml) was then refluxed with stirring for 10 hours TLC- (Benzene: ethyl acetate, 10:1) showed that the reaction was complete. The traces of theophylline salt was filtrated from the hot xylene suspension and washed with dichloromethane (20 ml). The organic layer was washed with 20% aqueous potassium iodide (2x10 ml) to remove the remaining traces of the mercury salt washed with water (2x10 ml.) dried over anhydrous magnesium sulphate and the solvent was removed to give after silica gel column chromatography (Benzene: ethyl acetate: acetone, 9:1:1) as eluent the acetylated nucleoside 16 (0.22-gm, 52 % yield) as a syrup. R_f (0.72); FTIR. (film) (ν_{\max} cm⁻¹), 1720 (C=O), 2246 (CN); 1565 (NO₂), 1310 (C-N_{tert}), 1695 (C=O benzoate).

1(2'-O-trifluoroacetyl-5'-O-benzoyl-3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) indole (17)

Following the same procedure for the preparation of compound (16) the benzoylated sugar (109) (0.45, 1.08 mmol) was stirred under reflux in xylene with indole mercury (II) salt (0.48 g, 1.109 mmol) for 10 hr to give (17) as syrup (0.251g, 51.3 %) R_f, 0.31 (Benzene: ethyl acetate, 10:1); FTIR (film) cm⁻¹, 1560, 1380 (NO₂), 2100 (C≡N), 1620 (C=C), 1370 (C-N_{tert}).

7(3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) theophylline (18)

To a solution of (16) (0.1 g, 0.15 mmol) and (0.5 gm, 0.1 mmol) of sodium methoxide in methanol (10 ml) was refluxed for 1 hour with stirring. The solvent was removed under reduced pressure to give a syrup (0.05 gm, 70% yield). This syrup was purified by column and eluted with a mixture of (chloroform: methanol, 10:1) chromatography to give a syrup (18) (0.045 g, 67 % yield). R_f (0.58), FTIR (film) (cm⁻¹), 3450 (O-H); 1708 (C=O), 1529, 1382 (NO₂), 2144 (C≡N). ¹H NMR (MeOD) δ (ppm): 3.587 (br s, OH), 3.72-3.84 (2H, m, H^o₅), 3.75 (s, CH₂NO₂), 5.31 (d, H^o₂), 5.28 (dd, H^o₄), 5.32 (d, H^o₁), 1.55 (6H, s, N-CH₃), 8.1 (1H, s, H₈) ¹³C-NMR (MeOD) δ (ppm): 91.11 (C^o₁), 67.58 (C^o₅), 73.54 (C^o₃), 80.36, 81.12 (C^o₂, C^o₄), 122.7 (C^o₃-C≡N), 71.66 (CH₂NO₂), 35.6, 38.3 (2N-CH₃), 166, 175 (C=O), 135.2-159.5 (C₄, C₅ and C₈)

1(3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) indole (21)

This compound was prepared under the similar condition as for (18) to afford (21) as a syrup. (0.081 g, 52 %) R_f, 0.45 (Chloroform : methanol 10:1); FTIR (film) cm⁻¹, 3400 (O-H), 2100 (C≡N), 1620 (C=C), 1570 (NO₂) 1370 (C-N_{tert}); ¹H NMR (MeOD) δ (ppm): 3.45 (br s, OH), 3.72-3.87 (2H, m, H^o₅), 3.82 (s, CH₂NO₂), 4.66 (d, H^o₂), 4.93 (dd, H^o₄), 5.89 (d, H^o₁), 6.3 (1H, d, H₂), 6.7 (1H, d, H₃), 7.4-7.5 (4H, H₄, H₅, H₆ and H₇ (aromatic)) ¹³C-NMR (MeOD) δ (ppm): 89.88 (C^o₁), 66.95 (C^o₅), 72.67 (C^o₃), 80.22, 81.35 (C^o₂, C^o₄), 123 (C^o₃-C≡N), 71.33 (CH₂NO₂), 76.36 (C-N), 127-148 (C-aromatic)

2-19 1(3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuranosyl) uracil (19)

This compound was prepared under the similar condition as for (18) to afford (19) as a syrup. (0.023 g, 48 %) R_f , 0.77 (Chloroform : methanol 10:1); FTIR (film) (cm^{-1}), 3400 (OH); 1748 (C=O). 2125 (C≡N). $^1\text{H-NMR}$ (MeOD) δ (ppm): 3.42 (br s, OH), 3.78-3.86 (2H, m, H°_5}), 3.88 (s, CH_2NO_2), 4.55 (d, H°_2}), 4.89 (dd, H°_4}), 5.83 (d, H°_1}), 6.45 (1H, d, H_2), 6.7 (1H, d, H_3), 10.52 (br s, NH). $^{13}\text{C-NMR}$ (MeOD) δ (ppm): 89.5 (C°_1}), 67.33 (C°_5}), 72.41 (C°_3}), 80.45, 81.76

(C°_2} , C°_4}), 122.1 ($\text{C}^{\circ_3}\text{-C}\equiv\text{N}$), 70.30 (CH_2NO_2), 165, 179 (C=O), 134-157 (C_2 and C_4)

1(3'-C-cyano-3'-deoxy-3'-C-nitromethyl- α -D-ribofuran osyl) cytosine (20)

This compound was prepared under the similar condition as for (18) to afford (20) as a syrup. (0.027 g, 49 %) (R_f = 0.73) (Chloroform : methanol 10:1); FTIR (film) ($\nu_{\text{max}} \text{cm}^{-1}$) 1581 (N-O), 1748 (C=O), 3317-3433 (OH & NH) 2126 (C≡N).

$^1\text{H-NMR}$ (MeOD) δ (ppm): 3.46 (br s, OH), 3.75-3.88 (2H, m, H°_5}), 3.83 (s, CH_2NO_2), 4.52 (d, H°_2}), 4.88 (dd, H°_4}), 5.87 (d, H°_1}), 6.1 (1H, d, H_2), 6.2 (1H, d, H_3), 10.23 (brs, NH)

$^{13}\text{C-NMR}$ (MeOD) δ (ppm): 87.88 (C°_1}), 68.25 (C°_5}), 72.85 (C°_3}), 80.32, 81.65 (C°_2} , C°_4}), 121.4 ($\text{C}^{\circ_3}\text{-C}\equiv\text{N}$), 70.62 (CH_2NO_2), 173.25 (C=O), 135.3-158.6 (C_2 and C_4)

3-C-[cyano(ethoxycarbonyl)methylene]-3-deoxy-1,2:5,6-di-O-isopropylidene- α -D-glucohexofuranose⁽¹²⁾ (22)

Compound (2) (7 g), dissolved in benzene (100 mL) was treated with ethyl cyanoacetate (3.1 mL) for 12 h with constant stirring at room temperature, in the presence of 0.2 M sodium hydroxide (12 mL) and tetrabutyl ammonium bromide (0.7 g). The aqueous layer was extracted three times with benzene (10 mL). The combined benzene layers were dried (magnesium sulfate) and evaporated to afford a brown syrup. The syrup was passed through a short column of silica gel with 20:1 (V:V) benzene – ethyl acetate as eluent. The solvent was removed under reduced pressure to afford (22) as a syrup that crystallized (8.2 g, 82%). A sample was recrystallized from 2-propanol-hexane to afford white needles, m.p 90-91°C, TLC (Benzene : ethyl acetate, 10:1) (R_f = 0.7) FTIR (film) ($\nu_{\text{max}} \text{cm}^{-1}$) 1720 (C=O), 1640 (C=C).

3-C-cyano-3-C-[cyano(ethoxycarbonyl)methyl]-3-deoxy-1,2:5,6-di-O-isopropylidene- α -D-glucohexofuranose⁽¹²⁾ (23)

A solution of (23) (2.5 g) in benzene (10 mL) was treated with potassium cyanide (1 g) for 2h with stirring at room temperature, in the presence of 0.2 M sodium hydroxide (2 mL) and tetrabutyl ammonium hydrogen sulfate (0.05 g) TLC (benzene : ethyl acetate, 10:1) showed that the reaction was complete.

The aqueous layer was extracted twice with benzene (5 mL), and the combined organic layers were dried (magnesium sulfate) and evaporated to a syrup.

Fractionation of the syrup by column chromatography with 20:1 (V/V) benzene – ethyl acetate as the eluent afforded a trace amount of 3-C-cyano-1,2:5,6-di-O-isopropylidene- α -D-glucofuranose, and a less-polar component that crystallized. Recrystallization of the less polar component from 2-propanol-hexane afforded (23) as white crystals (1.75g, 65%) m.p 92-93°C, FTIR (film) ($\nu_{\text{max}} \text{cm}^{-1}$) 1750 (C=O) 2210 weak (CN).

3-C-cyano-3-C-[cyano(ethoxycarbonyl)methyl]-3-deoxy-1,2-O-isopropylidene- α -D-glucohexofuranose (24)

This compound was prepared under the similar condition as for (6) to afford (24) as a syrup . (2.1 g , 90 %) ($R_f = 0.27$) (Chloroform: methanol 10:1) FTIR (film) (ν_{\max} cm^{-1}) 2100 (CN), 1710 (C=O) 3400 (O-H) .

3-C-cyano-3-C-[cyano(ethoxycarbonyl)methyl]-3-deoxy-1,2-O-isopropylidene- α -D-ribofuranose (25)

This compound was prepared under the similar condition as for (7) to afford (25) as syrup . (1.35 g, 76 %) ($R_f = 0.27$) (Chloroform: methanol 10:1) FTIR (film) (ν_{\max} cm^{-1}) 1748 (C=O), 3470 (O-H) .

5-O-benzoyl-3-C-cyano-3-C-[cyano(ethoxycarbonyl)methyl]-3-deoxy-1,2-O-isopropylidene - α -D-ribofuranose(26)

This compound was prepared under the similar condition as for (8) to afford (26) as a syrup . (0.45 g , 50 %) R_f , 0.75 (Chloroform : methanol 10:1) FTIR (film) (ν_{\max} cm^{-1}) 1700 (C=O) , 2100 (CN) .

1,2-Ditrifluoro-O-acetyl-5-O-benzoyl-3-C-cyano-3-C-[cyano(ethoxycarbonyl)methyl]-3-deoxy-1,2-O-isopropylidene - α -D-ribofuranose (27)

This compound was prepared under the similar condition as for (9) to afford (27) as syrup . (0.6 g , 86%) ($R_f = 0.58$) (Chloroform : methanol 10:1) FTIR(film) (ν_{\max} cm^{-1}) 1700 (C=O) , 2215 (C=N) .

7 (2'-O-trifluoro acetyl-5'-O-benzoyl-3'-C-cyano-3'-C-[cyano(ethoxycarbonyl)methyl]-3'-deoxy- α -D-ribofuranosyl) theophylline (28)

This compound was prepared under the similar condition as for (16) to afford (28) as syrup. (0.027 g, 52 %) $R_f = 0.72$ (Chloroform : methanol 10:1) FTIR (film) (ν_{\max} cm^{-1}) 1740 (C=O) , 1323 (C-N_{tert}) .

1(2'-O-trifluoro acetyl-5'-O-benzoyl-3'-C-cyano-3'-C - [cyano(ethoxycarbonyl)methyl]-3'-deoxy- α -D-ribofuranosyl) indole (29)

This compound was prepared under the similar condition as for (17) to afford (29) as a syrup . (0.27 g , 52 %) (R_f , 0.72) (Chloroform : methanol 10:1) FTIR (film) (ν_{\max} cm^{-1}) 1700 (C=O) , 1300 (C-N_{tert}) .

1(2'-O-trifluoroacetyl-5'-O-benzoyl-3'-C-cyano-3'-C -[cyano (carboxyl) methyl] -3'-deoxy- α -D-ribofuranosyl) trimethyl silyl Uracil (30)

This compound was prepared under the similar condition as for (14) to afford (30) as a syrup . (0.21 g , 50 %) R_f , 0.55 (Chloroform : methanol 10:1) ; FTIR (film) (ν_{\max} cm^{-1}) 1745 (C=O) , 2215 (C=N) .

1(2'-O-acetyl-5'-O-benzoyl-3'-C-cyano-3'-C-[cyano (carboxyl) methyl] -3'-deoxy- α -D-ribofuranosyl)N-trimethyl silyl cytosine (31)

This compound was prepared under the similar condition as for (15) to afford (31) as a syrup . (0.21 g , 50 %) R_f , 0.55 (Chloroform : methanol 10:1) ; FTIR (film) (ν_{\max} cm^{-1}) 1700 (C=O) , 2240 (C=N) .

7(3'-C-cyano-3'-C-[cyano(carboxyl)methyl]-3'-deoxy- α -D- ribofuranosyl) theophylline (32)

This compound was prepared under the similar condition as for (18) to afford (32) as a syrup . (0.055 g , 66 %) R_f , 0.36 (Chloroform : methanol 10:1) ; FTIR (film) cm^{-1} ,

3500 (O-H) , 1750 (C=O) , 2100 (C≡N) , 1620 (C=C) ,2600 (COOH) 1370 (C-N_{tert}); ¹HNMR (MeOD) δ (ppm) : 3.43 (br s ,OH) , 3.76-3.85 (2H , m , H^δ₅) , , 4.53 (d , H^δ₂) , 4.92 (dd , H^δ₄) ,5.85 (d , H^δ₁) , 3.28 (1H ,s , CNCHCO) 1.03 (6H , s , N-CH₃) ,8.5 (1H ,s, H₈) ,10.55 (1H ,s , COOH)¹³C-NMR (MeOD) δ (ppm) : 87.52 (C^δ₁),67.65 (C^δ₅), 73.32 (C^δ₃),80.25 , 81.29

(C^δ₂ , C^δ₄) , 122.2 (C^δ₃-C≡N) , 117 (CH- C≡N) , 182 (COOH) , 35.5 , 38.2 (2N-CH₃) ,169 , 177 (C=O) , 135.1-158.4 (C₄ , C₅ and C₈)

1(3'-C-cyano-3'-C-[cyano(carboxyl)methyl]-3'-deoxy-α-D-ribofuranosyl) indole (33)

This compound was prepared under the similar condition as for (21) to afford (33) as a syrup . (0.028 g , 45 %) R_f , 0.45 (Chloroform : methanol 10:1) ; FTIR (film) cm⁻¹, 3500 (O-H) , 1750 (C=O) , 2100 (C≡N) , 1620 (C=C) ,2600 (COOH) 1370 (C-N_{tert}); ¹HNMR (MeOD) δ (ppm) : 3.46 (br s ,OH) , 3.71-3.78 (2H , m , H^δ₅) , , 4.56 (d , H^δ₂) , 4.82 (dd , H^δ₄) ,5.93 (d , H^δ₁) , 3.25 (1H ,s , CNCHCO) ,10.46 (1H ,s , COOH) , 6.2 (1H , d , H₂) , 6.8 (1H , d , H₃) , 7.35-7.55 (4H ,H₄ , H₅ ,H₆ and H₇ (aromatic)) . ¹³C-NMR (MeOD) δ (ppm) : 92.83 (C^δ₁),65.85 (C^δ₅), 72.25 (C^δ₃),81.30 , 82.46 (C^δ₂ , C^δ₄) , 124.2 (C^δ₃-C≡N) , 119.67 (CH- C≡N) , 186 (COOH) , 73.2 (C-N) , 110-143 (C-aromatic)

1 (3'-C-cyano-3'-C-[cyano(carboxyl)methyl] -3'-deoxy-α-D- ribofuranosyl) uracil (34)

This compound was prepared under the similar condition as for (19) to afford (34) as a syrup . (0.035 g ,63 %) R_f , 0.61 (Chloroform : methanol 10:1) ; FTIR (film) cm⁻¹, 3500 (O-H) , 1750 (C=O) , 2100 (C≡N) , 1620 (C=C) ,2600 (COOH) 1370 (C-N_{tert}); ¹HNMR (MeOD) δ (ppm) : 3.46 (br s ,OH) , 3.77-3.91 (2H , m , H^δ₅) , , 4.55 (d , H^δ₂) , 4.82 (dd , H^δ₄) ,5.82 (d , H^δ₁) , 3.31 (1H ,s , CNCHCO) ,11.53 (1H ,s , COOH) ,10.53 (br s ,NH) , 6.4 (1H , d , H₂) , 6.6 (1H , d , H₃) . ¹³C-NMR (MeOD) δ (ppm) : 88.25 (C^δ₁),66.87 (C^δ₅), 72.48 (C^δ₃),82.25 , 83.29 (C^δ₂ , C^δ₄) , 121.3 (C^δ₃-C≡N) , 187 (COOH) , 165 , 178.5 (C=O) , 135.1-158.4 (C₂ and C₄).

1(3'-C-cyano-3'-C-[cyano(carboxyl)methyl] -3'-deoxy-α-D-ribofuranosyl) cytosine 35

This compound was prepared under the similar condition as for (20) to afford (35) as a syrup (0.026 g , 50.1 %) R_f , 0.54 (Chloroform : methanol 10:1) . FTIR (film) cm⁻¹, 3373 (O-H) , 1700 (C=O) , 2148 (C≡N) , 1602 (C=C) ,2600 (COOH) 1384 (C-N_{tert}); ¹HNMR (MeOD) δ (ppm) : 3.42 (br s ,OH) , 3.72-3.79 (2H , m , H^δ₅) , , 4.45 (d , H^δ₂) , 4.85 (dd , H^δ₄) ,5.93 (d , H^δ₁) , 3.4 8 (1H ,s , CNCHCO) ,10.48 (1H ,s , COOH) , 11.25 (br s ,NH) ¹³C-NMR (MeOD) δ (ppm) : 91.41 (C^δ₁), 69.55 (C^δ₅), 73.20 (C^δ₃),80.56 , 81.24 (C^δ₂ , C^δ₄) , 121.8 (C^δ₃-C≡N) , 183.5 (COOH) , 167 , 178 (C=O) ,127-135 (C₂ and C₄)

Results & Discussion

The characteristic absorption bands of the prepared compounds are shown in (Table 3-1) The appearance of the hydroxyl group absorption band at >3400 cm⁻¹ was utilized to confirm the structure of diacetone glucose (1) while the absence of this band indicates the formation of the 3-keto derivative (2) which also showed the carbonyl absorption band at 1749 cm⁻¹ . In the spectrum of (3) 1560 cm⁻¹ , 1380 cm⁻¹ and 3400 cm⁻¹ bands were attributed to the formation of the 3-C-nitromethyl allofuranose derivative (3) . The nitromethylene derivative(4) structure was confirmed by the appearance of C=C stretching at 1674 cm⁻¹.The absence of the 1674 cm⁻¹ band indicated that the Michael type addition to (4) had resulted in 3-C-nitromethyl derivative (5) . The hydroxyl group

absorption band 3450 cm^{-1} indicated the complete hydrolysis of 5,6-isopropylidene group which gave the diol (6 & 24).

Similar absorption band was shown for the 5-OH derivative (7 & 25). The absence of the 3450 cm^{-1} band was attributed to the formation of the 5-benzoate derivative

(108&126) which gave an absorption band at 3100 cm^{-1} for aromatic C-H, at 1710 cm^{-1} for carbonyl group and at 1590 cm^{-1} for C=C ring stretching. The 1,2-diacetate derivative (9 & 27) showed aromatic and aliphatic C-H stretching the region $3100\text{-}2850\text{ cm}^{-1}$ and two absorption bands at $1700\text{-}1750\text{ cm}^{-1}$ for the carbonyl group at C-1, C-2, and C-5 (the two acetate and benzoate group). The FTIR spectra of the nucleoside (16 & 28) are shown in respectively. In comparison with the FTIR spectrum of modified carbohydrate moiety (9 & 27), the theophylline nucleoside (16 & 28) showed weak band for the C-H stretching at $2850\text{-}2950\text{ cm}^{-1}$ because of the absence of one acetate group at C-1, while the uracil nucleoside (14 & 30) showed in addition to the mentioned weak band at $2850\text{-}2950\text{ cm}^{-1}$, the appearance of the NH stretching band at 3350 cm^{-1} which was attributed to the free NH group of the uracil. The FTIR spectrum showed that hydrolysis was complete since the stretching band at $3200\text{-}3400$ for (O-H & N-H) group for free nucleoside.

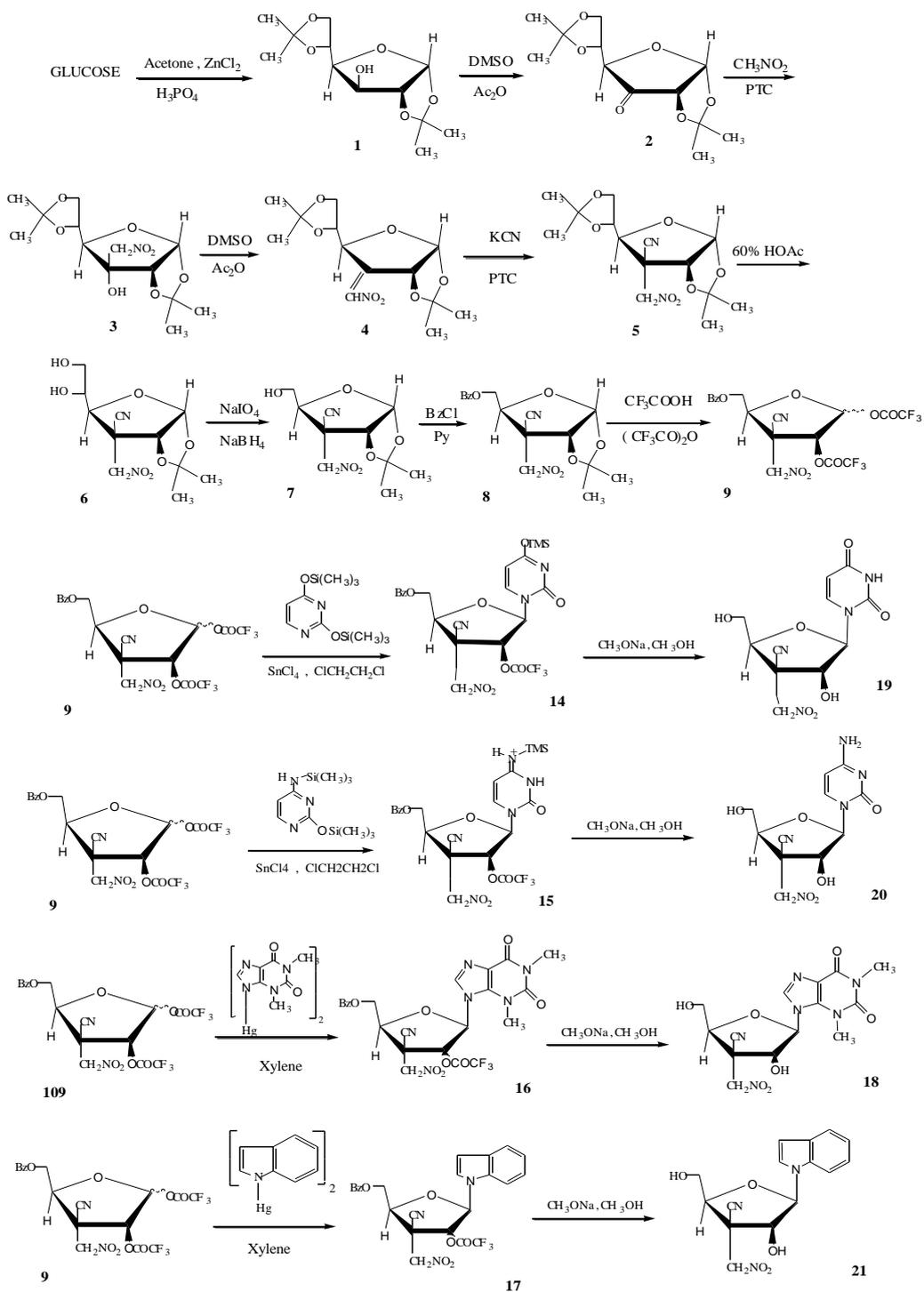
The structure of some of the prepared compounds were confirmed by $^1\text{H NMR}$ as shown in table (1). It was obvious that the anomeric proton H-1 appeared as a doublet in the region $\delta 5.32\text{-}5.93$.

The $^1\text{H NMR}$ spectrum of (18) demonstrated the H-4 as a doublet at $\delta 5.28$, H-5 at $\delta 3.72\text{-}3.84$ and at $\delta 5.32$, H-2.

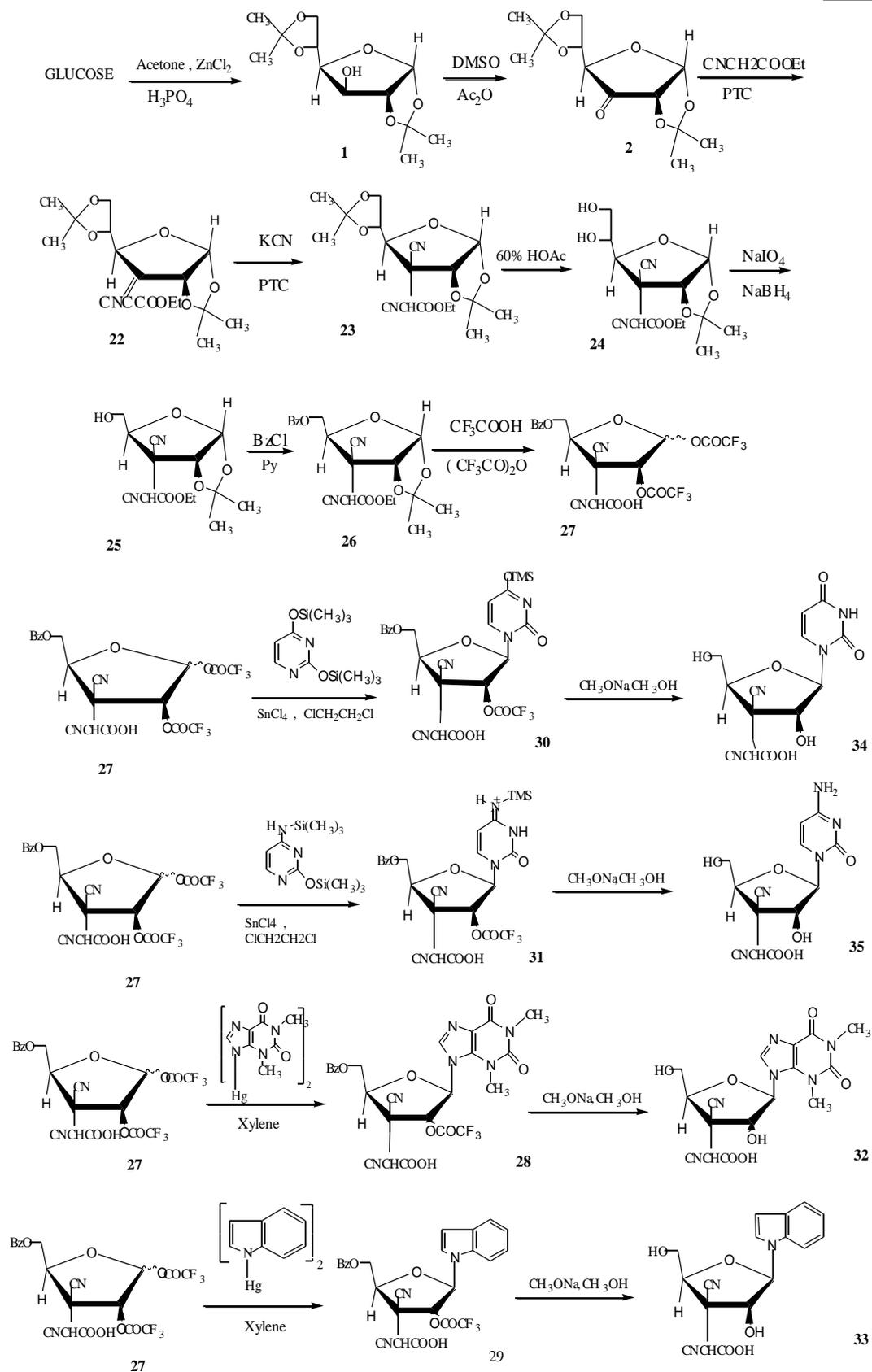
The signal at $\delta 3.45\text{-}3.52$ was attributed to the proton of the hydroxyl group in, H-4 and 2H-5 proton appeared as multiplets in the region $\delta 4.93\text{-}5.3$. The signal at $\delta 7.4\text{-}7.5$ was attributed to the aromatic proton in (21). The signal at $\delta 3.82$ was attributed to proton of CH_2NO_2 .

The $^1\text{H NMR}$ spectrum of (34) showed the demonstrated the H-4 as a doublet at $\delta 4.82$, H-5 at $\delta 3.77\text{-}3.91$ and at $\delta 4.55$, H-2. The signal at $\delta 3.42$ in was attributed to the proton of the hydroxyl group in (35), H-4 and 2H-5 proton appeared as multiplets in the region $\delta 3.72\text{-}4.85$. The signal at $\delta 11.25$ was attributed to the amino proton. The signal at $\delta 3.84$ was attributed to proton of CNCHCO .

The structure of some of the prepared compounds were also determined by $^{13}\text{C NMR}$ Spectroscopy (Table 2). For example The $^{13}\text{C NMR}$ spectrum of (21) showed the presence of signals for C-1, C-2, C-3, C-4, C-5, aromatic carbon, carbon cyanaide and CH_2NO_2 in the expected chemical shift ⁽¹⁵⁾.



Scheme A



Scheme B

**Table (1) ¹HNMR chemical shift data in δ values) for
**final nucleosides
and nucleotides****

Comp.	H-1	H-2	H-4	H-5	CH ₂ NO ₂	COOH	Others
18	5.32	5.31	5.28	3.72-3.84	3.75	-----	1.55 (6H , s , NCH ₃ of theophylline)
19	5.83	4.55	4.89	3.78-3.68	3.88	-----	10.52 (brs , NH of uracil)
20	5.87	4.52	4.88	3.75-3.88	3.83	-----	10.23 (brs , NH) of cytosine
21	5.89	4.66	4.93	3.72-3.87	3.82	-----	7.4-7.5 (aromatic proton of indole)
32	5.83	4.53	4.92	3.76-3.85	-----	10.55	1.03 (6H , s , NCH ₃ of theophylline)
33	5.93	4.56	4.82	3.71-3.78	-----	10.46	7.35-7.55 (aromatic proton of indole)
34	5.82	4.55	4.82	3.77-3.91	-----	11.53	10.53 (brs , NH of uracil)
35	5.93	4.45	4.85	3.72-3.79	-----	10.48	10.25 (brs , NH) of cytosine

Comp.	C'-1	C'-2	C'-3	C'-4	C'-5	C≡N	CH ₂ NO	Others
18	91.11	80.36	73.54	81.12	67.58	122.7	71.66	135.2-159.2 aromatic carbon of theophyllin
19	89.88	80.45	81.76	81.76	67.33	122.1	70.30	134-157 C ₂ & C ₄ of uracil
20	89.50	80.32	72.85	81.65	68.25	121.4	70.62	135.3-158.6 C ₂ & C ₄ of cytosine
21	87.88	80.22	72.67	81.35	66.95	123.9	71.33	127-148 C-aromatic indole
32	87.52	80.26	73.32	81.29	67.65	122.3	-----	169,177 carbonyl of theophylline
33	92.83	81.31	72.25	82.46	65.85	124.5	-----	186 carboxylic carbon , 110-143 aromatic i
34	88.25	82.25	72.48	83.29	66.87	121.3	-----	135.1-158 C ₂ & C ₄ of uracil
35	91.41	80.56	72.00	81.24	69.55	121.8	-----	127-135 C ₂ & C ₄ of cytosine

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تحضير مماثلات نيوكليوسيد و نيوكليوتيد جديدة

مقداد ارحيم كاظم جامعة القادسية /كلية العلوم

يوسف علي الفتاحي جامعة بغداد/كلية العلوم

الخلاصة

اظهرت مماثلات النيوكليوسيدات و النيوكليوتيدات المحتوية على تفرع في جزء السكر سواء كانت (طبيعية او محضرة) فعالية بايولوجية و مضادة للسرطان و الفايروسات و البكتيريا . يتضمن البحث تحضير نوعين من المركبات الجديدة :

النوع الاول : مماثلات نيوكليوسايد تحتوي على تفرع [3'-دايوكسي-(3'-C-سيانو-3'-نايترومثيل)]

النوع الثاني : مماثلات نيوكليوتايد تحتوي على تفرع [3'-دايوكسي-(3'-C-سيانو(كاربوكسي) ميثيل-3'-سيانو)]

للحصول على هذه المماثلات تطلب وضع استراتيجيات تسهل الوصول للهدف ، وقد اختيرت المادة الاولية 1,2,5:6-ثنائي-O- ايزوبروبيلدين-D- α -كلوكوفورانوز الذي يحتوي على مجموعة الهيدروكسيل حرة في موقع ذرة الكربون 3 الوحيدة غير المحمية . عند اكسدة (1) بثنائي ميثيل سلفوكسيد وانهريد الخليك تكون المشتق الكيتوني (2) .

لتكوين النوع الاول من المماثلات مخطط تم اضافة نيتروميثان للمشتق (2) فتكون المشتق (3) الذي يتحول الى المشتق (4) عند معاملته مع ثنائي ميثيل سلفوكسيد وانهريد الخليك . ومن اجل الحصول على مشتق سكر الرايبوز الخماسي الذي يمثل الجزء السكري في النيوكليوسيد تم ازالة مجموعة الحماية (الايزوبروبيلدين) في الموقع 6,5 باستخدام حامض الخليك يتبعها عملية اكسدة واختزال نحصل على المشتق (7) . ان حماية مجموعة الهيدروكسيل في الموقع 5- باستخدام كلوريد البنزويل يوفر الفرصة المناسبة لازاحة مجموعة الاستيل في الموقع 1,2- حيث يتكون المشتق (8) الذي يتحول الى المشتق (9) بالتفاعل مع حامض الخليك وثلاثي فلوريد حامض الخليك . حيث يؤدي تفاعل كل من المشتق (9) مع ملح الزئبق للثيوفلين و الاندول و مشتق السيلابيل لليوراسيل و مشتق السيلابيل سايتوسين الى مماثلات نيوكليوسايد (14) و (15) و (16) و (17) .

ان تسخين هذه المشتقات كل على حدة مع ميثوكسيد الصوديوم في الميثانول تحت مكثف اعطى المشتق (18) و(19) و(20) و(21) .

حصل على النوع الثاني (مماثلات النيوكليوتايد من معاملة المشتق (2) مع سيانو اسيتات الاثيل اولا حيث تكون (22) تبع ذلك اضافة سيانيد البوتاسيوم في وسط قاعدي ليعطي المشتق (23) وبازالة مجموعة الحماية في الموقع 6,5- باستخدام حامض الخليك يتبعها عملية اكسدة و اختزال لهذا الموقع فيتكون المشتق (25) . ان تفاعل كلوريد البنزويل مع المشتق (25) يؤدي الى حماية مجموعة الهيدروكسيل في الموقع 5- اذ تكون المشتق (26) ثم تحول الى مشتق ثلاثي فلورو الاستيل (27) بمعاملته مع حامض الخليك وثلاثي فلورو انهريد الخليك .

ان تكاثف المشتق (27) مع ملح الزئبق للثيوفلين و الاندول و مشتق السيلابيل لليوراسيل و مشتق السيلابيل سايتوسين اعطى مماثل النيوكليوتايد (28) و (29) و(30) و(31) على التوالي وقد تم الحصول على مماثل النيوكليوتايد الحر (32) و (33) و(34) و(35) من التحليل القاعدي الكحولي للمشتق لمماثل النيوكليوتايد .

تم تشخيص المركبات المحضرة بواسطة الطرق الطيفية : الاشعة تحت الحمراء و الرنين النووي المغناطيسي للبروتون و نظير الكربون -13 و كروماتوغرافيا الطبقة الرقيقة . ان الهدف من تحضير مماثلات النيوكليوسيد و النيوكليوتايد المتفرعة هو احتمالية ان تمتلك خواص مضادات السرطان و المضادات الحيوية و الفيروسات .