Synthesis of new Nucleoside & Nucleotide Analogues

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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); isopropyiidene 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 trifluouro acetic anhydride in the presence of acetic acid gave the 1,2-di-O-trifloro 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 benzoy1 chloride protected the 5-hvdroxyl 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 ,^{13}CNMR ,^{1}HNMR (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].

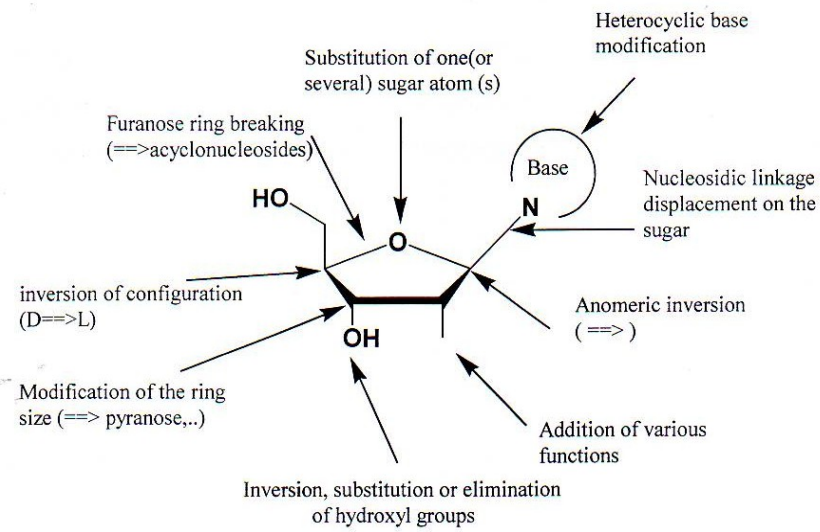

Fig. (1) : main structure modifications susceptible to transform a natural nucleoside (R = H or R = OH) in to one of its analogues .

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.
- ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker, Ultra Shield, 300 MHZ, Switzerland, Tetramethyl silane was used as an internal reference and $CDC1_3$, McOD or DMSO, as a solvent.
- TLC was preformed on aluminum sheets precoated with silica-gel F_{254} 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 disic) (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) (ν_{max} 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)(ν_{max} 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) (ν_{max} 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 (C≡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 (C≡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 metaperidate (1.32 g , 6.2 mmol) in 70 ml water .the resuting reaction maxture 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 aldehydo sugar was immedatly 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 %) (Rf =0.44),FTIR (film) (ν_{max} cm^{-1}) 1540 (N-O) , 2100 (C=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 ,8.3mmol) 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 resuting 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) (Rf =0.67) IR (film) (ν_{max} cm^{-1}) 2244 (C≡N) ,1595 (N-O) , 1695 (C=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 netralized 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 combaned 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 silated 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 filterate 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 (0.08m1) 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≡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 (0.08m1) 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)
(ύ $_{max}$ 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) theophyline16

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. Rf (0.72); FTIR. (film) ($\acute{\upsilon}_{max}$ cm^{-1}), 1720 (C=O), 2246 (CN); 1565 (NO_2), 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 %) Rf , 0.31 (Benzene: ethyl acetate, 10:1) ; FTIR (film) cm^{-1}, 1560 , 1380 (NO_2) , 2100 (C≡N) , 1620 (C=C) , 1370 (C-N_{tert}).

7(3′-*C*-cyano-3′-deoxy-3′-*C*-nitromethyl-α-D-ribofuranosyl) theophyline (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). Rf (0.58),FTIR (film) (cm^{-1}), 3450 (O-H); 1708(C=O) , 1529 , 1382 (NO_2) , 2144 (C≡N) . ^{1}HNMR (MeOD) δ (ppm) : 3.587 (br s ,OH) , 3.72-3.84 (2H , m , H_5') , 3.75 (s ,C**H**$_2$$NO_2$) , 5.31(d , H_2') , 5.28(dd , H_4') ,5.32 (d , H_1') , 1.55 (6H , s, N-CH_3) , 8.1 (1H , s , H8) ^{13}C-NMR (MeOD) δ (ppm) : 91.11 (C_1') , 67.58 (C_5') , 73.54 (C_3') ,80.36 , 81.12 (C_2' , C_4') , 122.7 (C_3'-**C**≡N) , 71.66 (**C**H_2NO_2) , 35.6 ,38.3 (2N-**C**H_3) ,166 , 175 (**C**=O) , 135.2-159.5 (C_4 , C_5 and C_8)

1(3′-*C*-cyano-3′-deoxy-3′-*C*-nitromethyl-α-D-ribo furanosyl) 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 (NO_2 **)** 1370 (C-N_{tert}); ^{1}HNMR (MeOD) δ (ppm) : 3.45 (br s ,OH) , 3.72-3.87 (2H , m , H_5') , 3.82 (s ,C**H**$_2$$NO_2$) , 4.66 (d , H_2') , 4.93 (dd , H_4') ,5.89 (d , H_1') , 6.3 (1H , d , H_2) , 6.7 (1H , d ,H_3) , 7.4 -7.5 (4H ,H_4 , H_5 ,H_6 and H_7 (aromatic)) ^{13}C-NMR (MeOD) δ (ppm) : 89.88 (C_1') ,66.95 (C_5') , 72.67 (C_3') ,80.22 , 81.35 (C_2' , C_4') , 123 (C_3'-**C**≡N) , 71.33 (**C**H_2NO_2) , 76.36 (C-N) , 127-148 (C-aromatic)

2-19 1(3′-*C*-cyano-3′-deoxy-3′-*C*-nitromethyl-α-D-ribofuran- osyl) 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}HNMR (MeOD) δ (ppm) : 3.42 (br s ,OH) , 3.78-3.86 (2H , m, H_5') , 3.88 (s ,C**H**$_2$NO$_2$) , 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}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 (C_3'-**C**≡N) , 70.30 (**C**H$_2$NO$_2$) , 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 %) (Rf= 0.73) (Chloroform : methanol 10:1); FTIR (film) (ν_{max} cm^{-1}) 1581 (N-O) , 1748 (C=O) ,3317-3433 (OH & NH) 2126 (C≡N) .

^{1}HNMR (MeOD) δ (ppm) : 3.46 (br s ,OH) , 3.75-3.88 (2H , m, H_5') , 3.83 (s ,C**H**$_2$NO$_2$) , 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}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 (C_3'-**C**≡N) , 70.62 (**C**H$_2$NO$_2$) , 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 presense of 0.2 M sodium hydroxide (12 mL) and tetrabutyl ammonium bromide (0.7 g) .The aqoues 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)(Rf = 0.7) FTIR (film) (ν_{max} 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 presense of 0.2 M sodium hydroxide (2 mL) and tetrabutylammonium 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 (5mL) ,and the combaned 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 . Recrytallization of the less polar component from 2-propanol-hexane afford (23) as white crystals (1.75g, 65 %) m.p 92-93 °C , FTIR (film) (ν_{max} 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 %) Rf , 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-trifloroacetyl-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 %) Rf, 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 %) Rf , 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 (CO**OH)** 1370 (C-N_{tert}); ^{1}HNMR (MeOD) δ (ppm) : 3.43 (br s ,OH) , 3.76-3.85 (2H , m , H_5') , , 4.53 (d , H_2') , 4.92 (dd , H_4') ,5.85 (d , H_1') , 3.28 (1H ,s , CNC**H**CO) 1.03 (6H , s , N-CH_3) ,8.5 (1H ,s, H8) ,10.55 (1H ,s , COOH)^{13}C-NMR (MeOD) δ (ppm) : 87.52 (C_1'),67.65 (C_5') , 73.32 (C_3') ,80.25 , 81.29 (C_2' , C_4') , 122.2 (C_3-**C**≡N) , 117 (CH- **C**≡N) , 182 (**C**OOH) , 35.5 , 38.2 (2N-**C**H_3) ,169 , 177 (**C**=O) , 135.1-158.4 (C_4 , C_5 and C_8)

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 (CO**OH)** 1370 (C-N_{tert}); ^{1}HNMR (MeOD) δ (ppm) : 3.46 (br s ,OH) , 3.71-3.78 (2H , m, H_5') , , 4.56 (d , H_2') , 4.82 (dd , H_4') ,5.93 (d , H_1') , 3.25 (1H ,s , CNC**H**CO),10.46 (1H ,s , COOH) , 6.2 (1H , d , H_2) , 6.8 (1H , d ,H_3) , 7.35-7.55 (4H ,H_4 , H_5 ,H_6 and H_7 (aromatic)) . ^{13}C-NMR (MeOD) δ (ppm) : 92.83 (C_1'),65.85 (C_5') , 72.25 (C_3') ,81.30 , 82.46 (C_2' , C_4') , 124.2 (C_3'-**C**≡N) , 119.67 (CH- **C**≡N) , 186 (**C**OOH), 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 (CO**OH)** 1370 (C-N_{tert}); ^{1}HNMR (MeOD) δ (ppm) : 3.46 (br s ,OH) , 3.77-3.91 (2H , m, H_5') , , 4.55 (d , H_2') , 4.82 (dd , H_4') ,5.82 (d , H_1') , 3.31 (1H ,s , CNC**H**CO),11.53 (1H ,s , COO**H**) **,**10.53 (br s ,NH) , 6.4 (1H , d , H_2) , 6.6 (1H , d ,H_3) . ^{13}C-NMR (MeOD) δ (ppm) : 88.25 (C_1'),66.87 (C_5') , 72.48 (C_3') ,82.25 , 83.29 (C_2' , C_4') , 121.3 (C_3'-**C**≡N) , 187 (**C**OOH), 165 , 178.5 (**C**=O) , 135.1-158.4 (C_2 and C_4)**.**

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 (CO**OH)** 1384 (C-N_{tert}); ^{1}HNMR (MeOD) δ (ppm) : 3.42 (br s ,OH) , 3.72-3.79 (2H , m , H_5') , , 4.45 (d , H_2') , 4.85 (dd , H_4') ,5.93 (d , H_1') , 3.4 8 (1H ,s , CNC**H**CO),10.48 (1H ,s , COOH) , 11.25 (br s ,NH) ^{13}C-NMR (MeOD) δ (ppm) : 91.41 (C_1'), 69.55 (C_5') , 73.20 (C_3') ,80.56 , 81.24 (C_2' , C_4') , 121.8 (C_3'-**C**≡N) , 183.5 (**C**OOH), 167 , 178 (**C**=O),127-135 (C_2 and C_4)

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 abscense 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 3100-2850 cm^{-1} and two absorption bands at 1700-1750 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 streaching at 2850- 2950 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 2850-2950 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 3200-3400 for (O-H & N-H) group for free nucleoside .

The structure of some of th prepared compounds were confirmed by ^{1}HNMR as shown in table (1). It was obvious that the anomeric proton H-1 appeared as a doublet in the region δ5.32-5.93.

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

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

The ^{1}HNMR spectrum of (34) showed the demonstrated the H-4 as a doublet at δ 4.82 , H-5 at δ 3.77 –3.91 and at δ 4.55 ,H-2 . The signal at δ 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 δ 3.72 – 4.85 . The signal at δ 11.25 was attributed to the amino proton . The signal at δ 3.84 was attributed to proton of CNC**H**CO.

The structure of some of the prepared compounds were also determined by ^{13}C NMR Spectroscopy (Table2) . For example The ^{13}C NMR spectrum of (21) showed the presense of signals for C-1 , C-2 , C-3 ,C-4 , C-5 , aromatic carbon ,carbon cynaide and CH_2NO_2 in the expected chemical shift [15] .

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تحضير مماثلات نيوكليوسيد و نيوكليوتيد جديدة

الخلاصة

اظهرت مماثلات النيوكليوسيدات والنيوكليوتيدات المحتوية على تفرع في جزء السكر سواء كانت (طبيعية او محضرة) فعالية بايلوجية و مضادة للسرطان والفايروسات والبكتيريا .

يتضمن البحث تحضير نوعين من المركبات الجديدة :

[النوع الاول : مماثلات نيوكليوساید تحتوي على تفرع ٣'-دايوكسي-C٣'-سيانو-(٣'-نايتروميثيل)]

[النوع الثاني : مماثلات نيوكليوتايد تحتوي على تفرع ٣'-دايوكسي-C٣'-سيانو(كاربوكسي) مثيل-(٣'-سيانو)]

للحصول على هذه المماثلات تطلب وضع استراتيجية تسهل الوصول للهدف ، وقد اختيرت المادة الاولية ١،٢:٥،٦-ثنائي-O- ايزوبروبيلدين -D– كلوكوفيورانوز الذي يحتوي على مجموعة الهيدروكسيل حرة في موقع ذرة الكاربون ٣- الوحيدة غير المحمية . عند اكسدة (١) بثنائي مثيل سلفوكسيد α وانهدريد الخليك تكون المشتق الكيتوني (٢) .

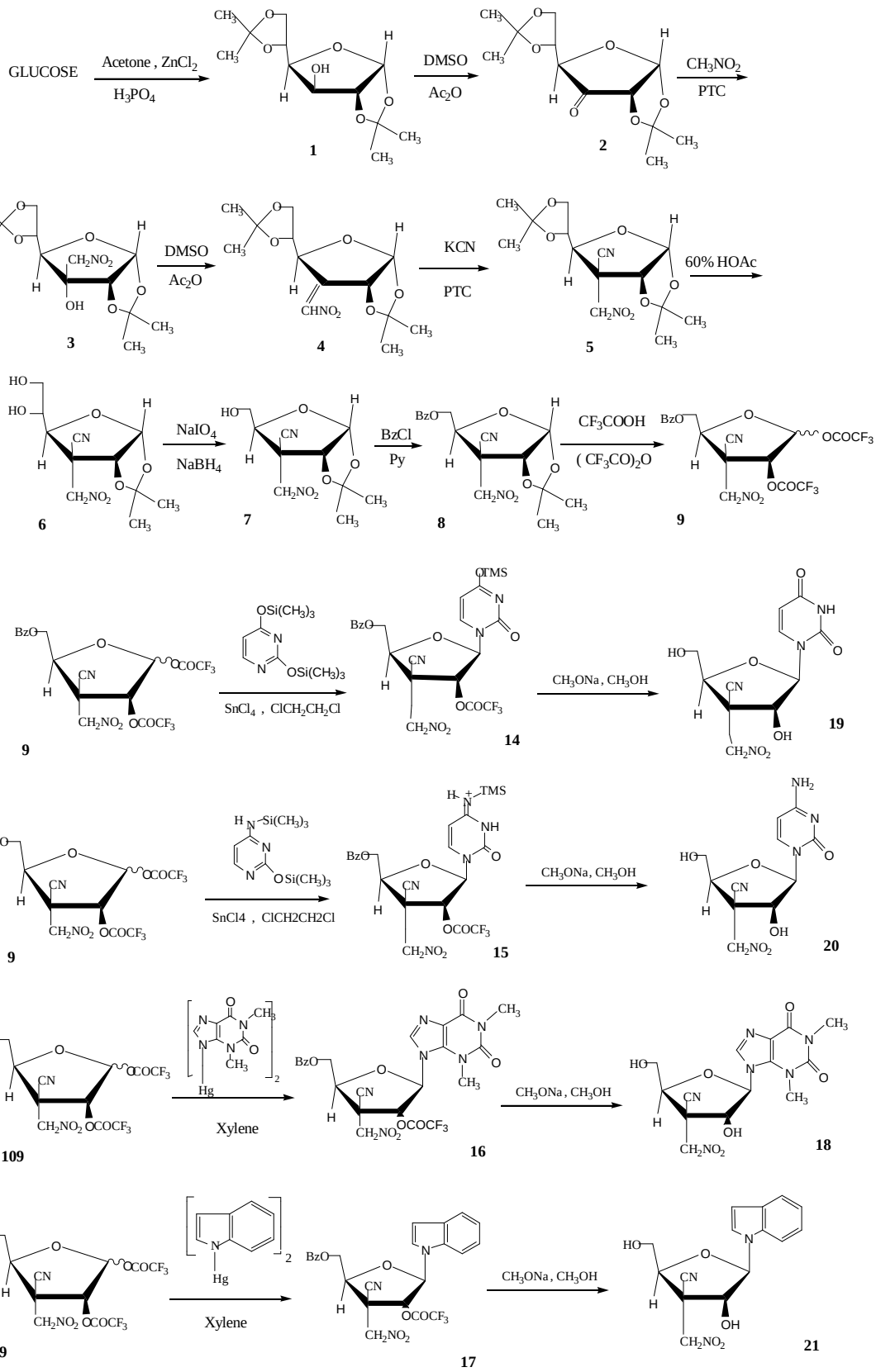
لتكوين النوع الاول من المماثلات مخطط تم اضافة نيتروميثان للمشتق (٢) فتكون المشتق (٣) الذي يتحول الى المشتق (٤) عند معاملته مع ثنائي مثيل سلفوكسيد وانهدريد الخليك . ومن اجل الحصول على مشتق سكر الرايبوز الخماسي الذي يمثل الجزء السكري في النيوكليوسيد تم ازاحة مجموعة الحماية (الايزوبروبليدين) في الموقع ٥،٦ باستخدام حامض الخليك يتبعها عملية اكسدة واختزال نحصل على المشتق (٧) .ان حماية مجموعة الهيدروكسيل في الموقع -٥ باستخدام كلوريد البنزويل يوفر الفرصة المناسبة لازاحة مجموعة الاستيل في الموقع -١،٢ حيث يتكون المشتق (٨) الذي يتحول الى المشتق (٩) بالتفاعل مع حامض الخليك وثلاثي فلوريد حامض الخليك . حيث يؤدي تفاعل كل من المشتق (٩) مع ملح الزئبق للثيوفلين و الاندول و مشتق السيلايل لليوراسيل و مشتق السيلايل سايتوسين الى مماثلات نيوكليوساید (١٤) و (١٥) و (١٦) و (١٧) .

ان تسخين هذه المشتقات كل على حدة مع ميثوكسيد الصوديوم في الميثانول تحت مكثف اعطى المشتق (١٨) و(١٩) و(٢٠) و(٢١) .

حصل على النوع الثاني (مماثلات النيوكليوتايد من معاملة المشتق (٢) مع سيانو اسيتات الاثيل اولا حيث تكون (٢٢) تبع ذلك اضافة سيانيد البوتاسيوم في وسط قاعدي ليعطي المشتق (٢٣) وبازالة مجموعة الحماية في الموقع -٥،٦ باستخدام حامض الخليك يتبعها عملية اكسدة و اختزال لهذا الموقع فيتكون المشتق (٢٥) . ان تفاعل كلوريد البنزويل مع المشتق (٢٥) يؤدي الى حماية مجموعة الهيدروكسيل في الموقع -٥ اذ تكون المشتق (٢٦) ثم تحول الى مشتق ثلاثي فلورو الاستيل (٢٧) بمعملته مع حامض الخليك وثلاثي فلورو انهدريد الخليك .

ان تكاثف المشتق (٢٧) مع ملح الزئبق للثيوفلين والاندول و مشتق السيلايل لليوراسيل و مشتق السيلايل سايتوسين اعطى مماثل النيوكليوتايد (٢٨) و (٢٩) و(٣٠) و(٣١) على التوالي وقد تم الحصول على مماثل النيوكليوتايد الحر (٣٢) و (٣٣) و(٣٤) و(٣٥) من التحليل القاعدي الكحولي للمشتق لمماثل النيوكليوتايد .

تم تشخيص المركبات المحضرة بواسطة الطرق الطيفية : الاشعة تحت الحمراء والرنين النووي المغناطيسي للبروتون و نظير الكاربون -١٣ وكروماتوغرافيا الطبقة الرقيقة . ان الهدف من تحضير مماثلات النيوكليوسيد و النيوكليوتايد المتفرعة هو احتمالية ان تمتلك خواص مضادات السرطان و المضادات الحيوية و الفيروسات .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9
KCN
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NaIO4
NaBH4
BzCl
Py
CF3COOH
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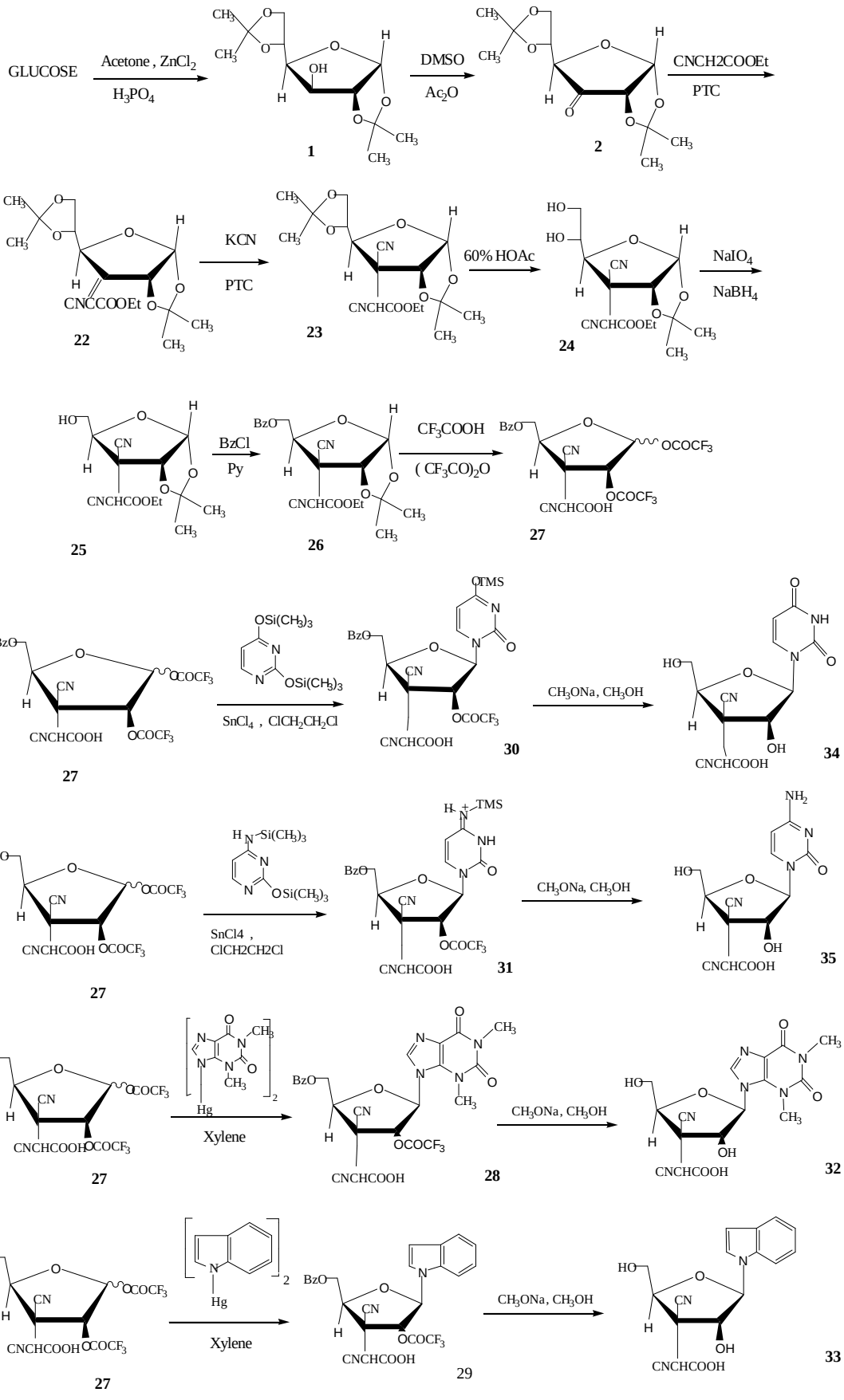
15
20
109
16
18
Xylene
17
21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1
2
KCN
PTC
60% HOAc
NaIO4
NaBH4
22
23
24
BzCl
Py
CF3COOH
(CF3CO)2O
25
26
27
SnCl4 , ClCH2CH2Cl
CH3ONa, CH3OH
30
34
31
35
Hg
Xylene
28
32
29
33

Scheme B

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

Comp.	H-1	H-2	H-4	H-5	CH_2NO_2	COOH	Others
١٨	5.32	5.31	5.28	3.72-3.84	3.75	-------	1.55 (6H , s , NCH_3 of theophylline)
١٩	5.83	4.55	4.89	3.78-3.68	3.88	-------	10.52 (brs , NH of uracil)
٢٠	5.87	4.52	4.88	3.75-3.88	3.83	-------	10.23 (brs , NH) of cytosine
٢١	5.89	4.66	4.93	3.72-3.87	3.82	-------	7.4-7.5 (aromatic proton of indole)
٣٢	5.83	4.53	4.92	3.76-3.85	-------	10.55	1.03 (6H , s , NCH_3 of theophylline)
٣٣	5.93	4.56	4.82	3.71-3.78	-------	10.46	7.35-7.55 (aromatic proton of indole)
٣٤	5.82	4.55	4.82	3.77-3.91	-------	11.53	10.53 (brs , NH of uracil)
٣٥	5.93	4.45	4.85	3.72-3.79	-------	10.48	10.25 (brs , NH) of cytosine

Table (3-3) ^{13}C chemical shift data (in δ values) for final nucleosides

Comp.	C′-1	C′-2	C′-3	C′-4	C′-5	C≡N	CH_2NO_2	Others
١٨	91.11	80.36	73.54	81.12	٦٧,٥٨	122.7	٧١,٦٦	135.2-159.2 aromatic carbon of theophylline
١٩	89.88	80.45	81.76	81.76	٦٧,٣٣	122.1	٧٠,٣٠	134-157 C_2 & C_4 of uracil
٢٠	89.50	80.32	72.85	81.65	٦٨,٢٥	121.4	70.62	135.3-158.6 C_2 & C_4 of cytosine
٢١	87.88	80.22	٧٢,٦٧	٨١,٣٥	66.95	123.9	71.33	127-148 C-aromatic indole
٣٢	87.52	80.26	73.32	٨١,٢٩	67.65	١٢٢,٣	------	169,177 carbonyl of theophylline
٣٣	92.83	81.31	72.25	82.46	٦٥,٨٥	124.5	------	186 carboxylic carbon , 110-143 aromatic indole
٣٤	88.25	82.25	٧٢,٤٨	83.29	٦٦,٨٧	121.3	------	135.1-158 C_2 & C_4 of uracil
٣٥	91.41	80.56	٧٢,٠٠	81.24	٦٩,٥٥	121.8	------	127-135 C_2 & C_4 of cytosine