Synthesis of Some New Cytidine and Inosine Derivatives

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The oxidation of cytidine **1** and inosine **5** by sodium metaperiodate followed by the reaction of the product with CH_3I in NaOH afforded 2',4'-dihydroxyhexopyranosyl derivatives **2** and **14** respectively. The reaction of thiophene-2-carboxylic acid or furfural with cytidine were taken place via cycloaddition afforded adducts **3** and **4** respectivily. The bromination of inosine **5** or its 2',3'-*O*-isopropylidine inosine **6** led to the formation of 8-bromoanalogue **7** and **8**, respectively. The reaction of 8-bromo-2',3'-*O*-isopropylidine inosine (**8**) with ethyl glycinate hydrochloride afforded 8-ethoxycarbonylmethylaminoinosine **9**. The alkylation of the compound **6** with urea led to the formation of 3-carbonylaminoinosine **10**. The oxidation of **6** with DCC afforded 4'-formyl derivative **11**, which on reaction with ethyl glycinate hydrochloride followed by reaction with sodium cyanoborohydride afforded **12**, while the treatment of dialdehyde inosine **13** with ethyl cyanoacetate in the presence of 0.5 N NaOH afforded compound **15**.

Key Words : Inosine dialdehyde, 8-Bromoinosine, 3-Carbonylaminoinosines, 2',4'-Dihydroxyhexopyranosyl hypoxanthine

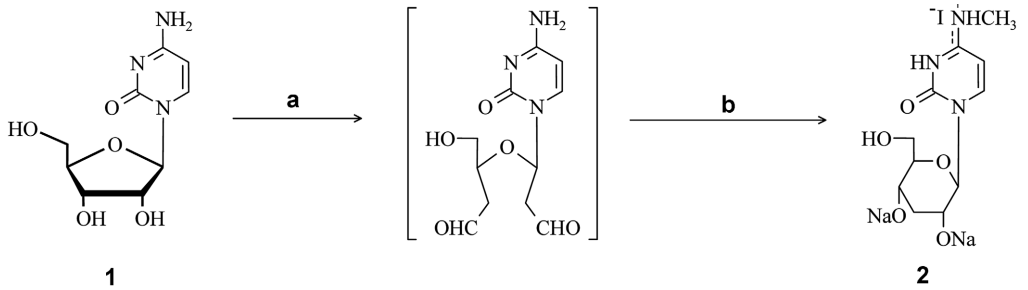
Introduction

Cytidine and inosine, which are considered as modified nucleosides,[1-11] are an important class of non-natural molecules. Owing to their importance as antitumor or antiviral agents, the syntheses and reactions of these compounds have attracted the attention of organic chemists, biochemists and medical chemists.[12,13] In the search for effective, selective, and non-toxic antiviral agents, a variety of strategies have been devised to design nucleoside analogs.[14,15] Herein, we studied the bromination of cytidine and inosine and the reaction of ethyl glycinate hydrochloride with bromo derivatives. Also, the alkylation of cytidine using urea and the oxidation of cytidine and inosine. We studied also the cycloaddition reaction of thiophene-2-carboxylic acid and furfural with cytidine. Finally, we reported some antimicrobial studies for new products.

Results and Discussion

It is important to mention that one of the most important reagents in investigations of the carbohydrate structure is sodium metaperiodate.[16] This reagent oxidatively cleaves C-C bond bearing adjacent OH or NH_2 groups in the carbohydrate molecule to form a dialdehyde in quantitative yield. The reaction of cytidine (**1**) with sodium metaperiodate in aqueous ethanol gave the crude dialdehyde as an intermediate, which was allowed to react with excess methyl iodide in the presence of 1.0 N NaOH to give compound **2** as shown in Scheme 1. The structure of compound **2** was determined by mass spectral analysis to be $C_{11}H_{16}INa_2N_3O_5$ (M^+, 443; M^++2, 445). The fragmentation of mass spectrum showed a peak at m/z 340, 254 (M^+- sugar moiety), 237, 180 and 127. The infrared spectrum of such compound showed absorption bands at υ_{max} 1644 cm^{-1} for (CONH) group, 1375 cm^{-1} (CH_3).

Cycloaddition reactions on the double bond of the heterobase ring of cytidine were conducted with some selected cyclodienes, especially 2-carboxylic thiophene and furfural. These reactions were taken into our interest due to its large area of valuable interests over the last three decades, while a large number of metals, ligands and dienophiles have been described through such reactions with dienophile substituents.[17,18] [2+4] Cycloadditions of reactive dienophiles with a large variety of substituted furans have been the topic of numerous publications ever since the pioneering work of



1 **2**

Scheme 1. Reagents (yield): a) $NaIO_4$, H_2O; b) CH_3I, 0 N NaOH, Dowex 50 (H^+), r.t. (69%).

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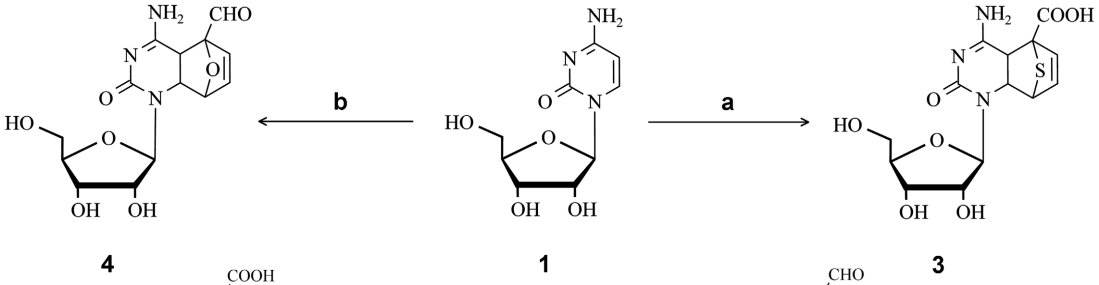
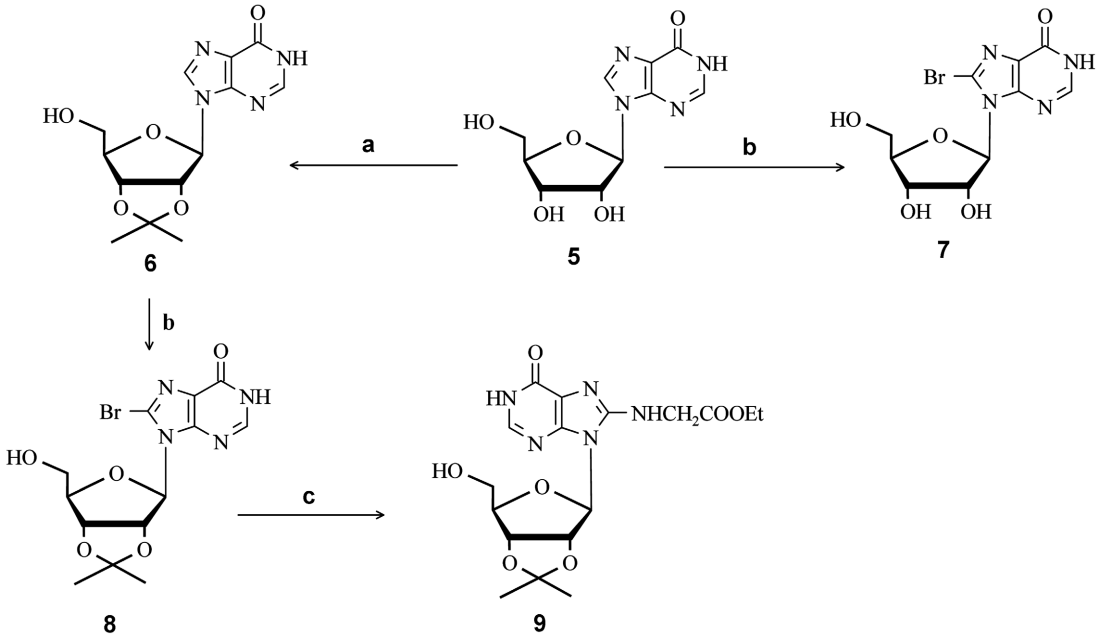
Diels and Alder.[19]

The cytosine ring looks as suitable reactant with the selected dienophiles, while the suitable positions of both (NH_2) group at C-4 and N-1 of the ring in addition to the ribose moiety resulted in combined effects from both mesomeric and inductive effects respectively. Such reasonable effect enhances a dipole carbons 5,6-favoring Diels-Alder reactions with cyclodienes such as 2-carboxylic thiophene and furfural. It was reported that Lewis acids were considered as suitable catalysts for such cycloaddition reactions especially with dienes because it performed one of the most effective agents for the creating new chiral centers during the formation of six membered ring. The optimum Diels-Alder reactions were achieved using $FeCl_3$ wherein adducts **3**, **4** were obtained in 53% and 66% yields with 2-carboxylic thiophene and furfural both of underwent cycloaddition with cytidine at 120 ºC for 12 and 16 hrs respectively. The reactants were dissolved in dry dichloroethane in the presence of $FeCl_3$ as the suitable Lewis acid together with silicon dioxide (SiO_2) as a catalyst to form the corresponding cycloadducts **3**, **4** respectively as shown in Scheme 2. The infrared spectrum for compound **3** showed absorption bands at ν_{max} 3530 cm^{-1} (OH), 3340 cm^{-1} (NH_2), 2950 cm^{-1} (CH aliphatic), 1696 cm^{-1} (C=O) of carboxylic group, 1620 cm^{-1} (C=C) and the molecular formula was determined by mass spectrum to be $C_{14}H_{17}N_3O_7S$ (M^+, 371). The fragmentation of mass spectrum showed a peak at m/z 353, which is corresponding to M^++1-(OH), a peak at m/z 238 corresponds the heterobase + 2-carboxylic thiophene and a peak at m/z 193 is equivalent to heterobase ring after loss of carboxylic group. The infrared spectrum for compound **4** showed absorption bands at ν_{max} 3510 cm^{-1} (OH), 3360 cm^{-1} (NH_2), 2930 cm^{-1} (CH aliphatic), 1720 cm^{-1} (CHO), 1600 cm^{-1} (C=C) and the molecular weight is confirmed by mass spectrum to be $C_{14}H_{17}N_3O_7$, (M^+, 339) and has fragmentation ions at m/z 207 represented heterobase +1 and m/z 96 corresponds to the furfural part.

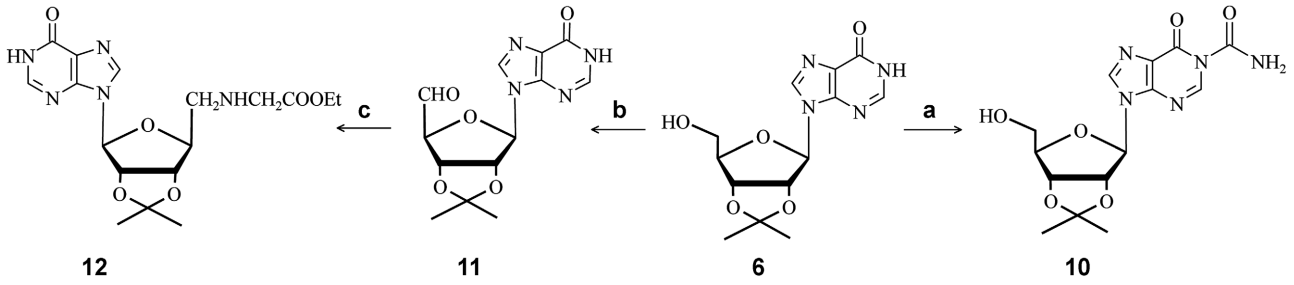
2',3'-O-Isopropylideneinosine (**6**) was prepared according to the same reported method that used with cytidine[20] by the reaction of inosine (**5**) with dry acetone, cupric sulfate, and sulphuric acid to afford **6** (Scheme 3). The infrared spectrum for compound **6** showed absorption bands at ν_{max} 3560 cm^{-1} (OH), 3310 cm^{-1} (NH), 3180 cm^{-1} (CH aromatic), 2920 cm^{-1} (CH aliphatic), 1630 cm^{-1} (C=O) of amide group, 1610 cm^{-1}



Scheme 2. Reagents (yield): (**a**)  , $SiO_2/FeCl_3/CH_2ClCH_2Cl$, 120 °C, 12 hr (53%); (**b**)  , CH_2ClCH_2Cl, $FeCl_3$, SiO_2, 120 °C, 16 hr. (63%).



Scheme 3. Reagents (yield): (**a**) $CuSO_4$, Conc. H_2SO_4, acetone, stir., 48 hr., r.t. (92%); (**b**) Br_2/CCl_4, 0.5 N HNO_3, dioxane, 8 hr., r.t. (80); (**c**) NH_3^+(Cl) CH_2COOEt, CH_3OH, TEA, reflux 8 hr., (72%)



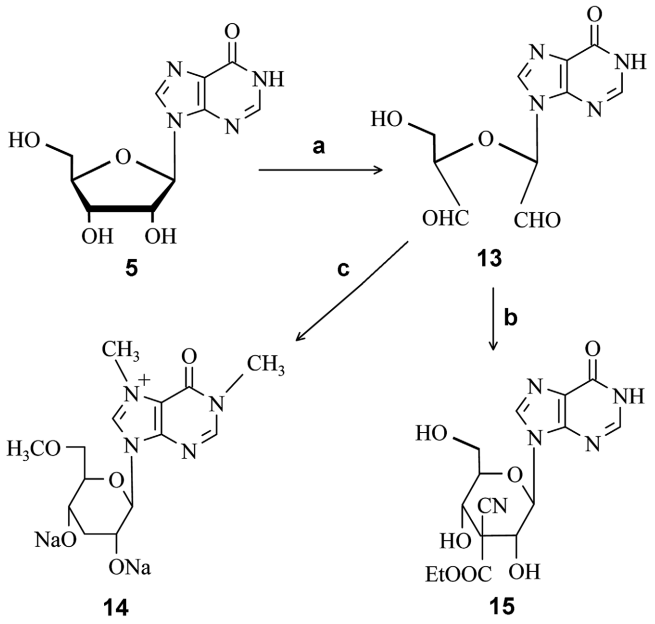
Scheme 4. Reagents (yield): (**a**) Urea, DMF, TEA, reflux 17 hr., (67%); (**b**) DCC, DMSO, pyridine, TEA, benzene, r.t. (92%); (**c**) ethyl glycinate hydrochloride, $NaBH_3(CN)$, HCOOH, CH_3OH, stir. 7 hr., r.t. (43%).

(C=C), while the molecular formula was elucidated by mass spectrum to be $C_{13}H_{16}N_4O_5$, (M^+, 308) and has fragmentation ions at m/z 274, 268, 237. Preparation of 8-bromo-inosine[21] **7** and 8-bromo-*2',3'-O*-isopropylideneinosine **8** were also accomplished by reacting inosine (**5**) and *2',3'-O*-isopropylidene inosine (**6**) successively, with a bromine solution in the presence of both of 1,4-dioxane and carbon tetrachloride[22] (Scheme 3). The two products were afforded in a quantitative yields and the structure of compound **8** was confirmed by spectroscopic analysis The infrared spectrum showed absorption bands at ν_{max} 3550 cm^{-1} (OH), 3320 cm^{-1} (NH), 3130 cm^{-1} (CH aromatic), 2940 cm^{-1} (CH aliphatic), 1630 cm^{-1} (C=O) of amide group and ^1H NMR spectrum showed the absence of the specific signal peak at δ 8.52 which characterizing the proton at position 8 of the purine ring, also showed a signal peak at δ 8.12 for CH-2. On the other hand, 8-bromo-*2',3'-O*-isopropylidene inosine (**8**) was reacted with ethyl glycinate hydrochloride in basic conditions by addition of drops of triethylamine then the reactants were refluxed for 7 hrs, and the residual product was purified on a column of silica gel to yield compound **9** (Scheme 3). ^1H NMR spectrum of the resulted product **9** revealed a broad multiplet peak at δ 1.16-1.19 which corresponds to six protons of isopropylidene group, a triplet peak at δ 1.25 represents methyl group, quartet peak at δ 3.45 represents a methylene group of $COOCH_2CH_3$ and a singlet peak at δ 3.52 for a methylene group, a singlet peak at δ 8.3 corresponds to NH-CH_2 and abroad singlet peak at δ 10.24 corresponds one proton of NH of the purine ring.

Nevertheless, inosine was refluxed with urea in basic conditions (drops of triethylamine) for 17 hrs to afford compound **10** as shown in Scheme 4 in 67% yield. The infrared spectrum for compound **10** showed absorption bands at ν_{max} 3530 cm^{-1} (OH), 3340 cm^{-1} (NH), 3180 cm^{-1} (CH aromatic), 2950-2930 cm^{-1} (CH aliphatic), 1660 cm^{-1} (C=O), 1630 cm^{-1} (C=O) of amide group and ^1H NMR spectrum of the discussed compound **10** showed a singlet peak at δ 5.63 represents OH-5', a peak at δ 8.12 represents the CH-8, a peak at δ 8.01 represents CH-2 and a peak at δ 8.6 which is characteristic to the side chain NH_2 of purine ring and the molecular weight is confirmed by mass spectrum to be $C_{14}H_{17}N_5O_6$, (M^+, 351) and another fragments at 307 (M^+-$CONH_2$), 293 (M^+-$NHCONH_2$), 279, 239 and 167. *2',3'-O*-Isopropylidene inosine (**6**) was oxidized by *N,N'*-dicyclohexylcarbodiimide (DCC) and trifluoroacetic acid in the presence of dimethylsulfoxide (DMSO), which led to convert the 5'-alcoholic group of the sugar moiety into the corresponding 5'-formyl derivative **11** which its molecular formula was elucidated by mass spectrum to be $C_{13}H_{14}N_4O_5$, (M^+, 306) and has fragmentation ions at m/z 264 (M^+-$C(CH_3)_2$), 225, 191, 135.9 (M^++ 1- sugar moiety) and 111. The infrared spectrum for compounds **11** showed absorption bands at ν_{max} 3320 cm^{-1} (NH), 3190 cm^{-1} (CH aromatic), 2940 cm^{-1} (CH aliphatic), 1720 cm^{-1} (CHO), 1630 cm^{-1} (C=O) of amide group. Compound **11** were reacted with ethyl glycinate hydrochloride and the produced Schiff base intermediate was reduced *in situ* by sodium cyanoborohydride to give compound **12** as shown in Scheme 4. The molecular weight of compound **12** is confirmed by mass spectrum to be $C_{17}H_{23}N_5O_6$, (M^+, 393) and has fragmentation ions at m/z 364 due to loss of CH_2-CH_3, m/z 307 (M^++1 -$CH_2COOC_2H_5$), 291 (M^+-$NHCH_2COOC_2H_5$), 279. The infrared spectrum that showed absorption bands at ν_{max} 3350 cm^{-1} (NH), 3310 cm^{-1} (NHCO), 3180 cm^{-1} (CH aromatic), 2970-2930 cm^{-1} (CH aliphatic), 1760 cm^{-1} (C=O), 1630 cm^{-1} (C=O) of amide group.

Inosine dialdehyde (IDA),[23,24] is the periodate-oxidation product of inosine, which is highly active against several transplantable murine tumors including L1210 and P388 leukemias and a number of variants of L1210 resistant to commonly, used cancer chemotherapeutic agents.

Furthermore, the reaction of inosine **5** with sodium meta-periodate in aqueous ethanol gave the crude dialdehyde **13**. ^1H NMR spectrum for the dialdehyde inosine derivative **13** revealed a singlet peak at δ 9.2, a singlet peak at δ 9.6 represents (CHO-3') and (CHO-2') respectively, a singlet peak at δ 11.82 represents NH of purine ring, and two singlet peak at δ 8.47 and δ 8.45 represents CH-2, CH-8. The infrared spectrum that showed absorption bands at ν_{max} 3480 cm^{-1} (OH), 3320 cm^{-1} (NHCO), 3180 cm^{-1} (CH aromatic), 2950 cm^{-1} (CH aliphatic), 1720 cm^{-1} (CHO), 1630 cm^{-1} (C=O) of amide group. Compound **13** was allowed to react with excess methyl iodide in the presence of 1.0 N NaOH to give compound **14** as shown in Scheme 5. The molecular weight of compound **14** was determined by mass spectrum to be $C_{14}H_{19}Na_2N_4O_5$, (M^++1, 370) and has fragmentation ions at m/z 250, 237, 170, and 164. The infrared spectrum showed absorption bands at ν_{max} 3465 cm^{-1} (OH), 1652 cm^{-1} (C=O), 1365 cm^{-1} (CH_3). On the other side, compound **13** was further allowed to react with ethyl cyanoacetate in the presence of 0.5 N NaOH to give compound **15** (Scheme 5).



Scheme 5. Reagents (yield): (**a**) $NaIO_4$, H_2O, stir., r.t., 20 min (85%); (**b**) Ethyl cyanoacetate, 1.0 N NaOH, r.t. 4 hr., Dowex 50 (H^+), (76%); (**c**) CH_3I, 1.0 N NaOH, Dowex 50 (H^+), r.t. 4 hr., (70%)

The structure of such compound **15** was proved by mass spectral data to be compatible with the molecular formula $C_{15}H_{17}N_5O_7$ (M^+, 379) with fragmentation of mass spectrum showed a peak at m/z 334 which is corresponding to M^+-(OCH_2CH_3) and a peak at m/z 307 corresponds to (M^++1-CH_3CH_2COO) and also m/z 135 represents the heterobase. The infrared spectrum of compound **15** showed also absorption bands at υ_{max} 3431 cm^{-1} (OH), 2925 cm^{-1} for aliphatic group, 2241 cm^{-1} (CN), 1705 cm^{-1} (C=O) for ester, 1656 cm^{-1} (C=O) for amide group, and 3080 cm^{-1} for an aromatic ring.

Antimicrobial activity. The novel compounds **2-4**, **6**, **8**, and **10-15** were screened for their *in vitro* antimicrobial activity against gram positive bacteria such as (*staphylococcus aureus* and *Bacillus subtilis*), gram negative bacteria such as (*pseudomonas aeuroginosa*) and fungi (*Aspergillus niger, Penicillium funiculosum*) using the Cup-plate technique.[25,26] It was clear from Table 1 that the tested compound **6**, **8** and **15** showed considerable activity against *Aspergillus niger* as fungi , *staphylococcus aureus* as gram positive and *pseudomonas aeuroginosa* as Gram-negative respectively. Compounds **3**, **11**, **12** and **15** showed slight activity against *Bacillus subtilis*; **4** and **8** showed slight activity against *pseudomonas aeuroginosa*; **6**, **11** and **12** showed slight activity against *staphylococcus aureus*, and **5** showed slight activity against *Penicillium funiculosum* and no active against the rest of the tested microorganisms.

Table 1. Antimicrobial activity of the compounds considered

Compound*	** Microorganisms				
	1	2	3	4	5
2	–	–	–	–	–
3	–	–	+	–	–
4	–	+	–	+	–
6	+	–	–	++	–
8	++	+	–	+	+
10	+	–	–	–	–
11	–	–	+	–	–
12	+	–	+	–	–
13	–	–	–	–	–
14	–	–	–	–	–
15	–	++	+	–	–

*The solvent is dimethylsulphoxide (DMSO). **1: *Staphylococcus aureus* 2: *Pseudomonas aeuroginosa*. 3: *Bacillus subtilis*. 4: *Aspergillus niger*. 5: *Penicillium funiculosum*. +++: highly active, ++: moderately active, +: slightly active, –: inactive

Experimental Section

Melting points were determined with an Electro Thermal Mel-Temp II apparatus and were uncorrected. IR spectra were obtained in the solid state as potassium bromide disc using a Perkin-Elmer model 1430 spectrometer. ^{1}H NMR was recorded on a Varian/Gemini 200/MHZ spectrometer in DMSO-d_6 as a solvent and TMS as an internal standard (Chemical shift in δ, ppm). Mass spectra were measured on an instrument VG- 7035, spectra were recorded at 70 or 15 electron volt. Elemental analysis was performed at the Micro analytical center, Cairo University, Giza, Egypt. All solvents were purified and dried according to the general procedures given in A.I. Vogel (organic preparation)

1-(2',4'–Dihydroxy-5'-hydroxymethyl-β-D-hexopyranosyl sodium)-4-methyl-cytosinium iodide 2. To a stirred ice-cold solution of sodium metaperiodate (0.15 g, 0.70 mmol) in water (20 mL) was added of cytidine (**1**) (0.17 g, 0.70 mmol). After completion the addition of reactant, the solution was stirred at room temperature for 20 min then kept overnight in a refrigerator. The precipitate (A) of inorganic salts was filtered off and the filtrate was poured into vigorously stirred ethanol (20 mL), giving a heavy white precipitate (B). Precipitates (A) and (B) were combined and well washed with ethanol. The solid was discarded, and the filtrate of washing (ethanol) were combined and evaporated *in vacuo* to yield the dialdehyde. Without further purification, methyl iodide (0.3 mL), water (3.0 mL) and 1.0 N sodium hydroxide (1.0 mL) was added to the dialdehyde during vigorous stirring. After being kept the reaction mixture for 4 hrs at room temperature. Dowex 50 (H^+) was added and the mixture was also stirred for 1 hr at r.t. (*else then filtered off and extracted with methanol to dissolve the produced nucleoside*). The resin was removed by filtration. Evaporation of the filtrate to dryness gave pale yellow crystal, which were washed with a small quantity of ice-cold water to give **2** (0.20 g, 69%); m.p. > 300 °C; R_f: 0.64 in chloroform : methanol (80 : 20). IR (KBr) 3444, 1644, 1375, 1120, 837, 778 cm^{-1}; ^{1}H NMR δ 8.10 (d, 1H, $J_{6,5}$ = 7.4 Hz, H-6), 7.68, 7.69 (each s, 2H, 4-NH_2), 5.84 (d, 1H, H-5), 5.15 (s, 1H, H-1′), 4.99 (t, 1H, J = 4.7 Hz, OH-5′), 4.1-4.87 (m, 3H, H-2′, 3′, 4′), 3.7-3.9 (m, 2H, CH_2-5′); M.S: (M^+ 443, $C_{11}H_{16}INa_2N_3O_5$. Anal. calc. for $C_{11}H_{16}INa_2N_3O_5$: C, 29.80; H, 3.63; N, 9.47. Found: C, 30.02; H, 3.41; N, 9.16.

Reaction of cytidine with 2-carboxylic thiophene 3. Thiophene 2-carboxylic acid (0.60 g, 4.68 mmol) was added to a solution of cytidine (**1**) (0.15 g, 0.61 mmol) in dry dichloroethane (20 mL) in the presence of ferric chloride in water (5 mL) and few crystals of silicon dioxide. The reactants were heated under reflux for 12 hrs. The reaction mixture was followed by thin layer chromatography and the solvent was evaporated under vacuum, and the product was chromatographed on a column of silica gel using a mixture of ethylacetate: petroleum ether as eluent to afford a white crystal **3** (0.12 g, 53%) m.p. 195 °C; ^{1}H NMR: δ 12.23 (s, 1H, COOH), 7.88 (d, 1H, J= 7.4, =CH), 7.32, 7.34 (each s, 2H, 4-NH_2), 5.68 (d, 1H, =CH), 5.35 (d, 1H, $J_{1',2'}$ = 3.1 Hz, H-1′), 4.99-4.89 (t, 1H, J = 4.7 Hz, OH-5′), 4.10-4.77 (m, 3H, H-2′,3′,4′), 3.7-3.9 (m, 2H, CH_2-5′); M.S: (M^+ 371). Anal. calc. for $C_{14}H_{17}N_3O_7S$. C, 45.28; H, 4.61; N, 11.31. Found: C, 45.12; H, 4.53; N, 11.00.

Reaction of cytidine with furfural 4. Furfural (0.40 g, 4.16 mmol) was added to a solution of cytidine (**1**) (0.17 g, 0.69 mmol) in dry dichloroethane (20 mL) and in the presence of ferric chloride solution and few crystals of silicon dioxide. The mixture was heated under reflux for 16 hrs. The reaction was monitored by thin layer chromatography and the solvent was evaporated under vacuum, and the product was chromatographed on a column of silica gel using a mixture of dichloromethane: methanol as eluent to afford a white crystal **4** (0.15 g, 63%); m.p. 207 °C; ^{1}H NMR: δ 10.21 (s, 1H, CHO), 7.83 (d, 1H, J= 7.4, =CH), 7.30, 7.32 (each s, 2H, 4-NH_2), 5.64 (d, 1H, =CH), 5.34 (d, 1H, $J_{1',2'}$ = 3.1 Hz, H-1′), 4.97-4.88 (t, 1H, J = 4.7 Hz, OH-5′), 4.12-4.73 (m, 3H, H-2′,3′,4′), 3.7-3.9 (m, 2H, CH_2-5′) M.S: (M^+ 339). Anal. calc. for $C_{14}H_{17}N_3O_7$. C, 49.55; H, 5.05; N, 12.38. Found: C, 49.03; H, 4.89; N, 12.11.

***2′,3′-O*-Isopropylidene inosine (6).** A mixture of inosine (**5**) (0.25 g, 0.93 mmol), anhydrous cupric sulphate (0.5 g) and concentrated sulphuric acid (0.15 mL) in dry acetone (10 mL) was stirred for 48 hrs at room temperature. The precipitate formed was filtered and the filtrate was then neutralized with anhydrous calcium carbonate (10 g) and the mixture was filtered again. The filtrate was evaporated under reduced pressure. The residue was purified by crystallization from absolute methanol to give colorless crystal **6** (0.26 g, 92%); m.p. 138-140 °C R_f: 0.46 in chloroform : methanol 90 : 10; ^{1}H NMR: δ 8.08 (s, 1H, 2-CH), 8.04 (s, 1H, 8-CH), 5.70 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1′), 5.07 (dd, 1H, J = 3.4 Hz, 6.4 Hz, H-2′), 4.64- 5.54 (m, 3H, H-2′,3′,OH), 4.00-4.32 (m, 1H, H-3′), 3.37-4.00 (m, 3H, H- 4′,5′); MS: (M^+, 308). Anal. calc. for $C_{13}H_{16}N_4O_5$: C, 50.64; H, 5.23; N, 18.17. Found: C, 50.37; H, 5.19; N, 17.98.

8-Bromoinosine (7).[21] A solution of bromine (0.07 mL) in carbon tetrachloride (2.0 mL) was added to a mixture of inosine (**5**) (0.25 g, 0.81 mmol), few drops of 0.5 N nitric acid and dry dioxane (8.0 mL), then the mixture was stirred for 8 hrs at room temperature. The solvent was removed by evaporating *in vacuo* to dryness and the residue was re-evaporated three times with a mixture of ethanol-ether to remove any traces of water to give colorless crystal **7** (0.26 g, 80%); m.p. 196-198 °C; ^{1}H NMR: δ 8.07 (s, 1H, H-2), 5.81 (d, 1H, J = 3.4 Hz, H-1′), 4.76-5.17 (m, 1H, H-2′), 4.64-5.54 (m, 3H, H-2′,3′,OH), 4.00- 4.32 (m, 1H, H-3′), 3.37-4.00 (m, 3H, H- 4′,5′); Anal. calc. for $C_{10}H_{11}BrN_4O_5$: C, 34.59; H, 3.19; N, 16.13. Found: C, 34.11; H, 3.07; N, 15.86.

8-Bromo-*2′,3′-O*-isopropylidene inosine (8). This was prepared by the same previous method in (0.25 g, 80%) of **6**; m.p. 217-219 °C; R_f = 0.46 in chloroform: methanol (80 : 20), MS.: (M^+ 387.27, M^++2 389). Anal. calc. for $C_{13}H_{15}BrN_4O_5$: C, 40.31; H, 3.90; N, 14.46. Found: C, 40.23; H, 3.78; N, 14.03.

8-Ethoxycarbonylmethylamino-*2′,3′-O*-isopropylidene inosine (9). A solution of 8-bromo-*2′,3′-O*-isopropylidene inosine (**8**) (0.25 g, 0.64 mmol), in dry methanol (10 mL) was added drop by drop with stirring to a solution of ethyl glycinate hydrochloride (0.12 g, 0.85 mmol) in dry methanol (10 mL) then an small amount of triethylamine (0.1 mL) was added. The reaction mixture was stirred under heating reflux for 8 hrs and monitored by TLC. The solvent was evaporated to dryness in vacuo. The residue was purified on a column of silica gel using chloroform : methanol (90 : 10) for elution to give colorless gum **9** (0.14 g, 72%); R_f = 0.5 in chloroform: methanol (80 : 20). IR spectra showed bands at 3440, 3320, 2900, 1659, 1725, 1480, 1100 cm^{-1}; ^{1}H NMR: δ 10.24 (s, 1H, NH-ring), 8.3 (s, 1H, NH-CH_2), 8.12 (s, 1H, CH-2), 4.2-4.1 (m, 3H, H-2′,3′,4′), 3.45 (q, 2H, J = 7.4 Hz, CH_2-CH_3), 1.25-1.31 (m, 9H, C $(CH_3)_2$ +CH_3); MS: (M^+, 409). Anal. calc. for $C_{17}H_{23}N_5O_7$: C, 49.87; H, 5.66; N, 17.10. Found: C, 49.56; H, 5.43; N, 16.91.

3-Carbonylamino-*2′,3′-O*-isopropylidene inosine (10). Compound **6** (0.25 g, 0.81 mmol) was dissolved in DMF (dimethylformamide) (20.0 mL), and urea (0.14 g, 2.3 mmol) was added in the presence of drops of triethylamine. The reaction mixture was heated under refluxed for 17 hrs and the solvent was evaporated to dryness. The residue was chromatographed on a column of silica gel using chloroform : methanol (95 : 5) for elution to give colorless crystal **10** (0.21 g, 67%); m.p. 210-212 °C; R_f = 0.53 in chloroform: methanol (85 : 15). ^{1}H NMR δ 8.69 (s, 2H, NH_2), 8.12 (s, 1H, CH-8), 8.01 (s, 1H, CH-2), 5.63 (s, 1H, OH-5′), 4.32-4.21 (m, 3H, H-2′,3′,4′), 3.7-3.62 (m, 2H, CH_2-5′); MS: (M^+ 351). Anal. calc. for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.87; N, 19.93. Found: C, 47.53; H, 4.72; N, 19.46.

5′-Formyl-*2′,3′-O*-isopropylidene inosine (11). A solution of compound **6** (0.25 g, 0.81 mmol) in anhydrous DMSO was added to a mixture of dicyclohexyl-carbodiimide (0.42 g, 2.05 mmol), pyridine (0.025 mL) and trifluoroacetic acid (0.3 mL) in benzene (2.5 mL). The mixture was kept at room temperature overnight. Oxalic acid (0.2 g, 2.22 mmol) was then added to decompose the excess carbodiimide and after 1hr chloroform (25 mL) was added with water (25 mL), then dicyclourea was removed by filtration and the chloroform layer was washed by 1.0 N sodium hydrogen carbonate and evaporated to dryness. The residue was dissolved in hot methanol and allowed to cool very slowly to give colorless crystal **11** (0.23 g, 92%); m.p. 175-177 °C; R_f = 0.43 in chloroform : methanol (90 : 10); ^{1}H NMR δ 10.2 (s, 1H, NH), 9.1 (s, 1H, CHO), 8.4 (s, 1H, H-8),

8.3 (s, 1H, CH-2), 5.81 (d, 1H, J = 3.4Hz, H-1′), 4.2-4.13 (m, 3H, H-2′,3′,4′), 3.5-3.42 (m, 2H, CH_2-5′), 1.34 (s, 6H, $C(CH_3)_2$) MS: (M^+, 306). Anal. calc. for $C_{13}H_{14}N_4O_5$: C, 50.98; H, 4.60; N, 18.29. Found: C, 50.73; H, 4.46; N, 18.01.

5′-Deoxy-5′-ethoxycarbonylmethylamino-*2′,3′*-*O*-isopropylidene inosine (12). A mixture of 5′-formyl-*2′,3′-O*-isopropylidine inosine (**11**) (0.12 g, 0.39 mmol), ethyl glycinatehydrochloride (0.15 g, 1.07 mmol), sodium cyanoborohydride (0.03 g, 0.47 mmol) and formic acid (2.5 mL) in dry methanol (25 mL) were stirred for 7 hrs at room temperature then the mixture was filtered and few drops of triethylamine was added to the filtrate. The solvent was evaporated and the dry residue was purified on a column of silica gel using a mixture of chloroform : methanol (95 : 5) as eluent to give colorless crystal **12** (0.07 g, 43%); m.p. 236-238 ºC; R_f: 0.35 in chloroform : methanol (80 : 20); ^{1}H NMR: δ 10.24 (s, 1H, NH-ring), 8.12 (s, 1H, CH-2), 8.06 (s, 1H, CH-8), 6.12-6.09 (m, 1H, NH), 5.74 (d, 1H, J = 3.4 Hz, H-1′), 4.27-4.12 (m, 5H, H-2′,3′,4′, NCH_2C), 3.43 (q, 2H, J = 7.4 Hz, CH_2-CH_3), 2.7 (d, 2H, CH_2-NH), 1.21-1.29 (m, 9H, C $(CH_3)_2$+CH_3); MS: (M^+, 393). Anal. calc. for $C_{17}H_{23}N_5O_6$: C, 51.90; H, 5.89; N, 17.80. Found: C, 51.78; H, 5.81; N, 17.65.

2′,3′-Diformyl-2′,3′-deoxyinosine (13). To a stirred ice-cold solution of sodium meta periodate (0.07 g, 0.32 mmol) in water (20 mL) was added (0.5 g, 1.86 mmol) of inosine (**5**). After completion the addition of reactant, the solution was stirred at room temperature for 20 min then kept overnight in a refrigerator. The precipitate (A) of inorganic salts was filtered and the filtrate was poured into vigorously stirred ethanol (20 mL), giving a heavy white precipitate (B). The two parts of (A) and (B) were combined and well washed with ethanol. The solid was discarded, and the filtrates of washing were combined and evaporated *in vacuo* to give **13** (0.42 g, 85%); m.p. 148-50 ºC; R_f = 0.53 in chloroform : methanol (80 : 10); ^{1}H NMR δ 11.82 (s, 1H, NH), 9.66 (s, 1H, CHO-2′), 9.24 (s, 1H, CHO-3′), 8.45 (s, 1H, CH-2), 8.47 (s, 1H, CH-8), 6.38 (s, 1H, H-1′), 5.7 (s, 1H, OH-5′), 4.2-4.11 (m, 3H, H-2′,3′,4′), 3.5-3.32 (m, 2H, CH_2-5′). Anal. calc. for $C_{10}H_{10}N_4O_5$: C, 45.11; H, 3.78; N, 21.04. Found: C, 44.89; H, 3.69; N, 20.93.

9-(2′,4′-Dihydroxy-5′-methoxymethyl-β-D-hexopyranosylsodium)-3,8-dimethylhypoxanthium 14. Methyl iodide (1.0 mL), water (3.0 mL) and 1.0 N sodium hydroxide (1.0 mL) were added to the produced dialdehyde **13** (0.15 g, 0.56 mmol) during vigorous stirring. After being, kept the reaction mixture for 4 hrs at room temperature. Dowex 50 (H^+) was added and the mixture was also stirred for 1 hr. Else then filtered off and extracted with methanol to dissolve the produced nucleoside. The resin was removed by filtration. Evaporation of the filtrate to dryness gives pale yellow crystals, which were washed off with a small quantity of ice-cold water to give **14** (0.14 g, 70%); m.p.: 172-174 ºC; R_f = 0.42 in chloroform : methanol (90 : 10); ^{1}H NMR δ 8.54 (s, 1H, CH-8), 7.89 (s, 1H, CH-2), 5.15 (s, 1H, J = 7.4 Hz, H-1′), 4.99-4.87 (m, 1H, J = 4.7 Hz, H-5′), 4.1- 4.87 (m, 4H, H-2′,3′,4′), 3.7-3.9 (m, 2H, CH_2-6′), 3.67 (s, 3H, OCH_3-6′), 3.52 (s, 3H, NCH_3-7), 3.29 (s, 3H, NCH_3-1); MS: (M^+ 369.31). Anal. calc. for $C_{14}H_{19}Na_2N_4O_5$: C, 45.52; H, 5.18; N, 15.16. Found: C, 45.13; H, 5.06; N, 14.89.

9-(4′-Cyano-4′-ethoxycarbonyl-β-D-hexopyranosyl) hypoxanthine (15). Ethyl cyanoacetate (0.2 mL), water (3.0 mL) and 1.0 N sodium hydroxide (1.0 mL) were added to the dialdehyde **13** (0.15 g, 0.56 mmol) during vigorous stirring. After being kept the reaction mixture for 4 hrs at room temperature. Dowex 50 (H^+) was added and the mixture was also stirred for 1 hr. *(else then filtered off and extracted with methanol to dissolve the produced nucleoside)*. The resin was removed by filtration. Evaporation of the solvent from the filtrate to dryness gives pale yellow crystal, which were washed off with a small quantity of ice cold water to give **15** (0.17 g, 76%); m.p.: 237-239 ºC; R_f = 0.71 in ethyl acetate : ethanol (3 : 2); IR spectra showed bands at 3431, 3080, 2925, 2854, 2241, 1705, 1656, 1602, 1494, 1444, 1413, 1217, 1036 cm^{-1}; ^{1}H NMR: δ 10.21 (s, 1H, NH-ring), 8.09 (s, 1H, CH-2), 8.01 (s, 1H, CH-8), 5.78 (d, 1H, J = 3.4 Hz, H-1′), 4.38-4.31 (m, 1H, H-5′), 4.27-4.12 (m, 2H, H-2′,4′), 3.7-3.62 (m, 2H, CH_2-6′), 3.43 (q, 2H, J = 7.4 Hz, CH_2-CH_3), 1.24-1.29 (t, 3H, CH_3); MS: (M^+, 379). Anal. calc. for $C_{15}H_{17}N_5O_7$: C, 47.49; H, 4.51; N, 18.46. Found: C, 47.18; H, 4.32; N, 18.03.

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