SYNTHESIS OF PURINE AND PRIMIDINE NUCLEOSIDES ANALOGUES

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Abstract

The theophlline and thymine nucleosides analogues (8) and (9) were obtained via reaction of diacetone glucose (1) with dimethyl sulphoxide and acetic anhydride to give diacetone glucose-3-ulose derivative (2). Reaction of compound (2) with di-methylmalonate yielded the 3-C-dimethylmalonyl derivative (3). The isopropylidene at 5, 6-position was removed with acetic acid followed by periodate oxidation and borohydride reduction to give the ribo derivative (5). The 3, 5-dihydroxy groups were protected with benzoyl groups using benzoyl chloride to give the 3,5-dibenzoate derivatives (6). Treatment of compound (6) with mixture of acetic acid, acetic anhydride and few drops of sulfuric acid to gave the 1, 2-di-O-acetylated compound (7). When compound (7) was allowed to react with theophlline and silylated thymine, compounds (8) and (9) were obtained. Deprotection of compounds (8) and (9) under basic condition yield the new nucleosides analogues (10) and (11).

Introduction

The nucleosides and nucleotides as the building block of (DNA) and (RNA) play an important role in the molecular mechanisms of conversion, replication and transcription of the genetic information. Also the nucleoside analogues have been found to display a wide rang of biological activities[1-9]. Therefore nucleosides organic chemistry has been actively involved in the synthesis of nucleoside analogues[10-12] (NA). Stimulated my interest for synthesis of new nucleoside analogues.

Results and Discussion

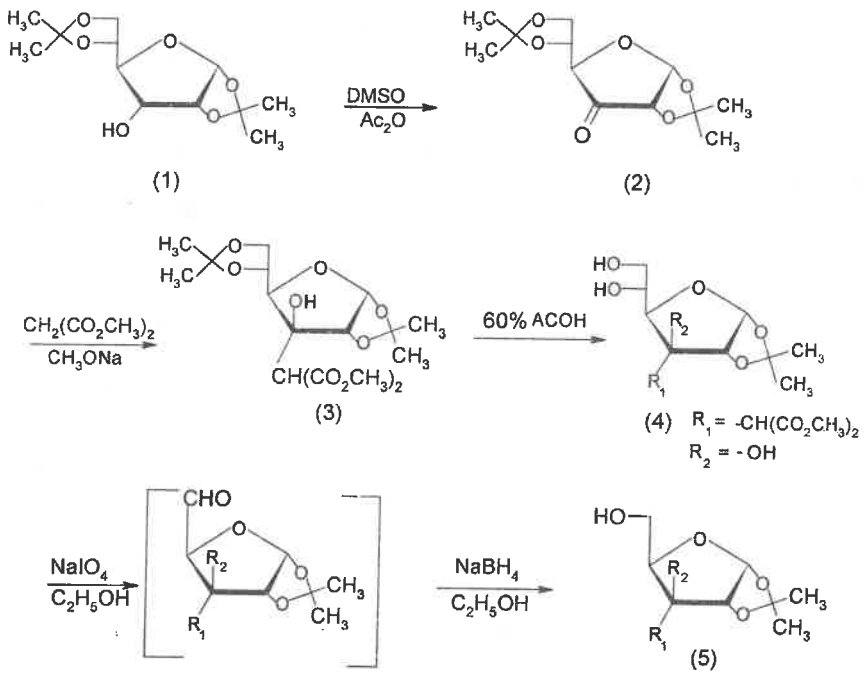
In this paper, i report here a method for the synthesis of purine and pyrimidine nucleosides analogues.

Starting from 1, 2: 5, 6– di-*O*- isopropylidene-α-D-glucofuranos -3-ulose[13, 14] (2) reacted with dimethylmalonate in the presence of sodium methoxid to give the malonate derivative (3) (56.71 % yield) as syrup. The structure of compound (3) was established based on the data of IR, ^{1}H-NMR spectra and elemental analysis. The IR spectrum of this compound showed a band of hydroxyl and ester groups. While their ^{1}H-NMR spectrum revealed the signal of hydroxyl group, four signals of methyl groups for isopropylidene and the signal of dimethylmalonyl . The isopropylidene at 5, 6-positions for compound (3) was removed by hydrolysis with acetic acid[15] (60 %) to afford the diol derivative (4) (70.87 % yield) as syrup. The IR spectrum of this compound showed a band of hydroxyl groups and ^{1}H-NMR

spectrum revealed the signal of hydroxyl groups with the disappearance of two -CH_3 signals.

Oxidation of compound (4) with sodium periodat in ethanol give aldehyed as intermediate followed by reduction immediately with sodium borohydride to give compound (5) (49.40 % yield) as syrup (*cf.* Scheme -1).

The structure of compound (5) was established based on the data of IR, ^{1}H-NMR spectra and elemental analysis. Protection of the primary hydroxyl group at three and five position was achieved by treatment of compound (5) with benzoyl chloride in a mixture of anhydrous pyridine and benzene to give the benzoate derivative (6) (59.67 % yield) as syrup. The IR spectrum of this compound showed a band of aromatic bonds with the disappearance ofs band of hydroxyl group and the ^{1}H-NMR spectrum revealed the signal of aromatic protons.



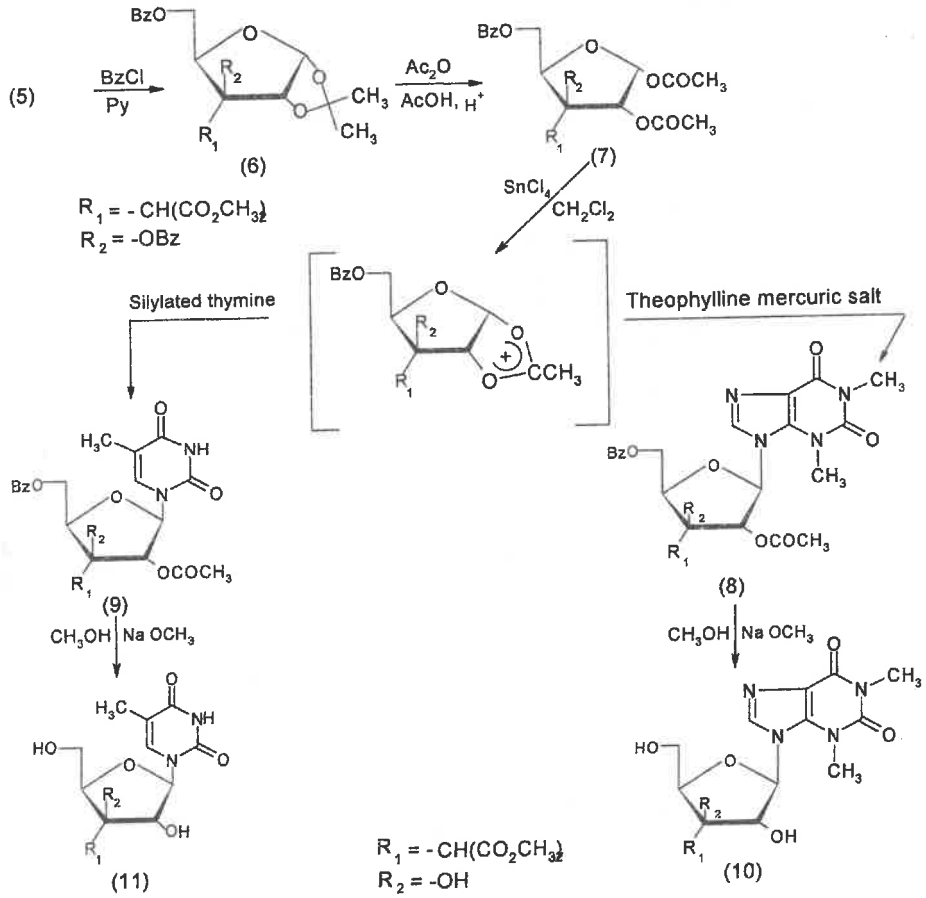
(Scheme -1)

The isopropylidine group at 1, 2 -position of compound (6) was removed and protected by using a mixture of concentrated acetic acid, acetic anh ydride and sulfuric acid(1:1: 0.05 v / v) to afford the 1, 2-di-*O*-acetylated compound (7) (73.07 % yield) as syrup. The ^{1}H-NMR spectrum revealed the signal for two -CH_3 of acetyl groups.

Synthesis of purine and pyrimidine nucleosides analogues (8) and (9) from compound (7) were obtained via modified Hilbert- Johnson procedure using anhydrous stannic chloride $SnCl_4$ as Friedel-Crafts catalyst[16-20]. The reaction

involves the conversion of the protected sugar into the reactive intermediate (electrophilic, 1, 2-acetyloxonium ion). The mercuric salt of theophlline and silylated thymine cane be reacted with equivalent amounts of (1, 2-acetyloxonium ion), peracylated sugar on warm, (thermodynamically controlled) to give β - anomers (8) (53.83 %) and (9) (56.05 % yield) as major products and α - anomers as minor products. The structure of compounds (8) and (9) was established based on the data of IR, ^{1}H-NMR spectra and elemental analysis.

When compounds (8) and (9) were allowed to react with sodium methoxide, the new unprotected purine and pyrimidine nucleosides analogues (10) and (11) were obtained (*cf*, Scheme-2). The IR spectrum of this compounds showed a band of hydroxyl groups, and the ^{1}H-NMR spectrum showed the disappearance of the signals of protected groups.



(Scheme -2)

Experimental procedure

Infrared spectra were recorded using Shimadzu-408(Nujol or thin film). ^{1}H-NMR spectra were recorded on Varian Em 390-90 MHZ, Gemini 200 and Brucker WP-80 spectrometer using $CDCl_3$ as the solvent and $(Me)_4Si$ as internal standard. Chemical shift are expressed as δ ppm. Elemental analyses were preformed at the Micro Analytical Center of Cairo University using Perkin Elemer 2400 CHN elemental analyzer. TLC was preformed on aluminum plates coated with 0.25 mm layer of silica gel f_{245} (fluka). Compound was detected by iodine vapors. Some synthesized compounds were purified by column chromatography using silica gel about (60-120) mesh. Solvent and liquid reagents were stored over Na, Mg SO4 and $CaCl_2$. Solvents were removed under reduced pressure using rotary evaporator. Compounds (1) and (2) were synthesized according to literature and other compounds were synthesized as follows:

3-hydroxy-3-*C*-dimethylmalonyl-1,2:5,6-di–*O*-isopropylidene-α-D-glucofuranos (3).

The 1,2 : 5,6 – di -*O*- isopropylidene-α-D-glucofuranos –3-ulose (2), (60 gm, 232.5 mmol) was dissolved in benzene (140 ml) and a solution of sodium methoxide (50 ml), followed by addition of dimethylmalonate (32 ml, 242.4 mmol) after stirring for 16 hours at room temperature, tlc showed that the reaction was completed. The benzene layer was separated and the aqueous layer was extracted with benzene (2 x 30 ml), the combined benzene extracts were dried over anhydrous ($MgSO_4$) and was evaporated under reduced pressure to give compound (3) (51.43 gm, 56.71 %) as syrup. IR ν_{max} (cm^{-1}) 3400 (OH); 2850-2950 (aliphatic C-H); and 1730 for ester groups. ^{1}H-NMR ($CDCl_3$) 1.4 -1.9 (12H, 4s, 4 -CH_3 isopropylidene); 2.8 (1H, d, -CH- malonate); 3.1(6H, s, 2-CH_3 malonate); 3.9 (H, s, -OH) and 4.9-6.0 (6H, m, H-1, H-2, H-4, H-5, H-6^a, H -6^b) 6.1(1H, d, H-1). Anal. Calc. For, $C_{17}H_{26}O_{10}$: C, 52.30; H, 6.66. Found: C, 52.45; H, 6.51.

3-hydroxy-3-*C*-dimethylmalonyl-1,2-*O*-isopropylidene-α-D-glucofur- anos (4).

Dissolved of compound (3) (50 gm, 128.2 mmol) in acetic acid (60 ml, 60 %), the mixture was stirred for 22 hours at room temperature. The reaction mixture was poured onto water (100 ml) and extracted with chloroform (3 x 50 ml), the organic layer was dried over ($MgSO_4$) and evaporated under a reduced pressure to give compound (5) (31.8 gm, 70.87 % yield) as syrup. IR ν_{max} (cm^{-1}) 3450 for hydroxyl groups; 2950, 2850 (aliphatic, C-H) and 1730 for ester groups.^{1}H-NMR δ:1.5, 1.6 (6H, 2s, CH_3 isopropylidene); 2.5 (1H, d, -CH-, malo); 3.2 (6H, s, 2-CH_3, malo); 4.0 (3H, s, –OHs); 5.0-6.0 (6H, m, H-1, H-2, H-4, H-5, H-6^a, H-6^b); Anal. Calc. For, $C_{14}H_{22}O_{10}$: C, 48.00; H, 6.28. Found: C, 47.91; H, 6.30.

3-*C*-dimethylmalonyl-1,2-*O*-isopropylidene- -α-D-xylofuranose(5).

Compound (4) (31 gm, 88.5 mmol) was dissolved in ethanol (100 ml), followed by addition of sodium periodate (20 gm, 93.4 mmol). The solution had been stirred at room temperature, tlc (Benzene : ethyl acetate 10:1) showed that the reaction was complete after 1 hour, ethylene glycol (4 ml) was added and the solution was stirred for 5 minutes. The resulting aldehydo sugar was immediately reduced by the addition of sodium borohydride (3.5 gm, 94.5 mmol), and stirred for 45 minutes.

The solid residue was removed by filtration and the filtrate was extracted with chloroform (3 x 50 ml), dried over anhydrous ($MgSO_4$) and the solvent was removed to give compound (5) (14.0 gm, 49.40 % yield) as syrup. IR ν_{max} (cm^{-1}) 3400 (OH); 2850-2950 (aliphatic C-H); and 1730 for ester groups. ^{1}H-NMR δ: 1.5 - 1.6 (6H, 2s, CH_3 isopropylidene); 2.8 (1H, d, -CH- malonate); 3.1(6H, s, 2-CH_3 malonate); 3.9 (2H, s, -OHs) and 4.9-6.0 (6H, m, H-1, H-2, H-4, H-5, H-6^a, H-6^b). Anal. Calc. For, $C_{13}H_{20}O_9$: C, 48.75; H, 6.25. Found: C, 48.61; H, 6.11.

3, 5-Di-*O*-benzoyl–3-*C*-dimethylmalonyl-1,2-*O*-isopropylidene -α-D-xylofuranose (6).

The compound (5) (13 gm, 40.6 mmol) was dissolved in a mixture of anhydrous pyridine (10 ml), and anhydrous benzene (80 ml), the mixture was cooled to 0 ^{0}C, followed by addition of benzoyl chloride (12.0ml, 85.4 mmol), and the resulting mixture was stirred for 20 hours at room temperature. The mixture was poured onto cooled water (100 ml) and extracted with chloroform (3 x 40 ml), the organic layer was separated then dried over anhydrous ($MgSO_4$), filtrated and concentrated under reduced pressure. Traces of pyridine were removed by co evaporation with dry toluene (3 x 10 ml) to afford syrup. This syrup was purified on a silica gel column chromatography eluted with the mixture (Benzene: ethylacetate, 10:1) to give compound (6) (12.8 gm, 59.67 % yield) as syrup. IR ν_{max} (cm^{-1}) 3030 (aromatic, C-H); 2850, 2950 (aliphatic,C-H); 1720 for ester groups; and 1580, 1600 (aromatic, C=C). ^{1}H-NMR δ: 1.5, 1.6 (6H, 2s, 2 -CH_3, iso); 2.7 (1H, d, -CH- malo); 3.0 (6H, s, 2 -CH_3, malo); 4.7-5.9 (5H, m, 1H, H-2, H-4, H5^a, H5^b); and 7.1-8.1(10H, m, aromatic protons). Anal. Calc. For, $C_{27}H_{28}O_{11}$:
C, 61.63; H, 5.30. Found: C, 61.45; H, 5.42.

3, 5-Di-*O*-benzoyl–3-*C*- dimethylmalonyl-1,2-di-*O*-acetyl-α-D-xylofuranose (7).

Compound (6) (12 gm, 22.7 mmol) was dissolved in a concentrated acetic acid (20 ml), acetic anhydride (20 ml) and sulfuric acid (0.02 ml). The resulting solution was stirred for 11 hours at room temperature. The reaction mixture was poured onto water (100 ml) and extracted with chloroform (3 x 50 ml), the organic layer was dried over ($MgSO_4$) and evaporated under reduced pressure to afford syrup. This syrup was purified on a silica gel column chromatography eluted with mixture of (Benzene: ethyl acetate, 10:1) to give compound (7) (9.5 gm, 73.07 % yield) as syrup. IR ν_{max} (cm^{-1}) 3020 (aromatic, C-H); 2850, 2950 (aliphatic, C-H); 1700, 1720 for ester groups; and 1570,1610 for (aromatic, C=C). ^{1}H-NMR δ: 2.2, 2.3 (6H, 2s, 2 -$COCH_3$); 2.6 (1H, d, -CH-, malo); 3.0 (6H, s, 2-CH_3, malo); 4.6-6.0 (5H, m, 1H, H-2, H-4, H-5^a, H-5^b); and 6.9-8.2 (10H, m, aromatic protons). Anal. Calc. For, $C_{28}H_{28}O_{13}$: C, 58.74; H, 4.89. Found: C, 58.99; H, 4.90.

1(3′,5′-Di-*O*-Benzoyl-3′-*C*-dimethylmalonyl-2′-*O*-acetyl-β-D-xylofuranosyl) theophlline(8).

Compound (7) (4.0 gm, 6.9 mmol) and mercuric salt of theophlline (4.0 gm, 7.1 mmol) was dissolved in anhydrous dichloromethane (40 ml) and anhydrous stannic chloride (0.09 ml). The mixture was stirred at 40 ^{0}C, which time, tlc (benzene: ethyl

acetate, 10:1) showed that the reaction was complete after 13 hours. The reaction mixture was poured onto (40 ml) water and extracted with dichloromethane (3 x 50 ml). The organic layer was dried over anhydrous ($MgSO_4$) and the solvent was removed to afford syrup. This syrup product was purified on a silica gel column chromatography using (chloroform: acetone, 10:1) as eluent to give two different fractions the first one (2.6 gm, 53.83 %) and the second (0.2 gm, 4.14 %) as syrup. IR ν_{max} (cm^{-1}) 3030 (aromatic, C-H); 2850, 2950 (aliphatic, C-H); 1680, 1720 for carbonyl groups; and 1575, 1620 for (aromatic, C=C). ^{1}H-NMR δ:1.5 (6H, s, CH_3 of theophlline); 2.0 (3H, s, $COCH_3$);2.5(1H, d, -CH-, malo); 3.4 (6H, s, CH_3,malo); 4.6-6.0 (4H, m, H-1′ , H-2′, H-4′, H-5$^{a'}$, H-5$^{b'}$); and 6.9-8.3 (11H, m, aromatic protons). Anal. Calc. For, $C_{33}H_{32}N_4O_{13}$: C, 57.22; H, 4.62; N,8.09. Found: C, 57.40; H, 4.59; N, 8.27.

1(3′,5′-Di-*O*-Benzoyl-3′-*C*-dimethylmalonyl-2′-*O*-acetyl-β-D-xylofura- nosyl) thymine(9).

The acetylated sugar (7) (4.0 gm, 6.9 mmol) and silylated thymine (2.0 gm, 7.4 mmol) were dissolved in anhydrous dichloromethane (40 ml) and anhydrous stannic chloride (0.09ml). The mixture was stirred at 40 ^{0}C for 15 hours to afford syrup. This syrup purified on a silica gel column using (chloroform: acetone, 10:1) as eluent to give two was different fraction, the first one (2.5 gm, 56.05 % yield) and the second (0.15 gm, 3.36 %yield) as syrup. IR ν_{max} (cm^{-1}) 3300 (-NH-); 3040 (aromatic, C-H); 2850, 2950 (aliphatic, C-H); 1690, 1720 for carbonyl groups; and 1575, 1620 for (aromatic, C=C). ^{1}H-NMR δ: 2.2 (3H, s, -$COCH_3$); 2.8 (1H, d, -CH-, malo); 3.2 (6H, s, 2 -CH_3,malo); 4.6-5.9 (5H, m, H-1′, H-2′, H-4′, H-5$^{a'}$, H-5$^{b'}$); 6.2-6.7 (2H, m, H-5, H-6) and 7.1-8.3 (11H, m, br, aromatic protons and amid NH). Anal. Calc. For, $C_{31}H_{30}N_2O_{13}$: C, 58.30; H, 4.70; N, 4.38. Found: C, 58.21; H, 4.66; N, 4.46.

1(3′-*C*-dimethylmalonyl-β-D-xylofuranosyl) theophlline and thymine (10) and (11).

Compound (8) (2.0 gm, 2.8 mmol) was dissolved in sodium methoxide (50 ml, 0.1 mol) the mixture was stirred at room temperature, tlc, showed that the reaction was complete after 16 hours and then evaporated fore near to dryness. The residue was purified on a silica gel column chromatography using (chloroform: ethanol, 10:1) as an eluent to give compound (10) (0.7gm, 55.11 % yield) as syrup. IR ν_{max} (cm^{-1}) 3450 (-OH);3010 (aromatic, C-H); 2850, 2950 (aliphatic, C-H); 1700 for carbonyl groups; and 1575, 1640 for (aromatic,N=C, C=C). ^{1}H-NMR δ:1.7 (6H, s, 2CH_3 of theophlline); 2.6 (1H, d, -CH-, malo); 3.4 (6H, s, CH_3,malo); 4.0 (3H, s, -OHs) 4.8-6.0 (4H, m, H-1′ , H-2′, H-4′, H-5$^{a'}$, H-5$^{b'}$); and 6.9-8.3 (11H, m, aromatic protons). Anal. Calc. For, $C_{17}H_{22}N_4O_{10}$: C, 46.15; H, 4.97; N,12.66. Found: C, 46.71; H, 5.10; N, 12.75.

Also compound (9) (2.0 gm, 3.1 mmol) was dissolved in sodium methoxide (50 ml, 0.1 mol) the mixture was stirred at room temperature, tlc showed that the reaction was complete after 18 hours and then evaporated fore near to dryness. The residue was purified on a silica gel column chromatography using (chloroform: ethanol, 10:1) as an eluent to give compound (11) (0.7gm, 57.85 % yield) as syrup.

IR ν_{max} (cm^{-1}) 3200, 3450 (OH, -NH-); 3010 (=C-H); 2850, 2950 (aliphatic, C-H); 1690, 1710 for carbonyl groups; and 1620 for (aliphatic, C=C). ^{1}H-NMR δ: 2.8 (1H, d, -CH-, malo); 3.2 (6H, s, 2 -CH_3,malo); 4.6-5.9 (5H, m, H-1$^{/}$, H-2$^{/}$, H-4$^{/}$, H-5$^{a/}$, H-5$^{b/}$); 6.0-6.5 (2H, m, H-5, H-6) and 7.5-8.0 (1H, br, amid NH). Anal. Calc. For, $C_{15}H_{20}N_2O_{10}$ C, 46.39; H, 5.15; N, 7.21. Found: C, 46.50; H, 5.61; N, 7.34.

Conclusion:

According to the literature of nucleoside analogues compounds which showed a biological activity we expect a biological activity for our derivative. For future study we recommend a biological study and bio-assay for compounds.

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