Synthesis of 3’-3’-gem – di – C Nitromethyl Nucleoside Analogues of Possible Biological Activity
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ABSTRACT
Synthesis of new nucleoside analogues of the type : 3’, 3’- gem – di – C nitromethyl , expected to have useful application in the chemotherapeutic treatment of AIDS , cancer and microbial infections. The synthesis involved the condensation of the appropriate sugar derivative ( i.e . 3’, 3’- gem – di – C nitromethyl – 1- ribofuranoside ) with nitrogen bases , such as , uracil and theophylline following a multi step scheme starting from diacetone golucose (1) (scheme 1).The prepared compound were identified by spectroscopic methods ; ir , mass , 1H and 13C nmr.

INTRODUCTION
The 2’, 3’ – dideoxy nucleosides have shown importance in several established chemotherapies (anticancer , antiviral and antibacterial) and other attractive field like immunomodulation or regulation of gene expression which may constitute new therapeutic approaches (13). The board application of modified nucleosides especially in the inhibition of the human immunodeficiency virus (HIV)(4,5), have targeted the investigation for utilizing new nucleoside analogues .Efforts have primarily focused on modification of the carbohydrate portion of the natural nucleosides because cellular kinases are more tolerant of these changes than changes within the base moiety(6). These observations, have led us to commit the synthesis of a variety of nucleoside analogues containing 3’, 3’- gem – di – C nitromethyl substituent at the sugar portion .

MATERIAL and METHOD
Melting points were determined using electrothermal melting point apparatus and are uncorrected .IR spectra were recorded using either , Shimadzu (408) Jasco (J – 0085 ) infrared spectrophotometer .' H – NMR and 13C – NMR spectra were determined on a Varian XR – 3005 (pharm 300) spectrometer at 299.908 and 75.4118 MHz respectively . CDC13 or DMSO was used as the solvent and TMS as internal standard . General mass spectrometer model 511 valzar was used for recording mass spectra .TLC was performed on glass plants coated with 0.25mm layer of silica gel (Fluka) and spot were detected by iodine vapour. Chromatography was carried out with silica gel 60 ( Fluka ). Uracil from Merk Company . 1.2 : 5.6 – Di – O – Isopropylidene – α – D – glucofuranose (1) was prepared from 1.2: 5.6 – Di – O – Isopropylidene – α – D – ribo – hexofuranose – 3 – ulose (2) was prepared as previously described (19,20).

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1.2 : 5.6 – Di – O – Isopropylidene – 3 – C – nitromethyl – 2 – D – allofurano (3) 
and 3 – deoxy – 1.2 : 5.6 – di – O – isopropylidene – 3 ,3 – gem – di – C – nitromethyl – 2 – D – ribo – hexofuranose (5) 
were prepared as previously (9).

3 – Deoxy – 1.2 : 5.6 – di – O – isopropylidene – 3 – C – nitromethylene – 2 – D – ribo – hexofuranose (4) 
was prepared according to the method described (10). Bis (theophylline – 7 
– yl) mercury (10) was prepared as in the method described (10). 
2,4 – Bis (trimethylyl uracil (13) was synthesized as described before (15).

3 – Deoxy – 1.2 : 5.6 – di – O – isopropylidene – 3 ,3 – gem – di – C – nitromethyl – 2 – D – ribo – hexofuranose (6)

3 – Deoxy – 1.2 : 5.6 – di – O – isopropylidene – 3 ,3 – gem – di – C – nitromethyl – 2 – D – ribohexofuranose (5) 
(8.3g, 22.9 mmol) was dissolved in methanol and IN HSOr (30ml) 
was added. The reaction mixture was left to stand at room temperature 
for 3hr. The resulting mixture was then neutralized by 
adding solid sodium hydrogen carbonate and 
then extracted with chloroform. The 
chloroform extract was dried over anhydrous 
sodium sulphate and 
gave upon evaporation the 
diol (6) as a syrup 
(6.0g, 80.4%).IR (smear) ν (3450)cm⁻¹ (OH).

3 – Deoxy – 1 , 2 – O – isopropylidene – 3 ,3 – gem – di – C – nitromethyl – 2 – D – ribo – furanose (7)

The diol (6) (2g, 6.2mmol) was dissolved in 
ethanol (40ml) and well – stirred, 
then saturated solution of sodium hydrogen 
carbonate (2ml) was added followed by 
a solution of sodium metaperiodate (1.32g, 6.2 
mmol) in 70 ml water the resulting reaction 
mixture was stirred 3hr after which the excess 
sodium metaperiodate was destroyed by adding 
fe few drops of ethylene glycol. The resulting 
aldehyd sugar was immediately reduced with 
sodium borohydride (0.12g). After the reaction 
mixture was kept with stirring for 4 hr, acetone 
(0.5ml) 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, dried over 
anhydrous sodium sulphate the solvent was 
removed to give (7) as a syrup (1.39g, 
76.8%).IR (smear) ν (3450)cm⁻¹ (OH).¹H – NMR δ 
5.82 (d, 1H, H – 1), 7.42 – 4.70 (q, 1H, H – 4), 4.66 – 4.60 (d, 1H, H – 2), 3.40 (s, 4H, 
2CH₃NO₂), 3.53 (d, 2H, H – 5), 5.0 – 5.5, 1.38 – 
1.23 (s, 6H, 2CH₃).¹³C – NMR δ 104.32 (C – 
1), 81.95 (C – 2), 58.74, 58.40 (C – 3), 81.74 
(C – 4), 84.19 (C – 5), 80.31, 78.42 
(CH₃NO₂), 110.51 (O – CMe₂ – O), 26.52, 
26.06 ... C (CH₃)₂.

5 – O – Benzoyl – 3 – deoxy – 1.2 – O – isopropylidene – 3,3 – gem – di – 
C – nitromethyl – 2 – D – ribo – furanose (8)

The 3 – deoxy – 1.2 – O – isopropylidene – 
3 ,3 – gem – di – C – nitromethyl – 2 – D 
ribofuranose (2) (2.4g, 8.2 mmol) was 
dissolved in anhydrous pyridine (6.7ml, 83 
mmol) after external cooling with ice, 
benzoyl chloride (0.96ml ; 8.3mmol) was 
added dropwise. The reaction mixture was 
kept at room temperature for 24 hr then 
iced water was added. The resulting syrup was 
extracted with petroleum ether (b.p. 40 – 60 
°C) then dried with anhydrous sodium 
sulphate, filtered and concentrated under 
reduced pressure. Traces of pyridine were 
removed by coevaporation with dry toluene. 
The benzoyl derivative (8) was obtained as 
syrup (1.6g, 50%).IR (smear) ν 
(3100 – 3000 cm⁻¹ (aromatic C – H) , (1710)cm⁻¹ (C = O), (1590) cm⁻¹, 
(C – H). Mass spectrum, gave M – 396 (5 – benzoxo 
benzoyl derivative 8) and m/e 261 ( – [ phCO₂CH₃ ]).¹H – NMR δ 
7.47 (m, 5H, aromatic), 5.95 (d, 1H, H – 1), 4.76 – 4.63 
(cm, H – 4, H – 5), 5.82 (d, H – 5), 4.58 (d, 1H, H – 2), 4.2 (d, 2H, CH₃NO₂), 3.95 (s, 2H, CH₂NO₂), 
1.50 – 1.32 (2s, 6H, 2CH₃).¹³C – NMR 
δ 133.31, 130.05, 129.85, 128.52, 128.38 
(aromatic ring carbons), 170.62 (ph – CO), 
105.30 (C – 1), 80.02 (C – 2), 60.49 (C – 3), 79.34 (C – 4), 77.83 (C – 5), 74.88, 
74.62 (CH₃NO₂).

1,2 – Di – O – acetyl – 5 – O – Benzoyl – 3 – deoxy – 3,3 – gem – di – 
C – nitromethyl – a – D – ribofuranosyl bromide (11)

The acetylated sugar (9) (1g, 2.2 mmol) was treated with 50%(W/V) hydrogen bromide in 
acetate (3ml) the solution was kept at 0°C for 
one hour then poured in to an ice cooled 
dichloromethane (50ml), washed with ice 
water, and then with saturated aqueous 
solution of sodium bicarbonate to remove the 
remaining acid. After a final wash with ice 
water, the dichloromethane layer was 
dried over anhydrous sodium sulphate and 
the solvent was removed to give (11) as syrup 
(0.9g, 95%). The isolated sugar bromide (11) 
was used directly for the nucleoside synthesis.
Synthesis of theophylline nucleoside analogue

7 – (2 – O – acetyl – 5 – O – benzoyl – 3 – deoxy – 3’ , 3’ – gem – di – C – nitromethyl – β – D – ribofuranosyl ) theophylline

The theophylline mercury salt (10) (0.55 g, 0.98mmol) was finely powdered, suspended in (150ml )sodium – dried xylene and the solvent was partially distilled to remove traces of water azeotropically. When the temperature of mixture was raised to 137 °C, the residual suspension was allowed to cool (below 50 °C) . The acetylated sugar (9) (0.44g, 1mmol ) and the reaction mixture was stirred at 23 °C overnight gave the 5,6 – di – isopropylidene group of the 3,3 – gem – di – C – nitromethyl – α – D – ribofuranosyl (5) in sulphuric acid in methanol (8) gave the monoisopropylidene derivative (6) as a syrup in 80.4% yield . Oxidation of the diol (6) with sodium periodate effected the cleavage of C5 – C6 Bond and resulting intermediate aldehyde derivative was reduced immediately with sodium borohydride to give the 3 – deoxy – 1,2 – O – isopropylidene – 3 , 3’ gem – di – C – nitromethyl – α – D – ribofuranosyl (8). The primary 5 – OH group in (7) was then protected by conversion to the 5 – benzate ester (8). Treatment of (7) with benzoxy chloride in pyridine overnight gave the 5 – O – benzoyl – 3 – deoxy – 1,2 – O – isopropylidene – 3 , 3’ gem – di – C – nitromethyl – α – D – ribofuranosyl (8) as syrup in 50% yield. The final step in the synthesis of the protected sugar moiety before carrying out the coupling reaction with nucleo – bases, was the removal of 5 – O – 1,2 – acetal of 5 – O – benzoyl derivative (8) with 99% trifluoroacetic acid followed by acetylation . Acetylation of the 1 – and 2 – hydroxyl groups was performed with acetic anhydride in pyridine which afforded 1,2 – di – O – acetyl – 5 – O – benzoyl – 3 – deoxy – 3 , 3’ gem – di – C – nitromethyl – α – D – ribofuranosyl (9) as a syrup in 90% yield.

2- Synthesis of nucleoside analogues

For the synthesis of 7 – (2’ – O – acetyl – 5’ – O – benzoyl – 3’ , 3’ – di – C – nitromethyl – α – D – ribo – furanosyl ) theophylline (12), the Koenigs – Knorr condensation method was followed. Thus treatment of 1,2 – di – O – acetyltrifuranosyl derivative (9) with anhydrous hydrogen bromide in dichloromethane readily afforded 1,2 – di – O bromide (11) which was used immediately because of its instability.
(Scheme 1).
The sugar bromide (11) was condensed with bis (theophylline – 7 – yl) mercury (10) as the activated base in anhydrous xylene under reflux which afforded the desired theophylline nucleoside analogue (12), after silica column chromatography, as a syrup in 52% yield. The theophylline (1,3 – dimethylxanthine) base has been used because of its availability and due to the fact that only one of its nitrogen atom (N – 7) is reactive, therefore mixture of different nucleoside analogues may be avoided. The reaction of theophylline with mercuric chloride as Lewis acid. The formation of 1,2-acetyloxonium ion determined the exclusive predominate position of attachment of mercury in the theophylline. It has also been demonstrated that the mercury derivative of theophylline (1,3–deoxyribofuranose derivative) involved the conversion of the protected sugar group in the theophylline. It has also been demonstrated that the mercury group from nitrogen by the reaction of theophylline with mercuric chloride in aqueous alkali afforded the mercury derivative rather than the chloromercury one, and it was assigned that N – 7 was the predominate position of attachment of mercury group in the theophylline. It has also been demonstrated that the mercury derivative of theophylline couple with acetylglucosyl halides at N – 7 (12, 14) and involves direct displacement of the mercury group from nitrogen by the incoming acetylglucosyl halide (11).

For the synthesis of 1',2'-O- acetyl -5' - O - benzoyl - 3' - deoxy - 3',5' - gem - di - C - mitromethyl - β - D - ribofuranose - 4' - (trimethylsilyl) uracil (14), the modified Hilbert – Johnson procedure using simply Friedel – Crafts catalysts like SnCl₄ was followed (15). The 1,2 – di – O – acetylribofuranose derivative (9) was coupled with the silylated uracil (13) in 1,2 – dichloroethane in the presence of anhydrous stannic chloride as Lewis acid. The reaction involved the conversion of the protected sugar (9) in to the reactive electrophilic 1,2-acetyloxonium ion followed by the silylated uracil (13) attack to afford the protected uracil nucleoside analogue (14) with the regeneration of the catalyst. The formation of 1,2-acetyloxonium ion determined the exclusive formation of the – β – anomer (14) (15, 16), which was separated as white semisolid and characterized by its ¹H NMR spectrum. Another fraction (14a) was separated on the silica gel column and identified from its ¹H NMR spectrum as being the desilylated nucleoside (14a).

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