

## 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 - ribofuranose) with nitrogen bases, such as uracil and theophyllin following a multi step scheme starting from diacetone glucose (1) (scheme 1). The prepared compound were identified by spectroscopic methods; IR, mass, <sup>1</sup>H and <sup>13</sup>C nmr.

### الخلاصة

تحضير مماثلات نيوكليوسيد جديدة من نوع 3', 3' - التوائم ثنائي - C - نيترومثيل محتمل الاستفادة منها في المعالجة الكيميائية (chemotherapy) لأمراض نقص المناعة والسرطان وللأمراض الجرثومية. تضمن التحضير تكثيف مشتقات الجزء السكري 3', 3' - التواء ثنائي - C - نيترومثيل - D - فيورانوز مع قواعد نتروجينية مثل اليوارسيل والثيوفلين باتباع مسار متعدد الخطوات يبدأ من ثنائي اسيتون كلوكوز (1) (المخطط 1). تم تشخيص المركبات المحضرة بواسطة الطرق الطيفية الأشعة تحت الحمراء والكتلة والرنين النووي المغناطيسي البروتوني ونظير الكربون 13.

### INTRODUCTION

The 2', 3' - didoxy 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<sup>(1,3)</sup>. The board application of modified nucleosides especially in the inhibition of the human immunodeficiency virus (HIV)<sup>(4,5)</sup>, 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<sup>(6)</sup>. 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. <sup>1</sup>H - NMR and <sup>13</sup>C - NMR spectra were determined on a Varian XR - 3005 (pharm 300) spectrometer at 299.908 and 75.4118 MHz respectively. CDCl<sub>3</sub> 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 plates coated with 0.25mm layer of silica gel (Fluka) and spot were detected by iodine vapour. Column 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<sup>(19,20)</sup>.

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1,2 : 5,6 - Di - Q - Isopropylidene - 3 - C - nitromethyl -  $\infty$  - D - allofuranose (3) and 3 - deoxy - 1,2 :5,6 - di - Q - isopropylidene - 3 ,3 - gem - di - C nitromethyl -  $\infty$  - D - ribo - hexofuranose (5) were prepared as previously <sup>(7)</sup> .

3 - Deoxy - 1,2 :5,6 - di - O - isopropylidone - 3 - C - nitromethylene -  $\infty$  - D - ribo - hexofuranose (4) was prepared according to the method described <sup>(21)</sup> .Bis (theophylline - 7 - yl )mercury (10) was prepared as in the method described <sup>(12)</sup> .2,4 - Bis (trimethylsilyl uracil) (13) was synthesized as described before <sup>(15)</sup> .

**3 - Deoxy - 1,2 :5,6 - di - O - isopropylidene - 3,3 - gem - di - C - nitromethyl -  $\infty$  - D - ribo - hexofuranose (6)**

3 - Deoxy - 1,2 :5,6 - di O - isopropylidene - 3,3 - gem - di - C nitromethyl -  $\infty$  - D - ribohexofuranose (5) (8.3g, 22.9 mmol ) was dissolved in methanol and IN H<sub>2</sub>SO<sub>4</sub>(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)v(3450)cm<sup>-1</sup>(OH) .

**3 - Deoxy - 1 , 2 - Q - isopropylidene - 3 ,3 - gem - di - C - nitromethyl -  $\infty$  - 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 metaaperidate was destroyed by adding few drops of ethylene glycol .The resulting aldehydo 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 )v(3450)cm<sup>-1</sup> (OH).<sup>1</sup>H - NMR  $\delta$  5.82(d,1H,H - 1 )4.72 - 4.70 (q,1H, H - 4 ) , 4.66 - 4.60 (d,1H,H - 2 ) ,4.10(s,4H , 2CH<sub>2</sub>NO<sub>2</sub>),3.53(d,2H,H - 5 ,H - 5' ,1.38 - 1.23(2s,6H,2CH<sub>3</sub>).<sup>13</sup>C - NMR  $\delta$  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 (CH<sub>2</sub>NO<sub>2</sub>),110.51(O - CMe<sub>2</sub> - O ) , 26.52 , 26.06 .. - C (CH<sub>3</sub>)<sub>2</sub> .

**5 - O - Benzoyl - 3 - deoxy - 1,2 - Q - isopropylidene - 3,3 - gem - di - C - nitromethyl -  $\infty$  - D - ribo - furanose (8)**

The 3 - deoxy - 1 , 2 - O - isopropylidene - 3 ,3 - gem - di - C nitromethyl -  $\infty$  - D ribofuranose (7) (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 pyridme were remove by coevaporation with dry toluene .The benzoate derivative (8) was obtained as syrup (1.6g , 50% ).IR (smear )v (3100 - 3000 ) cm<sup>-1</sup>(aromatic C - H ) ,(1710)cm<sup>-1</sup>( C = O ) , (1590 )cm<sup>-1</sup> , ( C - C ) . Mass spectrum , gave M - 396 ( 5 - benzoate derivative 8 ) ,and m/e 261 ( - [ phCO<sub>2</sub>CH<sub>2</sub>] ) .<sup>1</sup>H - NMR  $\delta$  7.47 (m , 5H , aromatic ) , 5.95 (d, 1H ,H - 1 ) , 4.76 - 4.63 (cm , H - 4 , H - 5 ,H - 5' ) ,4.58 (d,1H, H - 2 ) , 4.2 (d, 2H, CH<sub>2</sub>NO<sub>2</sub>),3.95(s,2H,CH<sub>2</sub>NO<sub>2</sub>), 1.50 - 1.32 (2s , 6H, 2CH<sub>3</sub>).<sup>13</sup>C - NMR  $\delta$ 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 ( CH<sub>2</sub>NO<sub>3</sub> ) .

**1,2 - Di - Q - acetyl - 5 - Q - 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 acetic (3ml) the solution was kept at 0°C for one hour then poured in to an ice cooled dichloromethane (50ml ) , washed with iced water , and then with saturated aqueous solution of sodium bicarbonate to remove the remaining acid .After a final wash with iced 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 - $\beta$ - 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 bromide (11) (0.9gm , 1.96mmol )in xylene was then added and the reaction mixture refluxed with vigorous stirring for 15 minute . The xylene layer was washed with 20% aqueous potassium iodide to remove the remaining traces of the mercuric salt , washed with water , dried over anhydrous sodium sulphate and the solvent was removed to give after silica gel column chromatography with chloroform as an eluent the acetylated nucleoside (12) (0.57g,52%Jas syrup .IR (smear )  $\nu$  (2850 - 2950 ) $\text{cm}^{-1}$ (C - H ) .

### Synthesis of protected uracil nucleoside analogue

#### **1 - ( 2 - O - Acetyl - 5 - O - Benzoyl - 3 ' - dideoxy - 3 ' , 3 ' - gem - di - C - nitromethyl - $\beta$ - D - ribofuranosyl ) - 4 - ( trimethylsilyl ) uracil (14)**

The acetylated sugar (9) (0.44g, 1mmol ) and silylated uracil (13) (0.29 g ,1.1mmol ) were dissolved in anhydrous 1,2 - dichloroethane (10ml) . Anhydrous stannic chloride (0.08ml , 0.684 mmol ) was then added in the presence of few pellets of molecular sieve 4A and reaction mixture was stirred at 23 °C , tlc (chloroform - methanol )9:1) showed the reaction was complete after 16 hrs . The reaction was then poured on an excess sodium bicarbonate solution and extracted with methylene chloride .The combined methylene chloride extracts was dried over anhydrous sodium sulphate , and the solvent was removed to give (14) (0.6 g , 40,3%),IR (smear) $\nu$ (2850 - 2950 ) $\text{cm}^{-1}$ (C - H ),(3350) $\text{cm}^{-1}$ (N - H ) .

The product was purified on silica gel column using chloroform as eluent .Two different fractions were isolated , the first one (14) (0.018g) as white semisolid which represented The silyated nucleoside  $^1\text{H NMR } \delta$  7.96 (s,5H,aromatic ),7.66 - 7.64 (2s,2H,CH=CH of uracil ),4.6 - 4.4 (cm,4H,H - 2 ,H - 4 , H - 5 ,H - 5 ' ), 4.2(d,4H,2CH<sub>2</sub>NO<sub>2</sub>),2.02

(s ,3H ,CH<sub>3</sub>), 0.90(s,9H,SiMe<sub>3</sub> ).The second fraction (14a) (0.02mg) obtained as syrup identified as the desilylated free nucleoside analogue  $^1\text{H NMR } \delta$  8.05 (m,5H , aromatic ),7.48 - 7.43 (2s,2H,CH=CH of uracil ),5.98(d,1H,H - 1 ),5.2 - 4.8(cm,4H,H - 2 ,H - 4 , H - 5 , H - 5 ' ) , 4.45 ( d , 2H , CH<sub>2</sub>NO<sub>2</sub> ) , 3.(d,2H ,CH<sub>2</sub>NO<sub>2</sub>),2.07(s,3H,CH<sub>3</sub>).

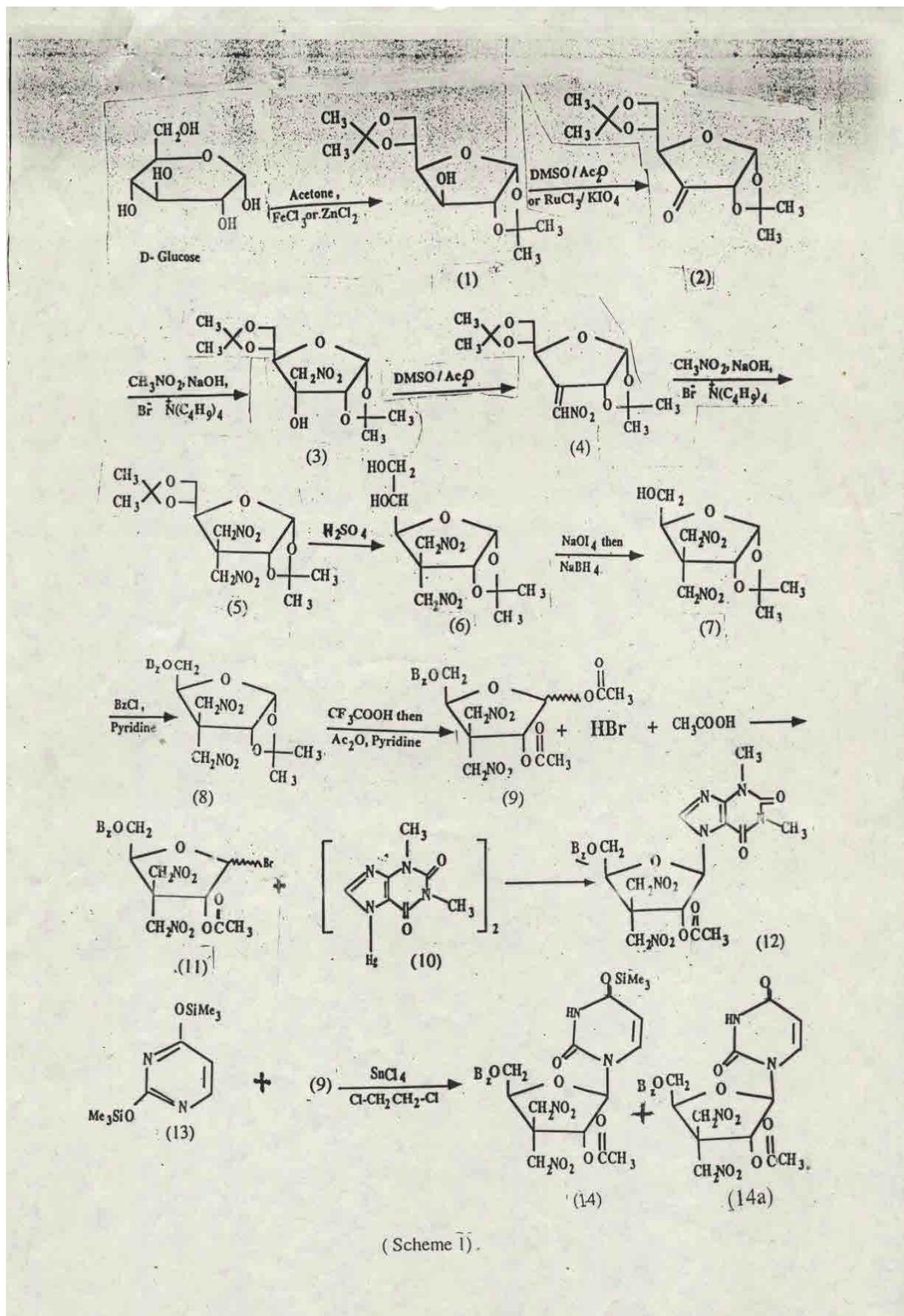
### RESULT and DISCUSSION

#### **1.Synthesis of the carbohydrate moiety of the nucleoside**

3 - Deoxy - 1,2 :5,6 - di - O - isopropylidene - 3 ,3 - di - gem - C - dinitromethyl -  $\alpha$  - D - ribofuranose (5) was obtained from D - glucose according to the method reported earlier by one of us <sup>(7)</sup> . Selective hydrolysis of the 5,6 - isopropylidene group of the 3,3 - gem - di - C - nitromethyl -  $\alpha$  - D - ribohexofuranose (5) with in sulphuric acid in methanol <sup>(8)</sup> gave the monoisopropylidene derivative (6) as a syrup in 80.4% yield .Oxidation of the diol (6) with sodium periodate effected the cleavage of C<sub>5</sub> - C<sub>6</sub> 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 -  $\alpha$  - D - ribofuranose <sup>(8)</sup> . The primary 5 - OH group in (7) was then protected by conversion to the 5 - benzoate ester (8) . Treatment of (7) with benzoyl chloride in pyridine <sup>(9)</sup> overnight gave the 5 - O - benzoyl - 3 - deoxy - 1,2 - O - isopropylidene - 3 ,3 - gem - di - C - nitromethyl -  $\alpha$  - D - ribofuranose (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 <sup>(9)</sup> which afforded 1,2 - di - O - acetyl - 5 - O - benzoyl - 3 - deoxy - 3 ,3 - gem - di - C - nitromethyl -  $\alpha$  - D - ribofuranose (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 -  $\alpha$  - D - ribo - furanosyl ) theophylline (12) , the Koenigs - Knorr condensation method was followed <sup>(10)</sup> . Thus treatment of 1,2 - di - O - acetylribofuranose derivative (9) with anhydrous hydrogen bromide in dichloromethane readily afforded 1,2 - di - O bromide(11) which was used immediately because of its instability .



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 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 acylglycosyl halides at N – 7<sup>(12-14)</sup> and involves direct displacement of the mercury group from nitrogen by the incoming acylglycosyl halide<sup>(11)</sup> .

For the synthesis of 1 – ( 2' – O – acetyl – 5' – O – benzoyl – 3' – deoxy – 3' ,3' – gem – di – C – mitromethy –  $\beta$  – D – ribofuranose ) – 4 – (trimethylsilyl) uracil (14), the modified Hilbert – Johnson procedure using simply Friedel – Crafts catalysts like SnCl<sub>4</sub> was followed<sup>(15)</sup> . 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 –  $\beta$  – anomer (14)<sup>(15-16)</sup> , which was separated as white semisolid and characterized by its <sup>1</sup>H NMR spectrum . Another fraction (14a) was separated on the silica gel column and identified from its <sup>1</sup>H NMR spectrum as being the desilylated nucleoside (14a) .

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