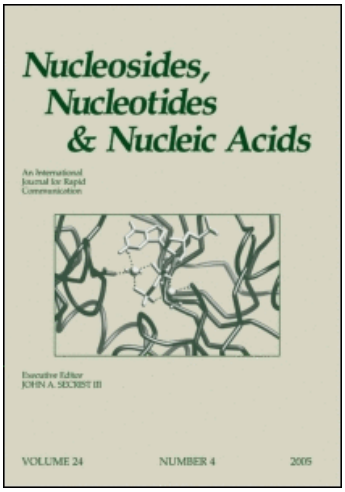

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The Synthesis and Antiviral Activity of (*E*)-5-(2-Nitrovinyl)uridine and (*E*)-5-(2-Nitrovinyl)-2'-deoxyuridine

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THE SYNTHESIS AND ANTIVIRAL ACTIVITY OF(*E*)-5-(2-NITROVINYL)URIDINE AND (*E*)-5-(2-NITROVINYL)-2'-DEOXYURIDINE**

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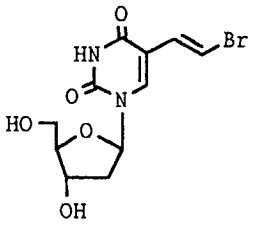
ABSTRACT: The protection of the sugar moiety of a 5-formyluracil nucleoside with acid-labile protecting groups allows for the deprotection of the sugar of a subsequently formed nucleoside possessing a 5-nitrovinyl side-chain. The synthesis and antiviral activity of (*E*)-5-(2-nitrovinyl)-uridine and (*E*)-5-(2-nitrovinyl)-2'-deoxyuridine are reported.

INTRODUCTION

More than a decade ago, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU, 1 was synthesized[1] and its antiviral activity against Herpes Simplex Virus Type 1[2] (HSV-1) and Varicella Zoster virus[3] (VZV) were reported. The minimum inhibitory concentrations (MIC) of BVDU were found to be 0.007 and 0.002 $\mu g\ ml^{-1}$ against these viruses respectively and with this lead many compounds have since been synthesized.[4] Although few of the



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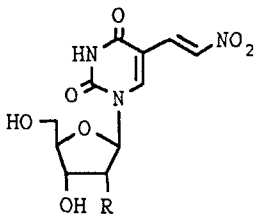


2 a R = OH

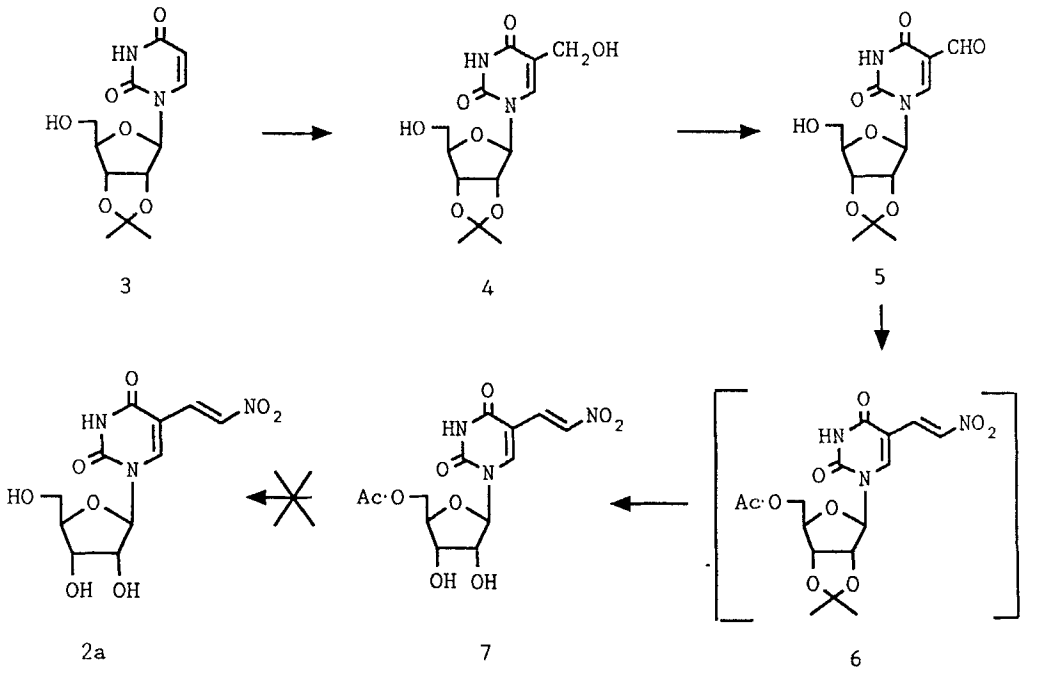
b R = H

** This paper is dedicated to the memory of Professor Tohru Ueda.

analogues obtained showed as good or better activity, the synthesis of 5-substituted vinylic 2'-deoxynucleoside analogues are still occasionally reported.[5,6] Few of the published analogues were ribonucleosides and therefore we determined to synthesize a series of such analogues. Previous attempts to synthesize (*E*)-5-(2-nitrovinyl)uridine (2a) had failed because of the use of unsuitable protecting groups, as the nitrovinyl side-chain proved to be unstable to base.[7] However, we found that acid deprotection could be achieved and thus we here describe the synthesis and antiviral properties of the ribo- and 2'-deoxyribonucleosides of (*E*)-5-(2-nitrovinyl)uracil.

RESULTS AND DISCUSSION

In a previous paper we described two initial routes to the synthesis of 2a.[7] 2',3'-*O*-Isopropylideneuridine (3) can be hydroxymethylated at the



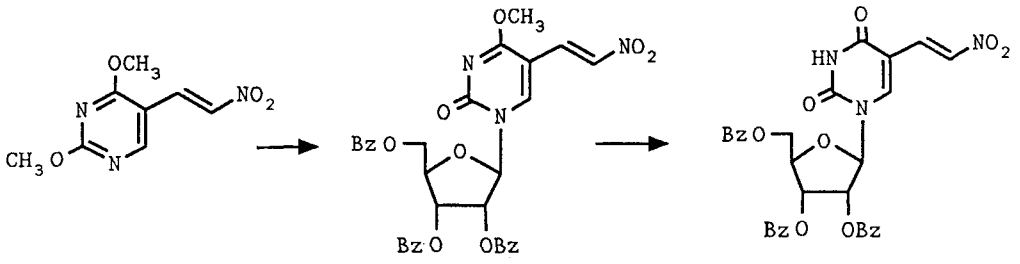
3 4 5

6 7 2a

5-position[8,9] and the resulting nucleoside 4 then oxidized using

pyridinium dichromate in dichloromethane and dimethylformamide. During the generation of the nitrovinyl side chain from 5, the 5'-hydroxyl group was protected as the acetyl ester to give 6 which was not isolated but was deprotected with acid to 7. Attempts to remove the 5'-*O*-acetyl moiety with potassium carbonate/methanol or ammonia/methanol were not successful.

Alternatively, we found that (*E*)-5-(2-nitrovinyl)-2,4-dimethoxypyrimidine (8) could be condensed with the sugar

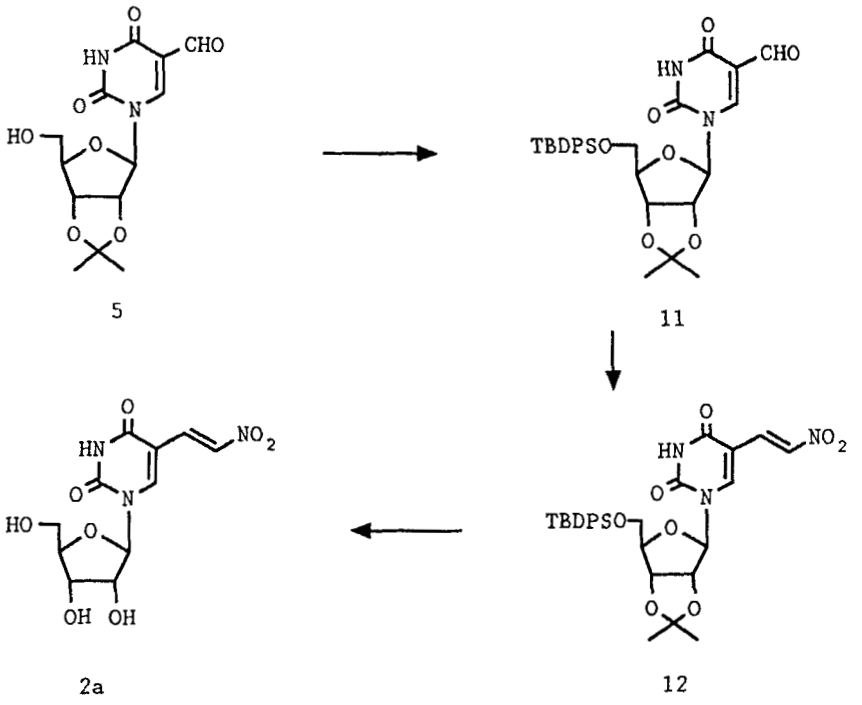


8 9 10

2a

1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose to give 9. Although this could be demethylated at C-4 to give compound 10, the benzoyl sugar protecting groups could not be removed without the destruction of the nitrovinyl side-chain.

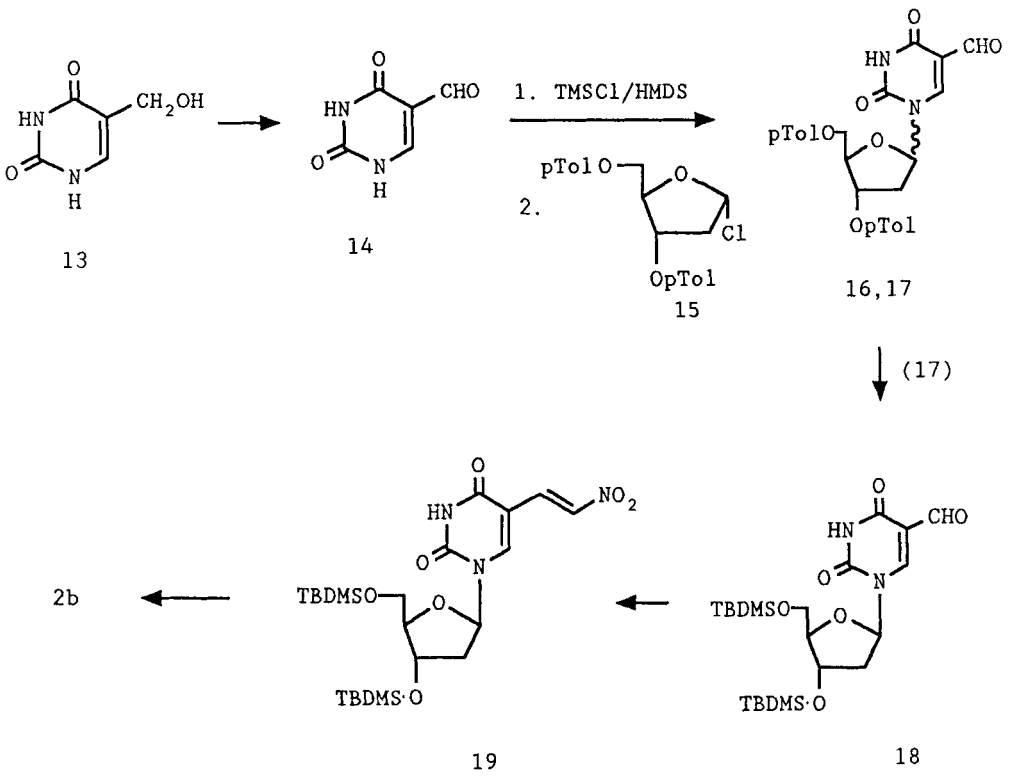
It was therefore decided to protect the sugar with an acid labile protecting group. Compound 5 was protected as its *t*-butyldiphenylsilyl ether and compound 11 was obtained in good yield. Under the conditions reported previously for the generation of the nitrovinyl side-chain, namely nitromethane and triethylamine followed by treatment with acetic anhydride, the nitrovinyl nucleoside 12 was obtained. This was then deprotected with 50% aqueous trifluoroacetic acid to give 2a as a pale yellow crystalline solid.



5 11

2a 12

Commercially available 5-hydroxymethyluracil[10] (13) was used as the starting material for the preparation of the 2'-deoxynucleoside, 2b. This base was oxidised by aqueous potassium persulphate to 5-formyluracil (14).[11] The published procedure[12] for the synthesis of 5-formyl-2'-deoxyuridine starts from the dimethyl acetal of 5-formyluracil, obtained by the action of methanol and *p*-toluene sulphonic acid on 14. This is then condensed with the chlorosugar 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (15) in chloroform[13] to give the α and β anomers of 5-formyl-3',5'-di-*O*-*p*-toluoyl-2'-deoxyuridine (16 and 17 respectively). We were unable to repeat the preparation of the dimethyl acetal,[14] each time we obtained a good recovery of 5-formyl uracil. As an alternative, the bis(trimethylsilyl) derivative of 14 was reacted directly with the chlorosugar 15. The α and β anomers of the product were partially separated by fractional crystallization from acetone although the overall



13 14 15 16,17 18 19

yield of pure separated anomers was not high.

The *p*-toluoyl protecting groups of the pure β anomer 17 were then removed using sodium methoxide in methanol and the free 3'- and 5'-hydroxyl groups were then immediately reprotected as their *t*-butyldimethylsilyl ethers by *t*-butyldimethylchlorosilane to give the *bis*(TBDMS) nucleoside 18.

Compound 19 was then readily obtained using the standard method for the generation of the nitrovinyl side-chain and this could then be deprotected to give the required nucleoside 2b by acid hydrolysis using *p*-toluene sulphonic acid in methanol.

BIOLOGICAL RESULTS

(*E*)-5-(2-Nitrovinyl)uridine (2a) was found to be inactive against HSV-2, HCMV and HIV with an IC_{50} (μM) > 100 μM and for VZV > 40 μM.

(*E*)-5-(2-Nitrovinyl)-2'-deoxyuridine (2b) was found to have an IC_{50} (μM) > 100 against HSV-2, HCMV, HIV and influenza-A and > 40 against VZV. Cytotoxicity ($CCID_{50}$) in vero cells = 72 μM.

EXPERIMENTAL

Ultraviolet spectra were recorded with a Perkin Elmer 552 spectrophotometer and were run in spectroscopic grade ethanol. Mass spectra were determined with a Kratos MS80 mass spectrometer with a DS55 data system employing automatic digital readout of data. For Fast Atom Bombardement (FAB) a 3-NOBA matrix with Na doping or a 3-mercapto-1,2-propanediol matrix were used. The ^{1}H-NMR spectra (s:singlet, d:doublet, t:triplet, b:broad, m:multiplet) were recorded on either a Jeol FX90 (90 MHz) or a Jeol GX270 (270 MHz) spectrometer. Precoated Merck silica gel 60 F_{252} plates were used for TLC and the spots were examined with a UV light (254 nm) and a sulphuric acid-cysteine spray. Column chromatography was performed using Kieselgel 60, 70-230 mesh ASTM, type 7734, supplied by E. Merck A.G., Darmstadt, Germany. Columns were packed under gravity. Pyridine and triethylamine were heated under reflux over CaH_2 and then distilled. THF was dried with potassium/benzophenone then distilled.

5'-*O*-(*tert*-Butyldiphenylsilyl)-5-formyl-2',3'-*O*-isopropylideneuridine (11) To a stirred solution of 5-formyl-2',3'-*O*-isopropylideneuridine (3.00 g, 9.60 mmol) in dry pyridine (40 ml) was slowly added a solution of *tert*- butyldiphenylchlorosilane (3.17 g, 11.53 mmol) in dry pyridine (15 ml). After stirring at room temperature for 10 hours the solution was evaporated to dryness *in vacuo* and the resulting gum purified by column chromatography with elution in chloroform to give the product as a white foam (4.10 g, 78%). UV λ_{max} 292.0 nm, ϵ= 8350. ^{1}H-NMR δ(DMSO-d_6) 11.80(1-H,bs,N-H), 9.72(1-H,s,CHO). 8.48(1-H,s,H-6), 7.80-7.20(10-H,m,2 Ph), 5.84(1-H,d,H-1'), 5.03(1-H,m,H-2'), 4.62(1-H,m,H-3'), 4.25(1-H,m,H-4'), 3.84(2-H,m,H-5'), 1.47(3-H,s,CH_3), 1.26(3-H,s,CH_3), 0.97(9-H,s,^{t}Bu). FAB mass spectrum (3-NOBA) m/e 493 (M-^{t}Bu)$^+$, 551 (M+H)$^+$. Elemental analysis $C_{29}H_{34}N_2O_7Si_2$ calculated C, 63.25; H, 6.22; N, 5.08; found C, 63.0; H, 6.1; N,5.35.

5'-*O*-(*tert*-Butyldiphenylsilyl)-2',3'-*O*-isopropylidene-(*E*)-5-(2-nitrovinyl) uridine (12) To a solution of 11 (4.00 g, 7.26 mmol) in ethanol (100 ml) and nitromethane (80 ml) was added triethylamine (40 ml). After 2 hours at room temperature, TLC in 85:15 toluene/acetone showed complete conversion to a slower running nucleoside. The reaction mixture was evaporated to dryness *in vacuo* and acetic anhydride (100 ml) added. After 5 hours at room temperature the solution was once again evaporated to dryness *in vacuo*, coevaporated with methanol (3 x 25 ml) and the product isolated by short column chromatography as a yellow foam (3.91 g, 91%). UV λ_{max} 329.0 nm, ϵ= 17670. ^{1}H-NMR δ(DMSO-d_6) 11.97(1-H,s,N-H), 8.45(1-H,s,H-6), 8.19(1-H,d,vinylic H,J=14Hz), 7.84(1-H,d,vinylic H,J=14Hz), 7.75-7.25(10-H,m,2 Ph), 5.78(1-H,d,H-1'), 5.00(1-H,m,H-2'), 4.68(1-H,m,H-3'), 4.28(1-H,m,H-4'), 3.92(2-H,m,H-5'), 1.50(3-H,s,CH_3), 1.26(3-H,s,CH_3), 0.95(9-H,s,^{t}Bu). FAB mass spectrum (3-NOBA) m/e 594 (M+H)$^+$. Elemental analysis $C_{30}H_{35}N_3O_8Si$ calculated C, 60.60; H, 5.94; N, 7.07; found C, 60.5; H, 5.9; N, 6.8.

(*E*)-5-(2-Nitrovinyl)uridine (2a) A solution of 12 (1.00 g, 1.68 mmol) in 50% aqueous trifluoroacetic acid (50 ml) was stirred for 3 hours at room temperature after which time TLC in 90:10 chloroform/methanol showed total conversion to a more polar nucleoside. This was isolated by column chromatography with elution in 90:10 chloroform/methanol then recrystallized from ethanol to give the product as a pale yellow powder

(0.27 g, 50%). mp 231-233 °C. UV λ_{max} 333.0 nm, ϵ= 17400. ^{1}H-NMR δ(DMSO-d_6) 11.90(1-H,s,N-H), 8.68(1-H,s,H-6), 8.18(1-H,d,vinylic H,J=14 Hz), 7.30(1-H,d,vinylic H,J=14Hz), 5.75(1-H,d,H-1'), 5.60-4.80(3-H,m,3 x OH), 4.20-3.80(3-H,m,H-2',H-3',H-4'), 3.70-3.50(2-H,m,H-5'). FAB mass spectrum (3-mercapto-1,2-propanediol) m/e 424 $(M+matrix+H)^+$. Elemental analysis $C_{11}H_{13}N_3O_8$ calculated C, 41.91; H, 4.16; N, 13.33; found C, 42.1; H, 4.3; N, 13.0.

5-Formyl-3',5'-di-*O*-*p*-toluoyl-2'-deoxyuridine (17) 5-Formyluracil (1.00 g, 7.14 mmol) in hexamethyldisilazane (10 ml) and chlorotrimethylsilane (10 ml) was heated under reflux until a clear solution was obtained. The solution was evaporated to dryness *in vacuo* under high vacuum and the resulting oil then dissolved in chloroform (100 ml). To this stirred solution was added 15 (2.80 g, 7.20 mmol) and the solution stirred at room temperature for 20 hours then extracted with aqueous sodium bicarbonate (3 x 100 ml), saturated NaCl and dried ($MgSO_4$). The anomer mixture was purified by column chromatography with elution in a chloroform/methanol 100:0 to 95:5 gradient and the anomers partially separated by fractional crystallization from acetone to give 0.5 g of each pure anomer, total yield of pure separated anomers 1.00 g (29%). For the β anomer (17): mp 115-118 °C (Lit[12] 195-196 °C). UV λ_{max} 293.0 nm, ϵ= 9740. ^{1}H-NMR δ(DMSO-d_6) 11.87(1-H,s,N-H), 9.70(1-H,s,CHO), 8.42 (1-H,s,H-6), 7.93-7.85(4-H,m,arom), 7.37-7.28(4-H,m,arom), 6.22(1-H,t, H-1'), 5.38(1-H,s,H-3'), 4.61(3-H,m,H-4',H-5'), 2.39(3-H,s,CH_3), 2.37 (3-H,s,CH_3). Elemental analysis $C_{26}H_{24}N_2O_8$ calculated C, 63.48; H, 4.91; N, 5.68; found C, 63.2; H, 5.0; N, 5.5.

3',5'-*bis*(*tert*-Butyldimethylsilyl)-5-formyl-2'-deoxyuridine (18) To a stirred solution of 17 (0.30 g, 0.61 mmol) in dry methanol (80 ml) was added a solution of sodium (0.1 g) in dry methanol (20 ml). After 3 hours at room temperature the solvent was removed *in vacuo* and the solid coevaporated with dry pyridine (2 x 30 ml) then suspended in dry pyridine (40 ml) and *tert*-butyldimethylchlorosilane (0.92 g, 6.10 mmol) added. After 3 days at room temperature the solvent was removed *in vacuo* to give an oil from which the product was isolated by column chromatography with elution in 96:4 dichloromethane/ethanol to give the product as a white foam (0.22 g, 79%). UV λ_{max} 292.0 nm, ϵ=10550. ^{1}H-NMR δ(DMSO-d_6) 11.84(1-H,s,N-H), 9.79(1-H,s,CHO), 8.32(1-H,s,H-6), 6.04(1-H,t,H-1'), 4.35(1-H,m,H-3'), 3.92(1-H,m,H-4'), 3.78(2-H,m,H-5'), 2.27(2-H,m,H-2'), 0.86(18-H,s,2 ^{t}Bu), 0.07(12-H,s,4 $SiMe_3$). FAB mass spectrum m/e 485 $(M+H)^+$, 507 $(M+Na)^+$. Elemental analysis $C_{22}H_{40}N_2O_6Si$ calculated C, 57.86; H, 8.83; N, 6.13; found C, 57.8; H, 8.9; N, 6.0.

3',5'-*bis*-(*tert*-Butyldimethylsilyl)-(*E*)-5-(2-nitrovinyl)-2'-deoxyuridine (19) To a stirred solution of 18 (0.37 g, 0.76 mmol) in dry ethanol (10 ml) were added nitromethane (8 ml) and triethylamine (8 ml) and the solution stirred for 2 hours then evaporated to dryness *in vacuo* and coevaporated twice with toluene. To the resulting foam were added acetic anhydride (10 ml) and triethylamine (0.50 ml) and the solution stirred overnight. The solution was taken to dryness and the product isolated by column chromatography with elution in dichloromethane/methanol 95:5 followed by recrystallization from ethanol to give the product as yellow crystals (0.24 g, 56%). mp 109-112 °C. UV λ_{max} 331.0nm, ϵ=18240. ^{1}H-NMR δ(DMSO-d_6) 11.94(1-H,s,N-H), 8.35(1-H,s,H-6), 8.20(1-H,d,vinylic H,J=13Hz), 7.92(1-H,d,vinylic H,J=13Hz), 6.10(1-H,t,H-1'), 4.37(1-H,

m,H-3'), 3.84(1-H,m,H-4'), 3.75(2-H,m,H-5'), 2.30(2-H,m,H-2'), 0.87 (18-H,s,2 ^{t}Bu), 0.08(12-H,d,2 $SiMe_3$). FAB mass spectrum m/e 528 $(M+H)^+$. Elemental analysis $C_{25}H_{41}N_3O_7Si$ calculated C, 52.34; H, 7.83; N, 7.96; found C, 52.45; H, 7.9; N, 8.1.

(*E*)-5-(2-Nitrovinyl)-2'-deoxyuridine (2b) To a solution of 19 (0.15 g, 0.284 mmol) in dry methanol (15 ml) was added *p*-toluenesulphonic acid (0.5 g). After 2 hours at room temperature, TLC in 90:10 chloroform/ methanol showed the appearence of a new nucleoside which was isolated by short column chromatography and recrystallization from acetone/hexane to give a pale yellow powder (0.065 g, 76%). mp 186-188 °C. UV λ_{max} 332.0 nm, ϵ=30010. ^{1}H-NMR δ(DMSO-d_6) 11.89(1-H,s,N-H), 8.60(1-H,s,H-6), 8.18 (1-H,d,vinylic H,J=13Hz), 7.91(1-H,d,vinylic H,J=13Hz), 6.09(1-H,d,H-1'), 5.30(1-H,d,3'-OH), 5.16(1-H,t,5'-OH), 4.25(1-H,t,H-3'), 3.81(1-H,d,H-4'), 3.66(1-H,m,H-5'), 2.20(2-H,t,H-2'). FAB mass spectrum (3-mercapto-1,2-propanediol) m/e 408 $(M+matrix+H)^+$. Elemental analysis $C_{11}H_{13}N_3O_7$ calculated C, 44.15; H, 4.38; N, 14.04; found C, 43.99; H, 4.34; N, 13.61.

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