Tetrahedron Vol 47, No 9, pp 1727-1736, 1991
Printed in Great Britain

0040-4020/91 $3 00+ 00

SYNTHESIS OF 3'-C-ETHYNYLNUCLEOSIDES OF THYMINE

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(*Received in UK* 14 *November* 1990)

Abstract.- Reaction of 1-[2,5-di-O-(t-butyldimethylsilyl)-β-D-erythro-pentofuranos-3-ulosyl]thymine with HC ≡ CMgBr gives a (25:1) mixture of 3'-C-ethynyl nucleosides of thymine having β-D-xylo and β-D-ribo configuration. Reaction of 1-(2'-deoxy-5'-O-trityl-β-D-glycero-pentofuran-3-ulosyl)thymine with HC ≡ CMgBr gives the corresponding 3'-C-ethynyl-β-D-threo-thymidine. The absolute configurations of the newly formed chiral centers at C-3' have been demonstrated by chemical means.

INTRODUCTION

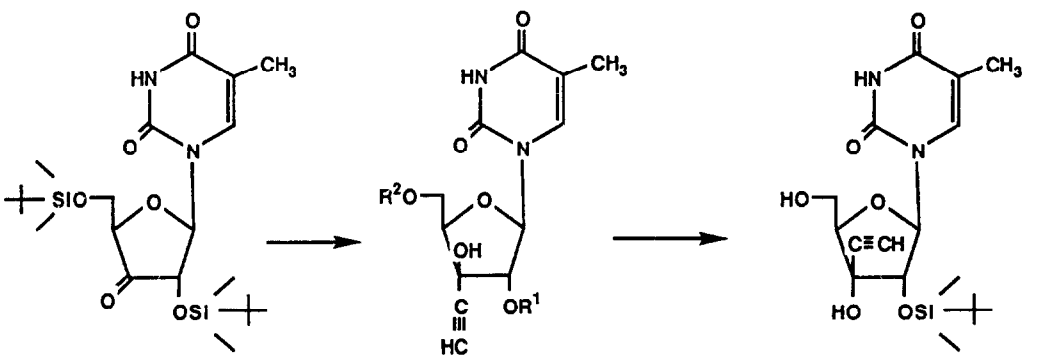
A number of branched-chain sugar nucleosides, both naturally occurring and synthetic show a variety of biological activities. For example, oxetanocin[1] is active against HIV and herpes viruses and the synthetic 3'-C-methylcytidine and 2' and 3'-C-methyladenosines are effective anti-vaccinia agents in mice[2] and also inhibit the growth of KB cells *in vitro*[3]. Other synthetic 2'-C- and 3'-C-branched-chain sugar nucleosides inhibit the growth of tumor cells[4-8] and bacteria[9] and are also inhibitors of a variety of enzymatic systems.[10]

A common method for the synthesis of branched-chain sugar nucleosides involves the reaction of branched-chain sugars with nucleic acid bases.[2-6,9,10] The most usual methods for the introduction of a C-branch in nucleosides involve the reaction of 2',3'-anhydronucleosides with a carbon nucleophile,[11,12] and the reaction of ketonucleosides with cyanide ion,[13,14] dimethylsulfoxonium methylide,[15] nitromethane[16] and organometallic reagents,[7,17-19] and Wittig reagents;[8,20] other procedures include the Michael addition to 3'-sulfone-2',3'-unsaturated nucleosides,[21] the ring contraction of a hexopyranosylnucleoside,[22] and several procedures using free-radical methodologies.[23,24]

In this paper we report the highly stereoselective synthesis of 3'-C-ethynyl derivatives of thymine nucleosides by reaction of ethynylmagnesium bromide with 3'-ketonucleosides of thymine.

RESULTS AND DISCUSSION

Reaction of ketonucleoside **1**[25] with ethynylmagnesium bromide in tetrahydrofuran gave a mixture of 3'-C-ethynyl nucleosides **2**(20%), **3**(53%) and **5**(3%). The stereochemistry of the major products **2** and **3** is β-D-xylo, and results from the approach of the Grignard reagent from the less hindered α face of the furanosulose ring. The cleavage of the 5'-O-silyl protecting groups of **3** and **5** can be explained by the ethynyl carbanions originating from the Grignard reagent, which is present in a large excess. Silylation of **3** with t-butyldimethylsilyl chloride, afforded **2** in quantitatively yield; on the other hand selective desilylation of **2**, by reaction with 80% aqueous acetic acid, gave **3** quantitatively.



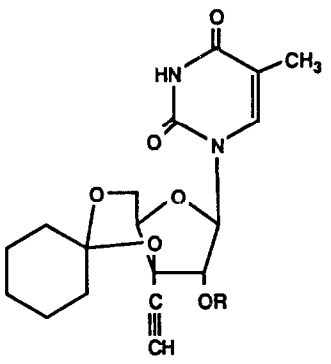
1 **2**, $R^1 = R^2 = Si$ **5**

3, $R^1 = Si$, $R^2 = H$

4, $R^1 = R^2 = H$

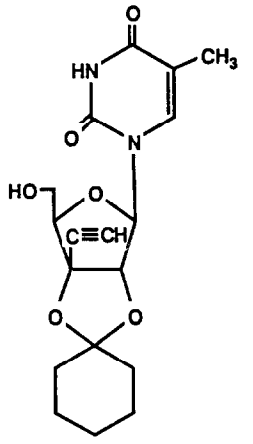
The absolute configuration of the new chiral center at C-3' of compounds **2**, **3** and **4** was assigned as follows: Deprotection of **2** with tetrabutylammonium fluoride trihydrate gave **4** in 85% yield. Reaction of **4** with cyclohexanone in the presence of sulfuric acid afforded a

cyclohexylidene derivative in 52% yield which was assigned as **6**. The formation of **6** instead of **8** was demonstrated by acetylation of the cyclohexylidene derivative with acetic anhydride and pyridine to afford **7** in 90% yield. The downfield shift of H-2' for **7** (δ = 5.23 ppm) with respect to the same proton of **6** (δ = 4.05-4.50 ppm) indicated that the acetylated hydroxyl group was the 2'-OH and, thus, that the cyclohexylidene ring was formed between the 3' and 5'-OH groups. This means that both hydroxyl groups are on the same face of the furanose ring, as in a β-D-xylo configuration.



6, R = H

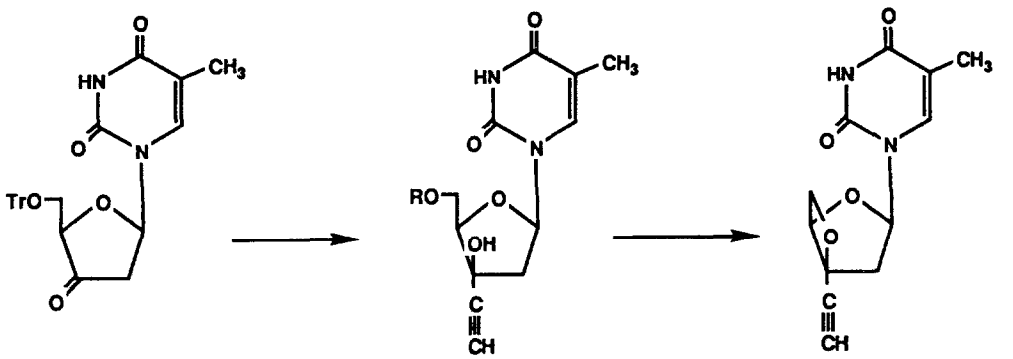
7, R = Ac



8

We then studied a similar reaction for ketonucleoside **9**.[26] Despite the reported instability of ketonucleoside **9** which, in the presence of acid or base,[26] or by treatment with MeMgCl or MeLi at -78°C in tetrahydrofuran[17], decomposes, the reaction of **9** with ethynylmagnesium bromide gave **10** in 67% yield. The different reactivity of HC ≡ CMgBr with respect to MeMgBr may be attributed to the higher nucleophilicity and lower basicity of the former. The formation of the β-D-threo nucleoside and thus the approach of the Grignard reagent from the less hindered α side of the furanosulose ring was demonstrated by transformation of **10** into the 3',5'-anhydro nucleoside **13**. Deprotection of **10** by reaction with aqueous acetic acid gave the fully deprotected **11** in 65% yield. Mesylation of **11** by reaction with mesyl chloride in pyridine gave **12** (92%), which by treatment under reflux with a 1M aqueous solution of NaOH afforded **13** in 99% yield. The formation of **13** and not the alternative O^2,5'-anhydronucleoside **14** was

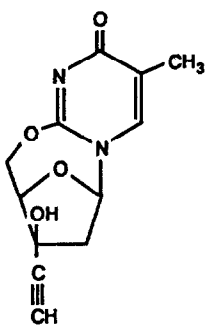
demonstrated by the UV spectrum of **13** which showed a λ_{max} 265 nm (ϵ, 9600) and λ_{min} 237 nm (ϵ, 2830). These values are in agreement with those measured for thymidine and 1-alkyl and 1-glycosylthymines[27] and are quite different from those measured for O^2,5'-anhydrothymidine λ_{max} 250 nm (ϵ, 10400), λ_{min} 218 nm (ϵ, 3500).[28]



9 **10**, R = $(C_6H_5)_3C$ **13**

11, R = H

12, R = CH_3SO_2



14

The stereochemistry of the above reactions of Grignard reagents with ketonucleosides of thymine is in agreement with those observed in most additions of carbon nucleophiles to 2' and 3'-ketonucleosides.[7,15-18] The major or exclusive products are always those resulting from the approach of the carbon nucleophile from the less hindered α face of the furanulose,

opposite to the heterocyclic base at the anomeric position. However, an exception has been reported.[19]

EXPERIMENTAL

Melting points were determined on a Reichert-Jung Thermovar microscope apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded with Varian EM-390 (^1H, 90 MHz), Bruker AM-200 (^1H, 200 MHz; ^{13}C, 50 MHz), and Varian XL-300 (^1H, 300 MHz; ^{13}C, 75 MHz) spectrometers using Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrophotometer. UV spectra were taken on a Perkin-Elmer 550 SE spectrophotometer. Analytical tlc plates were purchased from Merck. Preparative tlc was performed on glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). Flash columm chromatography was performed with silica gel 60 230-400 mesh (Merck). Compound were detected by UV light (254 nm) spraying the plates with 30% H_2SO_4 in ethanol and heating.

Reaction of ketonucleoside 5 with ethynylmagnesium bromide. A solution of ethynylmagnesium bromide in tetrahydrofuran was prepared as described[29] from magnesium turnings (2.4 g, 0.1 mol), ethyl bromide (8.2 mL, 0.11 mol) acetylene (excess) and tetrahydrofuran (90 mL) in a three necked flask equiped with gas inlet, dropping funnel and reflux condenser/ calcium chloride tube. To the above solution magnetically stirred at room temperature, a solution of **1** (2.3 g, 4.75 mmol) in tetrahydrofuran (50 mL) was added over a period of 30 min. During the addition, acetylene purified by the succesive passage through a column of neutral alumina and concentrated sulfuric acid, was bubbled through the solution. The bubbling was stopped after 3 hours, and the stirring was maintained overnight. The reaction flask was cooled in an ice bath and a saturated aqueous solution of ammonium chloride (200 mL) was added. The precipitate formed during the night was filtered and washed with chloroform (4 x 50 mL). The aqueous layer was extracted with chloroform (3 x 25 mL) and the combined organic layers were washed with water (2 x 25 mL), dried over anhydrous sodium sulfate, filtered and concentrated to dryness. The residue was purified by column chromatography using chloroform/methanol (99.5:0.5) as the eluent.

The first eluted compound was characterised as 1-[2,5-di-*O*-(tert-butyldimethylsilyl)-3-*C*-ethynyl-β-*D*-xylo-pentofuranosyl]thymine (**2**) (0.48 g, 20%), as a foam. IR (KBr) 3400 (OH), 3300 (≡C-H), 2105 cm^{-1} (C≡C); UV λ_{max} (MeOH) 264 nm (ϵ, 10400), λ_{min} 232 nm (ϵ, 2460); ^1H NMR ($CDCl_3$,

90 MHz): δ 0.75 (s, 18H, $(CH_3)_3CSi$), 1.74 (s, 3H, 5-CH_3), 2.46 (s, 1H, ≡CH), 3.87 (m, 2H, H-5'), 4.14 (m, 2H, H-2', H-4'), 5.43 (bs, 1H, 3'-OH), 5.75 (d, 1H, $J_{1',2'}$=1 Hz, H-1'), 7.57 (s, 1H, H-6), 9.05 (bs, 1H, 3-NH); ^{13}C NMR [$(CD_3)_2CO$, 75 MHz]: δ 5.27, 5.21, 4.60, 4.36 (CH_3-Si), 12.67 (5-CH_3), 18.53, 18.77 (C-Si), 26.16 [$(CH_3)_3C$], 62.44 (C-5'), 76.70 (C-3'), 79.93 (≡CH), 81.78 (-C≡), 83.93, 86.92 (C-2', C-4'), 92.73 (C-1'), 109.39 (C-5), 137.46 (C-6), 151.24 (C-2), 161.51 (C-4). Anal. Calcd. for $C_{24}H_{42}N_2O_6Si_2$: C, 58.89; H, 8.29; N, 5.49. Found: C, 58.53; H, 8.43; N, 5.60.

The second compound was eluted with chloroform/methanol (98:2) and was identified as 1-[2-O-(tert-butyldimethylsilyl)-3-C-ethynyl-β-D-xylo-pentofuranosyl]thymine (3) (1.0 g, 53%). mp 224-6° (from chloroform); IR (KBr) 3430, 3185 (OH, NH), 3300 (≡C-H), 2100 cm^{-1} (C≡C); UV λ_{max} (MeOH) 264 nm (ε, 10700), λ_{min} 231 nm (ε, 2240); ^1H NMR [$(CD_3)_2CO$, 90 MHz]: δ 0.88 (s, 9H, $(CH_3)_3C$ Si), 1.76 (s, 3H, 5-CH_3), 2.80 (bs, 1H, OH), 3.12 (s, 1H, ≡ CH), 4.04-4.21 (m, 4H, H-2', H-4', H-5'), 5.55 (bs, 1H, OH), 5.78 (d, 1H, $J_{1',2'}$ = 1 Hz, H-1'), 7.79 (q, 1H, J = 1Hz, H-6), 10 (bs, 1H, 3-NH); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz]· δ 4.52, 4.36 (CH_3-Si), 12.63 (5-CH_3), 18.60 (C-Si), 26.21 |$(CH_3)_3C$|, 61.00 (C-5'), 77.34 (C-3'), 77.93 (≡CH), 82.04 (-C≡), 84.15, 86.78 (C-2', C-4'), 92.75 (C-1'), 109.44 (C-5), 137.71 (C-6), 151.38 (C-2), 164.46 (C-4). Anal. Calcd. for $C_{18}H_{28}N_2O_6Si$: C, 54.52; H, 7.12; N, 7.07. Found: C, 54.50; H, 7.24; N, 7.18.

The third compound was eluted with chlroform/methanol (97:3) and was characterised as 1-[2-O-(tert-butyldimethylsilyl)-3-C-ethynyl-β-D-ribo-pentofuranosyl]thymine (5) (0.06 g, 3%). mp 177-9° (from CCl_4); IR (Nujol) 3400, 3250 (OH, NH), 3300 (≡C-H), 2100 cm^{-1} (C≡C); UV λ_{max} (MeOH) 264 nm (ε, 9400), λ_{min} 232 nm (ε, 2170); ^1H NMR [$(CD_3)_2CO$, 90 MHz]: δ 0.90 (s, 9H, $(CH_3)_3C$ Si), 1.83 (s, 3H, 5-CH_3), 2.70 (bs, 1H, OH), 3.12 (s, 1H, ≡CH), 3.53 (bs, 1H, OH), 4.00 (m, 3H, H-4', H-5'), 4.33 (d, 1H, $J_{1',2'}$ = 7 Hz, H-2'), 6.03 (d, 1H, H-1'), 7.62 (s, 1H, H-6). Anal. Calcd. for $C_{18}H_{28}N_2O_6Si$· C, 54.52; H, 7.12; N, 7.07. Found: C, 54.12; H, 7.25; N, 6.81.

Preparation of 2 from 3. A mixture of 3 (0.32 g, 0.81 mmol) pyridine (2 mL), and t-butyldimethylsilyl chloride (0.2 g, 1.3 mmol) was stirred at room temperature for 2 h. The solvent was evaporated to dryness and the residue, dissolved in chloroform (30 mL) was washed with cold 1N HCl (25 mL) and water (3 x 25 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated to dryness. The residue was

purified by column chromatography using chloroform/methanol (99:1) as the eluent to afford **2** (0.4 g, 97%).

Preparation of **3** from **2**. A solution of **2** (0.15 g, 0.29 mmol), acetic acid (4 mL) and water (1 mL) was stirred at room temperature for 30 min. The reaction mixture was concentrated and coevaporated with water (3 x 5 mL). The residue was purified by column chromatography using chloroform/acetone (3:1) as the eluent to give **3** (0.115 g, 99%).

1-(3-C-ethylnyl-β-D-xylo-pentofuranosyl)thymine (**4**). A mixture of **2** (0.511 g, 1 mmol), tetrahydrofuran (6 mL) and tetrabutylammonium fluoride trihydrate (0.95 g, 3 mmol) was stirred at room temperature for 3 h. The reaction mixture was filtered through a short column of silica gel (10 g) using tetrahydrofuran as the eluent. The filtrate was concentrated to dryness and the residue was purified by column chromatography using chloroform/ methanol (9:1) as the eluent to afford **4** (0.24 g, 85%) as a foam. IR (KBr) 3400, 3260 (OH, NH), 3295 (≡C-H), 2100 cm^{-1} (C≡C); UV λ_{max} (MeOH) 263 nm (ε, 8100); λ_{min} 231 nm (ε, 2500); ^1H NMR [$(CD_3)_2SO$, 200 MHz]: δ 1.74 (d, 3H, J = 1.1 Hz, 5-CH_3), 3.51 (s, 1H, ≡CH), 3.74 (m, 2H, H-5'), 3.89 (dd, 1H, $J_{1',2'}$ = 1.7, $J_{2',OH}$ = 6.0 Hz, H-2'), 3.97 (dd, 1H, H-4'), 4.86 (t, 1H, 5'-OH), 5.68 (d, 1H, H-1'), 6.09 (s, 1H, 3'-OH), 6.16 (d, 1H, 2'-OH), 7.60 (q, 1H, H-6), 10.81 (bs, 1H, 3-NH); ^{13}C NMR [$(CD_3)_2SO$, 75 MHz]: δ 12.69 (5-CH_3), 59.68 (C-5'), 75.11 (≡CH), 78.42 (C-3'), 79.33 (-C≡), 81.77, 86.86 (C-2', C-4'), 91.11 (C-1'), 108.72 (C-5), 137.18 (C-6), 150.72 (C-2), 164.20 (C-4). Anal. Calcd. for $C_{12}H_{14}N_2O_6$: C, 51.06; H, 5.00; N, 9.93. Found: C, 50.71; H, 5.27; N, 9.58.

1-(3,5-Cyclohexylidene-3-C-ethynyl-β-D-xylo-pentofuranosyl)thymine (**6**). A mixture of **4** (0.060 g, 0.21 mmol), cyclohexanone (0.16 mL, 1.5 mmol), concentrated sulfuric acid (0.02 g) and dry benzene (0.85 mL) was stirred at room temperature for 2 h. After cooling in an ice bath, a saturated aqueous solution of sodium hydrogen carbonate (2 mL) was added, and the mixture was extracted with ether (3 x 15 mL). The organic layer was washed with water, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was coevaporated twice with toluene to give **6** (0.040 g, 52%) as a foam; ^1H NMR (CD_3COCD_3, 90 MHz): δ 1.54 (m, 10H, Cyclohexylidene), 1.67 (s, 3H, 5-CH_3), 3.24 (s, 1H, ≡CH), 4.05-4.50 (m, 4H, H-2', H-4', H-5'), 5.30 (d, 1H, $J_{2',OH}$ = 6 Hz, 2'-OH), 5.86 (s, 1H, H-1'), 7.94 (s, 1H, H-6), 10.11 (bs, 1H, 3-NH); Anal. Calcd. for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.40; H, 6.23; N, 7.61.

1-(2-O-Acetyl-3,5-Cyclohexylidene-3-C-ethynyl-β-D-xylo-pentofuranosyl) thymine (7). To a solution of **6** (0.036 g, 0.1 mmol) in pyridine (2 mL) acetic anhydride (0.2 mL) was added and the mixture was stirred overnight at room temperature. The solution was poured into ice water (2 mL), stirred for 0.5 h, and extracted with chloroform (10 mL). The chloroform phase was washed with a saturated solution of $NaHCO_3$ and water, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was coevaporated with toluene and purified by column chromatography using chloroform/methanol (98:2) as the eluent to yield **7** (0.036 g, 90%) as a foam. ^1H NMR [$(CD_3)_2CO$, 90 MHz]: δ 1.6 (m, 10H, Cyclohexylidene), 1.83 (s, 3H, 5-CH_3), 2.10 (s, 3H, CH_3CO), 3.38 (s, H, ≡CH), 4.00-4.49 (m, 3H, H-4', H-5'), 5.23 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-2'), 5.95 (d, 1H, H-1'), 7.86 (s, 1H, H-6), 10.11 (bs, 1H, 3-NH). Anal. Calcd. for $C_{20}H_{24}N_2O_7$: C, 59.39; H, 5.98; N, 6.92. Found: C, 59.11; H, 6.07; N, 6.76.

1-(5-O-triphenylmethyl-2-deoxy-3-C-ethynyl-β-D-threo-pentofuranosyl) thymine (10). Ketonucleoside **9** (2 g, 4.14 mol) reacted with ethynylmagnesium bromide following the same procedure previously described for the reaction of **1** with that Grignard reagent. The residue was purified by column chromatography using chloroform/methanol (99:1) as the eluent to yield **10** (1.40 g, 67%) as a foam. IR (KBr) 3400 (broad, OH, NH), 3295 cm^{-1} (≡C-H); UV λ_{max} (MeOH) 262.5 nm (ε, 10000); λ_{min} 230.5 nm (ε, 5550); ^1H NMR ($CDCl_3$, 90 MHz): δ 1.76 (s, 3H, 5-CH_3), 2.40-2.90 (m, 2H, H-2'), 2.50 (s, 1H, ≡CH), 3.63 (d, 1H, $J_{4',5'}$ = 4 Hz, H-5'), 4.16 (t, 1H, H-4'), 4.32 (s, 1H, 3'-OH), 6.25 (dd, 1H, $J_{1',2'a}$ = 3, $J_{1',2'b}$ = 7.5 Hz, H-1'), 7.20-7.54 (m, 15H, trityl), 7.69 (s, 1H, H-6), 9.06 (bs, 1H, 3-NH). Anal. Calcd. for $C_{31}H_{28}N_2O_5$: C, 73.21; H, 5.55; N, 5.51. Found: C, 72.92; H, 5.50; N, 5.46.

1-(2-deoxy-3-C-ethynyl-β-D-threo-pentofuranosyl)thymine (11). A solution of **10** (0.51 g, 1 mmol) in acetic acid (4.8 mL) and water (1.2 mL) was stirred at room temperature for 14 h. The reaction mixture was concentrated under reduced pressure and coevaporated with a (1:1) mixture of ethanol and water (3 x 2 mL). The residue was purified by preparative tlc using ethyl acetate as the eluent to afford **11** (0.173 g, 65%). IR (KBr) 3400, 3260 (OH, NH), 3295 (≡CH), 2100 cm^{-1} (C≡C); UV λ_{max} (MeOH) 263 nm (ε, 9000); λ_{min} 231 nm (ε, 2830); ^1H NMR [$(CD_3)_2SO$, 200 MHz]: δ 1.74 (s, 3H, 5-NH), 2.21 (dd, 1H, $J_{1',2'a}$ = 2.8, $J_{2'a,2'b}$ = 14.4 Hz, H-2'a), 2,72 (dd, 1H, $J_{2',2'b}$ = 8.4 Hz, H-2'b), 3.54 (s, 1H, ≡CH), 3.72 (m, 2H, H-5'), 3,85 (dd, 1H, H-4'), 4.83 (t, 1H, 5'-OH), 6.11 (s, 1H, 3'-OH), 6.14 (dd, 1H, H-1'), 7.72 (q, 1H, H-6), 10.85 (bs, 1H, 3-NH). ^{13}C NMR [$(CD_3)_2SO$, 75 MHz]: δ 12.72 (5-CH_3), 47.02 (C-2'), 59.72 (C-5'), 70.38 (≡CH),

75.87 (C-3'), 83.15 (C-4'), 84.01 (-C≡), 88.40 (C-1'), 109.21 (C-5), 137.26 (C-6), 150.78 (C-2), 164.21 (C-4). Anal. Calcd. for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.04; H, 5.61; N, 10.22.

1-(2-Deoxy-3-C-ethynyl-5-O-methylsulfonyl-β-D-threo-pentofuranosyl) thymine (12). A solution of **11** (0.08 g, 0.3 mmol) in dichloromethane (15 mL) and pyridine (0.5 mL) was treated with methylsulfonyl chloride (0.075 mL, 0.96 mmol). The resulting mixture was stirred at room temperature for 1 h and water (1 mL) was added. The mixture was partitioned between dichloromethane (50 mL) and a 5% aqueous solution of $NaHCO_3$ (100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified through a short column of silica gel using chloroform/methanol (97:3) as the eluent to give **12** (0.095 g, 92%) as a foam. UV λ_{max} (MeOH) 262 nm (ε, 12000); λ_{min} 231 nm (ε, 4000); ^{1}H NMR (CD_3COCD_3, 90 MHz): δ 1.85 (s, 3H, 5-CH_3), 2.54 (dd, 1H, $J_{1',2'a}$ = 3, $J_{2'a,2'b}$ = 15 Hz, H-2'a), 2.95 (dd, 1H, $J_{1',2'b}$ = 8 Hz, H-2'b), 3.16 (2s, 4H, CH_3SO_2, ≡CH), 4.28 (dd, 1H, H-4'), 4.63 (m, 2H, H-5'), 5.66 (bs, 1H, 3'-OH), 6.33 (dd, 1H, H-1'), 7.80 (s, 1H, H-6), 10.28 (bs, 1H, 3-NH). Anal. Calcd. for $C_{13}H_{16}N_2O_7S$: C, 45.34; H, 4.68; N 8.14. Found: C, 45.22; H, 4.75; N, 8.01.

1-(3,5-Anhydro-2-deoxy-3-C-ethynyl-β-D-threo-pentofuranosyl)thymine (13). A mixture of **12** (0.074 g, 0.215 mmol), ethanol (9 mL), water (2.1 mL), and a 1 M aqueous solution of NaOH (0.9 mL) was refluxed for 1 h, allowed to cool to room temperature and brought to pH = 9 by addition of a 2.5 M aqueous solution of acetic acid. The solution was concentrated to dryness and the white precipitate was triturated with ethanol (10 mL). The mixture was filtered and the filtrate was evaporated to dryness. The residue was treated with chloroform (7 mL) and the mixture filtered. The filtrate was evaporated to dryness to afford **13** (0.053 g, 99%) as a foam. IR (KBr) 3415, 3220 (broad, OH, NH), 2100 cm^{-1} (C≡C); UV λ_{max} (MeOH) 265 nm (ε, 9600); λ_{min} 237 nm (ε, 2830); ^{1}H NMR ($CDCl_3$, 90 MHz): δ 1.95 (s, 3H, 5-CH_3), 2.76 (d, 2H, H-2'), 3.00 (s, 1H, ≡CH), 4.13 (dd, 1H, H-4'), 4.85 (m, 2H, H-5'), 6.78 (t, 1H, $J_{1',2'}$ = 5.6 Hz, H-1'), 7.92 (s, 1H, H-6), 8.50 (bs, 1H, 3-NH). Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87, N, 11.29. Found: C, 58.01; H, 4.93; N, 11.17.

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