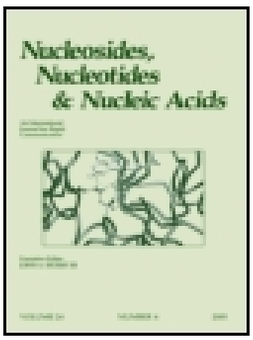


Nucleosides and Nucleotides

ISSN: 0732-8311 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/lncn19

The Synthesis and Biological Activity of 1-(2-Deoxy-4-Thio-α;-L-Threo-Pentofuranosyl)Thymine

Kamal N. Tiwari , John A. Montgomery & John A. Secrist III

To cite this article: Kamal N. Tiwari , John A. Montgomery & John A. Secrist III (1993) The Synthesis and Biological Activity of 1-(2-Deoxy-4-Thio-α;-L-Threo-Pentofuranosyl)Thymine, Nucleosides and Nucleotides, 12:8, 841-846, DOI: 10.1080/07328319308018554

To link to this article: http://dx.doi.org/10.1080/07328319308018554

NUCLEOSIDES & NUCLEOTIDES, 12(8), 841-846 (1993)

THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1-(2-DEOXY-4-THIO-α-L-*THREO*-PENTOFURANOSYL)THYMINE

Kamal N. Tiwari, John A. Montgomery, and John A. Secrist III*

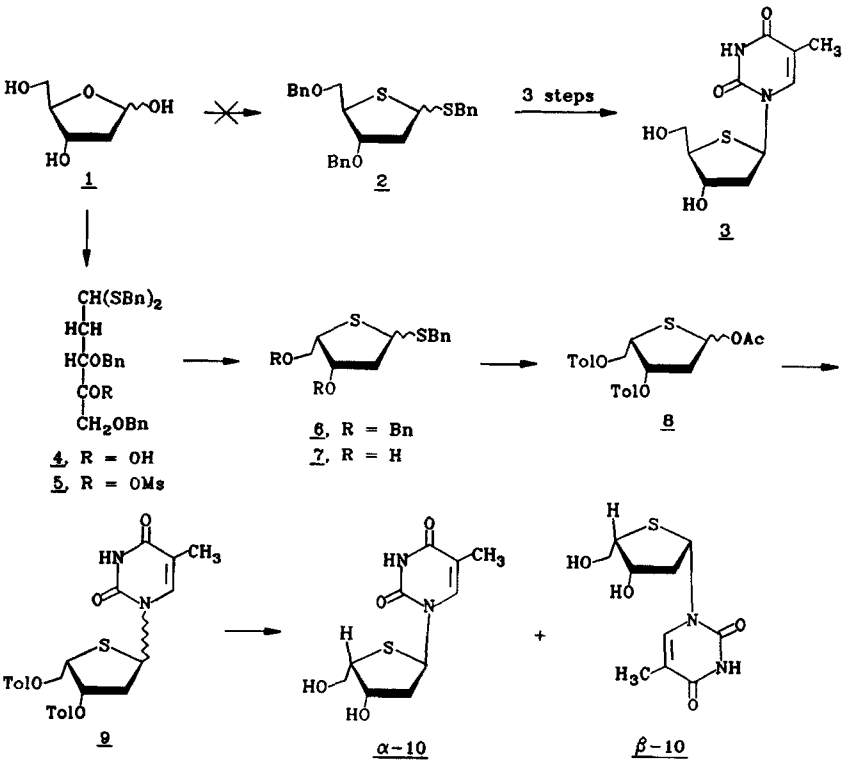
Southern Research Institute, Organic Chemistry Research Department,
Birmingham, Alabama 35255-5305

Abstract: The procedure of Huang and Hui (*Nucleosides & Nucleotides* **1993**, *12*, 139-147) was found to give benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-α-L-*threo*-pentofuranoside (**6**) rather than the claimed D-*erythro* isomer. This sugar was converted to an anomeric mixture of the thymine nucleosides. The mixture was separated and the α-anomer (**α-10**) was found to be cytotoxic, whereas the β-anomer (**β-10**) was inactive.

INTRODUCTION

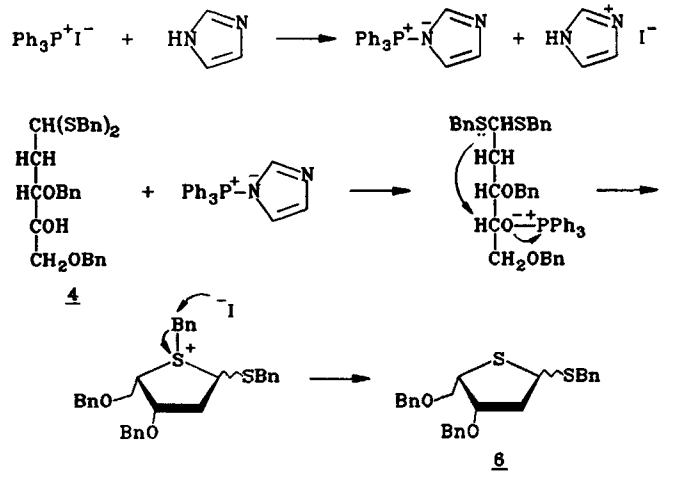
As a part of an ongoing program to develop new antiviral and anticancer agents we have synthesized and evaluated the biologic activity of a number of 4′-thio-2′,3′-dideoxy-, 4′-thio-2′-deoxy- and 4′-thioribonucleosides.[1-3] For the synthesis of 4′-thiothymidine and other 2′-deoxy-4′-thioribonucleosides, we prepared methyl 2-deoxy-4-thio-D-ribofuranose from L-arabinose by the fourteen-step synthesis of Fu and Bobek.[4] A recent publication by Huang and Hui claims a facile five-step synthesis of 1-acetoxy-3,5-di-*O*-benzyl 2-deoxy-4-thio-D-ribofuranose, which they converted to the corresponding nucleoside of thymidine.[5] We have now repeated the procedure of Huang and Hui for the preparation of the blocked 2-deoxy-4-thio-D-ribofuranose derivative, which we further converted to the 1-acetoxy-3,5-di-*O*-toluoyl derivative. This latter material was clearly different from authentic methyl 2-deoxy-4-thio-3,5-di-*O*-benzyl-D-*erythro*-pentofuranose prepared by us from methyl 2-deoxy-4-thio-D-*erythro*-pentofuranose.[2] Conversion of this new sugar derivative to the thymine nucleoside gave a compound different from 4′-thiothymidine.[2,6]

The key intermediate, 3,5-di-*O*-benzyl-2-deoxy-D-*erythro*-pentose dithiobenzylacetal (**4**) was prepared and converted to a 4-thiosugar by treatment with triphenylphosphine, iodine, and imidazole as described (Scheme I).[5] Compound **4** was also mesylated and converted to **6** by the procedure of Dyson *et al.*[8] The sugars prepared by the two procedures were identical and must be benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-L-*threo*-pentofuranoside (**6**)



SCHEME I

rather than the desired D-*erythro*-pentofuranoside (2-deoxy-D-ribofuranoside **2**). The ^{1}H NMR data for **6** agrees with that of Dyson *et al.*[8] Scheme II presents a plausible explanation[9] for the formation of **6** from **4**. Compound **6** was debenzylated with boron tribromide to **7**, which was then converted to 1-*O*-acetyl-2-deoxy-4-thio-3,5-di-*O*-toluoyl-L-*threo*-pentofuranoside (**8**), the ^{1}H NMR spectrum of which was different from the D-*erythro*-pentofuranoside previously prepared.[2] Silylated thymine was then allowed to react with **8** as previously described[2] to give 1-(2-deoxy-4-thio-3,5-di-*O*-toluoyl-L-*threo*-pentofuranosyl)thymine (**9**), which was deacylated with methanolic sodium methoxide to give **10**. The α and β anomers of **10** were separated by preparative TLC and identified by ^{1}H NMR. The spectra of the anomers are very similar to those of the corresponding 2′-deoxy-4′-thioribonucleosides[2] and the anomeric configurations were assigned on the basis of two observations. First, the difference in chemical shift between H-2′ and H-2″ is greater in the spectra of the nucleosides in which the pyrimidine is on the same side of the furanose ring as the 3′-OH (α-anomer of 4′-thiothymidine and β-**10**) than



SCHEME II

it is in the spectra of the nucleosides in which they are on opposite sides of the ring (4-thiothymidine and **α-10**). Second, the chemical shift of H-4′ in the spectra of the nucleosides in which the pyrimidine is on the same side of the ring (α-anomer of 4-thiothymidine and **α-10**) is downfield from H-4′ in the spectra of the nucleosides in which they are on opposite sides of the ring (4-thiothymidine and **β-10**).

The α-anomer of **10** was cytotoxic to both H.Ep.-2 and CCRF-CEM cells in culture[2] with an IC_{50} of 19 μM, whereas the β-anomer showed no activity at the highest level tested (155 μM), reminiscent of the biologic activity of α-arabinofuranosyladenine and its 8-aza analog.[10,11] In both cases the CH_2OH attached to C-4 of the furanose is trans rather than cis to the base of the nucleoside.

EXPERIMENTAL SECTION

All evaporations were carried out *in vacuo* with a rotary evaporator or by short-path distillation into a dry ice-acetone cooled receiver under high vacuum. Analytical samples were normally dried *in vacuo* over P_2O_5 at room temperature for 16 h. Analtech precoated (250 μM) silica gel G(F) plates were used for TLC analysis. The spots were detected by irradiation with a mineral light and/or by charring after spraying with saturated $(NH_4)_2SO_4$. All analytical samples were TLC homogeneous. Melting points were determined with a Kofler-Heizbank apparatus unless otherwise specified. Purifications by flash chromatography were carried out on Merck silica gel 60 (230-400 mesh), using the slurry method of column packing. The UV absorption spectra were determined in 0.1

N HCl (pH 1) pH 7 buffer, and 0.1 *N* NaOH (pH 13) with a Cary 17 spectrophotometer. The maxima are reported in nanometers (ϵ x 10^{-3} M^{-1} cm^{-1}). The NMR spectra in Me_2SO-d_6 or $CDCl_3$ with tetramethylsilane and in D_2O with acetone as internal standards were determined with a Nicolet NT 300 NB spectrometer operating at 300.635 MHz. Chemical shifts (δ) quoted in the case of multiplets were measured from the approximate center. Where necessary, the chemical shift and coupling constant values for the non-first order parts of the spectra were obtained from simulated spectra by employing the General Electric/Nicolet ITRACAL program for iterative analysis. The mass spectral data were obtained with a Varian-MAT 311A mass spectrometer in the fast atom bombardment mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

1-*O*-Acetyl-2-deoxy-4-thio-3,5-di-*O*-toluoyl-L-*threo*-pentofuranoside (8). A solution of **6** (436 mg) in dichloromethane (15 mL) was treated at -78 °C under argon with a solution (1 M) of boron tribromide in dichloromethane (6 mL). After stirring for 0.5 h at -78 °C, methanol (4 mL) was added followed by pyridine (4 mL) to neutralize the reaction mixture, and solution was evaporated to dryness with coevaporation using dry pyridine (3 x 20 mL). The brown crude **7** was dissolved in pyridine (25 mL) and to this solution at 0 °C was added p-toluoyl chloride (460 mg) dropwise with stirring. After 24 h the pyridine was removed *in vacuo* and the residue was extracted with chloroform, washed with HCl (2 M), sodium carbonate (M) and water, dried ($MgSO_4$) and concentrated to dryness. The brown syrup was passed through a short bed of silica gel on a filter funnel and washed first with cyclohexane and then with cyclohexane-ethyl acetate (4:1) to obtain a light yellow syrup (400 mg) that was dissolved in a mixture of acetic anhydride (4 mL) and acetic acid (4 mL) containing mercuric acetate (1 g). The solution was kept at 45-50 °C for 30 min, then water (50 mL) and $CHCl_3$ (50 mL) were added. The organic layer was dried, evaporated, and the residue was purified with silica gel (cyclohexane-ethyl acetate, 5:1) to give **8** (282 mg, 66%); MS *z/e* 435 $(M + Li)^+$, 369 $(M - OAc)^+$; ^1H NMR ($CDCl_3$) δ 1.98 (s, 3 H, CH_3CO), 2.30 (s, 3 H, CH_3CO), 2.40 (s, 3 H, CH_3 of toluoyl group), 2.42 (s, 3 H, CH_3 of toluoyl group), 2.44-2.80 (m, 4 H, H-2), 4.08-4.22 (m, 2 H, H-4), 4.28-4.68 (m, 4 H, H-5), 5.82-5.96 (m, 2 H, H-3), 6.16 (dd, 1 H, H-1, J = 4 and 6 Hz), 6.22 (dd, 1 H, H-1, $J_{1,2}$ = 2 Hz, $J_{1,2'}$ = 6 Hz), 7.12-7.36 (m, 8 H, H's of toluoyl group), 7.68-8.04 (m, 8, H's of toluoyl group).

1-(2-Deoxy-4-thio-3,5-di-*O*-toluoyl-L-*threo*-pentofuranosyl)thymine (9). To a suspension of **8** (428 mg, 1.0 mmol) and thymine (126 mg, 1.0 mmol) in anhydrous acetonitrile (30 mL) were added consecutively hexamethyldisilazane (HMDS, 161.5 mg, 1.0 mmol) and TMSCl (0.37 mL, 2.9 mmol). The resulting mixture was stirred at room temperature for 0.5 h before it was cooled to -78 °C, and trimethylsilyl trifluoromethanesulfonate (267 mg,

1.2 mmol) was added. The reaction mixture was stirred at -78 °C for another 1.5 h, after which time the reaction was essentially complete. The reaction mixture was warmed to room temperature, concentrated to a small volume (5 mL) diluted with methylene chloride (50 mL) then washed with water (15 mL) followed by saturated sodium bicarbonate and water. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography over silica gel (50 g, elution with $CHCl_3$-MeOH, 99:1) to afford a solid **9** as an α:β mixture that failed to resolve and was not crystallizable as a single anomer. TLC ($CHCl_3$-MeOH, 98:2) R_f 0.50; MS *z/e* 495 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 1.72 (d, 3 H, 5-CH_3, $J_{5\text{-}CH_3,6}$ = 1.4 Hz), 1.98 (d, 3 H, 5-CH_3, $J_{6,5\text{-}CH_3}$ = 1.4 Hz), 2.40 (s, 3 H, CH_3 of toluoyl), 2.42 (s, 3 H, CH_3 of toluoyl), 2.24-2.33 (m, 1 H, H-2′, α-anomer), 2.56-2.65 (m, 1 H, H-2′, β-anomer), 2.75-2.80 (m, 1 H, H-2′, β-anomer), 2.81-2.90 (m, 1 H, H-2′, α-anomer), 4.20-4.25 (m, 1 H, H-4′, β-anomer), 4.42-4.48 (m, 1 H, H-4′, α-anomer), 4.50-4.63 (m, 2 H, H-5′, α-anomer), 4.66-4.80 (m, 2 H, H-5′, β-anomer), 5.83 (m, 1 H, H-3′, β-anomer), 5.95 (m, 1 H, H-3′, α-anomer), 6.37 (dd, 1 H, H-1′, *J* = 4 Hz, β-anomer), 6.62 (t, 1 H, H-1′, *J* = 8 Hz, α-anomer), 7.22 (d, 4 H, H's of toluoyl group, *J* = 8 Hz), 7.25 (d, 4 H, H's of toluoyl group, *J* = 8 Hz), 7.50 (d, 1, H-6, $J_{6,5\text{-}CH_3}$ = 2 Hz), 7.92 (d, 1 H, H-6, $J_{6,5\text{-}CH_3}$ = 2 Hz), 7.80-7.96 (m, 8 H, H's of toluoyl group), 8.45 br d, 2 H, NH).

1-(2-Deoxy-4-thio-α-L-*threo*-pentofuranosyl)thymine (α-10) and 1-(2-Deoxy-4-thio-β-L-*threo*-pentofuranosyl)thymine (β-10). A solution of **9** (300 mg, 0.6 mmol) in anhydrous MeOH (50 mL) was stirred at room temperature with a freshly prepared solution of sodium methoxide (65 mg, 0.60 mmol) in MeOH (10 mL). A TLC aliquot (3 h, $CHCl_3$-MeOH, 95:5) showed complete consumption of starting material. The solution was rendered neutral with Dowex 50W x 8 (H^+) ion-exchange resin, and the resin was filtered off with MeOH washing. The filtrates were combined and evaporated to dryness, and methyl p-toluate was removed at 50 °C/0.01 torr. Preparative TLC ($CHCl_3$-MeOH, 9:1) of the crude mixture gave **α-10** (61 mg) and **β-10** (62 mg, total yield 79%). Compound **α-10**: mp 165-167 °C (EtOH), TLC $CHCl_3$-MeOH (9:1), R_f 0.45, MS *z/e* 259 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 1.75 (d, 3 H, 5-CH_3, $J_{5\text{-}CH_3,6}$ = 1.2 Hz), 2.10-2.25 (m, 2 H, H-2′), 3.48 (m, 1 H, H-5′), 3.76 (m, 1 H, H-5′), 3.98 (m, 1 H, H-4′), 4.42 (br, 1 H, H-3′), 4.80 (t, 1 H, 5′-OH, $J_{5'\text{-}OH,5''}$ = 5 Hz), 5.09 (d, 1 H, 3′-OH, $J_{3'\text{-}OH,3'}$ = 4 Hz), 6.37 (dd, 1 H, H-1′, $J_{1',2'}$ = 1.5 Hz, $J_{1',2''}$ = 4 Hz), 7.82 (d, 1 H, H-6, *J* = 1.5 Hz), 11.28 (s, 1 H, NH). *Anal.* Calcd for $C_{10}H_{14}N_2O_4S \cdot 0.5MeOH$: C, 45.98; H, 5.83; N, 10.21. Found: C, 45.64; H, 5.89; N, 10.07.

Compound **β-10**: mp 208-209 °C (EtOH); MS *z/e* 259 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 1.70 (d, 1 H, 5-CH_3, $J_{5\text{-}CH_3,6}$ = 1.2 Hz), 2.70 (m, 1 H, H-2′), 2.38 (m, 1 H, H-2′), 3.70 (m, 1 H, H-4′), 3.64 (m, 1 H, H-5′), 3.82 (m, 1 H, H-5′), 4.18 (m, 1 H,

H-3′), 4.86 (t, 1 H, 5′-OH), 5.38 (d, 1 H, 3′-OH, $J_{3'\text{-OH},3'}$ = 4 Hz), 6.14 (dd, 1 H, H-1′, $J_{1',2'}$ = 4 and $J_{1',2''}$ = 6 Hz), 8.20 (s, 1, H-6), 11.24 (s, 1 H, NH). *Anal.* Calcd for $C_{10}H_{14}H_2O_4S$: C, 46.50; H, 5.42; N, 10.85. Found: C, 46.21; H, 5.63; N, 10.42.

ACKNOWLEDGEMENT

This investigation was supported by the National Cancer Institute, National Institutes of Health, DHHS, Grant P01 CA34200. We are indebted to Drs. W. C. Coburn, J. M. Riordan, Mr. M. Kirk, Mrs. Christine Richards and Mrs. Rita Gray, for spectral and analytical data and to Drs. L. L. Bennett, Jr., W. B. Parker, and Mrs. Doris Adamson for cytotoxicity evaluations.

REFERENCES

(1) Secrist III, J. A.; Riggs, R. M.; Tiwari, K. N.; Montgomery, J. A. *J. Med. Chem.* **1992**, *35*, 533-538.

(2) Secrist III, J. A.; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A. *J. Med. Chem.* **1991**, *34*, 2361-2366.

(3) Miura, G. A.; Gordon, R. K.; Montgomery, J.A.; Chiang, P. K. *Purine and Pyrimidine Metabolism in Man V, Part B*, Nyhan, W. L.; Thompson, L. F.; Watts, R. W. E. (Eds.), Plenum Publishing Corp., **1986**, 667-672.

(4) Fu, Y.-L.; Bobek, M. *J. Org. Chem.* **1976**, *41*, 3831-3834.

(5) Huang, B.; Hui, Y. *Nucleosides, Nucleotides* **1993**, *12*, 139-147.

(6) 4′-Thiothymidine has been studied in detail by 500-MHz ^{1}H NMR spectroscopy and X-ray crystallography.[7]

(7) Koole, L. H.; Plavec, J.; Liu, H.; Vincent, B. R.; Dyson, M. R.; Coe, P. L.; Walker, R. T.; Hardy, G. W.; Rahim, S. G.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **1992**, *114*, 9936-9943.

(8) Dyson, M. R.; Coe, P. L.; Walker, R. T. *Carbohydr. Res.* **1991**, *216*, 237-248.

(9) Garegg, P. J. *Pure Appl. Chem.* **1984**, *56*, 845-858.

(10) Bennett, Jr., L. L.; Shannon, W. M.; Allan, P. W.; Arnett, G. *Ann. N. Y. Acad. Sci.* **1975**, *255*, 342-357.

(11) Bennett, Jr., L. L.; Hill, D. L. *Mol. Pharmacol.* **1975**, *11*, 803-808.

Received 4/15/93
Accepted 4/28/93