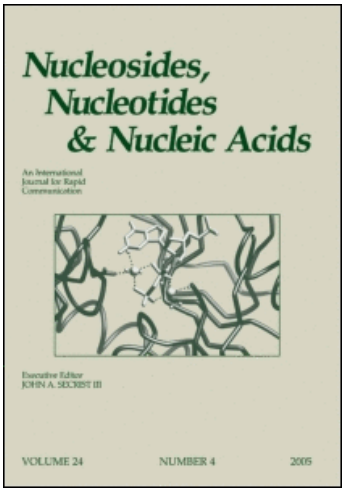


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biological Activity of Modified Thiopyrimidine Nucleosides

Adel M. Attia[ab]; Morsala A. Sallam[a]; Ahmed A. Almehdi[c]; Mohammed M. Abbasi[c]
[a] Department of Chemistry, Faculty of Education, Tanta University, Kafr, El-Sheikh, Egypt [b] Department of Chemistry, Faculty of Science, Qatar University, Doha, State of Qatar [c] Department of Chemistry, Faculty of Science, UAE University, Al-Ain, United Arab Emirates

To cite this Article Attia, Adel M. , Sallam, Morsala A. , Almehdi, Ahmed A. and Abbasi, Mohammed M.(1999) 'Synthesis and Biological Activity of Modified Thiopyrimidine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 18: 10, 2307 — 2315
To link to this Article: DOI: 10.1080/07328319908044883
URL: http://dx.doi.org/10.1080/07328319908044883

NUCLEOSIDES & NUCLEOTIDES, 18(10), 2307–2315 (1999)

SYNTHESIS AND BIOLOGICAL ACTIVITY OF MODIFIED THIOPYRIMIDINE NUCLEOSIDES

Adel M. Attia*[+] and Morsala A. Sallam
Department of Chemistry, Faculty of Education, Tanta University,
Kafr El- Sheikh, Egypt.

Ahmed A. Almehdi and Mohammed M. Abbasi*
Department of Chemistry, Faculty of Science, UAE University,
Al-Ain, United Arab Emirates.

Abstract: N^3-β-D-glucopyranosyl, galactopyranosyl and xylopyranosyl 6-methyl-2-methylthiouracil and their 5-bromo derivatives have been synthesized by coupling an α-acetobromosugar with the corresponding thiouracil. The new modified thiouridine analogues were evaluated for their inhibitory activity against Human Immunodeficiency Virus (HIV) replication in MT-4 cells as well as for their cytotoxicity.

Introduction

The 3`-azido-3`-deoxypyrimidine AZT is currently a licensed drug for the treatment of AIDS patient[1] and can be considered as an attractive basis for the design of new pyrimidine nucleosides as effective chemotherapeutic agents.[2,3] Pyrimidine nucleosides and their halogenated derivatives such as 5-iodo-2`-deoxyuridine and (E)-5-(2-halogenovinyl)-2`deoxyuridine have been in clinical against Herpes Simplex Virus Type I (HSV-1) and vericellazoster virus.[4] Structure activity relationships of 5-substituted-2`-deoxyuridine analogues have been studied[5,6] and many nucleoside analogues have shown broad spectrum antiviral activity.[7] In this

[+] Present Address: Department of Chemistry, Faculty of Science, Qatar University, P.O. Box 2713, Doha, State of Qatar.

study nucleoside analogues of 6-methyl-2-methylthiouracil and its 5-bromo derivative have been prepared and their effects on the replication of retro viruses and/or tumor cells were studied.

Chemistry

6-Methyl-2-thiouracil[8] **1a** was prepared by condensation of thiourea and ethyl acetoacetate in the presence of sodium ethoxide followed by methylation with methyl iodide in alcoholic sodium ethoxide. Bromination of **1a** with bromine in glacial acetic acid gave 5-bromo-6-methyl-2-methylthiouracil[9] **1b**. Compounds **1** reacted with tetra-*O*-acetyl-α-D-gluco- and galacto-pyranosyl bromides or tri-*O*-acetyl-α-D-xylopyranosyl bromide **2** in the presence of aqueous potassium carbonate in dimethylformamide at room temperature to give the corresponding 2-methylthiouracil nucleosides **3** (Scheme 1). The structures of **3** were confirmed by using elemental analyses and spectral data. The ^{1}H NMR spectrum of **3a** showed a doublet at δ 6.2 (*J* 8.2 Hz) assigned to the anomeric proton of the glucose moiety with a diaxial orientation with H-2` indicating the β-configuration and $^4C_1$(D) conformation. The other protons of the glucopyranose ring resonate at δ 3.9-5.3, while the four acetoxy groups appear as four singlets in the δ 1.9-2.2 region, and the two methyl groups of the aglycone at δ 2.4 and 2.6, in addition to the H-5 singlet at δ 6.4. Its ^{13}C NMR spectrum was characterized by a signal at δ 93.2 corresponding to the C-1` of the β-D-glucopyranose. The four signals appearing at δ 169.2-169.9 are due to four acetoxy carbonyl carbon atoms, while the four signals at δ 20.1-20.4 are attributed to the acetate methyl carbons . The methyl groups of the aglycone appear at δ 13.8 and 23.7. Another five signals at δ 61.7, 68.2, 70.4, 72.5 and 74.8 were assigned to C-6`, C-4`, C-2`, C-3` and C-5` respectively. The protected nucleosides **3** were deblocked by treatment with methanolic ammonia to give the free nucleosides **4** after chromatographic purification. TLC showed that single compounds were produced, and their structures were further confirmed by elemental analyses and spectral data. The ^{1}H NMR spectrum of **4c** showed the anomeric proton as a doublet at δ 5.7 (*J* 8.0 Hz), indicating the β-configuration of **4c**. The other six galactose

protons appeared as a multiplet at δ 3.3-3.8, while the four hydroxy groups resonated at δ 4.5-5.2 (exchangeable by D_2O). The ^{13}C NMR spectrum of **4c** was characterized by a signal at δ 96.9 corresponding to the C-1` atom of β-D-galactopyranose. Another five signals at δ 60.1, 67.8, 69.6, 73.2 and 75.9 were assigned to C-6`, C-4`, C-2`, C-3` and C-5` of the galactose moiety, respectively.

Biological Evaluation

As a part of our program directed towards the development of new pyridine and pyrimidine nucleosides with possible potential activity.[10-14] Compounds **3a-f** and **4a-d** were devoid of any activity against Human Immunodeficiency Virus (HIV) in MT-4 cells. At a concentration of $2x10^{-4}$ M galactosides analogues seem to be the best relative for this type of nucleosides. The substitution of bromine at 5-position did not change the activity at all, also the compounds did not show any significant anti-tumor activity *in vivo* in tumor implanted nude mice according to the NCI, NIH program.

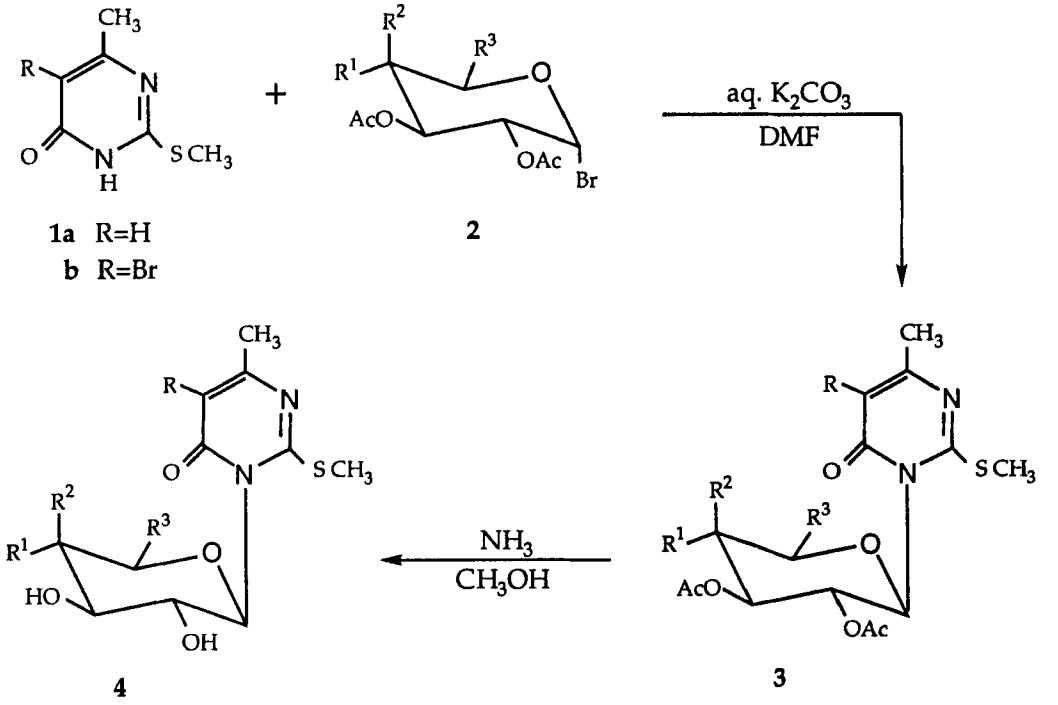
Experimental Section

All evaporations were carried out under reduced pressure at 40 °C. Melting points were uncorrected. TLC was carried out on aluminum sheet silica gel 60 F_{254} (Merck) detected by short UV light. IR Spectra were obtained (KBr) using Pye Unicam spectrometer 1000. ^1H NMR and ^{13}C NMR Spectra were measured on a Varian Gemini 200 MHz spectrometer in $CDCl_3$ or $(CD_3)_2SO$-d_6 using $SiMe_4$ as internal standard. Analytical data were obtained from the Microanalytical Center at Cairo University.

Compounds **1a** and **1b** were prepared following the literature procedures.[8,9]

***N*^{3}-(Per-*O*-acetyl-β-D-glycopyranosyl)-6-methyl-5-substituted-2-methylthio-uracil 3. General coupling procedure.**

To a solution of 5-substituted-6-methyl-2-methylthiouracil **1** (0.01 mol) in aqueous K_2CO_3 [0.01 mole in 6 mL of distilled water] and 15 mL dimethylformamide, a solution of per-*O*-acetyl-α-D-gluco- , galacto- or xylo-pyranosyl bromide **2** (0.011



1a R=H
b R=Br

2

3

4

	R	R^1	R^2	R^3
3 a	H	OAc	H	CH_2OAc
b	Br	OAc	H	CH_2OAc
c	H	H	OAc	CH_2OAc
d	Br	H	OAc	CH_2OAc
e	H	OAc	H	H
f	Br	OAc	H	H
4 a	H	OH	H	CH_2OH
b	Br	OH	H	CH_2OH
c	H	H	OH	CH_2OH
d	Br	H	OH	CH_2OH

Scheme 1

mol) in 30 mL of acetone was added. The reaction mixture was stirred at room temperature until the reaction was complete by TLC (18-24 h), using chloroform: petroleum ether 9:1, *v/v* (Rf 0.68-0.72 region), then evaporated under reduced pressure and the residue was washed with distilled water to remove the potassium bromide formed. The product was dried prior to crystallization from ethanol to afford pale yellow crystals.

3a: Yield 45%, mp152 °C; IR 1748(CO ester), 1620(CO pyrimidine) cm^{-1}; ^1H NMR δ 1.9-2.2(4s,12H,4CH_3CO), 2.4(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.9(m,2H, 2H-6`), 4.3(m,2H,H-5`and H-4`), 5.4(m,2H,H-3`and H-2`), 6.2(d,$J_{1'-2'}$ = 8.2 Hz,1H,H-1`), 6.4(s,1H, pyrimidine H-5); ^{13}C NMR δ 13.8(SCH_3), 20.1-20.4 (4CH_3), 23.7(CH_3), 61.7(C6`), 68.2(C4`), 70.4(C2`), 72.5(C3`), 74.8 (C5`), 93.2(C1`), 102.3(C6), 167.1(C5), 168.8(C4), 169.2-170.3(4CO ester), 171.2(C2); m/z 486 (Found: C,49.2; H,5.3; N,5.9. $C_{20}H_{26}N_2SO_{10}$ requires C, 49.4; H, 5.3; N, 5.8%).

3b: Yield 48%, mp 175 °C; IR 1744(CO ester), 1646(CO pyrimidine) cm^{-1}; ^1H NMR δ 1.8-2.1(4s,12H,4CH_3CO), 2.4(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.9(m,2H, 2H-6`), 4.4(m,2H,H-5`and H-4`), 5.3(m,2H,H-3`and H-2`), 6.0(d,$J_{1'-2'}$ = 8.0 Hz,1H,H-1`); ^{13}C NMR δ 14.2(SCH_3), 20.4-20.5(4CH_3), 24.2(CH_3), 61.8(C6`), 68.1(C4`), 70.0(C2`), 72.5(C3`), 72.8(C5`), 94.3(C1`), 101.1(C6), 162.9(C5), 167.9(C4), 168.8-170.0(4CO ester), 170.4(C2); m/z 565 (Found:C,42.3;H,4.1;N,4.7 $C_{20}H_{25}N_2BrSO_{10}$ requires C,42.5; H,4.4; N,4.9%).

3c: Yield 46%, mp 128 °C; IR 1756(CO ester), 1636(CO pyrimidine) cm^{-1}; ^1H NMR δ 1.9-2.2(4s,12H,4CH_3CO), 2.4(s,3H,CH_3), 2.6(s,3H,SCH_3), 4.1(m,3H,2H-6` and H-5`), 5.2(m,1H,H-4`), 5.5(m,2H,H-3` and H-2`), 6.1(d,$J_{1'-2'}$= 8.1 Hz,1H,H-1`), 6.4(s,1H, pyrimidine H-5); m/z 486 (Found: C, 49.7; H,5.0; N,5.8. $C_{20}H_{26}N_2SO_{10}$ requires C, 49.4; H, 5.3; N, 5.8%).

3d: Yield 44%, mp 150 °C; IR 1764(CO ester), 1645(CO pyrimidine) cm^{-1}; ^1H NMR δ 1.9-2.2(4s,12H,4CH_3CO), 2.4(s,3H,CH_3), 2.6(s,3H,SCH_3), 4.1(m,3H,2H-6`and H-5`), 5.2(dd,1H,H-4`), 5.4(d,1H,H-3`), 5.5(t,1H,H-2`),

5.9(d,$J_{1'-2'}$= 8.3 Hz,1H,H-1'); ^{13}C NMR δ 13.9(SCH_3), 20.3-20.5($4CH_3$), 23.7(CH_3), 61.2(C6'), 66.9(C4'), 67.9(C2'), 70.8(C3'), 71.5(C5'), 93.6(C1'), 102.3(C6), 167.1(C5), 169.1(C4), 169.2-170.1(4CO ester), 171.3(C2); m/z 565 (Found: C,42.6;H,4.6; N,4.7 $C_{20}H_{25}N_2BrSO_{10}$ requires C,42.5; H,4.4; N,4.9%).

3e: Yield 23%, mp 132 °C; IR1725(CO ester), 1640(CO pyrimidine) cm^{-1}; ^{1}H NMR δ 1.8-2.1(3s,9H,$3CH_3CO$), 2.3(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.7-4.1(m,2H,H-5'), 5.0(m,2H,H-4'and H-2'), 5.4(t,1H,H-3'), 6.3(d,$J_{1'-2'}$ = 8.1 Hz,1H,H-1'), 6.6(s,1H, pyrimidine H-5); ^{13}C NMR δ 13.7(SCH_3), 20.5-20.7($3CH_3$), 23.5(CH_3), 61.7(C5'), 68.5(C4'), 69.8(C2'), 70.9(C3'), 93.2(C1'), 102.3(C6), 167.3(C5), 168.7(C4), 169.3-169.7(3CO ester), 170.8(C2); m/z 414 (Found: C,49.1; H,5.1; N,6.5. $C_{17}H_{22}N_2SO_8$ requires C,49.3; H,5.3; N,6.8%).

3f: Yield 25%, mp 128 °C; IR 1746(CO ester), 1610(CO pyrimidine) cm^{-1}; ^{1}H NMR δ 1.9-2.1(3s,9H,$3CH_3CO$), 2.4(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.7(dd,2H, H-5'), 4.1(d,1H,H-4'), 5.2(t,1H,H-2'), 5.5(t,1H,H-3'), 6.4(d,$J_{1'-2'}$ = 7.7 Hz,1H,H-1'); m/z 493 (Found: C,41.2; H,4.0; N,5.3. $C_{17}H_{21}N_2BrSO_8$ requires C, 41.4; H, 4.2; N, 5.7%).

N^3-(β-D-Glycopyranosyl)-5-substituted-6-methyl-2-methylthiouracil 4.

General procedure for nucleoside deacylation.

Dry gaseous ammonia was passed through a solution of protected nucleoside **3** (0.5 g) in dry methanol (20 mL) at 0 °C for 0.5 h. The reaction mixture was stirred till completion (20-24h) as shown by TLC using $CHCl_3$: MeOH 9:1, *v:v*, (RF 0.60-64 region). The resulting mixture was then concentrated under reduced pressure to afford a solid residue that was crystallized from methanol to furnish colorless crystals.

4a: Yield 64%, mp 180 °C; IR 3454(OH), 1600(CO pyrimidine) cm^{-1}; ^{1}H NMR δ 2.3(s,3H,CH_3), 2.4(s,3H,SCH_3), 3.1-3.8(m,6H,2H-6',H-5',H-4',H-3'and H-2'), 4.6(t,1H,2'-OH), 5.1(d,1H,3'-OH), 5.2(d,1H,4'-OH), 5.4(d,1H,6'-OH), 5.8(d, $J_{1'-2'}$ = 7.8 Hz,1H,H-1'), 6.6(s,1H, pyrimidine H-5); ^{13}C NMR δ 13.3 (SCH_3),

23.2(CH_3), 60.4(C6`), 69.5(C4`), 72.5(C2`), 76.6(C3`), 77.6 (C5`), 96.3(C1`), 101.8(C6), 168.0(C5), 168.8(C4), 170.2(C2); m/z 318 (Found: C,45.2; H,5.5; N,8.9. $C_{12}H_{18}N_2SO_6$ requires C,45.3; H,5.7; N,8.8%).

4b: Yield 66%, mp 235 °C; IR 3644(OH), 1615(CO pyrimidine) cm^{-1}; ^1H NMR δ 2.4(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.1-3.7(m,6H,2H-6`,H-5`,H-4`,H-3`and H-2`), 4.6(t,1H,2`-OH), 5.0(d,1H,3`-OH), 5.2(d,1H,4`-OH), 5.4(d,1H,6`-OH), 5.9(d, $J_{1`-2`}$ = 7.9 Hz,1H,H-1`); ^{13}C NMR δ 13.7(SCH_3), 23.9(CH_3), 60.4(C6`), 69.4(C4`), 72.4(C2`), 76.7(C3`), 77.9(C5`), 96.9(C1`), 100.6(C6), 163.3(C5), 167.0(C4), 168.3(C2); m/z 397 (Found: C,36.1; H,4.6; N,7.1. $C_{12}H_{17}N_2BrSO_6$ requires C,36.3; H,4.3; N,7.0%).

4c: Yield 65%, mp 205 °C; IR 3456(OH), 1600(CO pyrimidine)cm^{-1}; ^1H NMR δ 2.3(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.2-3.8(m,6H,2H-6`,H-5`,H-4`,H-3`and H-2`), 4.4(d,1H,2`-OH), 4.6(t,1H,3`-OH), 4.8(d,1H,4`-OH), 5.1(d,1H,6`-OH), 5.7(d, $J_{1`-2`}$ = 8.0 Hz,1H,H-1`), 6.6(s,1H, pyrimidine H-5); ^{13}C NMR δ 13.3 (SCH_3), 23.2(CH_3), 60.1(C6`), 67.8(C4`), 69.6(C2`), 73.2(C3`), 75.9 (C5`), 96.9(C1`), 101.8(C6), 168.0(C5), 168.6(C4), 170.2(C2); m/z 318 (Found: C,45.6; H,5.6; N,8.8 $C_{12}H_{18}N_2SO_6$ requires C,45.3; H,5.7; N,8.8%).

4d: Yield 66%, mp 235 °C; IR 3390(OH), 1618(CO pyrimidine) cm^{-1}; ^1H NMR δ 2.3(s,3H,CH_3), 2.6(s,3H,SCH_3), 3.2-3.8(m,6H,2H-6`,H-5`,H-4`,H-3`and H-2`), 4.7(m,2H,2`-OH and 3`-OH), 4.9(d,1H,4`-OH), 5.2(d,1H,6`-OH), 5.8 (d,$J_{1`-2`}$ = 8.0 Hz,1H,H-1`); ^{13}C NMR δ 14.1(SCH_3), 24.3(CH_3), 60.6 (C6`), 68.4(C4`), 70.0(C2`), 73.7(C3`), 76.7(C5`), 97.9(C1`), 101.1(C6), 163.7(C5), 167.3(C4), 168.6(C2); m/z 397 (Found: C,36.1; H,4.6; N,7.2. $C_{12}H_{17}N_2BrSO_6$ requires C, 36.3; H, 4.3; N, 7.0%).

Acknowledgment

The authors are greatly indebted to Dr. John P. Bader, Dr. V. L. Narayanan and Dr. M. R. Boyd, AIDS Department & Drug Synthesis and Chemistry Branch of Health

and Human Services, NIH for their valuable *in vitro* anti-HIV and antitumor activities for our samples.

REFERENCES

1. Yarchoan, R; Weinhold, K. J; Lyerly, H. K; Gelmann E; Blum, R. M; Shearer, G. M; Mitsuya, H; Collins, J. M; Myer, C. E; Kelecker, R.W; Martcham, P. D; Duract, D. T; Nusinoff, L. S; Barry, D. W; Fischl, M. A; Gallo, R. C; Bolognesi, D. P; Broder S. *Lancet*, **1986**, *i*, 575.

2. Gallo, R. C; Sarin, P. S; Gelmann, E. P; Robert,G. M; Richardson, E; Kalyanaraman, V. S; Mann, D; Sidhu, G. D; Stahl, R. E; Zalla, P. S; Leibowitch, J; Provic, M. *Science (Washington, D. C.)* **1983**, *220*, 865.

3. De Clercq, E. *J. Med. Chem.*, **1986**, *29*, 1561.

4. De Clercq, E. *Pure Appl. Chem.*, **1986**, *55*, 623.

5. Goodchild, J; Poter, R. A; Roger R. H; Sim, I. S; Upton, R. M; Roger, M; Viney, J; Wadsworth, H. *J. Med. Chem.*, **1983**, *26*, 1252.

6. Wigerinck, P; Snoect, R; Claes, P; De Clercq, E; Herdewijn, P. A. *J. Med. Chem.*, **1991**, *34*, 2383.

7. Herdewijn, P. A. *Antiviral Res.*, **1992**, *19*, 1.

8. Carcia Novio, J. L; Lorentz, A; Soto, J. L. *Heterocycles*, **1982**, *19*, 305.

9. Hussain, S.M; El-Reedy, A. M; Rizk, A. M; Side El-Dien, K. A. *J. Heterocyl. Chem.*, **1987**, *24*, 1605.

10. Attia, A. M; Ibrahim, E. I; Hay, F. E; Abbasi, M. M; Mansour, H. A. *Nucleosides Nucleotides*, **1995**, *14*, 1581.

11. Attia, A. M; Ibrahim, E. I; Hay, F. E; Abbasi, M. M; Mansour, H. A. *Org. Prep. and Proc. Int.*, **1995**, *27*, 613.

12. Abdou, I. M; Strekowski, L; Attia, A. M. *Heterocycl. Commun.*, **1997**, *3*, 295.

13. Attia, A. M; Elgemeie, G. H; Shahada, L. A. *Tetrahedron*, **1997**, *53*, 17441.

14. Attia, A. M; Elgemeie, G. H; Alnaimi, I. S. *Nucleosides Nucleotides*, **1998**, *17*, 1355.

Received 10/22/98
Accepted 8/12/99