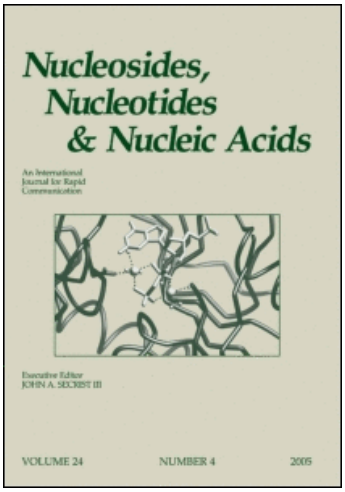


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A Facile Synthesis of 4'-Thio-2'-deoxypyrimidine Nucleosides and Preliminary Studies on Their Properties

Baoguo Huang[a]; Yongzheng Hui[a]
[a] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China

To cite this Article Huang, Baoguo and Hui, Yongzheng(1993) 'A Facile Synthesis of 4'-Thio-2'-deoxypyrimidine Nucleosides and Preliminary Studies on Their Properties', Nucleosides, Nucleotides and Nucleic Acids, 12: 2, 139 — 147
To link to this Article: DOI: 10.1080/07328319308021200
URL: http://dx.doi.org/10.1080/07328319308021200

NUCLEOSIDES & NUCLEOTIDES, 12(2), 139-147 (1993)

A FACILE SYNTHESIS OF 4'-THIO-2'-DEOXYPYRIMIDINE NUCLEOSIDES AND PRELIMINARY STUDIES ON THEIR PROPERTIES

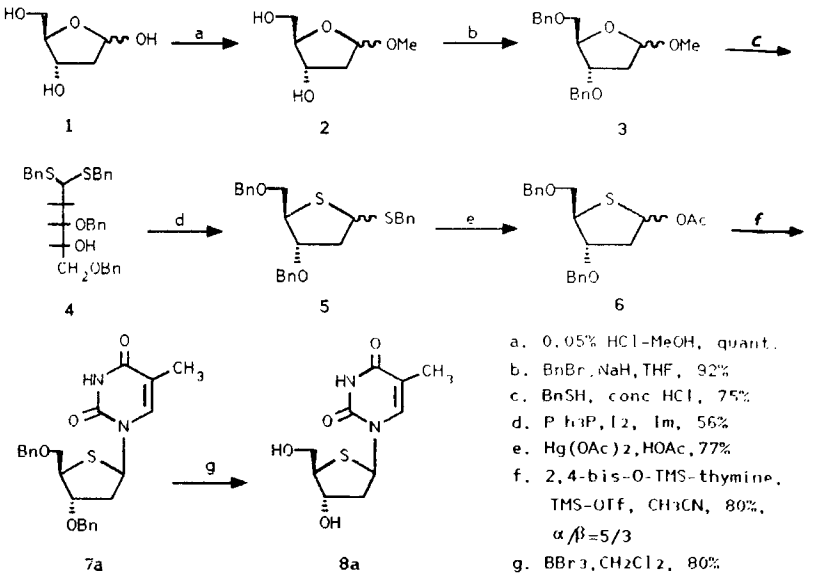
Baoguo Huang Yongzheng Hui*
Shanghai Institute of Organic Chemistry,
Chinese Academy of Sciences, Shanghai 200032, China

Abstract: Several 4'-thio-2'-deoxypyrimidine nucleosides were synthesized from 2-deoxy-D-ribose via 7 steps. The 4'-thio-nucleosides were obtained by reaction of the corresponding trimethylsilylated pyrimidine bases with 4-thiosugar(**6**), which has been synthesized in one step by treatment of 3,5-di-O-benzyl-2-deoxy-D-ribose dibenzyldithioacetal(**4**) with the Ph_3P-I_2-Im system. The chemical stability of 4'-thio-2'-deoxythymidine is also described. 5-Fluoro-2'-deoxyuridine (5-FDUR), 5-iodo-2'-deoxyuridine(5-IDUR) and their 4'-thio-analogs **8b**, **8c** were evaluated for antitumor activity. 5-Fluoro-4'-thio-2'-deoxyuridine(**8b**) was more inhibitory than 5-FDUR, especially at a concentration of 1ug/ml.

Introduction

4'-Thionucleosides, in which the furanose ring oxygen is replaced by sulfur, have been found to be of biological interest, e.g, as antiviral agents.[1]

Several derivatives of 4-thio-2-deoxyribose have been synthesized in low yield due to tediously long synthetic procedures.[2,3] Thus, there is a need for improved syntheses of some of the previously synthesized compounds as well as syntheses of some derivatives that have not yet been prepared. Recently, Walker and his co-workers



Scheme 1

described a method based on the Mitsunobu reaction for the synthesis of 4-thio-2-deoxyribose in 26% overall yield.[4] In this paper we report on a substantially improved synthesis of 4'-thio-2'-deoxypyrimidine nucleosides, illustrating a novel use of the Ph_3P-I_2-Im reagent system, which has been generally used for converting hydroxy into deoxyiodo compounds.[5] Preliminary results about their biological activity and stability are also presented.

Results and Discussion

To execute the synthesis of 4'-thio-2'-deoxynucleosides, 2-deoxy-D-ribose was chosen as the starting material. The synthetic route is illustrated in Scheme 1. A key intermediate in this synthesis was dibenzyldithioacetal(**4**). The synthesis of **4** began with 2-deoxy-D-ribose, which was changed into methyl 3,5-di-O-benzyl-2-deoxy-D-ribofuranoside (**3**) by methylation and benzylation. Treatment of **3** with benzylmercaptan and concentrated hydrochloric acid afforded **4**. Compound **4**, in which the free 4-hydroxyl group is available for

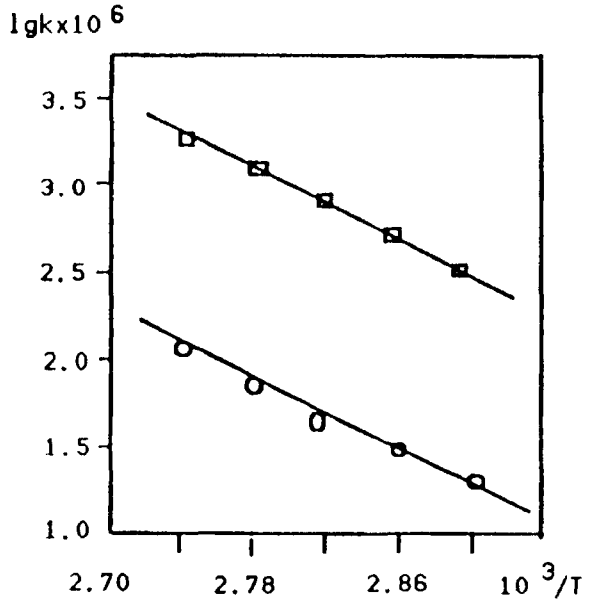


Figure 1. kinetics of acid hydrolysis of thymidine(○) and 4'-thiothymidine (□) [HCl]=1.0 N, [S]=1.0x10^{-5} M

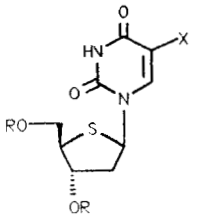
inversion of configuration, was then transformed into the protected 4-thio-2-deoxyriboside (**5**) via intramolecular condensation. Iodide (I^-), which is a good nucleophilic agent, replaced the 4-hydroxyl group of **4** with inversion of configuration, then acted as a good leaving group in the ring closure reaction. Those two reactions were performed in a single step by using the Ph_3P-I_2-Im system.

Thus, the 4-thio-2-deoxyribose derivative suitable for synthesis of nucleoside became readily available in large quantity in an overall yield of 39% from 2-deoxy-D-ribose.

For the convenience of synthesis of the corresponding nucleosides, **5** was converted to 1-O-acetyl-3,5-di-O-benzyl-4-thio-2-deoxyriboside (**6**), which enabled us to prepare nucleosides by the TMS-triflate procedure.[6] The ratio of β/α was equal to 3/5 when CH_3CN was used as the solvent. The β-anomer was identified by 2D NMR spectra and easily

separated from the anomeric mixture by silica gel chromatography. Boron tribromide was used to remove the benzyl groups from **7** to afford the target nucleoside **8a**.[7]

The glycosidic bond of 4'-thiothymidine is less stable than that of thymidine under acidic conditions. Figure 1 indicates that the hydrolysis rate for 4'-thiothymidine is 15-fold faster than thymidine, which may be explained as follows: the impulsion between the n-orbital of sulfur and the σ^*-orbital of the C-N bond is stronger than that of oxygen.



	7b	7c	8b	8c
R	Bn	Bn	H	H
X	F	I	F	I

Compound **6** was reacted with trimethylsilylated 5-fluorouracil or 5-iodouracil to give the corresponding derivatives of 4'-thio-2'-deoxynucleosides (**8b,c**). Because 5-fluoro-2'-deoxyuridine (5-FDUR) and 5-iodo-2'-deoxyuridine (5-IDUR) are anticancer or antiviral drugs, the study of the bioactivities of their 4'-thioanalogs is of interest. Preliminary results of inhibitory activity against L-1210 Leukemia cells are listed in Table 1. The two 4'-thio-2'-deoxynucleosides appear to be comparably cytotoxic to the corresponding normal nucleoside.

Experimental Section

General Procedure: ^{1}H NMR spectra were recorded with a Varian XL-200 (200 MHz) or Bruker AMX-600 (600 MHz) spectrometer relative to an internal tetramethylsilane reference. FAB mass spectra were obtained on a VG Quattro MS/MS spectrometer from samples dissolved in a suitable solvent with 3-nitrobenzyl

Table 1. Activity vs L-1210 Leukemia cell#

compound	inhibition yield(%)
5-FDUR	47.8, 70.8, 70
8b	72.3, 74.3, 68.4
5-IDUR	25.2, 38.9, 71.2
8c	19.0, 38.9, 71.2

The mixture of the test compounds and L-1210 cell were incubated for 24 h, then the living cell numbers were counted and the percentage inhibition was calculated. Efficacy was expressed as inhibition yield. Cell concentration = $1x10^5$ cell/well, concentration of test compound was 1, 10, 100 ug/ml, respectively.

alcohol or thioglycerol as matrix. Precoated, plastic-backed silica gel TLC plates (silica gel F_{254},0.2-mm thickness) were supplied by E. Merck, Darmstadt. Detection was achieved under UV light(254nm) or by spraying with 10% H_2SO_4 in methanol and heating. Column chromatography was performed on silica gel H, 200-300 mesh, from Qing Dao, China.

Methyl 2-deoxy-D-riboside (2):

2-Deoxy-D-ribose(0.5g, 37.3 mmol) was dissolved in methanolic hydrogen chloride(0.05%,17.5 mL). The solution was stirred at room temperature for 15 min. Silver carbonate was then added until the reaction mixture became neutral. The suspension was vigorously stirred for 15 min,passed through charcoal and the solvent was removed under reduced pressure to afford **2** as a colorless syrup, yield 0.53 g (96%).

$[\alpha]_D^{20}$=33.0°(c 0.2, $CHCl_3$), ^1H NMR(CDCl3):2.02-2.2(m,2H,H-2), 3.45 (s, 3H, OCH3), 3.65 (d, 2H, H-5), 4.02-4.1(m,2H,H-3,4),

5.10(t,1H,H-1), 4.20(s,1H,OH), 4.45(s,1H,OH),
FAB-MS(m/z): 150(M^++1), 297($2M^+$+1)

Methyl 3,5-di-O-benzyl-2-deoxy-D-riboside(3):

To an ice-cooled stirred solution of **2** (0.53 g) in 20 mL freshly distilled THF was added 0.35 g sodium hydride (80% dispersion in oil) in several portions under N_2, and the mixture was stirred for 15 min. After addition of 14.5 mg of tetrabutylammonium iodide, 1 mL of benzyl bromide was then added dropwise. When all the benzyl bromide was introduced, the ice bath was removed, the reaction mixture was allowed to warm to 25 °C and stirred for 2 h. The excess of benzyl bromide was consumed up by addition of methanol and the clean solution was evaporated under reduced pressure. The product **3** was obtained in 92% yield by flash chromatography.
$[\alpha]_D^{20}$ = 15.00°(c 1, $CHCl_3$), ^{1}H NMR($CDCl_3$): 2.10(m,2H,H-2), 3.08(m,1H,H-4), 3.30(s,3H,OCH_3),3.5(m,2H,H-5),4.4(m,1H,H-3), 4.50(m,4H,CH_2Ph), 5.1(t,1H,H-1), 7.3(m,10H,Ar)
FAB-MS(m/z): 328(M^+), 297(M^+-OCH_3)

3,5-Di-O-benzyl-2-deoxy-D-ribose dibenzyldithioacetal(4):

Compound **3** (1.07 g) was added to a mixture of concentrated hydrochloric acid (1.51 mL) and benzylmercaptan (1.39 mL).The mixture was stirred at room temperature overnight. After addition of water (20 mL) and chloroform (20 mL), the organic layer was washed suscessively with 0.1N $KHCO_3$ solution (3x 20mL) and water (3x20 mL) and then dried over anhydrous Na_2SO_4. The solvent was evaporated and the pure product was obtained in 75% yield by flash chromatography.
$[\alpha]_D^{20}$=-17.8°(c 1.98,$CHCl_3$),^{1}H NMR($CDCl_3$):2.05-2.15(m,2H,H-2), 2.37(s,1H,OH), 3.45(m,2H,H-5), 3.73-3.9(m,8H,CH_2Ph), 4.15(m,1H,H-4),4.38(m,1H,H-3),4.50(t,1H,H-1), 7.2-7.38(m,20H,Ar), FAB-MS(m/z): 653(M^++matrix), 421(M^+-BnS)

Benzyl 3,5-di-O-benzyl-1,4-dithio-2-deoxy-D-riboside (5):

Triphenylphosphine (850 mg), iodine (680 mg) and Im(290 mg) were added to a solution of **4**(588 mg) in toluene-acetonitrile (2:1, 10mL) and the mixture was kept at 90°C for 24 h. The solvent was evaporated, and the product was purified by flash chromatography to yield **5**, 226 mg(56%).

$[\alpha]_D^{20}$ =-34.9°(c 0.8, $CHCl_3$), ^1H NMR($CDCl_3$):2.0-2.3(m,2H,H-2), 3.50(d,2H,H-5),3.66(m,1H,H-4),3.80(m,2H,SCH_2Ph),4.22(m,1H,H-3) 4.50(m,4H,OCH_2Ph), 4.54(t,1H,H-1), 7.3(m,15H,Ar)
EI-MS(m/z): 436(M^+), 313(M^+-BnS), Anal. Calc for $C_{26}H_{28}O_2S_2$
C,71.56; H,6.42; S,14.68; Found: C,71.51; H,6.48; S,14.68

1-O-Acetyl-3,5-di-O-benzyl-4-thio-2-deoxy-D-ribose (6):
Compound 5(81.2 mg,0.186 mmol) and mercuric acetate (150 mg) were dissolved in a mixture of acetic anhydride (0.6 mL) and acetic acid (0.6 mL). The solution was kept at 45-50°C for 30 min, then water (15 mL) and chloroform (15 mL) were added. The organic layer was dried, evaporated and the residue was purified by preparative TLC to give 53 mg of the product(77%)
H NMR($CDCl_3$): 1.9(s,3H,$COCH_3$),2.3(m,2H,H-2),4.5(m,4H,CH_2Ph) 3.5-3.8(m,4H,H-3,4,5), 6.0(t,1H,H-1), 7.25(m,10H,Ar)
EI-MS(m/z): 373(M^++1), 313(M^+-OAc), Anal. Calc for $C_{21}H_{24}O_4S$
C, 67.71; H,6.51; S,8.61; Found: C,67.60; H,7.05; S,8.19

3',5'-Di-O-benzyl-4'-thio-2'-deoxythymidine (7a):
A mixture of thymine (126 mg), hexamethyldisilazane(5 mL) and ammonium sulfate(50 mg) was incubated at 120°C for 5 h. After removal of the solvent under reduced pressure, the silylated product was dissolved in absolute acetonitrile(5 mL), and 6 (240 mg) was added. To the mixture , trimethylsilyl triflate (0.5 mL) was added at -30°C. After 5 h the reaction mixture was warmed to 0°C, and solid sodium hydrogen carbonate was added until the mixture was neutral. The inorganic salt was removed by filtration, and the filtrate was evaporated and purified by column chromatography to give 225 mg of the product(80%) as a mixture of α- and β-anomer. Further separation by preparative TLC plate with $CHCl_3$:MeOH = 98:2 as elute gave α-anomer 140mg, β-anomer 85mg. The anomer configuration was determined by 2D ^1H NMR spectra as follows: for β-anomer 4'-thiothymidine, H-6 and H-3' showed mutal nOe's. In contrast, α-anomer of nucleoside nOe's was only observed between H-1' and H-3'.
β-anomer: ^1H NMR($CDCl_3$): 2.1(s,3H,CH_3),2.4(m,2H,H-2'), 3.6(m,2H,H-3',4'), 3.8(s,2H,H-5'),4.62(m,4H,OCH_2Ph), 6.4(dd,1H,H-1'),7.4(m,10H,Ar), 8.05(s,1H,H-6),

FAB-MS(m/z): 461(M^++Na), 439(M^++1). Anal. Calc for $C_{24}H_{26}N_2O_4S$: C, 65.75; H, 5.94; N, 63.93; S, 7.31; Found: C, 65.50; H, 6.10; N, 63.62; S, 7.05

α-anomer: ^{1}H NMR($CDCl_3$): 2.10(s,3H,CH_3), 2.30(m,2H,H-2′), 3.64(m,2H,H-3′,4′), 4.60(m,4H,OCH_2Ph), 3.78(m,2H,H-5′),6.25(t,1H,H-1′),7.38(m,10H,Ar),8.10(s,1H,H-6)

3′,5′-Di-O-benzyl-4′-thio-5-halouridine were synthesized and the anomer were identified in the same way as above.

3′,5′-Di-O-benzyl-4′-thio-2′-deoxy-5-fluorouridine(7b): 1H NMR($CDCl_3$): 2.20-2.40(m,2H,H-2′), 3.70(m,3H,H-4′,5′), 4.25(m,1H,H-3′),4.56(d,4H,CH_2Ph),6.44(t,1H,H-1′), 7.36(m,10H,Ar), 8.25(d,1H,H-6), 10.0(s,1H,N-H)

EI-MS(m/z): 443(M^++1), 533(M^++Bn)

3′,5′-Di-O-benzyl-4′-thio-2′-deoxy-5-iodouridine(7c): H NMR($CDCl_3$): 2.1-2.3(m,2H,H-2′),3.2-3.4(m,2H,H-5′), 4.10(m,1H,H-4′),4.3(d,1H,H-3′),4.4-4.6(m,4H,CH_2Ph), 6.25(t,1H,H-1′), EI-MS(m/z): 551(M^++1), 641(M^++Bn)

4′-Thio-2′-deoxythymidine (8a):

To a dry ice-cooled (-40°C), stirred solution of dibenzyl 4′-thiothymidine(83 mg) in dry dichloromethane, boron tribromide(0.2 mL) was added. After stirring for 1 h, the mixture was warmed to 0°C and sodium hydrogen carbonate was added, stirring was continued for 2h. The mixture was filtrated, and the filtrate was evaporated and purified to give 39 mg of the product(80%). ^{1}H NMR(D_2O):1.80(s,3H,CH_3),2.25(m,1H,H-2′), 2.65(m,1H,H-2′′), 3.63(q,1H,H-5′′), 3.75(m,2H,H-4′,5′), 4.45(q,1H,H-3′), 6.2(q,1H,H-1′), 8.15(s,1H,H-6), FAB-MS(m/z):259(M^++1),241(M^+-OH), Anal. Calc for $C_{10}H_{14}N_2O_4S$: C, 46.50; H, 5.42; N, 10.85; S, 12.4; Found: C, 46.22; H, 5.38; N, 10.80; S, 11.9

4′-Thio-2′-deoxy-5-fluorouridine(8b): yield,78%,^{1}H NMR(D_2O): 2.10-2.50(m,2H,H-2′), 3.70(q,1H,H-4′), 3.80(m,2H,H-5′), 4.55(q,1H,H-3′), 6.40(t,1H,H-1′),8.30(d,1H,H-6), 10.1(s,1H,N-H), FAB-MS(m/z): 263(M^++1), 245(M^+-OH)

Anal.Calc for $C_9H_{11}FN_2O_4S$: C,41.22; H,4.20; F,7.25; S,12.21; Found: C, 41.08; H, 4.30, F, 7.02; S, 11.80

4'-Thio-2'-deoxy-5-iodouridine (8c): yield, 82%,
^{1}H NMR(D_2O): 2.20-2.35(m,2H,H-2'), 3.60(m,2H,H-5'),
3.70(d,1H,H-4'), 4.10(m,1H,H-3'), 6.38(t,1H,H-1'),
8.55(s,1H,H-6),9.20(s,1H,N-H),FAB-MS(m/z): 371(M^++1),243(M^+-I)
Anal.Calc for $C_9H_{11}IN_2O_4S$: C,29.19; H,2.97; I,34.32; S,8.65;
Found: C, 29.01; H, 2.85; I, 34.02; S, 8.35

Kinetic measurement:

Thymidine(242 mg,1 mmol) or 4'-thiothymidine (258 mg, 1 mmol) was weighed into a 100ml volumetric flask and made up to volume with nitrogen-purged distilled water. From this stock solution, 40 uL was diluted with 4 mL 1.1M hydrochloric acid solution so that the concentration of the nucleoside was $1x10^{-4}$ M. The solutions were maintained in constant-temperature baths at 70, 75, 80, 85 and 90°C. The concentration of the nucleoside remained at pertinent interval time can be deduced from the peak area on HPLC. The results were shown in Figure 1.

Acknowledgement: We are grateful to Shanghai Institute of Materia Medica, Chinese Academy of Sciences for providing biological testing data.

References

1. a. D.J.Hoffman, R.L.Whistler, Biochemistry, 1970, 9, 2367
   b. N.Ototani, and R.L.Whistler, J.Med.Chem.,1974, 17, 535
2. Y.-L.Fu, and M.Bobek, J.Org.Chem., 1976, 41, 3831
3. Y.-L.Fu, and M.Bobek, Nucleic Acid Chemistry, eds. L.B.Townsend, and R.S.Tipson, Wiley, New York, 1978, p317
4. M.R.Dyson, P.L.Coe, and R.T.Walker, J.Chem.Soc.Chem. Commun., 1991, 741
5. P.J.Garegg, Pure Appl.Chem., 1984, 56, 845
6. T.Ogawa, K.Beppu,and S.Nakabayashi, Carbohydr.Res., 1981, C6-C9, 93
7. T.Mukaiyama,K.Suzuki,T.Yamada, and F.Tabusa, Tetrahedron, 1990, 46(1), 265,

Received 5/29/92
Accepted 11/5/92