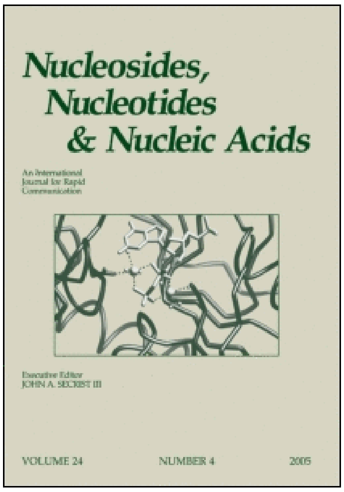

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Synthesis of 9-(2-Deoxy-β-D-ribofuranosyl)purine-2-thione

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NOTE

SYNTHESIS OF 9-(2-DEOXY–β-D-RIBOFURANOSYL)PURINE-2-THIONE

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Abstract: A synthesis of 9-(2-deoxy-β-D-ribofuranosyl)purine-2-thione was performed by desulfurization of 2'-deoxy-6-thioguanine to give 2-amino-9-(2-deoxy-β-D-ribofuranosyl)purine, diazotization with chloride replacement to give 2-chloro-9-(2-deoxy-β-D-ribofuranosyl)purine, and the replacement of chloride with sulfur using thiolacetic acid and deacetylation.

Several new complementary base pairs have been investigated for their replication and fidelity properties with DNA polymerases[1,2]. The goals are the modification of the genetic code and a new set of interactions between proteins and nucleic acid. A candidate base pair is 2-thiopurine/4-thiopyrimidine. A synthesis of the 2-thiopurine deoxynucleoside is presented in this communication, since a literature search did not reveal any previous report of a synthesis of the deoxynucleoside.

Figure 1 summarizes the synthesis of the title compound **6**. The plan of synthesis was to convert the 2-aminopurine deoxynucleoside **2** to the 2-chloropurine deoxynucleoside **4** by diazotization with chloride replacement and the replacement of chloride with sulfur. The 2-aminopurine deoxynucleoside **2** has been prepared by several methods[4,5,6,7]. Because of the simplicity of synthesizing 6-thioguanine deoxynucleoside[3], desulfuration using Raney Nickel was employed[5]. Protection of the 3' and 5' hydroxyl groups with the isobutyryl group was chosen because of solubility requirements in dichloromethane at -5 °C. The protected 2-aminopurine deoxynucleoside **3** was chlorinated using the nonaqueous diazotization method of Robins and Uznański[8]. A mixture of products was obtained, and after flash chromatography, the 2-chloropurine deoxynucleoside **4** was identified by the UV spectrum of the base[9]. Thiourea, hydrogen sulfide, and thiolacetic acid were investigated for the replacement of the chloride with sulfur. Only thiolacetic acid produced a significant amount of the 2-thiopurine deoxynucleoside. Most of the product was not the 2-thiopurine derivative. Increasing the concentration of thiolacetic acid above 0.1 mM rapidly produced depurination. Elementary analysis of the major product of the reaction of **4** with thiolacetic acid indicated it was the protected 2-(acetylthio)purine deoxynucleoside **5**. This suggested that debutyrylation and deacetylation could be carried out together. The crude mixture containing **5** was

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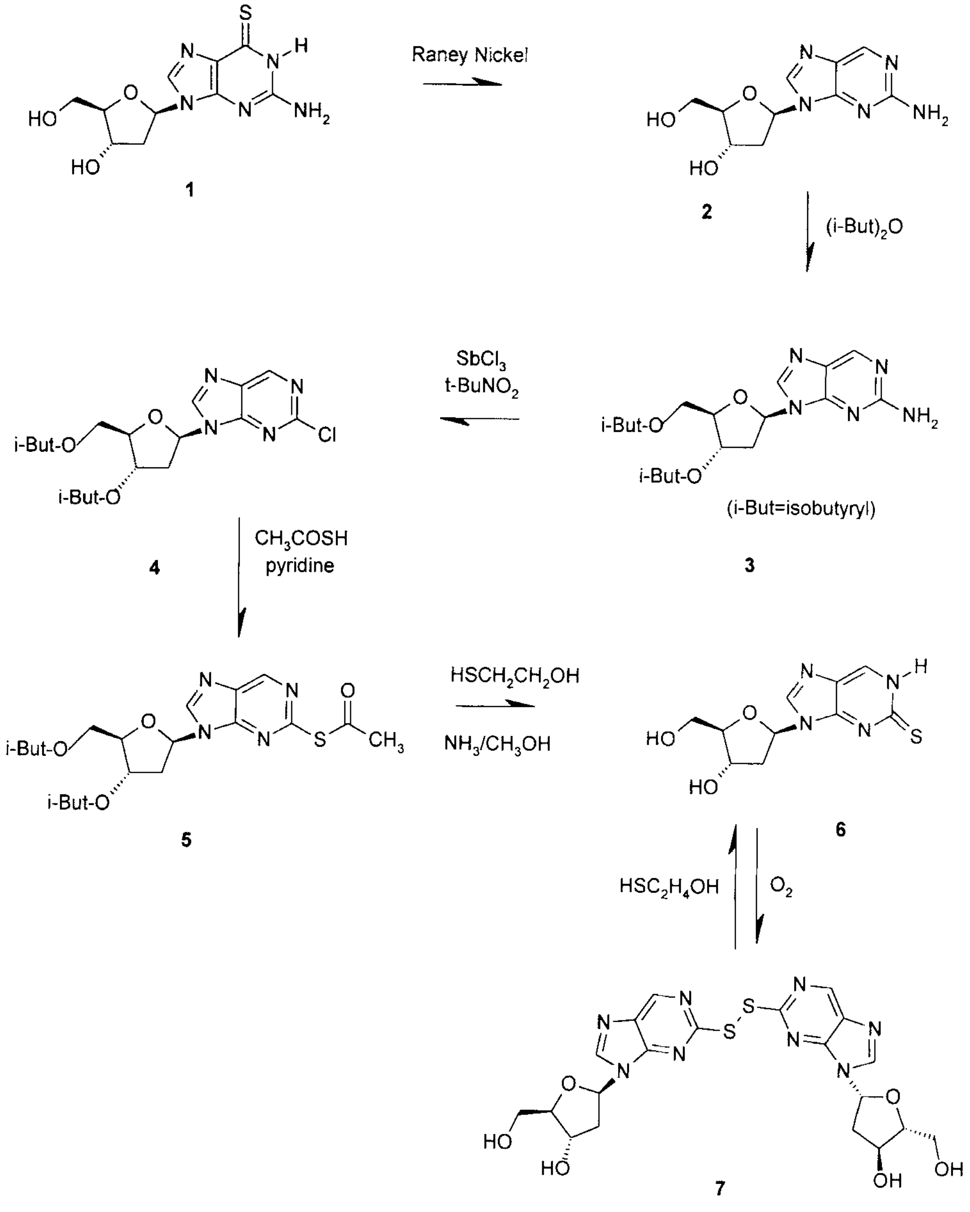
treated with methanol saturated with ammonia, and 2-mercaptoethanol was included to inhibit oxidative formation of disulfide **7**. The major product was isolated by HPLC or PLC . The NMR spectrum, UV spectrum[9], and TLC analysis of the base isolated from the major product by acid depurination were the same as authentic 2-thiopurine Mass spectral analysis of **6** by MADLI-TOF gave a major peak at m/z 269 $(M+H)^+$ and a very minor peak at 271, the corresponding ^{34}S isotope peak. If **6** is allowed to stand in methanol or water containing oxygen a new compound appears on TLC. This compound can be converted to **6** by the addition of mercaptoethanol. The results suggest the new compound is a dimer of **6**, structure **7**. The ^1H NMR spectrum of the 2-thiopurine deoxynucleoside showed a broad peak at 13.1 ppm. The broad spread and position indicate an aromatic amine hydrogen. If the sulfhydryl derivative was the predominant species no peak would have been observed since the quadrapole of sulfur would have eliminated the detection of a resonance. A published ^1H NMR spectrum of the 2-methylthio derivative has no peak above 9 pmm [10].

Experimental: All anhydrous solvents and reagents were obtained from Aldrich Chemical Co. 2-Thiopurine was obtained from Sigma-Aldrich and HPLC indicated a purity greater than 95%. TLC plates were obtained from Analtech. ^1H NMR spectra were obtained with a GE Omega 500 and were measured in $(CD_3)_2SO$ using $SiMe_4$ as the reference. UV data are reported in the format λ_{max} in nanometers ($\epsilon \times 10^{-3}$)

2-Amino-9-(2-deoxy-β-D-ribofuranosyl)purine (2): 2'-Deoxy-6-thioguanosine (1.6g, 5.65 mmol) was added to a 500 ml flask with stirrer. Water (120 ml) and ethanol (120 ml) were added and the mixture was brought to reflux with stirring. Raney Nickel (6g) was added and refluxing continued until the ratio of the optical density at 245nm to 335nm exceeded 3.5. More Raney Nickel was added if the ratio was not reached in one hour. The mixture was filtered hot. A yield of 77% ,1.3 g, of crude 2-amino- 9-(2-deoxy-β-D-ribofuranosyl)purine was recovered after evaporation. U.V: pH 7.0 236, 305 [9].

2-Amino-9-(2-deoxy-3,5-diisobutyryl-β-D-ribofuranosyl)purine (3): The 2-amino nucleoside (4.8 mmol) was added to a solution of pyridine (19 ml) and isobutyric anhydride (50 mmol). After completion of the reaction, about two hours as monitored by TLC, methanol (23 ml) was added and the solution allowed to remain at room temperature overnight. The solution was poured with stirring into 5% sodium bicarbonate (300 ml). The aqueous solution was extracted with chloroform (200, 100, 100 ml). The chloroform solution was dried with sodium sulfate and evaporated. The residue was dissolved in chloroform and flash chromatography performed with 5% methanol in chloroform. The protected 2-aminopurine deoxynucleoside was identified by its UV spectrum[9]. UV: pH 7.0 236, 306. The yield was 80% and the purity was greater than 90% by HPLC.



1
Raney Nickel
2
(i-But)$_2$O
3
(i-But=isobutyryl)
SbCl$_3$
t-BuNO$_2$
4
CH$_3$COSH
pyridine
5
HSCH$_2$CH$_2$OH
NH$_3$/CH$_3$OH
6
HSC$_2$H$_4$OH
O$_2$
7

FIGURE 1

2-Chloro-9-(2-deoxy-3,5-diisobutyryl-β-D-ribofuranosyl)purine (**4**): The protected 2'-deoxyribosyl- 2-aminopurine (0.5 mmol) was added to a jacketed vessel with stirrer. The compound was dried by repeated evaporation of anhydrous pyridine with dry nitrogen. Antimony trichloride (3.5 mmol) was added under nitrogen and the temperature lowered to -5 °C. Anhydrous dichloromethane (20 ml) was added and the mixture stirred for 15 minutes. *tert*-Butyl nitrite (4.6 mmol) was added and stirring continued at -5 °C. The extent of the reaction was followed by TLC, 5% methanol/95% chloroform. The product has an R_f=0.87. After about four hours the reaction was complete and the solution was poured into 6% sodium bicarbonate (200 ml) at 4 °C. After reaching room temperature, the aqueous mixture was extracted three times with chloroform (100 ml). The solution was dried with sodium sulfate and evaporated. The residue was dissolved in chloroform and purified by flash chromatography using 3% methanol in chloroform. The product was identified by the spectrum of the base[9]. U.V: pH 5.0 207 (4.2), 273 (3.82). The yield of the protected 2-chloropurine deoxynucleoside was 35% and 16% of the protected 2-aminopurine deoxynucleoside was recovered.

2-Acetylthio-9-(2-deoxy-3,5-diisobutyryl-β-D-ribofuranosyl)purine (**5**): The protected nucleoside of 2-chloropurine (0.8 mmol) was dissolved in chloroform (80 ml) and washed with 5% sodium bicarbonate (80 ml), water (40 ml), dried with sodium sulfate and evaporated. The washed compound (0.7 mmol) was placed in a 50 ml vessel and dried by evaporation of anhydrous pyridine with dry nitrogen. Thiolacetic acid (2.2 mmol) in anhydrous pyridine (24 ml) was added. The solution was purged with dry nitrogen and under a nitrogen atmosphere placed at 75 °C for three days. The progress of the reaction was followed by TLC, 5% methanol/95% chloroform. The major product has an R_f = 0.37. After returning to room temperature the solution was poured into 5% sodium bicarbonate (240 ml) with stirring. The aqueous solution was extracted with chloroform (100 ml, 60 ml, 60 ml). The chloroform dried with sodium sulfate and evaporated. A portion of the crude material was purified by silica gel chromatography using a gradient of methanol in chloroform of 0 to 5%. U.V: (Methanol) 292 (4.2). *Anal.* Calcd for $C_{20}H_{26}N_4O_6S_1$: C, 53.32; H, 5.82; N, 12.44; S, 7.12. Found C, 53.5; H, 5.65; N, 12.1; S, 7.3.

9-(2-deoxy-β-D-ribofuranosyl)purine-2-thione (**6**): The crude material containing the protected 2-thiolacetylpurine nucleoside was dried under vacuum. The dried residue was dissolved in 8ml of anhydrous methanol, saturated with ammonia at 4 °C. 2-Mercaptoethanol (1.05 mmol) was added. The solution was at room temperature for 24 hours. The solution was cooled to 4 °C and tributylamine (1.7mmole) added. The solution was evaporated. The titled compound was purified either on PLC, 30% methanol, 5% aqueous ammonia, 65% chloroform, or HPLC using a C_{18} reverse column with a solution of .02 M ammonium acetate and a gradient of acetonitrile, 0-20%. The yield from the protected 2-chloropurine nucleoside was 40%. MS(MALDI-TOF) 269 $(M+H)^+$, 271 $(M+H, {}^{35}S)^+$. 1H-NMR (DMSO-d6) δ 2.49,2.24 (m, m, 1, 1, H2', H2"), 3.53 (m, 2, H5', H5"), 3.8 (m, 1, H4'), 4.97 (t, 1, 5' OH), 5.3 (d, 1, 3' OH), 6.0 (t, 1, H1'), 8.3 (s,1,H8), 8.51 (s,1,H6), 13.1 (br s,1, H1). U.V: pH 5.0 241(3.8), 285 (4.0), 345 (3.0)[10]. *Anal.* Calcd for $C_{10}H_{12}N_4O_3S_1$ H_2O ; C, 41.95;H, 4.89; N, 19.58; S, 11.18. Found: C, 41.65;H, 4.72; N, 19.21; S, 11.35.

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REFERENCES

1. Piccirilli, J.A., Krauch, T., Moroney, S.E., Benner, S.A. *Nature*, **1990 343,** 33-34
2. Rappaport, H.P. *Biochemistry,* **1993 32**, 3047-3922
3. Kung, P., Jones, R.A., *Tetrahedron Lett.,* **1991 32**, 3919-3922
4. Milne, G.H., Townsend, L.G. *J. Med. Chem.*, 1974 17, 263-268
5. Fox, J.J., Wempen, I., Hampton, A., Doerr, I.L. *J. Amer. Chem. Soc.*, **1958 80**, 1669-1675
6. McLaughlin, L.W., Leong, T., Benseler, F,. Piel, N. *Nuc. Acids Res.*, **1988 16**, 5631-5644
7. Fujimoto, J., Nuesca, Z., Mazurek, M., Sowers, L.C. *Nucl. Acids Res.*, **1996 24**, 754-759
8. Robins, M.J., Uznański, B. *Can. J. Chem.,* **1981 59**, 2608-2611
9. Mason, S.F. *J. Chem. Soc.*, **1954**, 2071-2085
10. Seela, F., Chen, Y., Bindif, U., Kazimierczuk, Z. *Helvetica Chimica Acta*, **1994 77**, 194-202