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One-Flask Syntheses of 6-Thioguanosine and 2'-Deoxy-6-Thioguanosine

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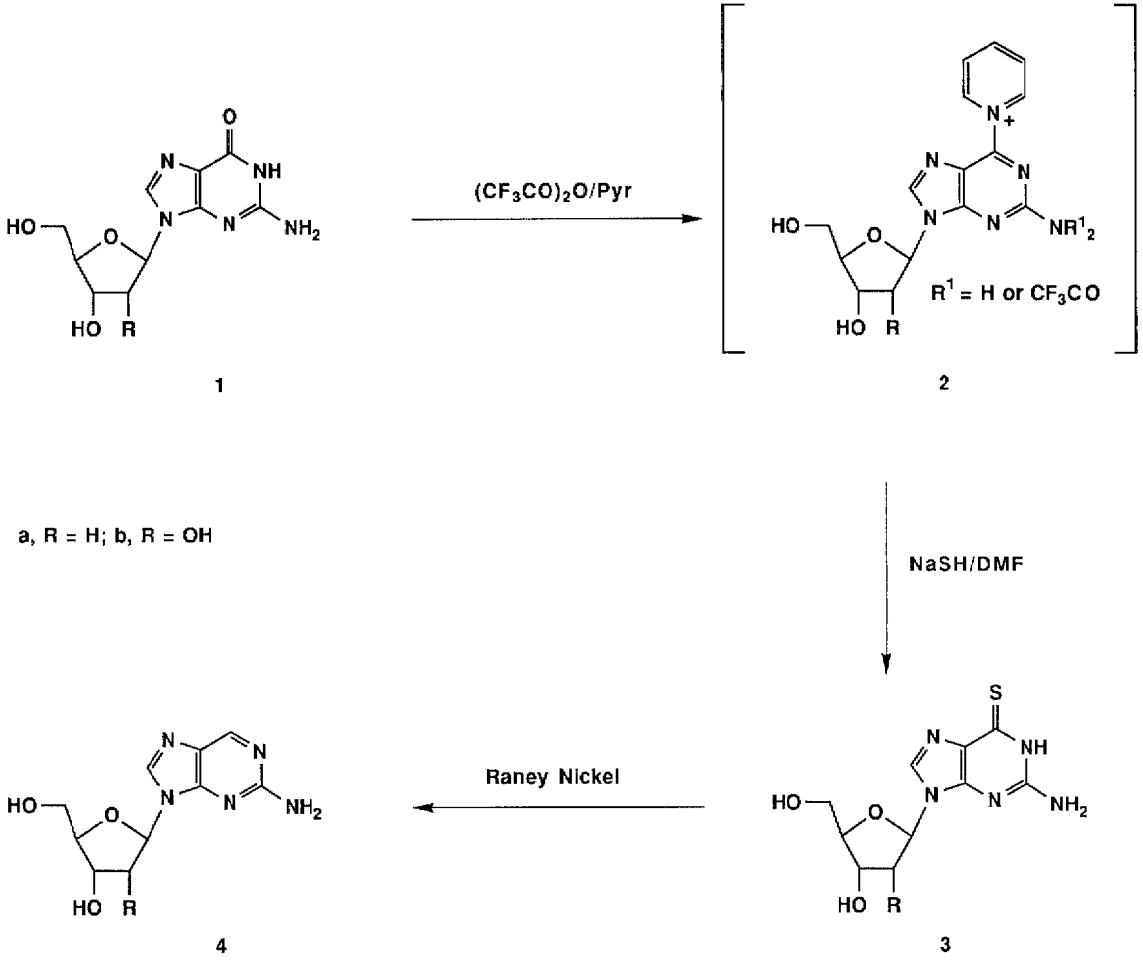
Abstract. We have previously reported that reaction of guanosine or 2'-deoxyguanosine with trifluoroacetic anhydride in pyridine gives a putative 6-pyridyl intermediate from which several 6-substituted derivatives may be obtained.[1] We now report high-yield conversion of guanosine and 2'-deoxyguanosine to the corresponding 6-thio compounds in a two-step, one-flask reaction via this 6-pyridyl intermediate. Standard Raney nickel treatment, as reported for the ribonucleoside,[2] then gives the 2-aminopurine nucleosides.

The significant antitumor activity of 2'-deoxy-6-thioguanosine (**3a**),[3,4] has led to the development of a number of synthetic routes to this and other 6-thiopurine nucleosides.[2,5-9] Each of these routes is multi-step and, with one exception,[7] employs a chemical coupling step for construction of the nucleoside. We now report conversion of guanosine or 2'-deoxyguanosine to the corresponding 6-thio derivative in a two-step, one-flask procedure that does not require use of protecting groups.

These syntheses make use of the facile conversion of guanine nucleosides to the corresponding 6-pyridyl derivatives (**2a** and **2b**) using trifluoroacetic anhydride in pyridine.[1] The 6-pyridyl group in **2** is susceptible to displacement by a variety of nucleophiles, although the yield is variable. Strongly basic nucleophiles such as alkoxide or ammonia generally give modest yields, while less basic nucleophiles such as pentafluorophenoxide have given high yields. We now report that NaSH can be used to effect high-yield conversion of **2** to **3**. The reaction is carried out simply by addition of a dimethylformamide suspension of NaSH to the reaction mixture containing **2**, and is complete in 24 h at room temperature. The 6-thio derivatives can be isolated by fractional crystallization from water, without chromatography, although the crystals obtained are rather brown.[10] Alternatively, either chromatography on silica gel using methanol as eluant, or reversed-phase chromatography, work well and give pale yellow material upon crystallization from water or water methanol mixtures. We have found that reversed-phase chromatography using acetonitrile/0.1 M triethylammonium acetate (TEAA) gradients is the most convenient procedure. In this way **3a** and **3b** were obtained in yields of 72 % (**3a**[11]) and 61 % (**3b**[12]).

This easy accessibility of **3a** allows it to be used in turn for conversion to the 2-aminopurine derivative **4a**. Desulfurization of **3a** using Raney nickel in water at 50°, as reported for **3b**,[2] gave **4a** in a yield of 73 %.[13] In addition to the antitumor activity of **3a**, both 6-thioguanine and 2-aminopurine nucleosides are of interest for incorporation into oligonucleotides.[14-18]


(CF3CO)2O/Pyr
R1 = H or CF3CO
NaSH/DMF
Raney Nickel
a, R = H; b, R = OH
1
2
3
4

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10. The reaction mixture contains a large quantity of salts, including the thio-acid derived from reaction of NaSH with trifluoroacetic anhydride. The latter is the major UV absorbing component of the mixture, and it is removed first upon fractional crystallization from water.
11 **2-Amino-6-mercapto-9-(2'-deoxy-β-D-*erythro*-pentofuranosyl)purine (3a).** To 0 53 g (2 mmol) of **1a**, dried three times by evaporation of pyridine, suspended in a 40 mL portion of dry pyridine and cooled in an ice bath under a nitrogen atmosphere, was added dropwise 2.3 mL (16 mmol) of trifluoroacetic anhydride. After 40 min, a suspension of 3 4 g (60 mmol) of NaSH in 60 mL of anhydrous DMF was added in portions. After a further 24 h, the reaction mixture was poured into 100 mL of 0.16 M ammonium bicarbonate, with vigorous stirring. The mixture was then concentrated to dryness and the residue triturated with methanol and filtered. The filtrate was again concentrated to dryness and the residue triturated with 0.1 M triethylammonium acetate (TEAA), filtered and the filtrate applied to a C18 Dynamax reversed-phase hplc column (41 4 mm x 25 cm). The product was eluted using a gradient of 2 to 13 % acetonitrile/0.1 M TEAA in 35 min at a flow rate of 10 mL/min. The product began to crystallize as it was eluted from the column. Further concentration of appropriate fractions and filtration, followed by recrystallization from methanol/water, gave 0.46 g (1.5 mmol, 72 %) of **3a**, mp 191-193°. UV (MeOH) λ_{max} 260, 345 nm; UV λ_{min} 240, 300 nm; ^{1}H NMR (DMSO-*d6*) δ 11.92 (br, 1), 8.1 (s, 1, H_8), 6 80 (br, 2, NH_2), 6.10 ("t", 1, J_{app}=6.83 Hz, $H_{1'}$), 5.29 (d, 1, J=3.52 Hz, 3'-OH), 4.95 (t, 1, J=5.02 Hz, 5'-OH), 4.33 (m, 1, $H_{3'}$), 3.80 (m, 1, $H_{4'}$), 3.53 (m, 2, $H_{5',5''}$), 2.49 & 2 23 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$). FAB MS (M+1) = 284. Anal. calcd. for $C_{10}H_{13}N_5O_3S$•3/4 H_2O: C, 40 46; H, 4.92, N, 23.60; S, 10 80. Found: C, 40.34; H, 4.60; N, 23.83; S, 10.51.
12 **2-Amino-6-mercapto-9-(β-D-ribofuranosyl)purine (3b).** To 0.57 g (2 mmol) of **1b**, dried three times by evaporation of pyridine, suspended in a 40 mL portion of dry pyridine and cooled in an ice bath under a nitrogen atmosphere, was added dropwise 3.4 mL (24 mmol) of trifluoroacetic anhydride. After 90 min, a suspension of 5.4 g (96 mmol) of NaSH in 50 mL of anhydrous DMF was added in portions. After a further 24 h, the reaction mixture was poured into a 100 mL portion of 0 24 M ammonium bicarbonate, with vigorous stirring The mixture was then concentrated to dryness and the residue triturated with methanol and filtered. The filtrate was again concentrated to dryness and the

residue triturated with 0.1 M triethylammonium acetate (TEAA), filtered and the filtrate applied to a C18 Dynamax reversed-phase hplc column (41.4 mm x 25 cm). The product was eluted using a gradient of 2 to 8 % acetonitrile/0.1 M TEAA in 35 min at a flow rate of 10 mL/min. The product began to crystallize as it was eluted from the column. Further concentration of appropriate fractions and filtration, followed by recrystallization from methanol/water, gave 0.34 g (1.2 mmol, 61 %) of **3b**, mp 214-216°. UV (MeOH) λ_{max} 260, 345 nm; UV λ_{min} 240, 300 nm; ^{1}H NMR (DMSO-d_6) δ 11.95 (br, 1), 8.13 (s, 1, H_8), 6.80 (br, 2, NH_2), 5.69 (d, 1, J=5.64 Hz, $H_{1'}$), 5.42 (d, 1, J=5.74 Hz, 2'-OH), 5.13 (d, 1, J=4.4 Hz, 3'-OH), 5.01 (t, 1, J=5.13 Hz, 5'-OH), 4.38 ("q", 1, J_{app}=5.4 Hz, $H_{2'}$), 4.08 ("q", 1, J_{app}=3.9 Hz, $H_{3'}$), 3.87 (m, 1, $H_{4'}$), 3.57 (m, 2, $H_{5',5''}$). FAB MS (M+1) = 300. Anal. calcd. for $C_{10}H_{13}N_5O_4S$•3/4 H_2O C, 38.39; H, 4.67; N, 22.39; S, 10.25 Found: C, 38.53; H, 4.49; N, 22.15; S, 10.10.

13. **2-Amino-9-(2'-deoxy-β-D-*erythro*-pentofuranosyl)purine (4a).** To 0.30 g of **3a** dissolved in a 50 mL portion of water was added 2 mL of a slurry of Raney nickel in water (50 %, Aldrich 22,167-8). The reaction was stirred at 50° for 13 h. TLC in CH_2Cl_2/MeOH (1/1) showed a single fluorescent spot (Rf 0.6). The reaction mixture was filtered, the filtrate concentrated to dryness and a solution of the residue in water was applied to a C18 Dynamax reversed-phase hplc column (21.4 x 25 cm). The product was eluted using a gradient of 0 to 10 % acetonitrile/water in 15 min, followed by a gradient of 10 to 17 % in 35 min, at a flow rate of 2.5 mL/min. Concentration of appropriate fractions gave 0.19 g (0.73 mmol, 73 %) of **4a**. Crystallization of a sample from methanol/ether gave mp 157-159°. UV (H_2O) λ_{max} 245, 305 nm; UV λ_{min} 233, 260 nm. ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1, H_6), 8.28 (s, 1, H_8), 6.54 (br, 2, NH_2), 6.26 ("t", 1, J_{app}=6.84 Hz, $H_{1'}$), 5.29 (d, 1, J=3.96 Hz, 3'-OH), 4.96 (t, 1, J=5.4 Hz, 5'-OH), 4.36 (m, 1, $H_{3'}$), 3.83 (m, 1, $H_{4'}$), 3.54 (m, 2, $H_{5',5''}$), 2.63 & 2.26 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$). FAB MS (M+1) = 252. Anal. calcd. for $C_{10}H_{13}N_5O_3$•3/4 H_2O: C, 45.36, H, 5.52; N, 26.45. Found: C, 45.53; H, 5.33, N, 26.33.

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