Preparation, Structural Characterization, and Acid-Catalyzed Isomerization of 3-, 7-, and 9-Benzyl-6-benzylthiopurines

Jim J. Huang*, Aris Ragouzeos, and Janet L. Rideout

Division of Organic Chemistry, Burroughs Wellcome Co.,
Research Triangle Park, NC 27709

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Benzylation of 6-benzylthiopurine was examined. Structural assignments of the products were determined by 1-D and 2-D nmr spectroscopy (HMQC, HMBC, and nOe). In the presence of base, the isomeric N3-, N7-, and N9-benzylated products **4**, **3**, and **2** were isolated; however, only 9-benzyl-6-benzylthiopurine (**2**) was obtained in the absence of base. In the latter case, the initially formed N3- and N7-isomers were, in the presence of acid, converted to 9-benzyl-6-benzylthiopurine (**2**) *via* a 6-benzylthiopurine intermediate as evidenced by analysis of the reaction over time using reversed-phase hplc.

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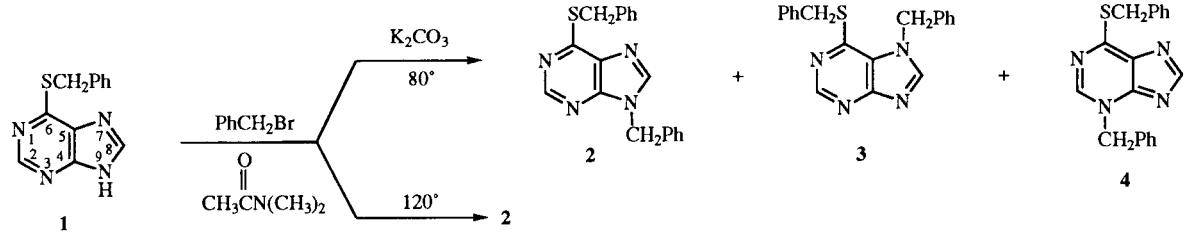
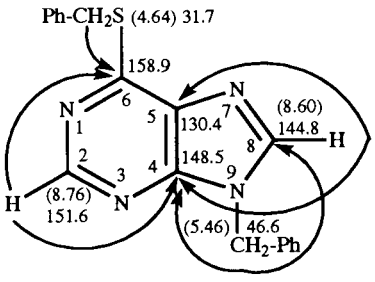
N-Alkylation reactions by alkyl halides are, in general, carried out in the presence of a base. Benzylation of 6-alkylthiopurines under basic conditions is reported to give a mixture of N9- and N7-benzyl derivatives [1-3]. In principle, the formation of additional N1- and N3-derivatives remains feasible. On this basis, the present work describes the reinvestigation of the benzylation of 6-benzylthiopurine and structural characterization of the reaction products by nmr spectroscopy.

In this study, 6-benzylthiopurine (**1**) [4] was prepared by reaction of 6-mercaptopurine with benzyl bromide in dimethylacetamide (DMAc) at room temperature. 6-Benzylthiopurine (**1**) was then treated with benzyl bromide in DMAc in the presence of anhydrous potassium carbonate at 80°. A ^{1}H nmr spectrum of the crude reaction mixture indicated the relative amounts of three isomeric products to be 9:3:2, as determined by integration of *N*- and *S*-benzyl methylene protons. Recrystallization of the crude reaction mixture gave the major product. The remaining two components were separated by column chromatography on silica gel (Scheme 1).

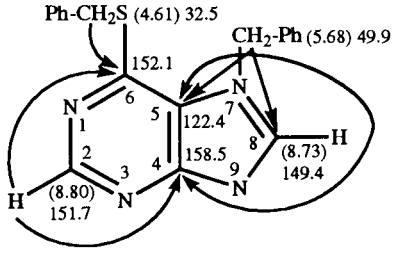
The structures of the three isolated isomers were determined by nmr spectroscopy. The assignment of the two methylene groups is based on the greater deshielding effect of a purine ring nitrogen relative to a sulfur atom. For the major benzylation product, **2**, the lower-field chemical shift at δ 5.46 (two proton singlet) was assigned to *N*-CH_2 protons and the upper-field shift at δ 4.64 (singlet, two protons) to *S*-CH_2 protons (Figure 1). The two one-proton singlets at δ 8.76 and δ 8.60 could not be assigned on the basis of chemical shift to H-2 and H-8 even though it has been reported that in purine itself the H-2 is more deshielded than the H-8 [5]. To this end, 2-D nmr techniques were used. By a proton-detected heteronuclear chemical shift correlation (HMQC) [6] experiment, the chemical shifts for the *N*-CH_2 and *S*-CH_2 carbons were obtained at δ 46.6 and 31.7, respectively. Further by a two-dimensional long-range heteronuclear multiple-bond shift correlation (HMBC) [7] experiment, the *S*-CH_2 protons resonating at δ 4.64 were three-bond correlated to the signal at δ 158.9, which must belong to C-6. The C-6 signal was also three-bond correlated to the proton resonating at δ 8.76, which then must be the H-2 proton. The H-2 proton was three-bond correlated to δ 148.5, which can now be assigned to C-4. The remaining singlet at δ 8.60 was assigned to H-8 and, by HMQC analysis, δ 144.8 was assigned to C-8. To determine the site of the *N*-CH_2Ph, the nuclear Overhauser effect (nOe) was studied. Irradiation of *N*-CH_2 protons gave 16% nOe to the H-8 proton resonating at δ 8.60, suggesting that this CH_2Ph group must be at either N7 or N9. By the analysis of the HMBC spectrum, the *N*-CH_2 protons were shown

Scheme 1

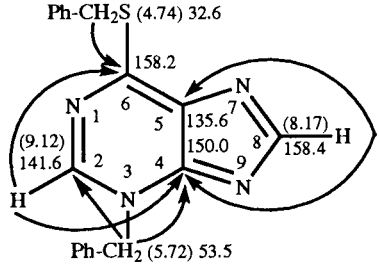




(**2**, 9-substituted)
Figure 1 [a]



(**3**, 7-substituted)
Figure 2 [a]



(**4**, 3-substituted)
Figure 3 [a]

[a] Chemical shifts for ^1H nmr (in parentheses) and ^{13}C nmr, both in DMSO-d_6, are expressed in δ (ppm), downfield from tetramethylsilane. The arrows represent three-bond correlation in HMBC experiments.

to be three-bond correlated to C-8 resonating at δ 144.8 and, most critically, to C-4 at δ 148.5. Thus, this CH_2Ph group was concluded to be at N9; that is, the major product of the benzylation was the N9-derivative **2** [4].

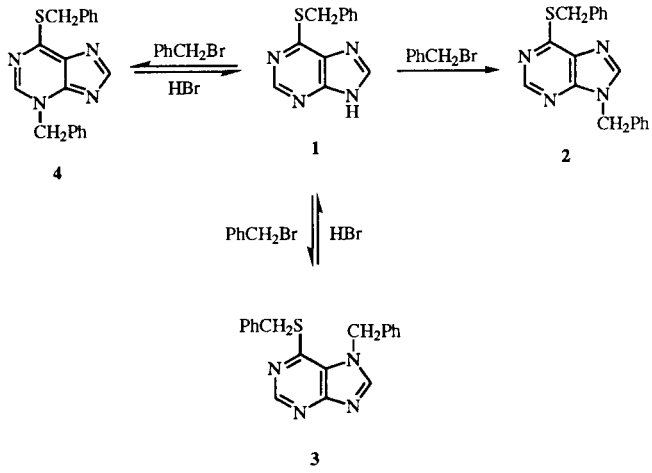
In an analogous manner, the early eluting minor product was identified as 7-benzylated isomer **3**: Irradiation of *N*-CH_2 resonating at δ 5.68 gave 21% nOe to the H-8 proton resonating at δ 8.73, suggesting the CH_2Ph group at either N7 or N9. An HMBC experiment indicated that the *N*-CH_2 protons were three-bond correlated to C-8 resonating at δ 149.4 and, most critically, to C-5 at δ 122.4 (Figure 2); thus this CH_2Ph group was established to be at N7 [4,8]. The second minor product was established to be the 3-benzylated isomer **4** as follows: Irradiation of *N*-CH_2 at δ 5.72 gave 24% nOe to the H-2 proton resonating at δ 9.12, suggesting the CH_2Ph group be at either N1 or N3. Based on HMBC analysis, the *N*-CH_2 protons were three-bond correlated to C-2 resonating at δ 141.6 and, most importantly, to C-4 at δ 150.0 (Figure 3); therefore this CH_2Ph group must be at N3 [1].

It is noteworthy that the N3-substituted isomer **4**, a "quinoid" purine, has significantly larger differences in chemical shifts between H-2 and H-8 ($\Delta\delta = 0.95$) than the "aromatic" purines, the N7- and N9-isomers, **3** and **2** ($\Delta\delta = 0.07$ and 0.16, respectively). This has also been reported in the cases of N7- and N9-methylated 6-dimethylaminopurines [9]. Furthermore, the methylene protons of the N3-benzyl group (on the pyrimidine moiety) resonate at lower field (δ 5.72) than those of the N7- and N9-benzyl groups on the imidazole ring of **3** and **2** at δ 5.68 and 5.46, respectively.

The isolation of the N9-, N7-, and N3-benzylated products from the base-catalyzed reaction in this study prompted the further investigation into the effect of base catalysis. Benzylation of 6-benzylthiopurine (**1**) in the absence of base was performed in DMAc at 120° for 24 hours. On reversed-phase hplc, the reaction mixture showed a single product. The product was isolated and characterized as **2**, 9-benzyl-6-benzylthiopurine. To study the mechanism, this reaction was repeated at room temperature. The reaction was largely incomplete after 24 hours; however, hplc analysis indicated the formation of N3-, N7-, and N9-benzylated products.

Close monitoring of the course of the reaction over the initial 4 hours and at 24 hours by hplc showed initially the N3-isomer **4** was the major product, whereas the N7- and N9-isomers were the minor products. These results suggest that the N3 atom is more susceptible toward alkylation [10]. Examination of the chromatographic data showed the amount of the N9-isomer **2** gradually increased while the amounts of the N7- and N3-isomers, **3**

Scheme 2



and **4** respectively, simultaneously decreased. In separate studies, all three isomers were individually found to be stable in DMAc or in DMAc/potassium carbonate at 110°; but in DMAc/hydrogen bromide, both N3- and N7-isomers underwent debenzylation to 6-benzylthiopurine (**1**), as detected by hplc, followed by a slow formation of the N9-isomer **2**. Under all conditions, the thermodynamically stable N9-isomer **2** did not revert to 6-benzylthiopurine (Scheme 2). These observations led to the conclusion that N3- and N7-benzylated derivatives **4** and **3** were initially formed and then subsequently debenzylated by hydrogen bromide catalysis. (The hydrogen bromide was generated during the reaction.) The phenomenon of the acid-catalyzed *N*-debenzylation of purines has been reported [11,12] and the migrating benzyl carbenium ion [12] is apparently stabilized by its delocalized charge.

In summary, the present study has established that the benzylation of 6-benzylthiopurine in the presence of base afforded the N3-, N7-, and N9-benzylated derivatives **4**, **3** and **2** with the major product being the N9-isomer. The formation of 1-benzyl-6-benzylthiopurine, while feasible, was not detected in this study; this is possibly due to the steric hindrance and the inductive effect of the 6-benzylthio substituent, which deactivates tlhe adjacent N1-position toward alkylation. In the absence of base, the reaction, when run to completion, gave exclusively the N9-isomer **2** although longer reaction time and higher reaction temperatures were required than when a base was present.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. The nmr spectra were acquired on a Varian Unity 400 with a Nalorac Cryogenic Corp Z•SPEC™ MD-400-3 3 mm probe at a sample concentration of 3 mg in 160 µl of DMSO-d_6; chemical shifts are reported in δ (ppm) downfield from tetramethylsilane. Spectral assignments were made by HMQC, HMBC, and nOe studies. The uv spectra were recorded with a Varian SuperScan 3 spectrophotometer. Column chromatography was performed on E. Merck silica gel (70-230 mesh). Reversed-phase hplc analysis was carried out on a PARTISIL-100 DS-2 column with a uv detector at 280 nm, a mobile phase methanol:water/8:2 v/v, and flow rate of 1.0 ml/min.

6-Benzylthiopurine (**1**).

A solution of 6-mercaptopurine (500 mg, 3.0 mmoles) and benzyl bromide (550 mg, 3.2 mmoles) in 10 ml of dimethylacetamide (DMAc) was stirred at room temperature overnight. After removal of the solvent, the oily residue was triturated with water to give 950 mg (85%) of the product, mp 168-171° (lit [4] mp 178-180°).

Benzylation of 6-Benzylthiopurine In the Presence of Base.

To a solution of 2.0 g (8.3 mmoles) of 6-benzylthiopurine (**1**) in 50 ml of DMAc was added 1.2 g (8.7 mmoles) of anhydrous potassium carbonate. After the mixture was heated at 85° for 0.5 hour, benzyl bromide (1.4 g, 8.2 mmoles) was added. The reaction was complete in 1 hour as determined by hplc. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with chloroform. The solution was dried over sodium carbonate, filtered, and evaporated to give 2.1 g of the benzylated mixture. Recrystallization from cyclohexane gave 0.9 g of 9-benzyl-6-benzylthiopurine (**2**). The cyclohexane filtrate was concentrated and chromatographed on silica gel eluted with chloroform followed by 5% methanol in chloroform to give 300 mg (total 43%) of 9-benzyl-6-benzylthiopurine (**2**), 150 mg (5%) of 7-benzyl-6-benzylthiopurine (**3**), and 40 mg (2%) of 3-benzyl-6-benzylthiopurine (**4**).

9-Benzyl-6-benzylthiopurine (**2**).

This compound had mp 101-103° (lit [4] mp 108°); ^{1}H nmr (DMSO-d_6): δ 4.64 (s, 2H, SCH_2), 5.46 (s, 2H, NCH_2), 7.22-7.45 (m, 10H, Ph), 8.60 (s, 1H, H-8), 8.76 (s, 1H, H-2); ^{13}C nmr (DMSO-d_6): δ 31.7 (SCH_2), 46.6 (NCH_2), 127.2 (Ph), 127.6 (Ph), 128.0 (Ph), 128.5 (Ph), 128.8 (Ph), 129.0 (Ph), 130.4 (C5), 136.5 (Ph), 137.9 (Ph), 144.8 (C8), 148.5 (C4), 151.6 (C2), 158.9 (C6); uv (*p*H 1): λ max 296 nm (ε = 18200), sh 288 (ε = 16200); uv (*p*H 13): λ max 295 nm (ε = 19100), sh 288 (ε = 10700); uv (ethanol): λ max 286 nm (ε = 20600), 294 (ε = 10400) {lit [4] *p*H 1: λ max 295 nm (ε = 18900), *p*H 13: λ max 294 nm (ε = 20500), sh 289; ethanol: λ max 286 nm (ε = 21300), sh 290}.

7-Benzyl-6-benzylthiopurine (**3**).

This compound had mp 117-120° (lit [4] mp 120°); ^{1}H nmr (DMSO-d_6): δ 4.61 (s, 2H, SCH_2), 5.68 (s, 2H, NCH_2), 7.07-7.35 (m, 10H, Ph), 8.73 (s, 1H, H-8), 8.80 (s, 1H, H-2); ^{13}C nmr (DMSO-d_6): δ 32.5 (SCH_2), 49.9 (NCH_2), 122.4 (C5), 126.4 (Ph), 127.3 (Ph), 127.9 (Ph), 128.5 (Ph), 128.9 (Ph), 129.1 (Ph), 137.0 (Ph), 137.2 (Ph), 149.4 (C8), 151.7 (C2), 152.1 (C6), 158.5 (C4); uv (*p*H 1): λ max 306 nm (ε = 12700); uv (*p*H 13): λ max 300 nm (ε = 12000); uv (ethanol): λ max 294 nm (ε = 13600), sh 303 (ε = 12300) {lit [4] *p*H 1: λ max 305 nm (ε = 13100), *p*H 13: λ max 300 nm (ε = 13700), sh 289; ethanol: λ max 295 nm (ε = 14900), sh 290}.

3-Benzyl-6-benzylthiopurine (**4**).

This compound had mp 148-149° (lit [1] mp 147-148°); ^{1}H nmr (DMSO-d_6): δ 4.74 (s, 2H, SCH_2), 5.72 (s, 2H, NCH_2), 7.23-7.52 (m, 10H, Ph), 8.17 (s, 1H, H-8), 9.12 (s, 1H, H-2); ^{13}C nmr (DMSO-d_6): δ 32.6 (SCH_2), 53.5 (NCH_2), 127.4 (Ph), 128.5 (Ph, two carbons), 128.6 (Ph), 128.8 (Ph), 129.1 (Ph), 135.2 (Ph), 135.6 (C5), 137.6 (Ph), 141.6 (C2), 150.0 (C4), 158.2 (C6), 158.4 (C8); uv (*p*H 1): λ max 238 nm (ε = 8700), 275 (ε = 6300), 321 (ε = 27200); uv (*p*H 13): unstable; uv (ethanol): λ max 243 nm (ε = 13300), 318 (ε = 20500) {lit [1] *p*H 1: λ max 234 nm (ε = 9600), 275 (ε = 6000), 321 (ε = 29200)}.

Benzylation of 6-Benzylthiopurine In the Absence of Base.

A solution of 2.0 g (8.26 mmoles) of 6-benzylthiopurine (**1**) and 1.4 g (8.2 mmoles) of benzyl bromide in 20 ml of DMAc was heated at 120° for 24 hours. The solution was concentrated *in vacuo* to an oil, which was subsequently dissolved in water. The resulting solution was made basic with sodium carbonate and was extracted with chloroform. The chloroform solution

was dried over sodium carbonate and was concentrated to dryness. Recrystallization of the residual solid from cyclohexane gave 1.7 g (62%) of 9-benzyl-6-benzylthiopurine (**2**).

When the above reaction was performed at room temperature for 24 hours, hplc analysis of the reaction mixture showed mostly unreacted 6-benzylthiopurine (**1**) plus small amounts of N3-, N7-, and N9-benzylated derivatives **4**, **3**, and **2**, respectively in the ratio of 33:1:2. No further workup was pursued.

Stability Studies of 3-, 7-, and 9-Benzyl-6-benzylthiopurine.

A small sample (3 mg) of each of 3-, 7-, and 9-benzyl-6-benzylthiopurine was dissolved in 1 ml of DMAc individually. Aliquots (20 µl) were removed at 0.5 hour intervals during the first 4 hours and at the end of 24 hours for analysis by hplc. Stability at 110° was also determined in the same manner after cooling. Components in the reactions were identified by comparison of the retention times.

One drop of 48% aqueous hydrobromic acid was added to a solution of each isomer (3 mg) dissolved in 1 ml of DMAc. Hplc analysis, as above, was used to monitor stability of these isomers at ambient temperature and at 110°. Similarly, stability of each isomer in a DMAc solution containing potassium carbonate (5 mg) was analyzed at ambient temperature and at 110°.

Observations from the above experiments were: (1) In DMAc or in DMAc/potassium carbonate, each isomer was stable at room temperature or at 110°; (2) In DMAc/hydrogen bromide, each isomer was stable at room temperature. At 110°, only the N9-isomer was stable; the N3- and N7-isomers **4** and **3** were isomerized to the N9-isomer **2** and a small amount of 6-benzylthiopurine (**1**).

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