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Reactions of SH-Substituted Purine Bases with Glycidol

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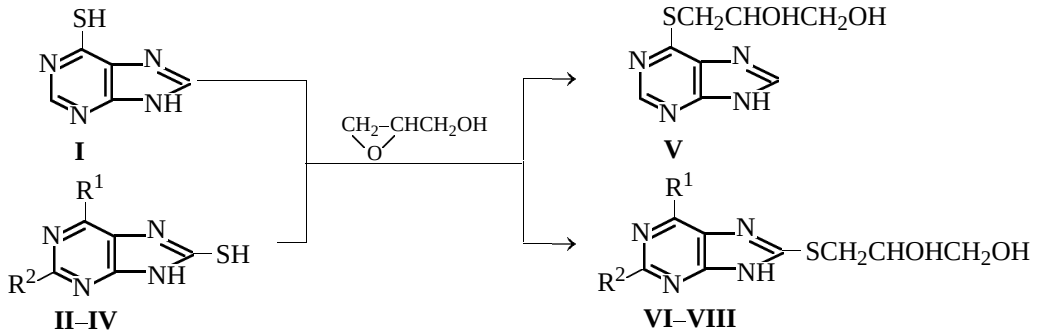
Abstract — The regioselectivity of S-alkylation of SH-substituted purine bases with glycidol tends to grow with decreasing temperature. A procedure was developed for epoxidation of these compounds in wet liquid ammonia. The products were tested for immunostimulating activity.

Proceeding with a search for synthetic routes to nonglycoside analogs of nucleosides exhibiting enhanced biological activity [1–3], we studied the regioselectivity of glycidol addition to SH-substituted purine bases. It is known that glycidol adds to 6-purinethiol in alcohol in the presence of a base at room temperature at the SH group to form the corresponding thioether [4]. It is also known that SH-substituted purine bases can be alkylated with alkyl halides both at the SH group and at N atoms of the purine system; the relative contribution of S-alkylation grows with decreasing temperature [5, 6]. A similar trend could not be ruled out with glycidol, despite the fact that, according to our recent data [7], 6-purinethiol and 8-hypoxanthinethiol are regiospecifically alkylated with acrylonitrile in liquid NH_3 at the 9-N atom [7].

To check this assumption, we studied the regioselectivity of glycidol addition to purine-6-thiol **I**, hypoxanthine-8-thiol **II**, adenine-8-thiol **III**, and guanine-8-thiol **IV** in various solvents in the presence of bases at various temperatures. Additionally, we developed an epoxidation procedure in wet liquid ammonia, allowing the reactions to be performed at temperatures from –35 to –40°C. The reaction does not occur in anhydrous liquid ammonia. The reaction progress was monitored by TLC until the conversion of the starting SH-substituted purine bases was complete.

We found that compound **I** reacted with glycidol in DMF in the presence of potassium carbonate at 75°C nonregioselectively to give 20% of **V** and a mixture of N-alkylated isomers; their separation was not attempted. When the reaction was performed at room temperature, the regioselectivity of formation of **V** increased to 60%, and 70% regioselectivity was attained in liquid ammonia at a temperature from –35 to –40°C. Identification of the reaction products as *S*- or *N*-isomers was based on the hypsochromic shift of the UV absorption band in the case of *S*-isomers [8].



R^1 = OH, R^2 = H (**II, VI**); R^1 = NH_2, R^2 = H (**III, VII**), R^1 = OH, R^2 = NH_2 (**IV, VIII**).

The reactions of **II** and **IV** with glycidol in aqueous solution in the presence of NaOH at room temperature yield compounds **VI** and **VIII** with 70 and 75% regioselectivity, respectively. The same regioselectivity was obtained with **II** in wet liquid ammonia. The reaction of **III**, which is less active than **I**, **II**, and **IV**, was performed in DMF in the presence of NaOH at 60–70°C; compound **VII** was obtained in 80% yield.

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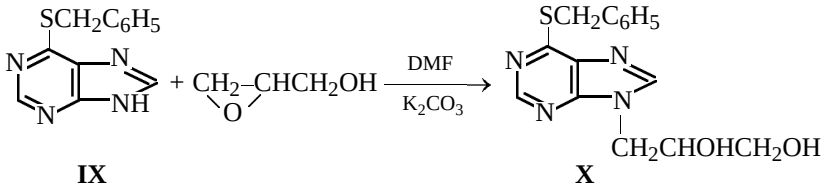
Thus, the regioselectivity of S-alkylation of **I**–**IV** with glycidol tends to grow with decreasing temperature, but does not exceed 70% even at temperatures from –35 to –40°C.

To perform N-alkylation of SH-purine bases with glycidol, it is necessary to protect the SH group. As we found previously [1], 8-benzylthioadenine is regioselectively alkylated with glycidol at the 3-N-position of the purine system owing to the steric effect of the benzylthio group [1]. Using this approach, we performed the reaction of 6-benzylthiopurine **IX** with glycidol and obtained compound **X**.



IX **X**

The structure of **X** was confirmed by NMR and UV spectroscopy. The absence of the bathochromic shift of the band with λ_{max} 293 nm in the case of **X** relative to **IX** unambiguously indicates that glycidol adds to the 9-N atom of the purine system. The NMR spectrum contains signals characteristic of the 9-*N*-dihydroxypropyl substituent.

The immunostimulating activity of the compounds prepared was assessed using as the model the immunodepression caused by a known immunodepressant, azathioprine. Experiments were performed with SVA line mice; the test samples were administered parentherally in the dose of 0.5 mg kg^{-1} over a period of 8 days against the background of immunodepression. All the samples showed moderate stimulating activity: the death percentage decreased to 50–70%, against 80% in the control group.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Bruker AC-200 spectrometer in DMSO-d_6 (internal reference TMS), and the UV spectra, on a Specord spectrophotometer. The TLC analysis was performed on Silufol UV-254 plates in the system *n*-butanol–ethanol–water, 4 : 1 : 1.5.

6-(2,3-Dihydroxypropylthio)purine V. A mixture of 0.01 mol of **I** and 0.02 mol of glycidol in 50 ml of absolute DMF in the presence of a catalytic amount of anhydrous K_2CO_3 was stirred at room temperature for 12 h. After distilling off DMF in a vacuum, the residue was treated with ethanol. The precipitate was filtered off, recrystallized from water, and dried at 100–110°C. Yield of **V** 0.006 mol (60%), mp 195–197°C (dec.) (published data: mp 196–197°C [4], 178–179°C [9]), R_f 0.50. ^{1}H NMR spectrum, δ, ppm: 3.50 t (2H, CH_2OH), 3.40–3.80 m (3H, SCH_2C*H*OH), 8.10 s (1H, C^{2}H), 8.45 s (1H, C^{8}H). UV spectrum (H_2O, pH 7), nm: λ_{max} 290.

A 0.2-g portion of glycidol was added to a solution of 0.5 g of **I** in 50 ml of liquid ammonia containing 10% H_2O. The solution was stirred for 2 h at a temperature from –35 to –40°C. After evaporation of ammonia, the residue was acidified with HCl to pH 5. The precipitate was recrystallized from water. Yield of **V** 0.52 g (70%).

6-Hydroxy-8-(2,3-dihydroxypropylthio)purine VI. A 0.015-mol portion of glycidol was added to a solution of 0.01 mol of **II** in 50 ml of water, containing 0.012 mol of NaOH. The solution was stirred at room temperature for 3 h and neutralized with HCl. Water was evaporated in a vacuum to a minimal volume. The precipitate was filtered off, washed with water, and dried at 100–110°C. Yield of **VI** 0.007 mol (70%), mp 180–185°C (dec.), R_f 0.45. ^{1}H NMR spectrum, δ, ppm: 3.20–3.65 m (5H, CH_2OH, C*H*OH, SCH_2), 4.80 m (1H, CH_2O*H*), 5.10 m (1H, CHO*H*), 8.10 s (1H, C^{2}H). UV spectrum (H_2O, pH 1), nm: λ^1_{max} 273, λ^2_{max} 278. Found, %: C 39.41, 39.57; H 4.23, 4.35; N 23.08, 23.20; S 12.92, 13.11. $C_8H_{10}N_4O_3S$. Calculated, %: C 39.67; H 4.16; N 23.13; S 13.23.

The reaction of **II** with glycidol in wet liquid ammonia was performed similarly to **I**. Yield of **VI** 71%.

2-Amino-6-hydroxy-8-(2,3-dihydroxypropylthio)purine VIII. The reaction of **IV** with glycidol was performed similarly to preparation of **VI**. Yield of **VIII** 0.0075 mol (75%), mp 196–198°C, R_f 0.40. ^{1}H NMR spectrum, δ, ppm: 3.10–3.80 m (5H, CH_2OH, C*H*OH, SCH_2), 4.80 m (1H, CH_2O*H*), 5.10 m (1H, CHO*H*), 6.40 s (2H, NH_2). UV spectrum (H_2O, pH

11), nm: λ_{max} 282. Found, %: C 37.40, 37.43; H 4.10, 4.05; N 27.18, 27.19; S 12.30, 12.39. $C_8H_{11}N_5O_3S$. Calculated, %: C 37.35; H 4.31; N 27.22; S 12.46.

2-Amino-8-(2,3-dihydroxypropylthio)purine VII. To a solution of 0.01 mol of **III** in 50 ml of absolute DMF, we added 5 mg of NaOH and 0.01 mol of glycidol. The solution was stirred at 75–80°C for 6 h. The solvent was vacuum-evaporated, and the residue was recrystallized from water. Yield of **VII** 0.008 mol (80%), mp 203–204°C, R_f 0.48. ^{1}H NMR spectrum, δ, ppm: 3.10–3.80 m (5H, C*H*$_2$OH, C*H*OH, SCH$_2$), 4.70 m (2H, CH$_2$O*H*), 5.12 m (1H, CHO*H*), 6.95 s (2H, NH$_2$), 8.05 s (1H, C^{2}H). UV spectrum (H$_2$O, pH 7), nm: λ_{max} 283. Found, %: C 39.75, 39.64; H 4.40, 4.54; N 28.83, 28.97; S 13.03, 13.19. $C_8H_{11}N_5O_2S$. Calculated, %: C 39.83; H 4.59; N 29.03; S 13.29.

6-Benzylthio-9-(2,3-dihydroxypropyl)purine X. The reaction of **IX** with glycidol was performed for 20 h similarly to the preparation of **V**. Yield of **X** 0.005 mol (50%), mp 170–175°C, R_f 0.65. ^{1}H NMR spectrum, δ, ppm: 3.14 s (2H, SCH$_2$), 3.40–3.70 m (2H, C*H*$_2$OH), 3.90–4.10 m (2H, NCH$_2$), 5.20–5.50 m (5H, C$_6$H$_5$), 8.2 s (1H, C^{2}H), 8.60 s (1H, C^{8}H). UV spectrum (H$_2$O, pH 7), nm: λ_{max} 293. Found, %: C 56.81, 56.89; H 5.01, 4.92; N 17.50, 17.64; S 9.87, 10.12. $C_{15}H_{16}N_4O_2S$. Calculated, %: C 56.95; H 5.10; N 17.71; S 10.13.

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