# Aminoacid and Protein Conjugates with Biologically Active Purines (1)

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Condensation products of amino acids and proteins with biologically active purines have been obtained to study their potential tumor inhibitory activity and as models for the preparation of conjugates with tumor specific antibodies. Among the new compounds are 2-glycinyl-6-methylmercaptopurine (**5**), 2-glycinyl-6-mercaptopurine (**6**), 2-glycinyl-6-chloropurine (**16**), 2-glycinyl-6-hydroxylaminopurine (**18**), 2-glycinylpurine (**8**) and 2-glycinyl-6-methylpurine (**7**). 9-Alkylpurines led to new purine derivatives, such as 6-chloro-9*H*-purine-9-acetic acid (**22**) and 6-hydroxylamino-9*H*-purine-9-acetylhydroxamic acid (**23**). These derivatives were coupled to bovine serum albumin (BSA) to yield conjugates. The amino acid-purines 6-cysteinyl (**27**) and 6-glutathionyl-*S*-purine 3-oxide (**28**) were obtained from 6-chloropurine 3-oxide (**26**). Other protein-purine 3-oxide conjugates were obtained with 6-mercaptopurine 3-oxide (**29**) and 6-bromomethylpurine 3-oxide (**33**).

Interaction of glutamic acid with 6-chloropurine was reported to yield *N*-(6-purinyl)-L-glutamic acid which showed antitumor activity (4). The coupling of the highly reactive 6-trichloromethylpurine (5) with amino acids and also proteins were later investigated. In the latter case, 6-trichloromethylpurine combines readily with proteins by a peptide linkage (purinoyl residues attached to the $\epsilon$-amino group of lysine [6]) yielding conjugates which induce tumor regression (7-10).

Reaction products of purine antimetabolites with serum proteins have now been prepared either by direct coupling of reactive purines with bovine serum albumin (BSA) or through acylpurines (2-glycinyl, and 9*H*-purine-9-acetic acid derivatives) conjugated to BSA using a carbodiimide as a coupling agent. The products are intended to be used as potential antitumor agents and as models for conjugates with specific tumor antibodies.

It is known that specific antibodies may be localized on the surface of tumor cells (11) and act as "homing carrier" for therapeutic agents (12). Alkylating agents have been bound electrostatically to specific tumor antibodies (13); in contrast, the purine-protein conjugates we have obtained are covalently linked.

The antimetabolites 2-fluoro-6-methylmercaptopurine (**1**) and 2-fluoro-6-mercaptopurine (**2**) (14,15) were each treated with glycine and the respective 2-glycinyl-6-methylmercaptopurine (**5**) and 2-glycinyl-6-mercaptopurine (**6**) were formed (Table I). Coupling to BSA was facilitated by the use of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) (16,17), affording the corresponding conjugates (**9**), (**10**) (Table III). Compound **1** was also incubated with BSA at 40° to yield the conjugate (**13**).

In a similar manner, we have prepared 2-glycinyl-6-methylpurine (**7**) from 2-fluoro-6-methylpurine (**3**) (14), a derivative of the cytotoxic 6-methylpurine (18). Condensation of glycine with 2-fluoropurine (**4**) gave 2-glycinylpurine (**8**). Protein conjugates such as (**11**) and (**12**) with a 2-aminopurine determinant group were prepared because of the mutagenic properties of this group (19).

An improved route for the preparation of 2-glycinyl-6-mercaptopurine, **6**, (Scheme I) consists of the reaction of 2-chloro-6-mercaptopurine (**15**) (20) (obtained by treatment of 2,6-dichloropurine (**14**) with sodium hydrosulfide in almost quantitative yield) and glycine. Chlorination of **6** in a methanol-hydrochloric acid solution

Scheme I

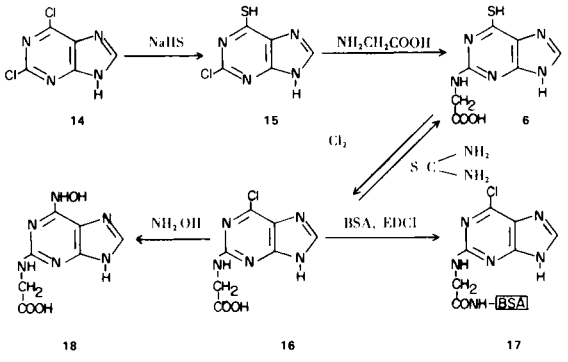

Table I

Formation of 2-Glycinylpurines



| Starting Material | $R_1$ | $R_2$ | Amount g., (mmole) | Potassium carbonate, g., (mmole) | Glycine g. (mmole) | Reaction time (hours) | Reaction product | $R_3$ | Yield, g. (%) | M.p., °C |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | F | $SCH_3$ | 0.37 (2) | 0.28 (2) | 0.56 (8) | 3 | **5** (a) | $SCH_3$ | 0.43 (90) | > 300 |
| **2** | F | SH | 0.20 (1.2) | 0.28 (2) | 0.28 (4) | 3 | **6** (a,c) | SH | 0.20 (74) | > 300 |
| **3** | F | $CH_3$ | 1.0 (6.6) | 0.83 (6) | 0.90 (12) | 5 | **7** (b,c) | $CH_3$ | 0.38 (36) | > 280 |
| **4** | F | H | 1.0 (7.2) | 0.88 (6.3) | 0.96 (12.8) | 8 | **8** (b,c) | H | 0.89 (64) | 200 dec |
| **15** | Cl | SH | 9.0 (45) | 8.4 (60) | 6.8 (90) | 24 | **6** | SH | 7.2 (66) | - |

(a) Precipitates upon addition of formic acid to *p*H 4. (b) Precipitates on adding acetic acid to *p*H 4. (c) Recrystallized from water.

Scheme 2



Scheme 3



Scheme 4



gave 2-glycinyl-6-chloropurine (**16**), which was transformed to the starting material **6** by treatment with thiourea. Compound **16** reacted with bovine serum albumin in the presence of EDCI to yield a conjugate of BSA (**17**). Interaction of **16** with ethanolic hydroxylamine afforded 2-glycinyl-6-hydroxylaminopurine (**18**) which, upon treatment with *N* sodium hydroxide, gave a dark red solution; the 2,2′-glycinyl-6,6′-azoxypurine which probably formed proved to be unstable and could

Table Ia

| | Formula | Analyses Calcd. C | H | N | S | Found C | H | N | S |
|---|---|---|---|---|---|---|---|---|---|
| **5** | $C_8H_9N_5O_2S$ | 40.16 | 3.76 | 29.28 | 13.39 | 39.96 | 3.76 | 29.07 | 13.21 |
| **6** | $C_7H_7N_5O_3S \cdot 1/3\ H_2O$ | 36.36 | 3.31 | 30.30 | 13.85 | 36.55 | 3.02 | 30.41 | 13.72 |
| **7** | $C_8H_9N_5O_2$ | 46.37 | 4.38 | 33.80 | | 46.36 | 4.43 | 33.81 | |
| **8** | $C_7H_7N_5O_2 \cdot 1/6\ H_2O$ | 42.85 | 3.77 | 35.70 | | 42.95 | 3.81 | 35.44 | |

not be isolated. Similar instability of azoxypurine derivatives has been observed (21-23). Unlike the described ease (24) with which methylmercaptopurines were transformed into the respective hydroxylamino derivatives, 2-glycinyl-6-methylmercaptopurine, **5**, was not converted into **18** even by prolonged reaction with ethanolic hydroxylamine and the use of hydroxylamine hydrochloride as a catalyst.

The amino acid-purine or protein-purine condensation products thus far reported in the literature (4-10) have been linked *via* a peptide bond at the $C_6$ of $C_2$ position. The desirability of obtaining amino acid and protein conjugates with linkage at the N-9 leaving free the $C_2$, $C_6$ and $C_8$ positions which are involved in metabolic processes prompted the preparation of 9-substituted purines with biologically-active groups at $C_6$ (mercapto, chloro, hydroxylamino) for subsequent condensation with proteins. The known 6-mercapto-9*H*-purine-9-acetic acid (**19**) (25) was condensed with bovine serum albumin to yield the conjugate (**20**) (Scheme 2). Treatment of 6-mercapto-9*H*-purine-9-acetic acid, **19**, with chlorine or the hydrolysis of ethyl(6-chloro-9*H*-purin-9-yl) acetate (**21**) (25) yielded 6-chloro-9*H*-purine-9-acetic acid (**22**) which upon reaction with ethanolic hydroxylamine afforded 6-hydroxylamino-9*H*-purine-9-acetylhydroxamic acid (**23**). Compound **23** was also obtained from the ethyl ester **21** and hydroxylamine. The known 9-acetic acid derivative (**24**) (25) was obtained by Raney nickel treatment of the mercapto compound, **19**.

As purines are good determinant groups when attached to proteins, the use of oncogenic purines, such as purine *N*-oxides (26), attached to proteins might elicit an immunoprotective response against the oncogenic activity of these purines. We have coupled 6-mercaptopurine 3-oxide (**29**) (27) to BSA. Treatment of 6-chloropurine 3-oxide (**26**) (28) with cysteine in methanol gave 6-cysteinyl-*S*-purine 3-oxide (**27**) (Scheme 3). Under similar conditions, reaction of **26** with glutathione afforded 6-glutathionyl-*S*-purine 3-oxide (**28**). 6-Mercaptopurine 3-oxide **29** readily reacted with chloroacetic acid in aqueous alkaline solution, giving 6-carboxy-methylmercaptopurine 3-oxide (**30**) (Scheme 4). This compound was coupled to BSA using EDCI as a condensing agent to yield the corresponding conjugate (**31**). An alternative way to obtain a similar

Table II

Uv Spectral Data of Some Purine Derivatives

| *p*H | λ max, nm (ε x $10^{-3}$) (a) |
|---|---|
| | 2-Glycinyl-6-methylmercaptopurine (**5**) |
| 1 | 321 (11.3), 276 (8.9), 274 (10.4) |
| 7 | 318 (9.1), 248 (17.6) |
| 13 | 322 (8.8), 321 (24.2) |
| | 2-Glycinyl-6-mercaptopurine (**6**) |
| 2 | 346 (18.6), 260 (8.7) |
| 6 | 344 (19.4), 260 (8.7) |
| 9.5 | 327 (12.8), 276 (8.3), 250 (9.7) |
| | 2-Glycinyl-6-methylpurine (**7**) |
| 1.1 | 316 (4.9) |
| 5.5 | 312 (5.9), 245 (8.2) |
| 7.8 | 311 (6.4), 244 (8.7) |
| | 2-Glycinylpurine (**8**) |
| 2.2 | 320 (4.3), 245 (2.9) |
| 7.8 | 318 (5.9), 243 (8.1) |
| 13.1 | 316 (5.8), 273 (3.1) |
| | 2-Glycinyl-6-chloropurine (**16**) |
| 1.1 | 321 (6.2), 242 (8.9) |
| 5.5 | 318 (6.1), 247 (8.7) |
| 13.1 | 320 (5.9), 266-273 (3.9) |
| | 2-Glycinyl-6-hydroxylaminopurine (**18**) |
| 5 | 254, 286 (unstable) |
| | 6-Chloro-9*H*-Purine-9-Acetic Acid (**22**) |
| 1.1 | 265 (9.1) |
| 7.8 | 266 (9.2) |
| 13.1 | 261 (9.4) |
| | 6-Hydroxylamino-9*H*-Purine-9-Acetyl-hydroxamic Acid (**23**) |
| 2.2 | 266 (13.6) |
| 5.5 | 268 (11.0) |
| | 6-Cysteinyl-*S*-purine 3-Oxide (**27**) |
| 1 | 312 (8.4), 250 (8.3) |
| 7 | 310 (16.0), 245 (17.8) |
| 13 | 312 (17.6), 245 (16.8) |
| | 6-Glutathionyl-*S*-purine 3-Oxide (**28**) |
| 2 | 319 (16.4), 253 (7.6) |
| 7 | 320 (13.7)sh, 313 (14.7), 246 (14.9) |
| 10 | 322 (13.7)sh, 314 (14.7), 246 (15.2) |
| | 6-Carboxymethylmercaptopurine 3-Oxide (**30**) |
| 2.9 | 316 (18.3), 251 (9.1) |
| 6.6 | 312 (17.1), 245 (13.4) |
| 7.9 | 311 (17.2), 245 (15.5) |

(a) sh denotes shoulder

Table III

Conjugates of Purines and BSA (a)

| Compound | Amount (mmole) | Reaction Product | Number of purine residues/mole protein |
|---|---|---|---|
| **5** | 0.27 | 2-Glycinyl-(BSA)-[6-methylmercaptopurine] (**9**) | 10 |
| **6** | 0.25 | 2-Glycinyl-(BSA)-[6-mercaptopurine] (**10**) | 22 |
| **7** | 0.5 | 2-Glycinyl-(BSA)-[6-methylpurine] (**11**) | 6 |
| **8** | 0.5 | 2-Glycinyl-(BSA)-[6-purine] (**12**) | < 1 |
| **16** | 0.5 | 2-Glycinyl-(BSA)-[6-chloropurine] (**17**) | 23 |
| **19** | 0.5 | 9H-Acetyl-(BSA)-[6-mercaptopurine] (**20**) | 1 |
| **22** | 0.5 | 9H-Acetyl-(BSA)-[6-chloropurine] (**25**) | 8 |
| **30** | 0.27 | 6-Carboxy-(BSA)-[methylmercaptopurine 3-oxide] (**31**) | 3 |
| **29** | -- | 6-Carboxy-(BSA)-[methylmercaptopurine 3-oxide[ (b) (**31**) | 13 |
| **5** | -- | 2-(BSA)-[6-methylmercaptopurine] (c) (**13**) | 10 |
| **33** | -- | 6-Methylene-(BSA)-[purine 3-oxide] (c) (**34**) | 3 |

(a) For each compound 70 mg. (*ca.* 1 mmole) of BSA was used together with an equimolar amount of EDCI. (b) Formed with chloroacetylated BSA (**32**). (c) Formed by direct incubation with BSA.

conjugate involved the chloroacetylation of BSA (29) followed by reaction with **29**. 6-Bromomethylpurine 3-oxide (**33**) (30) was directly coupled to BSA by incubation at 37°. The ratio of purine:protein of the obtained conjugates are listed on Table III.

## EXPERIMENTAL

Ultraviolet absorption spectra were determined with Beckman recording spectrophotometers, DBG and DU. Paper electrophoresis was performed using a borate buffer, *p*H 9, and disc electrophoresis using a Canalco (Rockville, Maryland) apparatus. Buffer was Tris-Glycine buffer, *p*H 8.5. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems: concentrated ammonium hydroxide-water-isopropyl alcohol (10:20:70); 1-butanol-water-acetic acid (50:25:25); and 1 *M* ammonium acetate-ethanol (30:70). Melting points were determined with a Thomas Hoover and Mel-Temp melting point apparatus and were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan. 2,6-Dichloropurine was purchased from Sigma Chemical Company, St. Louis, Missouri, 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) was supplied by the Ott Chemical Co., Muskegon, Michigan, and bovine serum albumin (BSA), Fraction V, powder, was purchased from Miles Laboratories, Kankakee, Illinois.

General Procedure for Preparing 2-Glycinylpurines.

An aqueous, 10% solution of the 2-halogenopurine containing glycine and potassium carbonate was refluxed for several hours. The product was collected, washed with water and dried *in vacuo*. Details for the preparation of the individual compounds are found in Table I.

2-Glycinyl-6-chloropurine (**16**).

To a solution containing concentrated hydrochloric acid (40 ml.) and methanol (40 ml.) at -10°, 2-glycinyl-6-mercaptopurine (**6**, 10.0 g., 0.04 mole) was added slowly. Chlorine was bubbled through the suspension for 1 hour keeping the temperature below 0°. The resulting precipitate was collected by filtration, suspended in water (100 ml.) at 5°, and 50% sodium hydroxide was added dropwise until solution occurred. Adjusting the *p*H to 4 with 2 *N* hydrochloric acid gave a white precipitate, which after washing repeatedly with cold water, ethanol, and drying *in vacuo*, gave 6.0 g. (59%) of white crystals. Repeated alkali-acid treatment gave white micro needles m.p. 190° dec.

*Anal.* Calcd. for $C_7H_6ClN_5O_2$: C, 36.93; H, 2.66; Cl, 15.58; N, 30.77. Found: C, 36.85; H, 2.79; Cl, 15.62; N, 30.75.

2-Glycinyl-6-mercaptopurine (**6**).

2-Glycinyl-6-chloropurine (**16**, 50 mg., 0.22 mmole) and thiourea (20 mg., 0.25 mmole) were suspended in 50% aqueous ethanol (10 ml.) and refluxed for 3 hours. The light yellow precipitate which appeared was collected by filtration and washed with water, ethanol, and dried *in vacuo*, yield, 25 mg. (49%). The product was identical (paper chromatography, m.p., and uv spectra) to an analytical sample of 2-glycinyl-6-mercaptopurine **6**.

2-Glycinyl-6-hydroxylaminopurine (**18**).

A solution of 2-glycinyl-6-chloropurine (**16**, 0.15 g., 0.6 mmole) in 0.7 *M* ethanolic hydroxylamine (150 ml.) was refluxed for 2 hours and cooled. The resulting precipitate was thoroughly washed with water and ethanol and dried to yield micro needles (0.090 g., 70%) m.p. 175° dec. An aqueous solution of this material with ferric chloride solution gave an intense blue color (hydroxylamino function) and with 2 *N* sodium hydroxide, a dark red product (azoxypurine formation); from this reaction, however, no crystalline product could be isolated.

*Anal.* Calcd. for $C_7H_8N_5O_3 \cdot 1/2\ H_2O$: C, 36.05; H, 3.89; N, 36.04. Found: C, 36.10; H, 3.73; N, 35.76.

Treatment with Raney Nickel.

A suspension of 6-mercaptopurine-9*H*-acetic acid (25) (**19**, 0.20 g., 0.9 mmole) in water (40 ml.) and Raney Nickel (400 mg.) was refluxed for 3 hours. The suspension was filtered through celite, the residue extracted with hot water, and the combined filtrates evaporated to dryness under reduced pressure. A crystalline product was obtained (0.052 g., 31%) which was identical (m.p., uv, paper chromatography) to purine-9*H*-acetic acid (**24**).

6-Chloro-9*H*-purine-9-acetic Acid (**22**).

A solution of ethyl (6-chloro-9*H*-purin-9-yl)acetate (25) (**21**, 10 g., 0.042 mole) in *N* sodium hydroxide (100 ml.) was kept at 25° for 15 minutes. The solution was adjusted to *p*H 5 with 2 *N* sulfuric acid and kept for 18 hours at 25°. Upon addition of concentrated hydrochloric acid, a precipitate appeared, which was collected by filtration, washed with a little water and ethanol to yield 4.3 g. (48%) m.p. 205° dec. An analytical sample of thin prisms (rosettes) was obtained by repeated crystallization from ethanol m.p. 205° dec. The same compound **22**, was obtained with lower yield by chlorination of **19** in methanol and concentrated hydrochloric acid at 0°.

*Anal.* Calcd. for $C_7H_5ClN_4O_2$: C, 39.50; H, 2.37; N, 26.35; Cl, 16.67. Found: C, 39.36; H, 2.59; N, 26.21; Cl, 16.51.

6-Hydroxylamino-9*H*-purine-9-acetylhydroxamic Acid (**23**).

A solution of ethyl (6-chloro-9*H*-purin-9-yl)acetate (**21**, 1.2 g., 5 mmoles) in 0.6 *M* ethanolic hydroxylamine (300 ml.) was refluxed for 3 hours. The precipitate which appeared upon cooling was filtered off and discarded and the filtrate concentrated under reduced pressure to 50 ml. The resulting precipitate was washed with cold water and ethanol and dried *in vacuo* to yield 0.44 g. (36%) of white needles, m.p. 206° dec. Ferric chloride test was positive (presence of hydroxylamino function); 2 *N* sodium hydroxide gave a dark green solution (formation of an azoxy derivative, presumably by the acetylhydroxamic group).

The same compound was obtained in 72% yield from 6-chloropurine-9*H*-acetic acid **22** and ethanolic hydroxylamine upon refluxing for 1 hour.

*Anal.* Calcd. for $C_7H_8N_6O_3 \cdot H_2O$: C, 34.71; H, 4.16; N, 34.70. Found: C, 34.68; H, 4.15; N, 34.65.

Preparation of Conjugates of Purine Derivatives and Bovine Serum Albumin.

The purine derivative was suspended in water (2-3 ml.), and 2 *N* sodium hydroxide was added dropwise to effect solution. The *p*H was adjusted to 5 with *N* hydrochloric acid (*p*H 7.5 with compounds **5** and **6**). After filtration, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) was added. The solution was stirred for 1 minute at 25° and poured into a solution of bovine serum albumin (BSA) (70 mg.) in water (1 ml.). After standing 6 hours at 25°, the solution was dialyzed against 0.2 *M* sodium bicarbonate and against water and then lyophilized.

The number of purine residues per mole of BSA was determined spectrophotometrically (6) and is indicated on Table III along with details for the preparation of the individual conjugates.

Conjugate (**13**) from (**5**) and BSA.

2-Fluoro-6-methylmercaptopurine (**5**, 0.046 g., 0.25 mmole) was suspended in water (1 ml.); a 50% sodium hydroxide solution (0.10 ml.) was added, and the mixture was heated; most of the solid dissolved. The remaining suspension was added to a solution of BSA (0.070 g.) in water (1 ml.) with dimethylformamide (0.25 ml.). The reaction mixture was kept at 40° for 36 hours, filtered, and the filtrate dialyzed against a sodium carbonate-sodium bicarbonate buffer, *p*H 10.5, for 24 hours, and against running distilled water overnight, and then lyophilized.

6-Cysteinyl-S-purine 3-Oxide (**27**).

Cysteine (0.60 g., 5 mmoles) was added to a solution containing methanol (25 ml.) and *N* sodium hydroxide (5 ml.). To the resulting suspension, 6-chloropurine 3-oxide (29) (**26**, 0.85 g., 5 mmoles) was added. The reaction mixture was kept at 80° for 2 hours, after which a precipitate appeared. The crude product was cooled and the precipitate collected by filtration, washed with cold water and dried to yield 0.40 g. (31%) of a crystalline material m.p. > 300°. An analytical sample was obtained by repeated dilute ammonia/acetic acid treatment.

*Anal.* Calcd. for $C_8H_9N_5O_3S \cdot 1/2\ H_2O$: C, 36.36; H, 3.78; N, 26.51; S, 12.12. Found: C, 36.42; H, 3.74; N, 26.55; S, 12.12.

6-Gluthathionyl-S-purine 3-Oxide (**28**).

To a suspension of glutathione (1.53 g., 5 mmoles) in methanol (25 ml.) and *N* sodium hydroxide (5 ml.), (**26**, 0.85 g., 5 mmoles) was added. The suspension was refluxed for 3 hours. The resulting product was collected by filtration, washed with cold water and ethanol to yield 1.25 g. (57%) of crystalline material, m.p. 211° (effervencence).

*Anal.* Calcd. for $C_{15}H_{19}N_7O_7SNa \cdot 1/2\ H_2O$: C, 36.60; H, 4.48; N, 20.00; S, 6.52; Na, 4.68. Found: C, 36.81; H, 4.15; N, 20.54; S, 6.58; Na, 4.18.

6-Carboxymethylmercaptopurine 3-Oxide (**30**).

6-Mercaptopurine 3-oxide (28) (**29**, 5.0 g., 29.4 mmoles) was dissolved in 30 ml. of an aqueous solution of potassium hydroxide (3.36 g., 60 mmoles). A solution of chloroacetic acid (3.0 g., 31.7 mmoles) in water (10 ml.) was neutralized with 5 *N* potassium hydroxide and added slowly to the above solution. (If a precipitate appears, 5 *N* potassium hydroxide should be added dropwise to redissolve it.) The reaction mixture was refluxed for 90 minutes, cooled, and the *p*H adjusted to 2, with 5 *N* hydrochloric acid. The resulting precipitate was filtered off and washed with cold water. After drying, 3.53 g. (52%) of white needles were obtained, m.p. 255° (effervescence).

*Anal.* Calcd. for $C_7H_6N_4O_3S$: C, 37.16; H, 3.65; N, 24.77; S, 14.16. Found: C, 37.20; H, 2.71; N, 24.79; S, 14.18.

Reaction of Chloroacetic Anhydride and BSA.

BSA (0.070 g.) was dissolved in water (10 ml.) and the solution cooled to 5°. Chloroacetic anhydride (1.9 g., 11 mmoles) was added portionwise with vigorous stirring. The *p*H was carefully adjusted to 8 with 25% sodium hydroxide solution after each addition. After 30 minutes, the resulting solution was dialyzed against running distilled water; at the end of the dialysis, a white precipitate appeared. It was collected, and the excess water was removed by lyophilization. The chloroacetylated BSA (**32**) thus obtained was used for the next reaction.

Reaction of Chloroacetylated BSA (**32**) with 6-Mercaptopurine 3-Oxide (**29**).

Compound **29** (0.020 g., 0.12 mmole) was dissolved in a concentrated solution of sodium carbonate (2 ml.) and added to the chloroacetylated BSA prepared above. If needed, sodium bicarbonate is added to adjust the *p*H to 9. The yellow solution was kept at 25° for 24 hours, dialyzed against 0.2 *M* sodium bicarbonate solution and against distilled water. The conjugate (**31**) was isolated by lyophilization.

Conjugate of 6-Bromomethylpurine 3-Oxide (**33**) with BSA.

To a solution of BSA (0.070 g.) in water (1 ml.), sufficient sodium carbonate was added to adjust the *p*H to 10. To this solution, compound **33** (0.010 g., 0.044 mmole) was added, and the reaction mixture was incubated at 37° for 5 days, dialyzed against distilled water overnight and lyophylized to give compound (**34**).

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