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Nonclassical Antimetabolites XXII

Simulation of 5′-Phosphoribosyl Binding V. Inhibition of Succinoadenylate Kinosynthetase by 6-Mercapto-9-purinylalkanoic Acid Derivatives of 4- and 5-Aminosalicylic Acid

By B. R. BAKER and PRAFULLCHANDRA M. TANNA

4- and 5-(6-Mercapto-9H-purine-9-ylvaleramido)salicylic acids (VII and XII) were relatively good inhibitors of succinoadenylate kinosynthetase, being about one-fourth as effective as thioinosinic acid (IX). The only further structural change that allowed retention of inhibition was variation of the valeryl bridge. Removal of the phenolic hydroxyl of VII, replacement of carboxyl group of XII by nitro, or replacement of the salicylic acid moiety by γ-butyric acid led to a decrease in inhibitory properties, thus indicating that both the phenolic hydroxyl and the carboxyl of the salicylate moiety are complexed with the enzyme. The binding of VII and related molecules was finally traced to the acylamino salicylate moiety, and there was no purine binding. Whether the salicylate moiety is simulating the enzyme binding of the phosphate moiety cannot as yet be certain, but appears unlikely.

ALTHOUGH the simulation of the binding of the phosphate moiety of a nucleotide such as IX or X by a more weakly ionized moiety is a goal worthy of pursuit (1–4) for its utility in chemotherapy (1), the solution of this problem is not as simple as the initial results portended (1). 9H-Adenine-9-ylvaleric acid could mimic the ability of 5′-adenylic acid to inhibit both lactic dehydrogenase and glutamic dehydrogenase (1), but 1-uracilvaleric acid failed to mimic 2′-deoxyuridylate (X) in its binding to thymidylate synthetase (2); the latter result led to two detailed studies, namely, on the relative contribution of phosphate *versus* other oxygen functions of the 5′-phosphoribosyl moiety of 5′-adenylic acid when it inhibits succinoadenylate kinosynthetase (3) and on whether the phosphate moiety would complex to enzymes through hydrogen bonds only (4). It was concluded (4) that the most likely mode of binding of phosphate to an enzyme was by one anionic–cationic interaction and one hydrogen bond. There are four such possible modes of binding (I–IV), although III is merely an ionized form of II.

It is possible for the salicylate structure to

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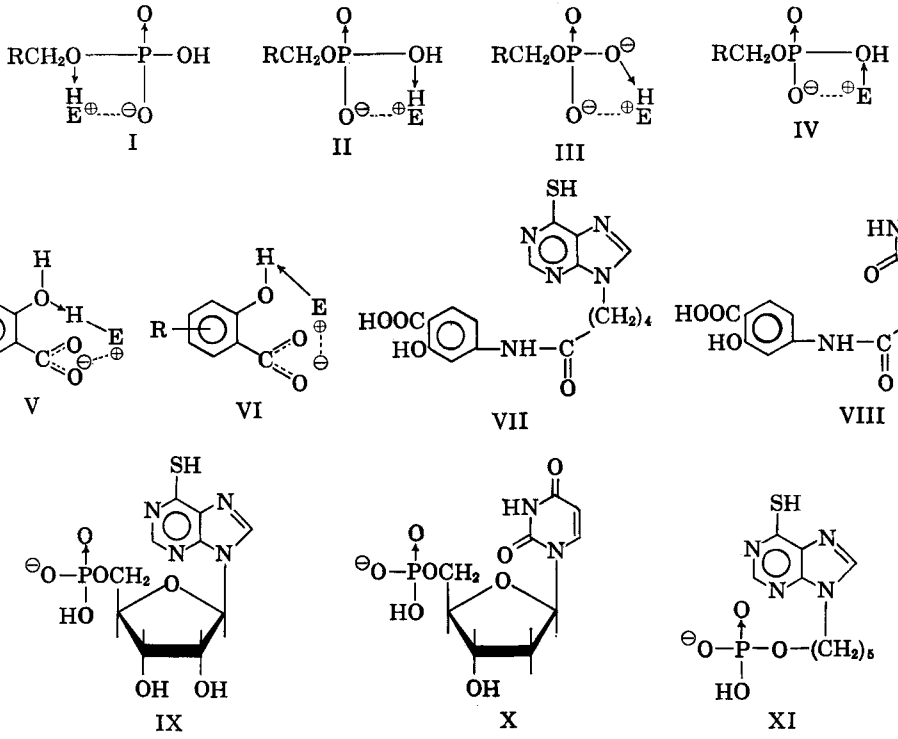


simulate binding of I–III to an enzyme (E) by structure V and to simulate binding of IV by structure VI. Although the salicylate moiety is only slightly larger than the methylene phosphate moiety, the latter has a greater degree of rotation of the two bonds about the $-CH_2-$ group compared to the bond to the phenyl ring of salicylate. Thus, although salicylate may have the functional groups necessary for simulation of phosphate binding, the substituted salicylate may not in all cases be able to assume the proper conformation for binding to an enzyme. Consideration of the latter fact led the authors to synthesize a molecule such as VII where 6-mercaptopurine is attached to *p*-aminosalicylic acid through a valeryl bridge, then investigate its inhibitory properties toward succinoadenylate kinosynthetase (inhibited by IX), even though the corresponding 1-uracilvaleramide derivative (VIII) did not simulate binding of 2′-deoxyuridylate (X) to thymidylate synthetase.

DISCUSSION

As can be seen in Table I, VII did show 50% inhibition of succinoadenylate kinosynthetase at four times the level required for thioinosinate (IX); however, VII was about twice as effective as 6-mercapto-9-purinylpentanol phosphate (XI) (3), whereas neither VII nor XI have the binding properties of the missing 2′-hydroxyl group of IX (3).

In order to determine some of the structural requirements of the purinyl salicylic acid (VII) for binding to succinoadenylate kinosynthetase, a number of related structures were investigated, the following five of which led to decreased activity (Table I).

(*a*) Salicylic acid was only about one-fourth as effective as VII, indicating that this moiety could bind to the enzyme, but that the remaining purinylvaleramide moiety of VII also contributed to binding in some fashion.

(*b*) Changing the amide linkage of VII from the 4-position to the 5-position (XII) allowed retention of activity.

(*c*) Shortening the bridge from the purine to the 5-aminosalicylic acid from valeryl (VII) to acetyl (XIII) allowed retention of activity. With an α-toluyl bridge (XIV), which has a more rigid demand on allowable conformations for binding, activity was still unchanged.

(*d*) Removal of the phenolic hydroxyl of VII, as in XV, or replacement of the carboxyl of VII by nitro (XVII) led to a decrease in activity, indicating that both the phenolic hydroxyl and an ionized carboxyl group are binding points to the enzyme. This conclusion is also supported by the decreased activity of XVIII.

(*e*) Alkylation of the thiol function as in XXXII allowed retention of activity.

The lack of specificity when the bridge between the 6-mercaptopurine and the salicylic acid moieties is varied, along with the almost equal inhibition of the 6-methylthiopurinyl salicylate (XXXII) and VII, made it seem unlikely that the purine ring was involved in binding to the enzyme. The only remaining structural feature was the amide linkage on the salicylate moiety. Therefore 4-(iodoacetamido)salicylic acid (XIX) (7) was checked as an inhibitor of succinoadenylate kinosynthetase; it was about as effective as VII, confirming that the

amide linkage made salicylic acid a better inhibitor either by direct binding of the amide or by increasing the binding of the salicylic moiety.

In order to determine whether a longer bridge between the aminosalicylate and 6-mercaptopurine moieties would allow both moieties to bind to the enzyme, the heptanoyl bridged compounds, XXXIII and XXXIV, were synthesized. As can be seen in Table I, these two compounds did not bind significantly better than VII.

The failure to achieve binding of both the 6-mercaptopurine and salicylate moieties of the candidate inhibitors to succinoadenylate kinosynthetase could be attributed to the inability of both moieties to bridge to their respective binding sites. It is also possible that the anomalous salicylate binding involves one or more of the binding points required for the 6-mercaptopurine moiety, thus making binding of the two moieties mutually exclusive.

The fact that 4-iodoacetamidosalicylic acid (XIX) reversibly complexes with succinoadenylate kinosynthetase about one-eleventh as well as substrate could be used for seeking an active-site-directed irreversible inhibitor (8) of this enzyme.

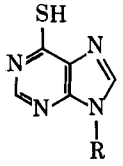
CHEMISTRY

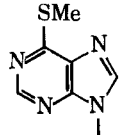
Methods.—Two routes can be envisioned for the synthesis of a purine bridged from its 9-position to the amino group of an aminobenzoic acid by an acyl group in a molecule such as XVI. One route would be activation of the carboxyl of a purine-9-alkanoic acid (XXIII) followed by coupling with an aminobenzoic acid. Alternately, an acylated aminobenzoic ester derivative bearing an ω-halogen group such as XXI could be condensed with 6-chloropurine, then converted to XVI *via* the ester (XXVI); this method was investigated first.

Reaction of 6-chloropurine (XX) with ethyl 4-(chloroacetamido)benzoate (XXI) (15) in dimethyl sulfoxide in the presence of potassium carbonate by the general method of Montgomery and Temple (9) gave a 75% yield of product (XXII). The latter was proved to be the 9-isomer by reaction with ethanolic diethylamine to give the 9-substituted-6-diethylaminopurine (XXIV) with an ultraviolet maximum of 277 mμ (1); the corresponding 7-isomer would have a maximum at a longer wavelength (10, 11).

When the chloropurine (XXII) was reacted with ethanolic ammonia for 1 hr. at 100°, selective conversion of the 6-chloro to 6-amino (XXV) took place in 88% yield without concomitant conversion of the ester to an amide (1). Reaction of XXII with thiourea in boiling ethanol proceeded smoothly to give a 92% yield of the 6-mercaptopurine derivative (XXVI). In contrast, selective conversion of the 6-chloropurine ester (XXII)

TABLE I.—INHIBITION OF SUCCINOADENYLATE KINOSYNTHETASE BY



Compd.	R[a]	m*M* Concn. Inhibitor	% Inhibition	Estimated *I*/*S*[b]
IX[c]	β-D-Ribofuranosyl-5′-phosphate	0.070	50	2.3
XI[c]	—$(CH_2)_5OPO(OH)_2$	0.83	50	27
VII	—$(CH_2)_4CO$-4-ASA	0.30	50	9.8
XII	—$(CH_2)_4CO$-5-ASA	0.29	50	9.5
XIII	—CH_2CO-5-ASA	0.30	50	9.8
XIV	*p*-$CH_2C_6H_4CO$-4-ASA	0.25	50	8.2
XV	—$(CH_2)_4CO$-PAB	0.25[d]	0	>24[e]
XVII	—$(CH_2)_4CONH(C_6H_3$-4-OH-3-$NO_2)$	0.25[d]	0	>24[e]
XVIII	—$CH_2CONH(CH_2)_3COOH$	0.60[d]	0	>60[e]
XXXIII	—$(CH_2)_6CO$-5-ASA	0.30	50	9.8
XXXIV	—$(CH_2)_6CO$-4-ASA	0.22	50	7.2
	Compd.			
V	Salicylic acid	1.4	50	82
XIX	Iodoacetyl-4-ASA[f]	0.35	50	11
XXXII	 $(CH_2)_4CO$—4—ASA	0.20	50	6.5

Succinoadenylate kinosynthetase was isolated from *Escherichia coli* B as previously described (3). Assays were performed with 30.6 μM inosinate, 3.75 mM L-aspartate, 100 μM guanosine triphosphate, 10 mM magnesium chloride, and 0.6 mg./ml. of streptomycin sulfate by the previously described modification (3) of the methods of Fromm (5) and Wyngaarden and Greenland (6). By plotting V_0/V_I against I for several concentrations of I, the concentration of I necessary to give 50% inhibition was determined ($V_0 - V_I = 2$), where V_0 = velocity of enzyme reaction with no inhibitor, V_I = velocity of reaction in the presence of inhibitor, and I = concentration of inhibitor (1). The authors acknowledge the technical assistance of Shirley Herrmann. [a] ASA, aminosalicylic acid; PAB, *p*-aminobenzoic acid. [b] I/S is the ratio of the concentrations of inhibitor to IMP showing 50% inhibition (I_{50}). [c] Data from *Reference 3*. [d] Maximum concentration still allowing light transmission. [e] Since 20% inhibition is readily detectable, the concentration necessary for 50% inhibition is estimated at four times greater than the concentration measured; I/S is calculated accordingly. [f] Preparation of this material previously recorded in *Reference 7*.

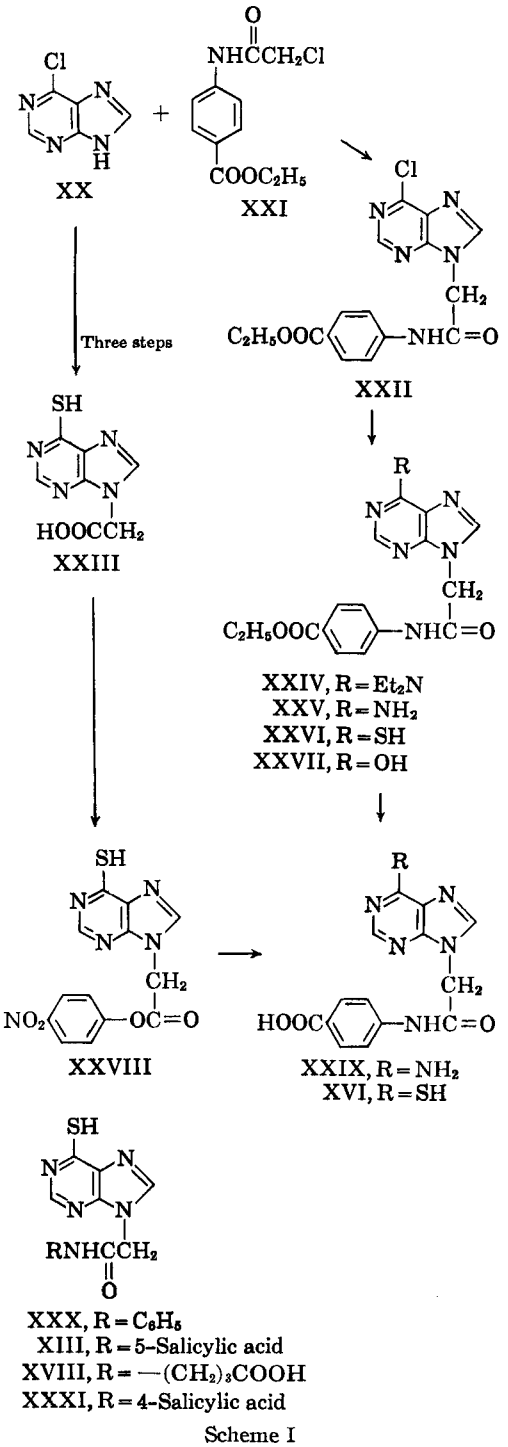
with 0.1 *N* hydrochloric acid in 85% ethanol (1) proceeded in only 12% yield to the hypoxanthine ester (XXVII); observations to be described later could be used to attribute the low yield to the abnormally high sensitivity of the amide linkage of XXVII to hydrolysis.

When the adenine ester (XXV) was allowed to react with 10% aqueous sodium hydroxide at room temperature until solution was complete (2.5 hr.), cleavage to 9H-adenine-9-ylacetic acid occurred in 83% yield. When the hydrolysis was attempted with 1.1 equivalents of sodium hydroxide in hot 2-methoxyethanol, a mixture of unchanged ester (XXV), 9H-adenine-9-ylacetic acid and the desired *p*-(9H-adenine-9-ylacetamido)benzoic acid (XXIX) was obtained; 3% of the unchanged ester (XXV) could be recovered due to its insolubility in aqueous sodium bicarbonate. Since 9H-adenine-9-ylacetic acid was readily soluble in 1 *N* hydrochloric acid and the *p*-(adenine-9-acetamido)benzoic acid (XXIX) was not, XXIX could be separated in 13% yield. The activation of the carbonyl group of acetic acid derivatives substituted on the α-position by the nitrogen of a heterocycle such as uracil has been previously observed (2). With the less basic *p* - (6 - mercapto - 9H - purine - 9 - ylacetamido)-benzoic ester (XXVI), base cleavage of the amide group was not as rapid; a study of nine different sets of reaction conditions showed that the best conditions for hydrolysis of XXVI and XVI were at room temperature with excess 1 *N* aqueous sodium hydroxide for 1 hr., in which case a 50% yield of XVI was obtained.

The second route started with 6-mercapto-9H-purine-9-ylacetic acid (XXIII), which is readily available from 6-chloropurine in three steps (9). Activation of the carboxyl group of XXIII to the mixed anhydride by reaction with ethyl chloroformate (12, 13) and triethylamine in *N,N*-dimethylformamide, followed by reaction with aniline at ambient temperature, gave a mixture of ethyl ester of XXIII and the desired anilide (XXX). Apparently, disproportionation (14) of the mixed anhydride to the ethyl ester and carbon dioxide occurs more readily than usual; such an easy disproportionation was previously observed with uracil-1-acetic acid (2). When the mixed anhydride was prepared at −40° and reacted with aniline at −20°, the desired anilide (XXX) was obtained in 62% yield. Unfortunately, the less reactive amino group of *p*-aminobenzoic acid failed to react at −20°, and at higher temperature, the ethyl ester of XXIII was obtained. (Scheme I.)

Since uracil-1-acetic acid also showed such a disproportionation, it was activated as its *p*-nitrophenyl ester and could then be converted to an amide (2). Reaction of 6-mercapto-9H-purine-9-ylacetic acid (XXIII) with *p*-nitrophenol in the presence of a water-soluble carbodiimide (16) gave a *p*-nitrophenyl ester (XXVIII) in 50% yield. The *p*-nitrophenyl ester (XXVIII) could be coupled with aniline in boiling *tert*-butyl alcohol to give a 78% yield of 6-mercapto-9H-purine-9-ylacetanilide (XXX). Similarly, the triethylammonium salts of *p*-aminobenzoic acid and 4-aminobutyric acid were condensed with the nitrophenyl ester (XXVIII) to give an over-all yield of XVI and XVIII of 32 and 43%, respectively, for the two steps. Although the 6-mercapto-9H-purine-9-ylacetyl derivative of 5-aminosalicylic acid (XIII) could be prepared in the same manner in 41% yield, the condensation with 4-aminosalicylic acid gave a product (XXXI) that could not be purified.

Since 6-mercapto-9H-purine-9-ylvaleric acid (1), 6-mercapto-9H-purine-9-ylheptanoic acid, and α-(6-mercapto-9H-purine-9-yl)-*p*-toluic acid (1) gave mixed anhydrides from ethyl chloroformate with



Scheme I

the normal stability, the amides, VII, XII, XIV, XV, XVII, XXXIII, and XXXIV (Table I), were prepared with no untoward difficulties other than the differences in problems of purification.

Synthesis.—Melting points were determined in capillary tubes in a Mel-Temp block, and those below 230° are corrected. Infrared spectra were determined in KBr pellets with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined with a Perkin-Elmer 202 spectrophotometer.

Ethyl p-(6-Chloro-9H-purine-9-ylacetamido)benzoate (XXII).—To a solution of 4.83 Gm. (20 mmoles) of XXI (15) in 40 ml. of reagent dimethyl sulfoxide was added 2.76 Gm. (20 mmoles) of powdered anhydrous potassium carbonate and 3.09 Gm. (20 mmoles) of 6-chloropurine. After being magnetically stirred for 6 hr. protected from moisture, the mixture was diluted with 50 ml. of water and extracted with chloroform (4 × 50 ml.). The combined extracts were washed with water, dried with magnesium sulfate, and spin-evaporated *in vacuo;* the last of the dimethyl sulfoxide was removed at 1 mm. The residue was triturated with 10 ml. of ethanol, collected on a filter, and washed with two 10-ml. portions of ethanol; yield, 5.41 Gm. (75%), m.p. 211–213° dec., that was suitable for further transformations. Recrystallization of a sample from 2-methoxyethanol–water gave the analytical sample, m.p. 220–223° dec.; $\lambda_{max.}$ (EtOH) 271 mμ; $\nu_{max.}$ 3300, 3200 (NH); 1720 (ester C=O); 1680, 1610, 1600, 1570, 1550 (amide I and II, C=C, C=N); 1270, 1180 (ester C—O—C); 860 cm.$^{-1}$ (*p*-C_6H_4).

Anal.—Calcd. for $C_{16}H_{14}ClN_5O_3$: C, 53.4; H, 3.92; N, 19.5. Found: C, 53.6; H, 3.90; N, 19.5.

Ethyl p-(6-Diethylamino-9H-purine-9-ylacetamido)benzoate (XXIV).—A solution of 180 mg. (0.5 mmole) of XXII and 110 mg. (1.5 mmoles) of diethylamine in 10 ml. of absolute ethanol was refluxed for 2 hr., then spin-evaporated *in vacuo.* Trituration of the residue with 10 ml. of water and recrystallization of the insoluble material from 50% aqueous ethanol gave 125 mg. (63% of pure product, m.p. 183–184°; $\lambda_{max.}$ (EtOH) 277 mμ; $\nu_{max.}$ 3300 (NH); 1720 (ester C=O); 1680, 1600, 1580, 1560, 1540 (amide I and II, C=C, C=N); 1270, 1175 (ester C—O—C); 860 cm.$^{-1}$ (*p*-C_6H_4).

Anal.—Calcd. for $C_{20}H_{24}N_6O_3$: C, 60.6; H, 6.10; N, 21.2. Found: C, 60.7; H, 6.00; N, 21.2.

Ethyl p-(9H-Adenine-9-ylacetamido)benzoate (XXV).—A mixture of 720 mg. (2 mmoles) of XXII and 20 ml. of ethanol previously saturated with ammonia at 0° was heated in a steel bomb at 100° for 1 hr. The bomb contents were spin-evaporated *in vacuo.* Trituration of the residue with water gave 600 mg. (88%) of product, m.p. 300–303° dec. Two recrystallizations of a sample from ethanol gave white needles, m.p. 314–315° dec.; $\lambda_{max.}$ (EtOH) 269 mμ; $\nu_{max.}$ 3320, 3150 (NH); 1700 (ester C=O); 1670, 1600, 1580, 1525 (amide I and II, C=C, C=N); 1280, 1170 (ester C—O—C); 855 cm.$^{-1}$ (*p*-C_6H_4).

Anal.—Calcd. for $C_{16}H_{16}N_6O_3$: C, 56.5; H, 4.74; N, 24.7. Found: C, 56.6; H, 4.86; N, 24.6.

Ethyl p-(6-Mercapto-9H-purine-9-ylacetamido)benzoate (XXVI).—A mixture of 720 mg. (2 mmoles) of XXII, 152 mg. (2 mmoles) of thiourea, and 25 ml. of ethanol was refluxed with magnetic stirring for 3 hr. When solution was nearly complete (0.5 hr.), the product began to separate. The cooled mixture was filtered and the buff-colored crystals were washed with water; yield, 662 mg. (92%), m.p. 323–327° dec. For analysis a sample was recrystallized from 75% aqueous ethanol to give opaque needles, m.p. 330–332° dec.; $\lambda_{max.}$ (EtOH) 273, 326 mμ; $\nu_{max.}$ 3300, 3150 (NH); 1710 (ester C=O); 1675, 1600, 1575, 1540 (amide I and II, C=C, C=N); 1280 (ester C—O—C); 855 cm.$^{-1}$ (*p*-C_6H_4).

Anal.—Calcd. for $C_{16}H_{15}N_5O_3$: C, 53.8; H, 4.24; N, 19.6. Found: C, 53.9; H, 4.46; N, 19.4.

Ethyl p-(6-Hydroxy-9H-purine-9-ylacetamido)benzoate (XXVII).—A solution of 360 mg. (1 mmole) of XXII in 50 ml. of ethanol and 5 ml. of aqueous 1 *N* hydrochloric acid was refluxed for 6 hr., then the solvent was spin-evaporated *in vacuo.* Trituration of the syrupy residue with 25 ml. of water gave a solid that was collected on a filter and washed with 5 ml. of acetone; yield, 127 mg. (37%) of crude product, m.p. 302–313° dec. Recrystallization from 25 ml. of 2-methoxyethanol gave 40 mg. (12%) of white crystals, m.p. 330–333° dec. A second recrystallization gave the analytical sample, m.p. 335–336° dec.; $\lambda_{max.}$ (EtOH) 270 mμ (broad); $\nu_{max.}$ 3250 (NH); 2800–2400 (acidic H); 1710 (ester C=O); 1660, 1600, 1580, 1540, 1525 (amide I and II, ring C=O, C=C, C=N); 1280 (ester C—O—C); 860 cm.$^{-1}$ (*p*-C_6H_4).

Anal.—Calcd. for $C_{16}H_{15}N_5O_4$: C, 56.3; H, 4.43; N, 20.5. Found: C, 56.6; H, 4.65; N, 20.3.

p-Nitrophenyl 6-Mercapto-9H-purine-9-yl-acetate (XXIII).—Ethyl 6-mercapto-9H-purine-9-ylacetate, m.p. 273–275° dec., was prepared in 27% over-all yield from 6-chloropurine according to the method of Montgomery and Temple (9); if the difficultly purifiable ethyl 6-chloro-9H-purine-9-ylacetate was not purified, but the crude ester reacted with thiourea, an over-all yield of 57% was obtained. An over-all yield of 30% and melting point greater than 260° has been recorded (9).

Saponification to XXIII (9) proceeded in 96% yield, m.p. 310–313° dec. A yield of 94% and melting point greater than 264° has been recorded (9).

A warm solution of 841 mg. (4 mmoles) of XXIII in 25 ml. of *N,N*-dimethylformamide was cooled to room temperature, then 616 mg. (4.4 mmoles) of *p*-nitrophenol and 907 mg. (4.4 mmoles) of dicyclohexylcarbodiimide were quickly added. After being magnetically stirred for 2.5 hr., the mixture was diluted with 25 ml. of ethyl acetate. The solid was collected on a filter and washed with ethyl acetate (3 × 5 ml.); yield, 2.1 Gm. of a mixture of XXVIII and dicyclohexylurea with $\nu_{max.}$ 1760 cm.$^{-1}$ (ester C=O) that could be separated only inefficiently and was best converted to amides directly as described later.

The pure nitrophenyl ester (XXVIII) was best prepared by using a water-soluble carbodiimide reagent, but the over-all yields of an amide such as XIII were lower than by the dicyclohexylcarbodiimide method.

A warm solution of 210 mg. (1 mmole) of XXIII in 6 ml. of *N,N*-dimethylformamide was cooled to room temperature; then 154 mg. (1.1 mmoles) of *p*-nitrophenol and 466 mg. (1.1 mmoles) of 1-cyclo-

hexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (16) were quickly added. After being magnetically stirred for 3.5 hr., the mixture was spin-evaporated *in vacuo* to a syrup. Trituration with 50% aqueous acetone gave a solid that was collected on a filter and washed with water (2 × 10 ml.), then acetone (5 × 10 ml.); yield, 166 mg. (50%), m.p. 263–265° dec. Recrystallization from *N,N*-dimethylformamide gave light yellow crystals of unchanged melting point; $\nu_{max.}$ 1760 (ester C=O); 1660, 1600, 1590, 1570 (C=C, C=N); 1520, 1350 (NO_2); 1220 (ester C—O—C); 860 cm.$^{-1}$ (*p*-C_6H_4).

Anal.—Calcd. for $C_{13}H_9N_5O_4S$: C, 47.2; H, 2.74; N, 21.2. Found: C, 47.2; H, 2.91; N, 21.4.

α - 6 - Mercapto - 9H - purine - 9 - ylacetanilide (XXX).—*Preparation A.*—A suspension of 331 mg. (1 mmole) of XXVIII (prepared by the soluble CMC carbodiimide method) (16) in 20 ml. of *tert*-butanol containing 0.5 ml. (5.5 mmoles) of aniline was refluxed for 22 hr. The solvent was spin-evaporated to dryness *in vacuo;* the residue was stirred with 25 ml. acetone and the white crystalline solid collected on a filter, then washed with acetone (3 × 10 ml.); yield, 222 mg. (78%), m.p. 338–342° dec. Recrystallization from aqueous *N,N*-dimethylformamide gave white crystals; yield, 151 mg. (53%), m.p. 350–353° dec. One more recrystallization gave the analytical sample of unchanged melting point; $\nu_{max.}$ 1660, 1600, 1575, 1540 (amide I and II, C=C, C=N); 755, 690 cm.$^{-1}$ (C_6H_5); $\lambda_{max.}$ 236, 326 mμ.

Anal.—Calcd. for $C_{13}H_{11}N_5OS$: C, 54.7; H, 3.88; N, 24.5. Found: C, 54.6; H, 4.00; N, 24.4.

Preparation B.—A warm solution of 105 mg. (0.5 mmole) of XXIII in 3 ml. of *N,N*-dimethylformamide was quickly cooled to about 25°, then 0.10 ml. (1.1 mmoles) of aniline and 155 mg. (0.75 mmole) of dicyclohexylcarbodiimide were added. After 5 hr. at ambient temperature, the mixture was diluted with 10 ml. of water, the solids were collected on a filter, then washed with water. The solids were stirred with 25 ml. of 0.1 *N* aqueous sodium hydroxide for about 30 min., then the mixture was filtered to remove dicyclohexylurea. Acidification of the filtrate gave 78 mg. (48%) of product, m.p. 350–355° dec. Reprecipitation from basic solution by addition of acid gave an analytical sample as buff crystals, m.p. 350–353° dec., identical with *Preparation A.*

Preparation C.—A hot solution of 105 mg. (0.5 mmole) of XXIII in 2 ml. of *N,N*-dimethylformamide was cooled to room temperature, then 0.14 ml. (1 mmole) of triethylamine was added. To the magnetically stirred mixture cooled to −40° and protected from moisture was added 0.06 ml. (0.6 mmole) of ethyl chloroformate. After being stirred at −40° for 20 min., 0.5 ml. (5.5 mmoles) of aniline was added. The reaction mixture was stirred at −20° for 6.5 hr. and then overnight at room temperature. Then 10 ml. of cold water was added to the reaction mixture, and the pH was adjusted to 1 with 1 *N* hydrochloric acid. The colorless solid was collected on a filter, washed with 5% sodium bicarbonate solution (3 × 5 ml.), water (3 × 5 ml.), and finally acetone (2 × 5 ml.); yield, 88 mg. (62%), m.p. 344–348° dec. Recrystallization from aqueous *N,N*-dimethylformamide gave needles, m.p. 350–353 dec., identical with *Preparation A.*

p - (6 - Mercapto - 9H - purine - 9 - ylacetamido)benzoic Acid (XVI).—A solution of 178 mg. (0.5 mmole) of XXVI in 4 ml. of 1 *N* aqueous sodium hydroxide was allowed to stand at room temperature for 1 hr. Acidification to pH 1 with 1 *N* aqueous hydrochloric acid gave a precipitate that was collected on a filter and washed with water; yield, 98 mg. (60%), m.p. 312–316° dec. A clarified solution of 88 mg. of crude product in 5% aqueous sodium bicarbonate gave on acidification, 77 mg. (50%) of pure product, m.p. 314–316° dec. The melting point was unchanged after one more precipitation; $\nu_{max.}$ 3300 (NH); 2800–2200 (broad acidic H); 1700 (carboxyl C=O); 1680, 1600, 1540, 1525 (amide I and II, C=C, C=N); 850 cm.$^{-1}$ (*p*-C_6H_4); $\lambda_{max.}$ 268, 326 mμ.

See Table I for analytical data; this is listed in Table I as method *A.*

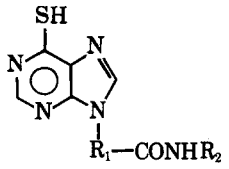
With 2-methoxyethanol as a diluent and reaction at 30–90° with varying base concentrations and reaction times, yields were 0–34%. Saponification with 10% sodium hydroxide at ambient temperature for 24 hr. gave a 66% yield of the cleavage product (XXIII). Similarly, 6 *N* sodium hydroxide on the steam bath for 3 hr. gave 50% of XXIII.

5 - (6 - Mercapto - 9H - purine - 9 - ylacetamido)salicylic Acid (XIII).—A mixture of the crude *p*-nitrophenyl ester (XXVIII, contains dicyclohexylurea) from 40 mg. (0.2 mmole) of XXIII, 76.6 mg. (0.5 mmole) of 5-aminosalicylic acid, 0.14 ml. (1 mmole) of triethylamine, and 5 ml. of *tert*-butyl alcohol was gently refluxed with magnetic stirring for 20 hr. The solvent was removed by spin-evaporation *in vacuo.* The residue was triturated with 10 ml. of acetone to remove *p*-nitrophenol, the mixture filtered, then the solid was washed with acetone. The solid was stirred with 5% aqueous sodium bicarbonate for about 30 min.; the mixture was filtered from dicyclohexylurea, then the filtrate was acidified with 1 *N* hydrochloric acid. The product was collected on a filter and washed with water; yield, 27 mg. (41% from XXIII), m.p. 310–313° dec. For analysis, the sample was reprecipitated once more from aqueous sodium bicarbonate to give a buff-colored solid, m.p. 314–316° dec., $\nu_{max.}$ 3400 (NH); 2600–2300 (broad acidic H); 1700–1660 (broad), 1600, 1540 cm.$^{-1}$ (broad) (C=O, NH, C=C); $\lambda_{max.}$ (EtOH) 250, 325 mμ.

Other compounds prepared in this way are listed in Table II under method *B.*

5 - (6 - Mercapto - 9H - purine - 9 - ylvaleramido)salicylic Acid (XII).—A hot solution of 126 mg. (0.5 mmole) of 6-mercapto-9H-purine-9-ylvaleric acid (1) in 3 ml. of *N,N*-dimethylformamide was cooled to room temperature, then 0.21 ml. (1.5 mmoles) of triethylamine was added. To the magnetically stirred mixture cooled in an ice–salt bath to −5° and protected from moisture was added 0.10 ml. (1 mmole) of ethyl chloroformate. After being stirred at −5° for 20 min., a cold solution of 76.6 mg. (0.5 mmole) of 5-aminosalicylic acid and 0.14 ml. (1 mmole) of triethylamine in 1 ml. of *N,N*-dimethylformamide was added. After being stirred

TABLE II.—PHYSICAL PROPERTIES OF



Compd.[a]	R_1	R_2	Method	% Yield	M.p., °C. dec.	Anal. Calcd.	Anal. Found
VII	$-(CH_2)_4-$	4-SA[b]	*C*	86	212–216	C, 52.8 H, 4.43 N, 18.0	53.1 4.76 17.6
XII	$-(CH_2)_4-$	5-SA[b]	*C*	67	255–259	C, 52.8 H, 4.43 N, 18.0	52.6 4.49 18.0
XIII	$-CH_2-$	5-SA[b]	*B*	41	314–316	C, 48.8 H, 3.21 N, 20.3	48.9 3.49 20.3
XIV	$-p$-$CH_2C_6H_4-$	4-SA[b]	*C*	43	253–257	C, 57.1 H, 3.59 N, 16.6	57.0 3.75 16.9
XV	$-(CH_2)_4-$	p-C_6H_4COOH	*C*	19	280–283[c]	C, 55.1 H, 4.62 N, 18.8	54.9 4.86 18.4
XVI	$-CH_2-$	p-C_6H_4COOH	*A*	50	314–316	C, 51.1 H, 3.36 N, 21.3	50.9 3.45 21.0
			B	32	314–316	... ...	... ...
XVII	$-(CH_2)_4-$	$-C_6H_3$-4-OH-3-NO_2	*C*	43	248–250[d]	C, 49.5 H, 4.16 N, 21.6	49.2 4.24 21.4
XVIII	$-CH_2-$	$-(CH_2)_3COOH$	*B*	43	266–269	C, 44.8 H, 4.44 N, 23.7	44.8 4.60 23.6
XXXVI	$-(CH_2)_4-$	C_6H_5-	*C*	81	298–302[d]	C, 58.8 H, 5.25 N, 21.4	58.7 5.34 21.6
XXXIII	$-(CH_2)_6-$	5-SA[b]	*C*	34	258–261[e]	C, 54.9 H, 5.09 N, 16.9	54.9 5.20 17.0
XXXIV	$-(CH_2)_6-$	4-SA[b]	*C*	39	235–236[e]	C, 54.9 H, 5.09 N, 16.9	54.9 5.26 17.1
XXXV	$-(CH_2)_6-$	C_6H_5-	*C*	93	270–271[e]	C, 60.9 H, 5.97 N, 19.7	60.6 6.09 19.5

[a] All compounds had ultraviolet and infrared spectra that agreed with the assigned structures. [b] SA, salicylic acid. [c] Recrystallized from 50% aqueous ethanol. [d] Reprecipitated from 0.1 *N* aqueous sodium hydroxide with 1 *N* hydrochloric acid. [e] Recrystallized from aqueous *N*,*N*-dimethylformamide.

for 24 hr. at ambient temperature, the mixture was spin-evaporated *in vacuo* and the residue stirred with 20 ml. of water; after standing for about 18 hr., the gum had solidified. The solid (190 mg.) was collected on a filter and washed with water, then dissolved in 1% aqueous sodium bicarbonate. The clarified solution was acidified with 0.1 *N* hydrochloric acid, then the product was collected on a filter and washed with water; yield, 130 mg. (67%), m.p. 251–257° dec. One more reprecipitation gave a buff-colored solid, m.p. 255–259° dec.; $\nu_{max.}$ 3300 (OH, NH); 2700–2300 (broad acidic H); 1700–1650, 1600, 1590, 1650, 1540 cm.$^{-1}$ (C=O, NH, C=C, C=N); $\lambda_{max.}$ (EtOH) 250, 325 mμ.

Other compounds prepared by this method are listed in Table I under method *C*.

4 - (6 - Methylthio - 9H - purine - 9 - ylvaleramido)salicylic Acid (XXXII).—To a solution of 387 mg. (1 mmole) of VII in 30 ml. of 0.1 *N* aqueous sodium hydroxide was added 0.065 ml. (1 mmole) of iodomethane. After being stirred for 2 hr., the solution was acidified; the product was collected on a filter and washed with water; yield, 336 mg. (84%), m.p. 167–174° dec. The solid was dissolved in 5% aqueous sodium bicarbonate, then the filtered solution was acidified. The buff-colored solid was collected on a filter and washed with water; yield, 280 mg. (68%), m.p. 169–173° dec.; $\lambda_{max.}$ (EtOH) 270–295 mμ (broad); $\nu_{max.}$ 3300 (OH, NH); 2400–2100 (acidic H); 1660 (C=O); 1600, 1575, 1540 cm.$^{-1}$ (C=C, C=N, amide II).

Anal.—Calcd. for $C_{18}H_{19}N_5O_4S$: C, 53.9; H, 4.77; N, 17.4. Found: C, 53.6; H, 4.83; N, 17.2.

4 - (6 - Benzylthio - 9H - purine - 9 - ylvaleramido)salicylic Acid.—This compound was prepared from VII and α-chlorotoluene as described for the preparation of XXXII; crude yield, 412 mg. (82%), m.p. 160–164°. Recrystallization from aqueous *N,N*-dimethylformamide gave 240 mg. (48%), m.p. 180–181°. A second crop of 160 mg. (32%), m.p. 166–170°, was isolated. A second recrystallization of the first crop gave the analytical sample, m.p. 180–181°; $\lambda_{max.}$ (EtOH) 270–295 mμ (broad); $\nu_{max.}$ 3300 (broad OH, OH); 1680–1650 (C=O); 1600, 1560, 1540–1500 cm.$^{-1}$ (C=C, C=N, amide II).

Anal.—Calcd. for $C_{24}H_{23}N_5O_4S$: C, 60.4; H, 4.85; N, 14.7. Found: C, 60.2; H, 4.86; N, 14.5.

6 - Chloro - 9H - purine - 9 - ylheptanonitrile.—This compound was prepared from 6-chloropurine and 7-bromoheptanonitrile as described for the preparation of 6-chloro-9H-purine-9-ylvaleronitrile (1); it was obtained as an oil that could not be crystallized, but had the proper ultraviolet and infrared spectra and was probably contaminated with a lesser amount of the 7H-7-alkyl isomer. The crude material was used for the other transformations described below.

6 - Amino - 9H - purine - 9 - ylheptanonitrile.—A solution of 3.32 Gm. (12.7 mmoles) of crude 6-chloro-9H-purine-9-ylheptanonitrile in 44 ml. of ethanol previously saturated with ammonia at 0° was heated in a steel bomb at 110° for 1 hr. The bomb contents were spin-evaporated *in vacuo* and the residue partitioned between 20 ml. of water and 40 ml. of chloroform. The aqueous layer was further extracted with chloroform (2 × 40 ml.). The combined chloroform extracts were dried with magnesium sulfate, then spin-evaporated *in vacuo*. The residual syrup was dissolved in 5 ml. of ethyl acetate and cooled at 5°. The amorphous solid was collected on a filter and washed with two 5-ml. portions of ice cold ethyl acetate; yield, 1.69 Gm. (55%), m.p. 112–115°, with previous softening, that was suitable for the next step. Two recrystallizations from ethyl acetate gave an amorphous white solid, m.p. 135°, with previous softening; $\nu_{max.}$ 3300, 3150 (NH); 2250 (C≡N); 1660, 1600, 1570 cm.$^{-1}$ (NH, C=C, C=N); $\lambda_{max.}$ (EtOH) 262 mμ.

Anal.—Calcd. for $C_{12}H_{16}N_6$: C, 59.0; H, 6.60; N, 34.4. Found: C, 58.8; H, 6.66; N, 34.4.

6 - Amino - 9H - purine - 9 - ylheptanoic Acid.—A solution of 100 mg. (0.41 mmole) of 6-amino-9H-purine-9-ylheptanonitrile in 4 ml. of 12 *N* hydrochloric acid was refluxed for 5 hr., then spin-evaporated *in vacuo*. The residue was dissolved in 1 ml. of water and diluted with 2 ml. of saturated aqueous sodium acetate. The product was collected on a filter and washed with a small amount of water; yield, 60 mg. (56%), m.p. 220–223°. Recrystallization from water gave 37 mg. (34%) of white crystals, m.p. 230–234°; $\nu_{max.}$ 3300, 3150 (NH); 2700–2100 (acidic H); 1700 (carboxyl C=O); 1680, 1600, 1575 cm.$^{-1}$ (C=C, C=N, NH).

Anal.—Calcd. for $C_{12}H_{17}N_5O_2$: C, 54.7; H, 6.52; N, 26.6. Found: C, 54.4; H, 6.34; N, 26.3.

6 - Mercapto - 9H - purine - 9 - ylheptanoic Acid.—A mixture of 2.64 Gm. (0.01 mole) of 6-chloro-9H-purine-ylheptanonitrile, 30 ml. of ethanol, and 0.76 Gm. (0.01 mole) of thiourea was refluxed with magnetic stirring for 3 hr. The cooled mixture was filtered and the product washed with ethanol; yield, 1.36 Gm. (52%) of 6-mercapto-9H-purine-9-ylheptanonitrile, m.p. 245–250°; ν max. 2250 (C≡N); 1600, 1580, 1550 cm.$^{-1}$ (C=C, C=N); the nitrile was not purified further.

A solution of 261 mg. (1 mmole) of the nitrile in 10 ml. of 12 *N* hydrochloric acid was refluxed 13 hr., then spin-evaporated *in vacuo*. After addition of 5 ml. of water to the residue, the spin-evaporation was repeated. The residue was recrystallized from 50 ml. of water; yield, 100 mg. (36%), m.p. 232–233°. No attempt was made to obtain a second crop. A second recrystallization from water gave white crystals, m.p. 233–235°; $\lambda_{max.}$ (EtOH) 326 mμ; $\nu_{max.}$ 2800–2200 (acidic H); 1700 (carboxyl C=O); 1595, 1570, 1540 cm.$^{-1}$ (NH, C=C, C=N).

Anal.—Calcd. for $C_{12}H_{16}N_4O_2S$: C, 51.4; H, 5.76; N, 20.0. Found: C, 51.3; H, 5.69; N, 20.3.

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