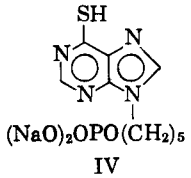
# Nonclassical Antimetabolites XXVII

## Simulation of 5′-Phosphoribosyl Binding VII. Analogs of 6-Mercapto-9H-purine-9-ylpentanol Phosphate and Their Evaluation as Inhibitors of Succinoadenylate Kinosynthetase

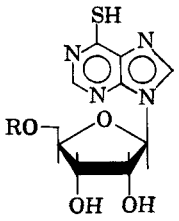
By B. R. BAKER* and PRAFULLCHANDRA M. TANNA

**6-Mercaptopurines substituted on the 9-position with 5′-carbamoyloxypentyl (IX), 5′-thionocarbamoyloxypentyl (X), valeramide (XI), valeryl-DL-aspartic acid (XIII), 5′-thioureidopentyl (XXIV), and 5′-dichloroacetamidopentyl (XXV) groups were synthesized as possible analogs of 6-mercapto-9H-purine-9-ylpentyl phosphate (IV). These six compounds along with the previously synthesized 5′-hydroxypentyl (V), 4′-carboxybutyl (VIII), *p*-carboxybenzyl (XXVI), and 2′-carboxyethyl (XXVII) groups substituted on the 9-position of 6-mercaptopurine, were compared to the pentanol phosphate (IV) as inhibitors of succinoadenylate kinosynthetase. The order of effectiveness was IV = XXVI = IX > XIII = VIII > XXIV = V > X = XXV = XI. Based on these results, it is proposed that the 5′-carbamoyloxy group of XXVI can simulate phosphate binding by its two oxygen functions binding to the enzyme in place of the oxygens of the two ionizable hydroxyl groups of the phosphate of IV. It is also proposed that the carbamoyloxy group does not bind to the enzyme as well as phosphate, but that the 5′-carbamoyloxypentyl group complexes to the enzyme in the ground-state staggered conformation and the pentanol phosphate complexes in a less-favored skew-like conformation.**

IN A PREVIOUS study, the relative contribution of phosphate and other oxygen-containing functions of the 5′-phosphoribosyl moiety of I to succinoadenylate kinosynthetase has been reported (1). It was observed that the pentanol phosphate analog (IV) of thioinosinate




IV




I, R = $(NaO)_2OP-$
II, R = H
III, R = $NH_2C(=O)-$

(I) was one-twelfth as effective as I in inhibiting the enzyme; in contrast, the corresponding nucleoside (II) was less than one-sixtieth as effective as the nucleotide (I) (1). Thus, the phosphate contributed more to the binding of 5′-phosphoribosyl moiety of thioinosinate (I) to succinoadenylate kinosynthetase than did the remainder of the oxygen functions in the moiety, as predicted earlier (2); of the three remaining oxygen functions of I, most of the binding was due to the 2′-hydroxyl group (1).

In another study attempting to simulate phosphate binding, the 5′-carbamate (III) of thioinosine (II) was synthesized and evaluated (3); III showed no inhibition of succinoadenylate kinosynthetase at the maximum concentration that could be measured, being less than one-fiftieth as effective as thioinosinate (I). Since IV had a simple 9-side-chain which was easier to modify than I and was a reasonably good inhibitor of the enzyme, some analogs of IV were synthesized that had relatively unionized side-chain functional groups for possible simulation of phosphate binding; the biological rational and need for such unionized analogs of nucleotides has been previously discussed (2). The synthesis and enzymic evaluation of these analogs are the subjects of this paper.

### DISCUSSION

Acylation of 6-mercapto-9H-purine-9-ylpentanol (V) (1) with phenyl chloroformate in pyridine gave the mixed carbonate (VI) in 98% yield; reaction of VI with amonia water afforded the desired 5′-carbamate (IX) (3).

The synthesis of thiocarbamate esters has been a relatively rare occurrence; the recent commercial availability of phenoxy thionocarbonyl chloride suggested that the thiocarbamate (X) might be synthesized in the same manner as the carbamate (IX). This did prove to be the case; the intermediate thiocarbonate (VII) was obtained as a crystalline solid in 73% yield that could be con-

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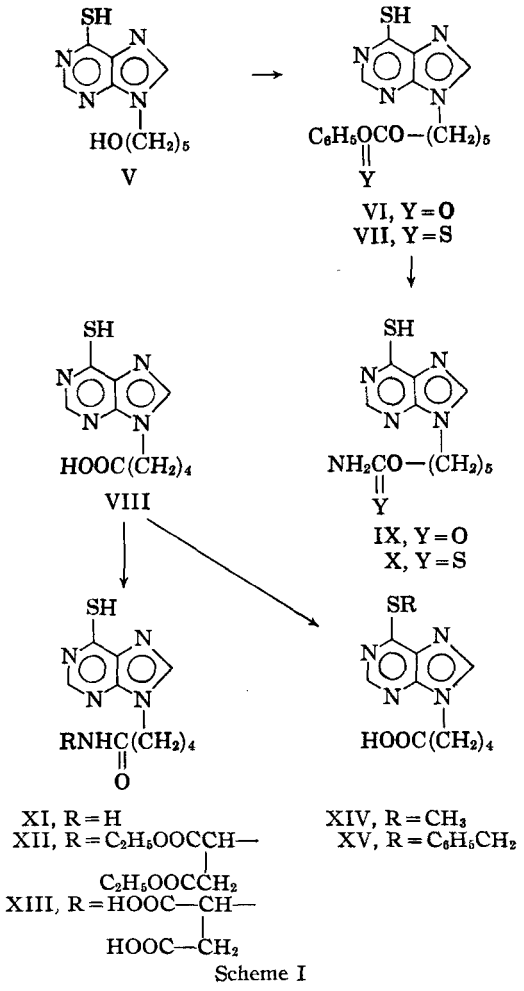
Previous paper: Baker, B. R., and Schwan, T. S., *J. Med. Chem.*, **9**, January(1966). Previous paper on phosphate simulation: Baker, B. R., Jackson, G. D. F., and Chheda, G. B., *J. Pharm. Sci.*, **54**, 1617(1965).

* Address inquiries to Department of Chemistry, University of California, Santa Barbara.

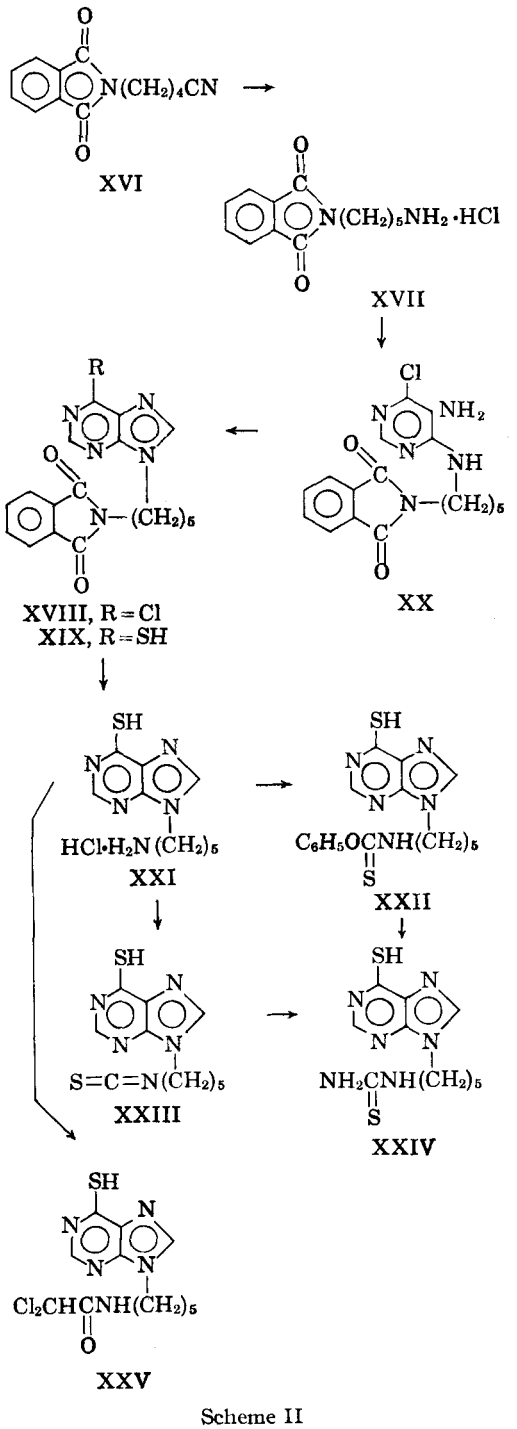



Scheme I

verted to the desired thiocarbamate (IX) with methanolic ammonia in excellent yield.

Since 6 - mercapto - 9H - purine - 9 - ylvaleric acid (VIII) (2) showed some inhibition of succinoadenylate kinosynthetase, four derivatives were prepared. Conversion of VIII to the mixed anhydride with ethyl chloroformate and triethylamine in *N,N*-dimethylformamide followed by treatment with ammonia gave the amide (XI). Similarly, reaction of this mixed anhydride with diethyl DL-aspartate gave the diester (XII) which was saponified to the aspartate derivative (XIII). Alkylation of VIII in aqueous sodium hydroxide with methyl iodide or benzyl chloride gave the 6-alkylthiopurine-9-valeric acids (XIV and XV) in 21 and 95% yields, respectively. (Scheme I.)

The 9-pentyl-6-mercaptopurine bearing a thiourea grouping on the terminal position of the alkyl group (XXIV) was selected for a candidate inhibitor since the thiourea group is more acidic than the carbamate group (3). The synthesis started with *N*-(4-cyanobutyl)phthalimide (XVI) (4). Hydrogenation of XVI in ethanol containing 1.4 equivalents of hydrochloric acid in the presence of platinum oxide catalyst proceeded smoothly to the amine hydrochloride (XVII) in 69% yield. Condensation of XVII with 5-amino-4,6-dichloropyrimidine in *n*-butanol in the presence of excess triethylamine (5, 6) afforded crystalline XX in 65% yield. Ring closure of XX with ethyl orthoformate in the presence of 1.3 equivalents of hydrochloric acid (7) afforded the 6-chloropurine derivative (XVIII) in 78% yield. The corresponding 6-




Scheme II

mercaptopurine derivative (XIX) was obtained from XVIII and thiourea in ethanol in 89% yield.

Cleavage of the phthalimido group of XIX with hydrazine initially presented some difficulties. If sufficient hot 2-methoxyethanol was employed to dissolve XIX completely, reaction was so slow that hydrazinolysis of the 6-mercapto group became a competitive reaction, as shown by the gradual appearance of an ultraviolet absorption peak at 271 mμ. The problem was resolved by treating a suspension of XX in hot 2-methoxyethanol with five equivalents of hydrazine; solution rapidly took place, then the intermediate *N*-aminophthalamide rapidly separated, which removed the compound from solution and slowed further hydrazinolysis of the 6-mercapto group. Treatment of this intermediate phthalamide with hot 1 *N* hydrochloric acid caused rapid formation of the insoluble phthalhydrazide; from the filtrate the desired amine hydrochloride (XXI) could be isolated in near quantitative yield.

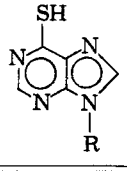
When the amine hydrochloride (XXI) in 50% aqueous acetone containing four equivalents of triethylamine was reacted with phenoxy thionocarbonyl chloride, a mixture of the thiocarbamate (XXII) and the isothiocyanate (XXIII) was obtained. Since both XXII and XXIII should give the thiourea (XXIV) on reaction with ammonia, the mixture was treated with methanolic ammonia at 0°. The desired thiourea (XXIV) was obtained in 45% over-all yield for the three steps from XX.

Another candidate inhibitor, 9-(dichloroacetamidopentyl)-6-mercaptopurine (XXV) was prepared from the aminopentyl-purine (XXI) and dichloroacetyl chloride in *N,N*-dimethylformamide in the presence of potassium carbonate. (Scheme II.)

**Enzyme Results.**—The relative abilities of the various 6-mercaptopurine derivatives to inhibit succinoadenylate kinosynthetase are listed in Table I. In a previous paper (1) it had been noted that thioinosinate (I) was greater than 60 times as effective as thioinosine (II), but I was only 12 times as effective as 6-mercaptopurine-9-pentanol phosphate (IV). Thus, the phosphate group of the ribosyl phosphate moiety contributed more to the binding to this enzyme than the other three oxygen functions. Note, however, that 6-mercaptopurine-9-pentanol (V) is a better inhibitor of the enzyme than thioinosine (II). It is unlikely that the hydroxyl group of V binds to the enzyme in place of the 2′, 3′, or 4′-oxygen functions of thioinosinate (I) since V would have to assume an energetically unfavorable conformation to do so, which would result in poorer binding than thioinosine (II).

A clue to a possible solution of this dilemma arose from the recent study on the mode of binding of inhibitors to thymidine kinase (12). It was noted that 1-(4′-hydroxybutyl) uracil would inhibit thymidine (deoxyuridine) kinase at a concentration where neither 1-(5′-hydroxypentyl) nor 1-(3′-hydroxypropyl) uracil showed any inhibition. This result was attributed to the fact that in its stable staggered conformation, the 4′-hydroxy of the 4′-hydroxybutyl group is closely juxtapositioned to the binding point for the 5′-hydroxyl group of

TABLE I.—INHIBITION OF SUCCINOADENYLATE KINOSYNTHETASE BY

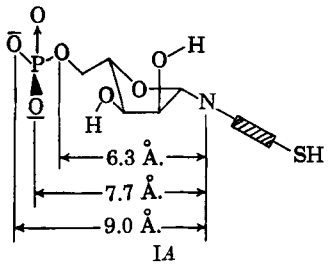



| Compd. | R | m*M* Concn. | Av. % Inhibition[a] | Estimated m*M* Concn. for 50% Inhibition[b] |
|---|---|---|---|---|
| I | Ribosyl[c]-5′-phosphate | 0.070[d] | 50 | 0.070 |
| II | Ribosyl[c] | 1.05[d] | 0 | >4.2[e] |
| III | Ribosyl[c]-5′-carbamate | 0.90[f] | 0 | >3.6[e] |
| IV | —$(CH_2)_5OPO(OH)_2$ | 0.83[d] | 50 | 0.83 |
| V | —$(CH_2)_5OH$ | 1.2[g] | 30(6) | 2.8 |
| VIII[h] | —$(CH_2)_4COOH$ | 1.0[i] | 46(6) | 1.2 |
| IX | —$(CH_2)_5OCONH_2$ | 1.0[g] | 52(9) | 0.93 |
| X | —$(CH_2)_5OCSNH_2$ | 0.96[g] | 17(4) | 4.3 |
| XI | —$(CH_2)_4CONH_2$ | 1.2[g] | 16(4) | 6.3 |
| XIII | —$(CH_2)_4CONHCHCOOH$ <br> $CH_2COOH$ | 0.90[i,j] | 40(6) | 1.3 |
| XXIV | —$(CH_2)_5NHCSNH_2$ | 0.96[g] | 28(8) | 2.5 |
| XXV | —$(CH_2)_5NHCOCHCl_2$ | 0.96[g] | 19(4) | 4.0 |
| XXVI[h] | —$CH_2C_6H_4COOH$—*p* | 0.83[i] | 50 | 0.83 |
| XXVII[k] | —$(CH_2)_2COOH$ | 0.80[i] | 0 | >3.2[e] |

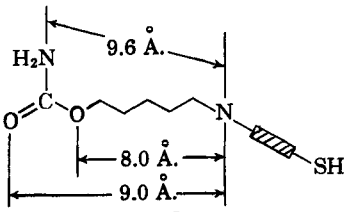
Succinoadenylate kinosynthetase from *E. coli* B was isolated and assayed with 30.6 μ*M* of IMP, 100 μ*M* of GTP, 3.75 m*M* of L-aspartate, and 10 m*M* of $MgCl_2$ in glycine buffer (pH 8.0) as previously described (1, 8, 9). The technical assistance of Shirley Humphrey with these assays is acknowledged. [a] The number of runs from which each average was derived is indicated in parentheses. In these cases, the assays were run at the highest concentration still allowing complete light transmission. [b] The concentration required for 50% inhibition is estimated by plotting $V_0/V_I$ against $I$ where $V_0$ = velocity without inhibitor, $V_I$ = velocity with inhibitor, and $I$ = concentration of inhibitor (10). If the 50% inhibition point ($V_0/V_I = 2$) could not be reached due to lack of light transmission, the line of the plot was extended to this point where $V_0/V_I = 2$; the lower is the maximum inhibition below 50% that could be reached, the less reliable is the estimated 50% inhibition point [c] 9-β-D-Ribofuranosyl. [d] Data from *Reference 1*. [e] Since 20% inhibition is readily detectable, the concentration necessary for 50% inhibition is estimated at 4 times greater than the concentration measured. [f] Data from *Reference 3*. [g] Solution of inhibitor made up in *N,N*-dimethylformamide and assay run in 10% aqueous *N,N*-dimethylformamide. [h] For preparation of this compound see *Reference 2*. [i] Solution of inhibitor made up in 0.1 *N* KOH, then adjusted to pH 8 with aqueous HCl. [j] *N*-Acetyl-DL-aspartic acid showed no inhibition at a concentration of 24 m*M*. [k] See *Reference 11* for preparation.

thymidine or 2′-deoxyuridine. In contrast, in order for the hydroxyl of the 5′-hydroxypentyl group to complex to this binding point, the pentyl group must approach the energetically unfavorable eclipsed conformation, this energy being obtained at the expense of the energy released in complex formation with the enzyme.

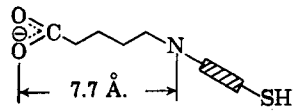
The dimensions of the energetically most favorable conformation of thioinosinate are shown in I*A*; the 5′-methylene group is assigned the equatorial conformation rather than the purine ring (12). Note that the distances from the center of the purine-$N^9$ atom to the center of ionizable oxygens of the phosphate are 7.7 and 9.0 Å. when the phosphate is extended to its longest conformation (2). The 5′-oxygen of the 6-mercaptopurine-9-pentanol (V) is 8.0 Å. from the $N^9$-nitrogen as can be seen from IX*A*; a slight twisting of $C_1'$—$C_2'$ bond to less than a skewed conformation brings this oxygen to 7.7 Å., the same position as one of the ionizable hydroxyl functions on the phosphate.



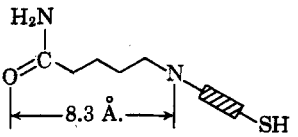

I*A*



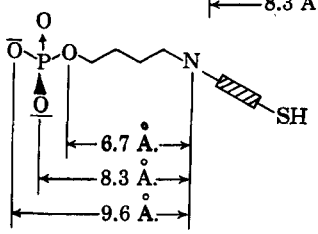

IX*A*



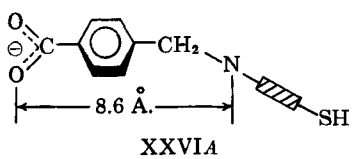

VIII*A*




XI*A*




XXVIII*A*




XXVI*A*

As a working hypothesis, it is proposed that 6-mercaptopurine-9-pentanol (V) binds more strongly to the enzyme than thioinosine (II), because of 5′-oxygen of V can bridge to the phosphate binding point of I at 7.7 Å., but the 5′-oxygen of II can only extend 6.3 Å. from $N^9$. The 5′-carbamate (IX) of 6-mercaptopurine-9-pentanol can then be in a position to complex with the sites for both ionizable hydroxyl groups of the phosphate, as seen in IX*A*; the amido $NH_2$-group is in about the same position as the oxygen of the P → O group. Therefore, the carbamate (IX*A*) could be expected to give an increment in binding over the pentanol (V). It was previously noted (3) that the 5′-carbamate (III) of thioinosine was ineffective as an inhibitor at a concentration of 0.90 m*M*; in contrast, the pentanol carbamate shows 50% inhibition at 0.93 m*M*. The carbamate oxygen of III should be able to complex with the enzyme in place of the ionizable hydroxyl that is 7.7 Å. from $N^9$; such a complex would force the amide $NH_2$-group of III in the unfavorable position on the enzyme which presumably forms an EH....$^-$OP interaction; this would be unfavorable since the EH hydrogen and NH hydrogen would have to occupy the same space.

That the C=O of the pentanol carbamate (IX*A*) contributes to binding by a C=O → HE bond is further suggested by the fact that the corresponding thiocarbamate (X) binds more poorly to the enzyme since sulfur is a poor electron donor for a hydrogen bond; note that the thiocarbamate (X) is about as effective as the pentanol (V). Further confirmation that a C=O properly placed can complex in place of the $PO^-$ group is shown with the valeramide (XI*A*); XI is about as effective as the pentanol (V).

Even though the pentanol carbamate (IX) inhibits almost as well as the pentanol phosphate (IV), it cannot be inferred that the carbamate group binds as well as the phosphate group. Note that the carbamate (IX) has the favorable *trans*-staggered conformation, whereas the pentanol phosphate (IV) must assume a less favorable conformation to simulate the binding of I*A*. Thus, the results can be rationalized by assuming that the carbamate binds less effectively than phosphate, but IV loses some net binding energy by having to fold to an energetically less favorable conformation. Such a hypothesis could be further strengthened by synthesis and evaluation of 6-mercaptopurine-9-butanol phosphate (XXVIII*A*); since the latter needs only a slight twisting of the $C_1'$—$C_2'$ bond to have the exact dimensions between $N^9$ and the ionizable phosphate hydroxyl groups, it should be a better inhibitor than IV due to its favorable ground-state conformation that closely approximates the conformation needed for complexing to the enzyme.

In the first study on phosphate simulation it was noted that 9-(4′-carboxybutyl)adenine and 9-(*p*-carboxybenzyl)adenine were as effective as 5′-adenylic acid as inhibitors of glutamic dehydrogenase (2); in this case, there was probably only a single ionizable phosphate hydroxyl that was binding to glutamic dehydrogenase, in contrast to the

apparent binding of both ionizable phosphate hydroxyl groups of inosinate to succinoadenylate kinosynthetase. Table I shows that 9-(*p*-carboxybenzyl)-6-mercaptopurine (XXVI) (2) can inhibit the enzyme as well as the pentanol phosphate (IV); again it should not be inferred that the carboxylate group of XXVI binds as well as the phosphate group of IV since the ground-state conformation of XXVI (XXVI*A*) is favorable for binding, but the ground-state conformation of IV is not. Therefore, it can be tentatively rationalized that the carboxylate of XXVI*A* binds to one of the phosphate binding points, but not both. Similarly, the carboxylate of the valeric side chain (VIII*A*) could possibly bind in place of the oxygen 7.7 Å. removed from $N^9$ in I*A*, the valeric acid being in its ground-state conformation. An important comparison is the lack of inhibition by 6-mercaptopurine-9-propionic acid (XXVII); this result shows that both VIII*A* and XXVI*A* have a properly positioned carboxylate for binding. Further confirmation of the mode of binding of XXVI*A* might be obtained by insertion of a hydroxyl group *ortho* to the carboxyl; this weakly acidic hydroxyl group is then juxtapositioned close to the 7.7 Å. oxygen of I*A*, and the resultant compound should be a better inhibitor. Such a salicylic acid derivative is unlikely to be so good an inhibitor as thioinosinate (I) since the 2′-hydroxyl of I also contributes appreciably to binding (1), but should be a better inhibitor than the pentanol phosphate (IV).

The dichloroacetamidopentyl purine (XXV) was synthesized on the basis of the proposal that the dichloroacetamido group of chloramphenicol may simulate the phosphate binding of uridylic acid by dipole interaction of the chloro groups at a receptor site that normally complexes the phosphate (13). Table I shows that XXV has the same magnitude of binding as the valeramide (XI); this result would indicate that only the C=O of the XXV side-chain is binding to the enzyme in the position where the 9.0 A. oxygen of IX*A* is proposed to bind. *A posteriori*, the proper conformational aspects were not considered. A better choice for enzymic evaluation would have been 9-(3′-dichloroacetamidopropyl)-6-mercaptopurine. The latter, in its most staggered conformation, has the two chloro groups 8.0 and 10.0 Å. from $N^9$; however, slight rotation about the two bonds of the carbonyl group, which requires little or no energy, would bring the chloro groups to 7.8 and 8.8 Å.

The thiourea group (XXIV) attached to the 5′-position of 9-pentyl-6-mercaptopurine was selected for enzymic evaluation on the basis that an NH group would be more acidic than that of a carbamate or an urea. If the P—$O^-$ group of thioinosinate were binding to a weakly basic group on the enzyme through a P—$O^-$....$H^+$E bond, then NHCS—NH....E could form a stronger bond than a hydrogen bond but weaker than a cationic–anionic interaction (14, 15). As can be seen in Table I, XXIV was about as effective as the pentanol (V), indicating a single interaction of the thiourea group with the enzyme, perhaps by the mode proposed; XXIV was considered to be an inhibitor too weak for further study, regardless of the mode of binding.

The final compound synthesized for enzymic evaluation was the 6-mercaptopurine-9-valeryl-DL-aspartate (XIII). This compound was selected as a possible "hybrid" inhibitor (16). Since L-aspartate is a cosubstrate for succinoadenylate kinosynthetase, there must be some binding points for one or both carboxyls on the enzyme that hold the $\alpha$-amino group one interatomic distance from the 6-position of the substrate, inosinate; it should be theoretically possible to construct an inhibitor that can bind both in the hypoxanthine region and the L-aspartate region. DL-Aspartate was selected since it cannot be predicted *a priori* whether such a bridge should be to D- or L-aspartate, even though the cosubstrate has the L-configuration. Table I shows that XIII was a better inhibitor than the valeramide (XI) indicating that XIII has an additional binding point or just forms a more energetically favorable complex with the enzyme with a single point of attachment in the side-chain. Considerable further study will be needed to ascertain whether XIII is a hybrid inhibitor and whether the valeryl bridge is optimum for maximum inhibition.

A comment is in order on why 1-(*p*-carboxybenzyl)-uracil did not inhibit thymidylate synthetase at 25 times the concentration of the substrate, 2′-deoxyuridylate (17), whereas 9-(*p*-carboxybenzyl)-6-mercaptopurine (XXVI) was one-twelfth as effective as thioinosinate as an inhibitor of succinoadenylate kinosynthetase (Table I). Note that thioinosinate (I) is a twelvefold better inhibitor of the kinosynthetase than the pentanol phosphate (IV) (1), but 60 times as much uracil-1-pentanol phosphate as substrate (2′-deoxyuridylate) is needed for 50% inhibition of thymidylate synthetase (18). Thus, the loss in binding by removal of the ribosyl oxygens (other than phosphate) is 5 times more severe for thymidylate synthetase than succinoadenylate kinosynthetase; this greater loss of binding can be due to a greater contribution by these oxygen functions to binding to thymidylate synthetase, or to the more severe requirements for an eclipsed-like conformation for binding to the enzyme, or both. Since these requirements for the kinosynthetase are less severe, the further studies suggested heretofore may lead to side chains on 6-mercaptopurine with better binding; if such better side chains are found, it would also be of interest to evaluate these side chains on uracil as inhibitors of thymidylate synthetase.

Finally, it should be emphasized that strong evidence for these proposed modes of binding for phosphate simulation is certainly not yet available. These proposed modes of binding should be considered working hypotheses for future design of compounds which may simulate phosphate binding more effectively. The stakes are high for finding relatively unionized groups that can simulate phosphate binding and can enter cells by passive diffusion; such inhibitors would not need activation for inhibition of enzymes operating on nucleotides and thus would not be subject to the type of drug resistance resulting by mutational loss of a kinase or pyrophosphorylase (2). Therefore, further study on simulation of phosphate binding—even though somewhat nebulous at this stage—is worthy of pursuit.

## EXPERIMENTAL

Melting points were determined in capillary tubes in a Mel-Temp block, and those below 230° are corrected. Infrared spectra were determined in

KBr pellet with a Perkin-Elmer spectrophotometer; ultraviolet spectra were determined in water, unless otherwise indicated, with a Perkin-Elmer 202 spectrophotometer. Thin - layer chromatograms (TLC) were run on Brinkmann Silica Gel G, and spots were detected with iodine vapor or by visual examination under ultraviolet light. Paper chromatograms were run on Whatman No. 1 paper by ascending technique, and spots were detected by visual examination under ultraviolet light.

**6 - Mercapto - 9H - purine - 9 - ylpentyl Phenyl Carbonate (VI).**—To a magnetically stirred suspension of 119 mg. (0.5 mmole) of V (1) in 3 ml. of reagent pyridine cooled in an ice bath and protected from moisture was added 0.07 ml. (0.53 mmole) of phenyl chloroformate. After being stirred in the ice bath for 30 min., the mixture was stirred at ambient temperature for 3.5 hr. The solvent was removed by spin-evaporation *in vacuo* and the residue was triturated with water; yield, 178 mg. (98%), m.p. 222–223° dec., that was suitable for further transformation. Recrystallization from ethanol afforded 130 mg. (72%), m.p. 233–234° dec. One more recrystallization gave the analytical sample, m.p. 237–238° dec. $\nu$ max. 1755 (ester C=O); 1600, 1575, 1540 (C=C, C=N); 755, 710 cm.$^{-1}$ ($C_6H_5$).

*Anal.*—Calcd. for $C_{17}H_{18}N_4O_3S$: C, 57.0; H, 5.07; N, 15.6. Found: C, 57.3; H, 5.25; N, 15.3.

**O-(6-Mercapto-9H-purine-9-ylpentyl) Carbamate (IX).**—A solution of 358 mg. (1 mmole) of VI in 80 ml. of concentrated ammonia water was allowed to stand for about 18 hr., then spin evaporated *in vacuo*. Trituration of the residue with ether (2 × 10 ml.) gave 262 mg. (93%) of product, m.p. 235–236°. Recrystallization from 50 ml. of 50% ethanol afforded 235 mg. (84%) of white crystals, m.p. 237–238°. $\nu$ max. 3500, 3350 (NH); 1690 (carbamate C=O); 1600, 1570, 1540 (C=C, C=N); no carbophenoxy bands near 1755, 755, or 710 cm.$^{-1}$.

*Anal.*—Calcd. for $C_{11}H_{15}N_5O_2S$: C, 47.0; H, 5.38; N, 24.9. Found: C, 46.9; H, 5.47; N, 24.7.

**9-(5'-Hydroxypentyl)hypoxanthine.**—A solution of 2.5 Gm. (10.9 mmoles) of 5-amino-6-chloro-4-(5'-hydroxypentyl)pyrimidine (1) in 10 ml. of ethyl orthoformate and 10 ml. of acetic anhydride was refluxed for 3 hr. (6), then spin evaporated *in vacuo*. The residual glassy 6-chloropurine derivative was refluxed with 25 ml. of 1 *N* hydrochloric acid for 2.5 hr., then spin evaporated *in vacuo*. The residue was dissolved in 20 ml. of saturated aqueous sodium acetate. After standing overnight at 5°, the mixture was filtered; yield, 0.87 Gm. (36%), m.p. 206–210°. Recrystallization from water gave white crystals, m.p. 212–214°. $\nu$ max. 3500 (NH, OH); 1700, 1590, 1550 cm.$^{-1}$ (C=O, C=C, C=N).

*Anal.*—Calcd. for $C_{10}H_{14}N_4O_2$: C, 54.0; H, 6.35; N, 25.2. Found: C, 53.9; H, 6.24; N, 25.0.

**O-(6-Hydroxy-9H-purine-9-ylpentyl) Carbamate.**—Treatment of 111 mg. (0.5 mmole) of the aforementioned compound with phenyl chloroformate, then aqueous ammonia as described for the preparation of IX, gave 110 mg. (83% over-all yield) of product, m.p. 206–210°. Two recrystallizations from 95% ethanol afforded white crystals, m.p. 215–217°. $\nu$ max. 3460, 3350 (NH); 1700, 1620, 1590, 1550 (NH, C=O, C=C, C=N); 1220 cm.$^{-1}$ (ester C—O—C).

*Anal.*—Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.8; H, 5.70; N, 26.4. Found: C, 49.5; H, 5.63; N, 26.3.

**6-Mercapto-9H-purine-9-ylpentyl Phenyl Thionocarbonate (VII).**—This compound was prepared from V (1) and phenoxy thionocarbonyl chloride (Pierce Chemical Co.) as described for VI, except that the pyridine was removed in a 30° bath; yield, 544 mg. (73%), m.p. 191–192°. Recrystallization from ethanol gave buff-colored crystals, m.p. 198–200°. $\lambda$ max. (EtOH): 326 m$\mu$; $\nu$ max. 2600–2200 (broad acidic NH); 1600, 1560, 1540 (C=C, C=N); 1280, 1200 (ester C—O—C); 775, 690 cm.$^{-1}$ ($C_6H_5$). The compound moved as a single spot on TLC in benzene–methanol (3:1) with the same $R_f$ as VI. However, the infrared spectrum showed no carbonyl absorption near 1750 cm.$^{-1}$ characteristic of VI.

*Anal.*—Calcd. for $C_{17}H_{18}N_4O_2S_2$: C, 54.5; H, 4.84; S, 17.1. Found: C, 54.3; H, 5.01; S, 16.8.

**O-(6-Mercapto-9H-purine-9-ylpentyl) Thionocarbamate (X).**—A solution of 1.00 Gm. (2.67 mmoles) of VII in 60 ml. of methanol previously saturated with ammonia at 0° was allowed to stand at 0–5° for 5 hr., then spin evaporated *in vacuo*. Trituration of the residue with ether gave 0.81 Gm. (87%) of product, m.p. 230–232° dec. Recrystallization from *N,N*-dimethylformamide gave buff-colored crystals, m.p. 238–240° dec., which was unchanged after another recrystallization. The compound had $\lambda$ max. (EtOH) 326 m$\mu$ and $\nu$ max. 3350 (broad NH); 2800–2100 (broad acidic NH); 1610, 1590, 1560, 1540 (C=C, C=N); no $C_6H_5$ near 775 or 690 cm.$^{-1}$ and no C=O (characteristic of IX) near 1690 cm.$^{-1}$. The compound also traveled at a single spot on TLC with the same $R_f$ as IX in 3:1 benzene–methanol and was free of VII and V.

*Anal.*—Calcd. for $C_{11}H_{15}N_5OS_2 \cdot 0.62$ dimethylformamide: C, 45.1; H, 5.67; N, 22.9; S, 18.7. Found: C, 45.5; H, 5.12; N, 22.6; S, 19.0.

Further recrystallization from ethanol gave an alcohol solvate of the same melting point and same TLC pattern.

*Anal.*—Calcd. for $C_{11}H_{15}N_5OS_2 \cdot C_2H_5OH$: C, 45.5; H, 5.88; S, 18.6. Found: C, 45.6; H, 5.21; S, 18.5.

The infrared spectrum showing that no VII or IX were present, combined with TLC showing the same $R_f$ as VII, left little doubt as to the assigned structure, even though a solvent-free crystal form could not be obtained. After standing overnight at ambient temperature in excess 0.01 *N* sodium hydroxide, a solution of X gave the unchanged thionocarbamate (X) on acidification as shown by melting point and infrared spectrum.

**6-Mercapto-9H-purine-9-ylvaleramide (XI).**—A mixture of 126 mg. (0.5 mmole) of VIII (2) and 3 ml. of *N,N*-dimethylformamide was warmed to effect solution, then cooled to about 25°, and treated with 0.21 ml. (1.5 mmoles) of triethylamine. The mixture was cooled to −5° protected from moisture and magnetically stirred. Then 0.10 ml. (1 mmole) of ethyl chloroformate was added; after being stirred for 20 min., anhydrous ammonia was slowly bubbled through the mixture for 5 min. while the mixture was still in the ice-salt bath. Then the mixture was stirred at ambient temperature for 18 hr. Dilution with 10 ml. of cold water and acidification with 1 *N* hydrochloric acid gave a solid

that was collected on a filter. The solid was washed with cold 2% aqueous sodium bicarbonate then water; yield, 100 mg. (80%), m.p. 245–250°. Recrystallization from water gave white crystals, m.p. 248–251°. $\nu$ max. 3400, 3200 (NH); 1650 (amide C=O); 1600, 1560, 1540 cm.$^{-1}$ (C=C, C=N, amide NH).

*Anal.*—Calcd. for $C_{10}H_{13}N_5OS$: C, 47.8; H, 5.22; N, 27.9. Found: C, 47.7; H, 5.09; N, 27.8.

**6 - Mercapto - 9H - purine - 9 - ylvaleryl - DL-aspartic Acid (XIII).**—To a magnetically stirred suspension of 505 mg. (2 mmoles) of VIII in 8 ml. of *N,N*-dimethylformamide and 0.84 ml. (6 mmoles) of triethylamine protected from moisture and cooled in an ice bath was added 0.21 ml. (2.1 mmoles) of ethyl chloroformate. After being stirred an additional 20 min. at 0°, the mixture was treated with 452 mg. (2 mmoles) of diethyl DL-aspartate hydrochloride, then stirred at ambient temperature for 24 hr. Solvent was removed by spin evaporation *in vacuo*. The residual crude ester (XII) was dissolved in 15 ml. of 1 *N* aqueous sodium hydroxide. After standing for 4.5 hr. at ambient temperature, the solution was acidified to about pH 5, concentrated to about 5 ml. by spin evaporation *in vacuo*, then further acidified to pH 2 with 1 *N* hydrochloric acid. The product was collected on a filter and washed with 5 ml. of water, then acetone (2 × 5 ml.); yield, 440 mg. (60%), m.p. 212–213° dec. Recrystallization from water gave the analytical sample, m.p. 215–216° dec. $\lambda$ max. ($H_2O$) 323 m$\mu$; $\nu$ max. 3300 (NH); 2800–2200 (acidic H); 1750, 1725 (carboxyl C=O); 1650 (amide C=O); 1600, 1560, 1540 cm.$^{-1}$ (C=C, C=N, amide NH). The compound traveled on paper in 5% aqueous $K_2HPO_4$ as a single spot at $R_f$ 0.80.

*Anal.*—Calcd. for $C_{14}H_{17}N_5O_5S$: C, 45.8; H, 4.67; N, 19.1. Found: C, 45.8; H, 4.60; N, 18.9.

**6-Benzylthio-9H-purine-9-ylvaleric Acid (XV).**—To a stirred solution of 505 mg. (2 mmoles) of VIII (2) in 50 ml. of 0.1 *N* aqueous sodium hydroxide was added 0.35 ml. (3 mmoles) of $\alpha$-chlorotoluene. After being stirred for 2 hr., the mixture was clarified by filtration, then acidified to pH 2 with 1 *N* hydrochloric acid. The product was collected on a filter and washed with water; yield, 650 mg. (95%), m.p. 137–139°. Recrystallization from acetone gave white needles, m.p. 137–139°. $\lambda$ max. (pH 7, 11): 291 m$\mu$; $\nu$ max. 1720 (carboxyl C=O); 1680, 1575 (C=C, C=N); 695 cm.$^{-1}$ ($C_6H_5$).

*Anal.*—Calcd. for $C_{17}H_{18}N_4O_2S$: C, 59.7; H, 5.30; N, 16.3. Found: C, 59.5; H, 5.27; N, 16.1.

**6-Methylthio-9H-purine-9-ylvaleric acid (XIV).**—This was prepared similarly, except that the compound was recrystallized from water and obtained as white crystals, m.p. 123–125°.

*Anal.*—Calcd. for $C_{11}H_{14}N_4O_2S$: C, 49.6; H, 5.30; N, 21.0. Found: C, 49.4; H, 5.10; N, 21.1.

**N-(5'-Aminopentyl)phthalimide Hydrochloride (XVII).**—A solution of 4.93 Gm. (21.6 mmoles) of XVI (4) in 200 ml. of ethanol and 10 ml. of 3 *N* aqueous hydrochloric acid was shaken with hydrogen at 2–3 Atm. in the presence of 0.25 Gm. of platinum oxide catalyst; reduction was complete in 5 hr. The filtered solution was spin evaporated *in vacuo*. Trituration of the residue with ethyl acetate gave 4.00 Gm. (69%) of product, m.p. 201–203°, that was suitable for the next step. Recrystallization of a sample from ethanol–ethyl acetate afforded white crystals, m.p. 206–209°. $\nu$ max. 2800–2400 ($NH^+$); 1775, 1725 cm.$^{-1}$ (imide C=O).

*Anal.*—Calcd. for $C_{13}H_{16}N_2O_2 \cdot HCl$: C, 58.1; H, 6.38; N, 10.4. Found: C, 57.9; H, 6.40; N, 10.2.

**5 - Amino - 6 - chloro - 4 - (5' - phthalimidopentylamino)pyrimidine (XX).**—A solution of 269 mg. (1 mmole) of XVII, 164 mg. (1 mmole) of 5-amino-4,6-dichloropyrimidine, and 0.56 ml. (4 mmoles) of triethylamine in 10 ml. of butanol was refluxed for 20 hr., then spin evaporated *in vacuo*. Crystallization from aqueous acetone gave 235 mg. (65%) of product in two crops, m.p. 145–147°, that was suitable for further reaction. Recrystallization from the same solvent gave white needles, m.p. 155–157°. $\nu$ max. 3400, 3300 (NH); 1775, 1720 (imide C=O); 1640, 1590 (NH, C=C, C=N); 735, 720 cm.$^{-1}$ ($C_6H_4$).

*Anal.*—Calcd. for $C_{17}H_{18}ClN_5O_2$: C, 56.8; H, 5.05; N, 19.4. Found: C, 57.0; H, 5.05; N, 19.2.

**6-Chloro-9-(5'-phthalimidopentyl)purine (XVIII).**—To a magnetically stirred solution of 1.08 Gm. (3 mmoles) of XX in 12 ml. of ethyl orthoformate was added 0.35 ml. (3.9 mmoles) of 12 *N* aqueous hydrochloric acid over a period of about 3 min.; within 30 min. the crystalline product began to separate. After being stirred at ambient temperature for about 18 hr., the mixture was diluted with 15 ml. of ethyl acetate and filtered. The product was washed with ethyl acetate; yield, 680 mg. (61%), m.p. 147–149°. From the mother liquor was isolated an additional 182 mg. (total 76%) of product, m.p. 140–142°. Recrystallization of a sample of the first crop from ethanol gave white crystals, m.p. 147–149°. $\nu$ max. 1770, 1720 (imide C=O); 1590, 1560 (C=C, C=N); 720 ($C_6H_4$); no NH near 3300 cm.$^{-1}$.

*Anal.*—Calcd. for $C_{18}H_{16}ClN_5O_2$: C, 58.5; H, 4.37; N, 18.9. Found: C, 58.7; H, 4.32; N, 18.7.

**6 - Mercapto - 9 - (5' - phthalimidopentyl)purine (XIX).**—A solution of 648 mg. (1.75 mmoles) of XVIII and 266 mg. (3.5 mmoles) of thiourea in 35 ml. of ethanol was refluxed with magnetic stirring for 4 hr., during which time the product separated. The crystals were collected on a filter and washed with water (2 × 5 ml.), then ethanol (2 × 5 ml.); yield, 571 mg. (89%), m.p. 279–281°. Recrystallization from 2-methoxyethanol gave buff-colored crystals of unchanged m.p.; $\lambda$ max. (EtOH) 325 m$\mu$; $\nu$ max. 1770, 1720 (imide C=O); 1600, 1570, 1540 (C=C, C=N); 720, 710 cm.$^{-1}$ ($C_6H_4$).

*Anal.*—Calcd. for $C_{18}H_{17}N_5O_2S$: C, 59.0; H, 4.67; N, 19.1. Found: C, 58.8; H, 4.73; N, 18.9.

**6-Mercapto-9H-purine-9-ylpentylamine Hydrochloride (XXI).**—To a magnetically stirred suspension of 735 mg. (2 mmoles) of XIX in 15 ml. of 2-methoxyethanol heated in a bath to 95° was added 0.32 ml. (10 mmoles) of hydrazine. Solution was complete in 10 min., then the intermediate *N*-amino phthalamide began to separate; after an additional 5 min., the mixture was cooled, diluted with 40 ml. of ethyl acetate, and kept at 5° for 2 hr. The intermediate phthalamide was collected on a filter and washed with ethyl acetate; yield, 728 mg., m.p. 236–237° dec. $\nu$ max. 1650 (amide C=O); 1600, 1590–1560, 1525 (C=C, C=N, amide NH); no imide C=O near 1770 or 1720 cm.$^{-1}$.

The intermediate was suspended in 20 ml. of 1 *N*

aqueous hydrochloric acid and magnetically stirred in a bath at 80° for 15 min.; the insoluble phthalhydrazide (324 mg., 99%) was collected on a filter and washed with water. The filtrate was spin evaporated *in vacuo*. Recrystallization from aqueous acetone gave 402 mg. (74%) of crystals, m.p. 310–312° dec. Further recrystallization gave buff-colored crystals of unchanged melting point. λ max. (EtOH) 326 mμ; ν max. 2800–2100 ($NH^+$, acidic H); 1580, 1540 (C=O, C=N); no C=O between 1650 and 1720 $cm.^{-1}$.

*Anal.*—Calcd. for $C_{10}H_{15}N_5S \cdot HCl$: C, 43.9; H, 5.89; N, 25.6, S, 11.7. Found: C, 44.2; H, 6.18; N, 25.2; S, 11.5.

**N - (6 - Mercapto - 9H - purine - 9 - ylpentyl)-thiourea (XXIV).**—A solution of the crude XXI, obtained from 184 mg. (0.5 mmole) of XIX, in 5 ml. of 50% aqueous acetone was magnetically stirred in an ice bath; then 0.28 ml. (2 mmoles) of triethylamine was added, followed by 0.10 ml. of phenoxy thionocarbonyl chloride. After being stirred for 30 min. at 0° and 12 hr. at ambient temperature, the mixture was filtered and the intermediate XXII was washed with water; yield, 125 mg., m.p. 230°, with previous softening. ν max. 1590, 1560 (broad) (C=C, C=N); 1240 (broad ester C—O—C); 770, 680 $cm.^{-1}$ ($C_6H_5$); λ max. (EtOH); 326 mμ.

By concentration of the combined filtrate and washings *in vacuo*, there was obtained 45 mg. of the crude isothiocyanate, XXIII, m.p. 246–248° dec. λ max. (EtOH); ν max. 2200, 2100 (—N=C=S); 1600, 1570, 1540 (C=C, C=N); no ester C—O—C absorption near 1240 $cm.^{-1}$.

Both XXII and XXIII were combined and dissolved in 25 ml. of methanol previously saturated with ammonia at 0°. After 1.5 hr. at 0°, the mixture was spin evaporated *in vacuo*. Trituration of the residue with acetone gave 66 mg. (45% over-all yield from XIX) of product, m.p. 215–216°. Recrystallization from aqueous ethanol gave buff-colored crystals of unchanged melting point. λ max. (EtOH): 326 mμ; ν max. 3350, 3150 (NH); 1610, 1600, 1540 $cm.^{-1}$ (NH, C=C, C=N). The compound traveled as a single spot on TLC in benzene–methanol (3:1). It also moved as a single spot on paper chromatography in 5% $K_2HPO_4$ with $R_f$ 0.47.

*Anal.*—Calcd. for $C_{11}H_{16}N_6S_2$: C, 44.6; H, 5.45; N, 28.4. Found: C, 44.8; H, 5.64; N, 28.1.

**9 - (5′ - Dichloroacetamidopentyl) - 6 - mercaptopurine (XXV).**—A solution of 137 mg. (0.5 mmole) of XXI in 1 ml. of 1.0 *N* aqueous sodium hydroxide was spin evaporated *in vacuo*. After addition of 2 ml. of *N,N*-dimethylformamide to the residue, the spin evaporation *in vacuo* was repeated. The residue was partially dissolved in 1 ml. of *N,N*-dimethylformamide, cooled in an ice bath, then treated with 138 mg. of anhydrous potassium carbonate, followed by 0.19 ml. (2 mmoles) of dichloroacetyl chloride. After being stirred for 1 hr. in the ice bath, the mixture was diluted with 15 ml. of ice water. The crystalline solid was collected on a filter and washed with water; yield, 85 mg. (49%), m.p. 194–195° dec.

Recrystallization from aqueous acetone gave 64 mg. (37%) of analytical sample as buff-colored crystals, m.p. 205–207° dec. λ max. (EtOH): 326 mμ; ν max. 3280 (NH); 2700–2200 (acidic NH); 1660 (amide C=O); 1590, 1575, 1540 $cm.^{-1}$ (amide NH, C=C, C=N).

*Anal.*—Calcd. for $C_{12}H_{15}Cl_2N_5OS$: C, 41.4; H, 4.34; N, 20.1. Found: C, 41.6; H, 4.37; N, 20.3.

When XXI was reacted with dichloroacetyl chloride in aqueous acetone, as described for the preparation of XXII, the yield of XXV was about 25%.

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