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Irreversible Enzyme Inhibitors. CXXI.[1,2] Thymidine Phosphorylase. IX.[3] On the Nature and Dimensions of the Hydrophobic Bonding Region

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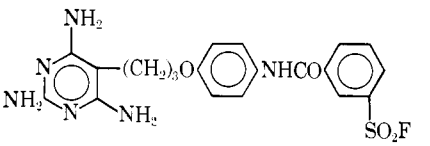
The dimensions of the hydrophobic bonding region of *E. coli* B thymidine phosphorylase have been studied with 25 derivatives of 6-anilinouracil (**4**) and four derivatives of 6-benzylaminouracil (**5**), where substituents have been placed on the benzene ring of these two compounds. One side of the benzyl group of **5** is hydrophobically bonded and the other side is not. Similarly, the opposite side of the phenyl group of **4** is hydrophobically bonded and the other is not; it is in these nonhydrophobic regions where the benzene rings should be substituted with appropriate leaving groups in order to convert **4** and **5** to active-site-directed irreversible inhibitors. The hydrophobic bonding region on the enzyme is fairly planar since bicyclic and tricyclic systems such as 3- and 4-aminobiphenyl, α- and β-naphthylamine, and 2-anthranylamino (**31**) attached to the 6 position of uracil are effectively complexed to the enzyme; in fact, **31** and 6-(2,3-dichloroanilino)uracil (**23**) are inhibitors that complex to thymidine phosphorylase 1100-fold more effectively than the substrate, 5-fluoro-2′-deoxyuridine (FUDR).

A hydrophobic region on an enzyme just adjacent to where the substrate is held in the active site has considerable utility in the design of active-site-directed irreversible inhibitors.[5] First, reversible binding can be considerably enhanced; for example, a phenylbutyl group placed on the 5 position of a 2,4-diaminopyrimidine can increase binding to dihydrofolic reductase by a factor of 40,000.[6,7] Second, such a hydrophobic bonding region adjacent to the active site can be expected to have undergone evolutionary changes[8] between species;[9,10] small differences can even occur in an enzyme from a tumor compared to the liver of the same animal.[11] Third, if an exo-type active-site-directed irreversible inhibitor is constructed that utilizes the hydrophobic bonding region, these small differences can be greatly magnified to give inhibitors that can inactivate an enzyme from a tumor with no inactivation of the enzyme from the liver of the same animal.[2,12] For example, the triaminopyrimidine moiety of **1** binds to the active site of dihydrofolic reductase, the phenoxypropyl moiety binds to the hydrophobic region of the



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enzyme, but the *m*-fluorosulfonylbenzamido group is in a polar region of the enzyme that promotes covalent bond formation between the sulfonyl fluoride group and the enzyme.[12] Since thymidine phosphorylase[13,14] has a hydrophobic bonding region adjacent to the active site,[3,15-18] a similar approach utilizing this region for tissue-specific inhibitors has been one of the endeavors of this laboratory.

In order to design an effective active-site-directed irreversible inhibitor of thymidine phosphorylase that utilizes the hydrophobic bonding region, it is useful to know what types of hydrophobic moieties will give maximum interaction with the enzyme; it is even more imperative to know where this hydrophobic region ends so that an appropriate leaving group can be

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and P. C. Huang, *J. Med. Chem.*, **11**, 495 (1968).

(3) For the previous paper on this enzyme see B. R. Baker and W. Rzeszotarski, *ibid.*, **10**, 1109 (1967), paper CIV of this series.

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(6) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, **54**, 1415 (1965).

(7) For a review on the binding to dihydrofolic reductase see ref 5, Chapter X.

(8) For a discussion of evolutionary changes in enzymes see ref 5, pp 184–190.

(9) (a) G. H. Hitchings and J. J. Burchall, *Advan. Enzymol.*, **27**, 417 (1965); (b) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, **1**, 126 (1965).

(10) B. R. Baker, *J. Med. Chem.*, **10**, 912 (1967), paper XCVII of this series.

(11) B. R. Baker, *ibid.*, **11**, 483 (1968), paper CXVII of this series.

(12) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **11**, 489 (1968), paper CXIX of this series.

(13) For the possible chemotherapeutic utility of a tissue-selective blockade of this enzyme, see B. R. Baker, *ibid.*, **10**, 297 (1967), paper LXXV of this series.

(14) For the possible chemotherapeutic utility of a tissue-selective blockade of this enzyme, see also ref 5, Chapter IV.

(15) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 302 (1967), paper LXXVI of this series.

(16) B. R. Baker, M. Kawazu, D. V. Santi, and T. J. Schwan, *ibid.*, **10**, 304 (1967), paper LXXVII of this series.

(17) B. R. Baker and M. Kawazu, *ibid.*, **10**, 316 (1967), paper LXXX of this series.

(18) B. R. Baker and M. Kawazu, *ibid.*, **10**, 311 (1967), paper LXXVIII of this series.

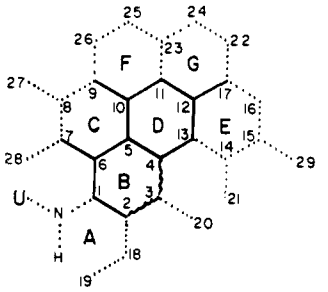
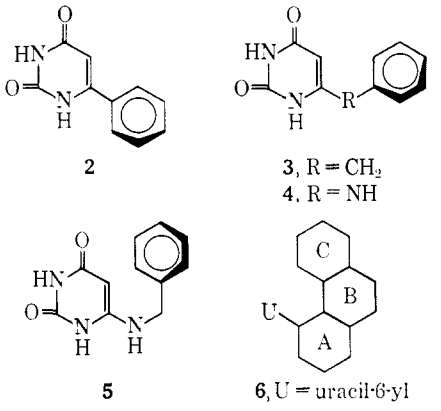


Figure 1.—A proposed map of the hydrophobic bonding region of *E. coli* B thymidine phosphorylase: ——, hydrophobic interaction; ····, no hydrophobic interaction; ∿∿, unknown.



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3, R = CH_2
4, R = NH

5

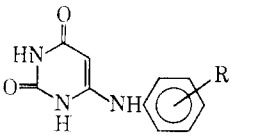
6, U = uracil-6-yl

placed on the inhibitor that is juxtaposed to a nucleophilic group in a hydrophilic area on the enzyme. A 6-phenyl group (**2**) on uracil gives hydrophobic interaction with enzyme only when it is held out of the plane of the uracil ring by *ortho* substituents.[16] As a working hypothesis we suggested[3] that the phenyl group of **3**[18] and **4**,[3] as well as **5**,[3] is also out of plane with the uracil ring as indicated; this working hypothesis has now been extended by the assumption that the benzene rings of **2**–**5** are all complexed in the same plane as indicated in the composite drawing (**6**) where A is the benzene ring of **2**, B is the benzene ring of **3** and **4**, C is the benzene ring of **5**, and U is the uracil-6-yl moiety that is out-of-plane with the ABC system. The subject of this paper is therefore the support of **6** as a working hypothesis and further mapping of the hydrophobic bonding based on this hypothesis (Figure 1).

Enzyme Results.—The inhibitors of thymidine phosphorylase in this study can be divided into two types: (a) 6-anilinouracils with substituents on the benzene ring in Table I, and (b) 6-benzylaminouracils with substituents on the benzylamino moiety in Table II. From these data emerge the mapping of the hydrophobic region as shown in Figure 1.

One of the conformations of 6-anilinouracil (**4**) would have coplanarity of the two rings. That these rings are not coplanar in the enzyme–inhibitor complex is shown by two lines of evidence. The phenyl group of 6-phenyluracil (**2**) complexes to the hydrophobic bonding region only when the 6-phenyl group is held out of coplanarity by *ortho* substituents, as previously discussed.[16] The second line of evidence arises by comparison of the binding of the 6-anilino group (**4**) with the *o*-methyl- (**8**) and 2,6-dimethylanilino (**18**) groups. The *o*-methyl group of **8** gives a 13-fold increment in binding, the reasons for which will be discussed later; introduction of the second methyl group (**18**) gives less than a twofold change in binding compared to **8**. Molecular models show that **18** cannot have the two rings coplanar due to the steric interaction of the two

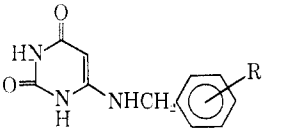
Table I
Inhibition of Thymidine Phosphorylase[a] by



No.	R	μM conen for 50% inhib	$([S]/[I])_{0.5}$[b]
6	H	40[c]	10
7	Hexahydro	1600	0.25
8	*o*-CH_3	3.1	130
9	*m*-CH_3	11	36
10	*p*-CH_3	21	19
11	*o*-C_2H_5	3.1	130
12	*p*-C_2H_5	15	27
13	*p*-C_4H_9-*n*	7.4	54
14	*p*-C_4H_9-*t*	72	5.5
15	2,3-$(CH_3)_2$	0.89	450
16	2,4-$(CH_3)_2$	1.5	270
17	2,5-$(CH_3)_2$	4.2	96
18	2,6-$(CH_3)_2$	1.8	220
19	*o*-Cl	2.1	190
20	*m*-Cl	6.2	65
21	*p*-Cl	14	29
22	*p*-Br	10	40
23	2,3-Cl_2	0.35	1100
24	*o*-OCH_3	20	20
25	*o*-OCH_2CH_3	24	17
26	*p*-OCH_2CH_3	6.7	60
27	2,3-Benzo	1.6	250
28	3,4-Benzo	2.2	180
29	*m*-C_6H_5	0.93	430
30	*p*-C_6H_5	1.9	210
31	4,5-(2,3-Naphtho)[d]	0.37	1100
32	N^6-CH_3	55[c]	7.3

[a] Thymidine phosphorylase was a 45–90% saturated ammonium sulfate fraction from *E. coli* B prepared and assayed with 400 μM 2′-deoxy-5-fluorouridine (FUDR) in arsenate–succinate buffer (pH 5.9) in the presence of 10% DMSO as previously described;[14a] the technical assistance of Pepper Caseria with these assays is acknowledged. [b] Ratio of concentration of 400 μM substrate to inhibitor giving 50% inhibition. [c] Data from ref 3. [d] 6-(2-Anthracylamino)uracil.

Table II
Inhibition of Thymidine Phosphorylase[a] by



No.	R	μM conen for 50% inhib	$([S]/[I])_{0.5}$[b]
5	H	6.2[c]	65
33	*o*-Cl	2.2	180
34	*m*-Cl	1.2	330
35	*p*-Cl	11	36
36	2,5-$(CH_3)_2$	6.7	60
37	N^6-CH_3	53	7.6
38	N^6-C_2H_5	37	11
39	N^6-$CH_2C_6H_5$	70	6
40	α-CH_3	28	14
41	α-C_6H_5	38	11
42	α-$CH_2C_6H_5$	78	5.1

[a]–[c] See corresponding footnotes in Table I.

o-methyl groups with the uracil ring. The two extremes in conformations of **18** that are possible are (a) the planes of the two rings are bent in a V shape with an angle of about 30°; (b) the plane of the benzene ring in (a) can then be rotated 90° so that the planes of the benzene and uracil rings are perpendicular. Thus, as shown in Figure 1, it is quite probable that the phenyl groups of 6-phenyluracil (**2**) and 6-anilinouracil (**4**) are in the same plane and actually overlap in the hydrophobic region, *i.e.*, the phenyl group of **2** would reside in area A and the phenyl group of **4** would reside in area B; thus, the phenyl rings of **2** and **4** have positions 1 and 2 in common in Figure 1. As will be shown later, positions 18 and 19 do not give a hydrophobic interaction with the enzyme; second, the N^6 of the uracil of 6-anilinouracil (**4**) is complexed in a polar region; third, no hydrophobic interaction occurs when this N^6 is substituted by methyl (**32**). Therefore, the only remaining carbons of 6-phenyluracil that could give a hydrophobic interaction with enzyme are at positions 1 and 2 in Figure 1.

The 13-fold increase in binding by the *o*-methyl group of 8 could be due to a hydrophobic interaction with the enzyme, or partial restriction of a coplanar ground-state conformation or both. If the major effect of the *o*-methyl group is due to the effect of the somewhat restricted conformation on the ground state of the molecule, then a similar effect should be seen with the more polar *o*-methoxy group (**24**); note that **24** is only a twofold better inhibitor than 6-anilinouracil (**4**), but that the *o*-methyl group gives a 13-fold increment in binding. Therefore, the maximum effect a single *ortho* substituent could have on restricting a coplanar ground-state conformation would be twofold. However, a 13-fold increment in binding is above the tenfold maximum that is thermodynamically possible from hydrophobic bonding of a single methyl group.[19] It follows that the 13-fold effect of the *o*-methyl group is probably due to a sixfold increment in hydrophobic bonding and a twofold increment in binding due to an *ortho* effect in common with the *o*-methoxy substituent on possible ground-state conformations. The hydrophobic bonding by the *o*-methyl group of 8 can be at position 7 or position 18 (Figure 1); that the methyl group is not at position 18 can be supported by the following considerations.

Thymidine phosphorylase is a reversible enzyme reaction that can condense 2-deoxy-α-D-ribofuranosyl 1-phosphate with thymine to give thymidine. The polar phosphate must be held on the enzyme so that N^1 of the thymine can readily attack C^1 of the sugar. For the highly polar α-phosphate group, which is a probable binding point,[13,14] to be able to position properly, it must be in contact with a polar region on the enzyme. It follows that the large hydrophobic region (Figure 1) must be in the opposite direction from where the phosphate of the cosubstrate is held as shown in **4**; thus the *o*-methyl of 8 should be complexed to position 7. Therefore, position 18 is not in a hydrophobic area since no increment in binding is observed between the *o*-methyl (**8**) and 2,6-dimethyl (**18**) derivatives.

That position 19 is not involved in hydrophobic bonding is indicated by a twofold increment in binding by the *o*-methoxyl group (**24**) over **4**; this twofold effect has been discussed earlier. Since the relatively polar ether oxygen of **24** would be repulsed from the hydrophobic position 7, it therefore should occupy the nonhydrophobic position 18; it follows that position 19 is not hydrophobic, else the methyl of the methoxy group of **24** should have shown an increment in binding.

Introduction of a 5-methyl group on the *o*-methyl derivative (**8**) to give **17** results in no increase in binding; therefore, position 20 where the 5-methyl group of **17** resides cannot be hydrophobic. In contrast, introduction of a 3-methyl (**15**) or 4-methyl (**16**) gives an increment in binding indicating that positions 10 and 13 are hydrophobic; this conclusion is further supported by the increments in binding of **9** and **10** compared to **4**.

Completely parallel results concerning positions 7, 10, and 13 were obtained by halogen substitution (**19**–**23**). In fact, 6-(2,3-dichloroanilino)uracil (**23**) is one of the two best inhibitors in Tables I and II, being complexed to thymidine phosphorylase 1100-fold better than the substrate (FUDR).

That positions 8 and 28 are not hydrophobic is shown by the fact that *o*-ethyl (**11**) does not bind any better than *o*-methyl (**8**). Further evidence that position 8, as well as position 9, are not in a hydrophobic region is shown by comparison of the α-naphthyl (**27**) and the 2,3-dimethylphenyl (**15**) groups; there is only a twofold difference in binding, indicating that position 8 or 9 makes a weak hydrophobic contribution. Since position 8 makes no hydrophobic contribution, this weak increment between **15** and **27** is probably due to a hydrophobic interaction at position 9.

Introduction of an *o*-chloro atom (**33**) on 6-benzylaminouracil (**5**) gives a threefold increment in binding; since it has already been shown that position 28 is not hydrophobic, the increment with **33** is probably due to a hydrophobic interaction at position 4. It is also highly probable that positions 5 and 6, which are in common between the 6-anilino group (area B) and the 6-benzylamino group (area C), are hydrophobic to account for the binding of these two benzene rings. No information is available on whether or not position 3 is hydrophobic; such an answer could be obtained by replacing the phenyl group of 6-anilinouracil (**4**) by a 3-pyridyl group; loss in binding by the replacement would indicate position 3 is in a hydrophobic region.

Position 26 is not hydrophobic since there is a twofold loss in binding when the *p*-chloro atom (**35**) is introduced onto 6-benzylaminouracil (**5**) (Table II). That either position 11 or 27 is hydrophobic is seen by the fivefold increment in binding when the *m*-chloro atom (**34**) is introduced on **5**. Since it has been established that positions 7, 8, 26, and 28 are not hydrophobic, it is unlikely that the adjacent 27 would be hydrophobic; that position 27 is not hydrophobic is further supported by **36** where the gain in binding by the 2-methyl group at position 4 is lost by the repulsion of the 5-methyl group from position 27. Therefore, the *m*-chloro atom of **34** logically interacts hydrophobically at position 11; additional evidence that position 11 is hydrophobic will be presented later.

Introduction of the *m*-phenyl group (**29**) on 6-anilinouracil (**4**) gives a 43-fold increment in binding in area F. Of this 43-fold increment in binding, position 10 can account for fourfold (**4** *vs.* **9**), and position 11 for

(19) For a discussion of the bonding forces that cause complex formation between an enzyme and its inhibitor, see ref 5, Chapter II.

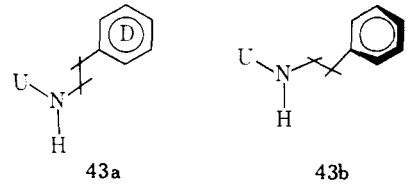
fivefold (**5** *vs.* **34**); the remaining twofold difference can be accounted for by a hydrophobic interaction at position 23. It is therefore unlikely that position 25 is in a hydrophobic area and it was already shown that positions 9 and 26 of area F were not.

Introduction of the *p*-phenyl group (**30**) on 6-anilinouracil (**4**) gives a 21-fold increment in binding in area E. Of this 23-fold increment in binding, twofold can be due to an interaction at position 13 (**4** *vs.* **10**); the remaining 11-fold can be accounted for by interaction of two additional carbon atoms which should logically be chosen from positions 12, 14, and 17. That positions 12 and 17 are probably hydrophobic is indicated by the sixfold increment in binding by the *p*-(*n*-Bu) (**13**) group on 6-anilinouracil (**4**); the *n*-butyl group of **13** could have a staggered conformation covering positions 13, 12, 17, and 22 or 13, 14, 15, and 29. Hydrophobic bonding at positions 13, 12, and 17 could account for the increments in binding by the *n*-Bu (**13**) and *p*-phenyl (**30**) moieties. Similarly, the sixfold increment in binding by the *p*-OEt group (**25**) on **4** can be accounted for by a hydrophobic interaction at positions 12 and 17 or 12 and 11. It follows by these comparisons that positions 14–16 are not hydrophobic. Since it is likely that positions 14–16 are not hydrophobic, then the extended 21 and 29 positions are probably not hydrophobic.

The 2-anthracylamino derivative (**31**) is a fivefold better inhibitor than the *p*-biphenylamino derivative (**30**) and sixfold better than the β-naphthylamino derivative (**28**). The anthracene derivative probably binds in areas B, D, and G. Thus the sixfold difference between **28** and **31** could be due to hydrophobic interaction at positions 23 and 17 and the fact that no binding occurs at positions 22 and 24.

That these hydrophobic interactions are with a relatively flat nonpolar area on the enzyme is further supported by the following: (a) the *t*-Bu group (**14**) with its three-dimensional bulk causes a threefold loss in binding compared to the *p*-methyl group (**10**) on **4**; (b) reduction of the benzene ring of **4** to hexahydro (**7**) with its nonplanar chair conformation gives a huge loss in binding; (c) the planar bicyclic and tricyclic structures of **27–31** are not only tolerated by the enzyme, but appropriate increments in binding occur through positions 10–13, 17, and 23.

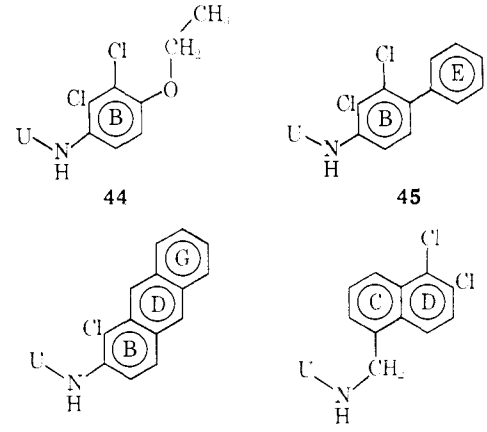
One compound that at first glance does not fit with the picture presented in Figure 1 is 6-phenethylaminouracil (**43**), which shows no hydrophobic bonding.[3] This compound in conformation **43a** could give a hydrophobic interaction of its benzene ring in area D and its ethyl group at positions 1 and 6 of the hydrophobic area; a benzene interaction in area D can be estimated from the binding of **28** and **33** to give a 50-fold increment. However, conformation of **43a** would have proton interactions between the methylene group



43a **43b**

(attached to the N) and the NH, as well as between this methylene and the phenyl ring; a more favorable ground-state conformation would be **43b** which would put the benzene ring out of plane with the hydrophobic region in Figure 1, thus giving a loss in binding as seen with **14**. Although as much as a 100-fold increment (2.8 kcal/mole) could be expected by **43** binding to the enzyme in conformation **43a**, a large part of this 2.8 kcal/mole may be required to convert **43** to the unfavorable conformation **43a**. Some of the studies planned with **44–47** may resolve this question further.

The two most powerful inhibitors in Tables I and II are 6-(2,3-dichloroanilino)uracil (**23**) and 6-(3-anthracylamino)uracil (**31**) both of which are complexed to thymidine phosphorylase 1100-fold better than the substrate, 5-fluoro-2′-deoxyuridine (FUDR). Based on the hydrophobic interactions indicated in Figure 1, it should be possible to obtain even more potent inhibitors. For example, introduction of the *p*-ethoxy group (**44**) could give a sixfold increment (4

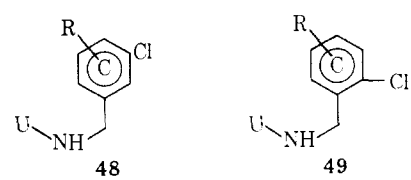


44 **45**

46 **47**

and **25**) over the parent **23**; thus **44** could be expected to be complexed 6600-fold better than FUDR. Similarly, introduction of the *p*-phenyl group (**45**) on **23** should give a further 20-fold increment (**4** *vs.* **30**) and **45** should complex 23,000-fold better than FUDR. Another example would be introduction of a chloro group (**46**) on **31** which should give a 20-fold increment (**4** *vs.* **19**) and **46** should complex 22,000-fold better than FUDR. Another type of structure that could give potent inhibitors would be **47**; studies on this type could also give more information on complexing in area E and G by appropriate substitution. Such studies are being pursued.

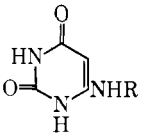
From Figure 1 also emerges the answer to one of the important questions posed at the beginning of this paper, *i.e.*, where does the hydrophobic region end so that appropriate leaving groups can be placed on the inhibitor that could lead to irreversible inhibition by covalent bond formation in a polar region of the enzyme. One important class emerges from the fact that positions 26–28 (Figure 1) are not in a hydrophobic area. 6-Benzylaminouracils should be constructed



48 **49**

that have a chloro group on the *ortho* (**49**) or *meta* position (**48**) which should hold the benzene group in one

TABLE III
PHYSICAL PROPERTIES OF

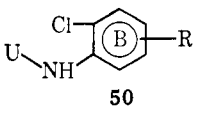


No.	R	Method (hr)	Amine ratio[a]	% yield	Mp, °C dec	Formula[j]
7	Cyclohexyl	C (12)	2	43	327–328[b,c]	$C_{10}H_{15}N_3O_2$
8	2-$CH_3C_6H_4$	A (0.5)	2	73	332–333[d]	$C_{11}H_{11}N_3O_2$
9	3-$CH_3C_6H_4$	A (0.5)	2	74	302–303[d]	$C_{11}H_{11}N_3O_2$
10	4-$CH_3C_6H_4$	A (0.5)	2	69	321–323[d,e]	$C_{11}H_{11}N_3O_2$
11	2-$C_2H_5C_6H_4$	C (12)	2	69	324–325[b]	$C_{12}H_{13}N_3O_2$
12	4-$C_2H_5C_6H_4$	C (4)	2	85	324–326[d]	$C_{12}H_{13}N_3O_2$
13	4-$C_6H_4C_4H_9$-*n*	B (12)	1	84	320–321[d]	$C_{16}H_{17}N_3O_2$
14	4-$C_6H_4C_4H_9$-*t*	B (4)	1	89	332–333[g]	$C_{14}H_{17}N_3O_2$
15	2,3-$C_6H_3(CH_3)_2$	C (12)[h]	2	63	325–326[b]	$C_{12}H_{13}N_3O_2$
16	2,4-$C_6H_3(CH_3)_2$	C (12)	2	69	316–317[b]	$C_{12}H_{13}N_3O_2$
17	2,5-$C_6H_3(CH_3)_2$	C (12)	2	87	321–322[b]	$C_{12}H_{13}N_3O_2$
18	2,6-$C_6H_3(CH_3)_2$	C (12)	2	60	346–347[d]	$C_{12}H_{13}N_3O_2$
19	2-ClC_6H_4	C (48)	2	75	312–314[b]	$C_{10}H_8ClN_3O_2$
20	3-ClC_6H_4	C (24)[h]	1	75	332–333[d]	$C_{10}H_8ClN_3O_2$
21	4-ClC_6H_4	C (4)	2	79	341–342[d]	$C_{10}H_8ClN_3O_2$
22	4-BrC_6H_4	D (12)	1	79	336–337[b,i]	$C_{10}H_8BrN_3O_2$
23	2,3-$C_6H_3Cl_2$	A (0.5)	2	80	322–323[d]	$C_{10}H_7Cl_2N_3O_2$
24	2-$CH_3OC_6H_4$	C (2)	1.2	76	296–298[b]	$C_{11}H_{11}N_3O_3$
25	2-$C_2H_5OC_6H_4$	C (2)	2	80	297–299[d]	$C_{12}H_{13}N_3O_3$
26	4-$C_2H_5OC_6H_4$	C (12)	2	88	330–332[d]	$C_{12}H_{13}N_3O_3$
27	α-Naphthyl	B (4)	1.2	71	326–327[g]	$C_{14}H_{11}N_3O_2$
28	β-Naphthyl	B (12)	2	75	338–339[d]	$C_{14}H_{11}N_3O_2$
29	3-$C_6H_5C_6H_4$	C (12)	1	60	321–323[b]	$C_{16}H_{13}N_3O_2$
30	4-$C_6H_4C_6H_4$	C (12)	1	75	>360[f]	$C_{16}H_{13}N_3O_2$
31	2-Anthracyl	D (3)	1.2	72	>360[f]	$C_{18}H_{13}N_3O_2$

[a] Ratio of amine to 6-chlorouracil. [b] Recrystallized from EtOH. [c] Lit.[21] mp 321–322°. [d] Recrystallized from HOAc. [e] Lit.[21] mp 327–328°. [f] Recrystallized from DMSO–EtOH. [g] Recrystallized from HOAc–H_2O. [h] Reaction run in 1:1 H_2O–MeOEtOH. [i] Lit.[21] mp 336–337°. [j] All compounds were analyzed for C, H, N, and the analytical values obtained were within ±0.4% of the calculated figures.

conformation by complexing at position 4 or 11 (Figure 1); the group for irreversible inhibition (R) should then be bridged to **48** and **49** so that it projects through position 26, 27, or 28 on Figure 1.

A second important class emerges from the fact that positions 18–21, 14, 15, and 29 (Figure 1) are not in a hydrophobic area. 6-Anilinouracils should be constructed that have a chloro group *ortho* (**50**) or *meta*



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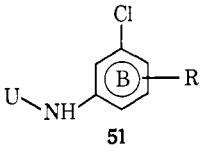

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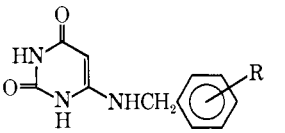
(**51**) in order to hold the phenyl ring in one conformation by complexing to position 7 or 10 (Figure 1), the group for irreversible inhibition (R) should then be bridged to **50** and **51** so that it projects through position 14, 18, or 20.

Substitution on 6-benzylaminouracil (**5**) (Table II) at the α position (**41–43**) or N^6 position (**37–39**) by methyl, phenyl, or benzyl led to a 4–12-fold loss in binding; however, this loss could be recouped by introduction of 2,3-dichloro substituents on the benzyl group. Thus a group for irreversible inhibition could be placed on these N^6 and α substituents; however, a better probability for good irreversible inhibition would emerge with compounds of type **48–51**. Such studies are being pursued.

TABLE IV
PHYSICAL PROPERTIES OF



No.	R	Reaction[a] time, hr	% yield	Mp, °C dec	Formula[h]
33	2-Cl	12	88	314–316[b]	$C_{11}H_{10}ClN_3O_2$
34	3-Cl	24	67	312–314[b]	$C_{11}H_{10}ClN_3O_2$
35	4-Cl	12	63	320–322[b]	$C_{11}H_{10}ClN_3O_2$
36	2,5-$(CH_3)_2$	24[c]	77	312–313[b]	$C_{13}H_{15}N_3O_2$
37	N^6-CH_3	48[c]	44	273–275[d]	$C_{12}H_{13}N_3O_2$
38	N^6-C_2H_5	48[c]	53	277–279[b]	$C_{13}H_{15}N_3O_2$
39	N^6-$CH_2C_6H_5$[e]	5[c]	75	205–207[f,g]	$C_{18}H_{17}N_3O_2 \cdot HCl$
40	α-CH_3	24	78	325–327[b]	$C_{12}H_{13}N_3O_2$
41	α-C_6H_5	8[c]	81	290–292[d]	$C_{17}H_{15}N_3O_2$
42	α-$CH_2C_6H_5$	3	29	318–320[d]	$C_{18}H_{17}N_3O_2$

[a] All compounds were made by method C with a 20% excess of amine unless otherwise indicated. [b] Recrystallized from HOAc. [c] Twofold ratio of amine. [d] Recrystallized from EtOH. [e] Hydrochloride salt. [f] Recrystallized from MeOH. [g] Does not decompose. [h] See footnote *j*, Table III.

Experimental Section[20]

The chemistry of the synthesis of the substituted 6-aminouracils by condensation of the appropriate amine with 6-chlorouracil[21]

(20) All analytical samples had ir and uv spectra in agreement with their assigned structures; all gave combustion values for C, H, and N within 0.4 of the theoretical percentage. Each moved as a single spot on tlc on Brinkmann silica gel GF with HOAc–C_6H_6 or DMF–C_6H_6 when viewed under uv light. Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected.

(21) A. Paul and D. Sen, *Indian J. Chem.*, **2**, 212 (1964).

or 2,4-dimethoxy-6-chloropyrimidine[3] was discussed previously.[3]

See Tables III and IV for the physical and analytical data for these compounds.

6-(2,3-Dichloroanilino)uracil (23) (Method A).—A mixture of 0.755 g (5 mmoles) of 6-chlorouracil[22] and 1.62 g (10 mmoles) of 2,3-dichloroaniline was heated in a bath at 200° for 30 min when the mixture resolidified. The cooled mixture was triturated with 250 ml of hot water. The product was collected on a filter and washed with hot water; yield 1.10 g (80%), mp 322–323° dec. Recrystallization from HOAc gave white crystals of unchanged melting point.

(22) B. L. Langley, British Patent 845,378 (1960); *Chem. Abstr.*, **55**, 6506 (1961).

6-(β-Naphthylamino)uracil (28) (Method B).—A mixture of 0.755 g (5 mmoles) of 6-chlorouracil,[22] 1.69 g (10 mmoles) of β-naphthylamine, 100 ml of H_2O, and 1 drop of 12 *N* HCl was refluxed with stirring for 12 hr. The hot mixture was filtered and the product washed with hot H_2O; yield 0.95 g (75%), mp 333–334° dec. Recrystallization from HOAc gave white crystals, mp 338–339° dec.

Method C was the same as method B, only the HCl was omitted.

Method D was the same as method C, only DMF was used as solvent. This method is ineffective if the amine is insufficiently reactive, such as 2,3-dichloroaniline. With this unreactive amine, the product was **6-dimethylaminouracil**, white crystals from HOAc, mp 312–314° dec. *Anal.* ($C_6H_9N_3O_2$) C, H, N.

Irreversible Enzyme Inhibitors. CXXII.[1,2] On the Nature and Dimensions of the Hydrophobic Bonding Region of Guanine Deaminase and Xanthine Oxidase[3]

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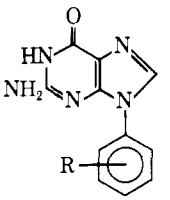
Thirty-four selected 9-substituted guanines have been studied as inhibitors of guanine deaminase and xanthine oxidase in order to map the hydrophobic bonding regions of these two enzymes; such maps aid in the design of active-site-directed irreversible inhibitors and in the design of more potent reversible inhibitors. These maps were remarkably similar for the two enzymes, the main difference being observed at the *para* position of 9-phenylguanine. The two most potent reversible inhibitors were 9-(*m*-benzamidophenyl)guanine (**20**) and 9-(*p*-phenylpropyloxyphenyl)guanine (**14**); these were complexed about 250-fold better than the substrate (guanine) to guanine deaminase and about 100-fold better than the substrate (hypoxanthine) to xanthine oxidase.

9-Phenylguanine (**1**) has been found to be a good inhibitor of both guanine deaminase[4] and xanthine oxidase,[5] being complexed 1.3- and 20-fold better than the respective substrate. That this 9-phenyl group interacts with the two enzymes by hydrophobic binding was then demonstrated.[6] In order to design an



1, R = H
11, R = *p*-OCH_3
16, R = *p*-COO^-
18, R = *m*-$NHCOCH_2Br$

active-site-directed irreversible inhibitor[7] from an inhibitor that also exhibits hydrophobic bonding, it is necessary to determine where the hydrophobic region on the enzyme ends; then a leaving group can be properly positioned to form a covalent bond with a nucleophilic center in a more polar region on the enzyme surface.

In addition to these dimensional studies on the hydrophobic bonding region, answers to two earlier questions were sought. In what manner does the *p*-OCH_3 substitnent of **11** give a 50-fold increment in binding to guanine deaminase?[6] In what manner does the *m*-bromoacetamido group of **18** give a 60-fold increment in binding to guanine deaminase[6] and a sevenfold increment in binding to xanthine oxidase? The results posed by these questions are the subject of this paper.[8]

Guanine Deaminase.—The inhibition results with 34 selected compounds on guanine deaminase are listed in Table I. The topography (Figure 1) of the hydrophobic bonding region of guanine deaminase will be discussed first; each position in the area containing the hydrophobic bonding region is numbered by position and each hexagon is lettered by area.

The 9-phenyl group (**1**) on guanine gives a 28-fold increment in binding over the 9-methyl group (**10**).[4] Such an increment would require hydrophobic interaction by only two or three of the six carbons. Since the 9-H of guanine binds to the enzyme as an electron acceptor,[9,10] it is clear that position 1 (Figure 1) is polar and not hydrophobic. Furthermore, one *meta* position of this phenyl (area A) is also not in a hydrophobic region because no loss in binding occurs with a *m*-amino group (**17**).[6] Thus the left side of area A is arbitrarily assigned to a hydrophobic region in positions 4–6. The pyrimidine portion of the guanine can then be either to the left of area A or flipped over to the right of area A.

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see B. R. Baker and W. Rzeszotarski, *J. Med. Chem.*, **11**, 639 (1968).

(3) For the previous paper on these enzymes see B. R. Baker and W. F. Wood, *ibid.*, **10**, 1106 (1967), paper CIII of the series.

(4) B. R. Baker and D. V. Santi, *ibid.*, **10**, 62 (1967), paper LXXIV of this series.

(5) B. R. Baker, *J. Pharm. Sci.*, **56**, 959 (1967), paper XCIII of this series.

(6) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1101 (1967), paper CII of this series.

(7) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(8) The chemotherapeutic reasons for studying guanine deaminase[9,10] and xanthine oxidase[11] have been previously discussed.

(9) See ref 7, p 101.

(10) B. R. Baker, *J. Med. Chem.*, **10**, 59 (1967), paper LXXIII of this series.

(11) B. R. Baker and J. L. Hendrickson, *J. Pharm. Sci.*, **56**, 955 (1967), paper XCII of this series.