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]

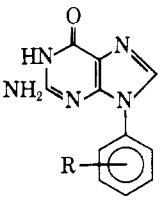
B. R. Baker and William F. Wood

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received February 26, 1968

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 substituent of **11** give a 50-fold increment in binding to guanine deaminse?[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.

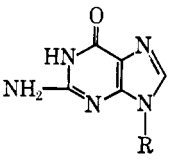
(9) See ref 7, p 101.

(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.

TABLE I

INHIBITION[a] OF GUANINE DEAMINASE[b] AND XANTHINE OXIDASE[c] BY



No.	R	Guanine deaminase I_{50},[d] μM	Guanine deaminase ([S]/[I])$_{0.5}$[e]	Xanthine oxidase I_{50},[d] μM	Xanthine oxidase ([S]/[I])$_{0.5}$[e]
1	C_6H_5	10[f]	1.3	0.41[g]	20
2	$C_6H_5CH_2$	370[f]	0.036	23[g]	0.34
3	$C_6H_5(CH_2)_2$[h]	190	0.071	16	0.50
4	$C_6H_5(CH_2)_3$	83	0.16	9.7	0.83
5	$C_6H_5(CH_2)_4$	77	0.17	13	0.62
6	*o*-FC_6H_4	21	0.63	0.62	13
7	*o*-ClC_6H_4	100	0.13	8.1	1.0
8	*o*-BrC_6H_4	120	0.11	7.7	1.0
9	α-Naphthyl	14	0.95	4.2	1.9
10	CH_3	275[f]	0.048	58[g]	0.14
11	*p*-$CH_3OC_6H_4$[h]	0.20	67	0.50	16
12	*p*-HOC_6H_4	1.0	13	0.21	38
13	*p*-$C_2H_5OC_6H_4$[h]	0.098	130	0.11	91
14	*p*-$C_6H_5(CH_2)_3OC_6H_4$	0.056	240	0.084	95
15	Dibenzofuran-3-yl	0.18	74	0.37	22
16	*p*-$HOOCC_6H_5$[h]	>200	<0.067	0.12	68
17	*m*-$NH_2C_6H_4$[h]	5.9	2.3	0.60	13
18	$C_6H_4NHCOCH_2Br$-*m*[i]	0.17	78	0.071	110
19	C_6H_4NHCHO-*m*	0.44	30	0.23	35
20	$C_6H_4NHCOC_6H_5$-*m*	0.050	270	0.072	110
21	$C_6H_4(C_4H_9$-*n*)-*p*	0.65	20	0.62	13
22	$C_6H_4(C_4H_9$-*t*)-*p*	9.3	1.4	1.8	4.5
23	$C_6H_4(C_3H_7$-*i*)-*p*	1.2	11	0.25	32
24	$C_6H_4CH_3$-*p*[h]	3.5	3.8	1.6	5.0
25	$C_6H_4C_2H_5$-*p*[h]	1.3	10	0.68	12
26	$C_6H_4CF_3$-*p*	5.3	2.6	1.3	6.2
27	$C_6H_4C_6H_5$-*p*	0.34	39	0.25	32
28	$C_6H_4C_6H_5$-*m*	0.24	56	0.082	100
29	$C_6H_4CH_3$-*m*[h]	2.0	6.7	0.24	33
30	β-Naphthyl[h]	0.22	59	0.41	20
31	*m*-$C_6H_4NHCOC_6H_4SO_2F$-*m*[j]	0.12	110	0.11	74
32	*m*-$C_6H_4NHCONHC_6H_4SO_2F$-*m*[j]	0.10	130	1.8	4.5
33	*p*-$C_6H_4NHCOC_6H_4SO_2F$-*p*[j]	18	0.73	0.70	12
34	*p*-$C_6H_4NHCOCH_2Br$[i]	13	1.0	1.9	4.3

[a] The technical assistance of Maureen Baker and Pepper Caseria with these assays is acknowledged. [b] Guanine deaminase (guanase) was a commercial preparation from rabbit liver that was assayed with 13.3 μM guanine in 0.05 M Tris buffer (pH 7.4) containing 3% DMSO as previously described.[10] [c] Xanthine oxidase was a commercial preparation from bovine milk that was assayed with 8.1 μM hypoxanthine in 0.05 M Tris buffer (pH 7.4) containing 10% DMSO as previously described.[11] [d] I_{50} = concentration for 50% inhibition. [e] Ratio of concentrations of substrate to inhibitor giving 50% inhibition. [f] Data from ref 4. [g] Data from ref 5. [h] Data from ref 6. [i] Data from ref 3. [j] Data from ref 14.

Introduction of an *o*-bromo (**8**) or *o*-chloro atom (**7**) gives an 8–10-fold loss in binding; that loss is not due to an electronic effect, but is steric, is indicated by the less than twofold loss in binding by the small *o*-fluoro atom (**6**). These results indicate that the phenyl ring of **1** is coplanar to the purine ring when complexed to the enzyme; in order for **7** and **8** to complex effectively, an 8–10-fold loss in binding energy could occur as a result of the energy needed to bring **7** and **8** to coplanarity. The α-naphthyl group (**9**) gives a sevenfold increment in binding over the *o*-chlorophenyl group (**7**); this increment can be accounted for by hydrophobic bonding of one or two carbons of the benzo moiety in area B. Since the *m*-methyl group (**29**) gives a fivefold increment over **1**, the additional binding by **9** over **7** is accounted for by a hydrophobic interaction at positions 9 and 10; it follows that positions 7 and 8 are not hydrophobic.

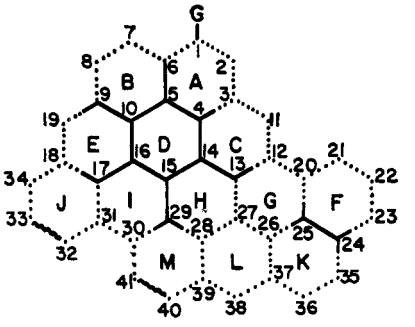


Figure 1.—A proposed map of the hydrophobic bonding region of rabbit liver guanine deaminase; G = 9-guanyl, —— = hydrophobic, ····· = not hydrophobic, ∿∿ = unknown.

Introduction of the *p*-methyl group (**24**) gives a threefold increment in binding by a hydrophobic interaction at position 14; that position 14 can also form a

nonpolar donor–acceptor complex with **11** will be discussed later. Increasing the chain from *p*-methyl (**24**) to *p*-ethyl (**25**) gives a threefold increment in binding due to interaction at position 13 or 15. That position 13 is probably hydrophobic will be discussed later with the binding of *m*-formamido group (**19**) and related amides.

The β-naphthyl group (**30**) gives a 45-fold increment in binding over the parent 9-phenyl group (**1**); fivefold of this increment is accounted for by hydrophobic interaction at position 10 (**1** *vs.* **29**) and the eightfold increment at positions 14 and 15 (**1** *vs.* **25**). Therefore hydrophobic interaction at position 16 is weak at best. That positions 16 and 17 are in a hydrophobic area is indicated by the 40-fold increment in binding given by the *m*-phenyl group (**28**) over **1**; sevenfold is accounted for by position 10 (**1** *vs.* **29**), the remaining sixfold can be accounted for by positions 9, 16, and 17 being hydrophobic and positions 18 and 19 not hydrophobic.

The *p*-phenyl group (**27**) gives a 29-fold increment in binding over the parent **1**; eightfold of this is accountable by the hydrophobic interaction at positions 14 and 15 (**1** *vs.* **25**). The remaining fourfold increment is accountable by hydrophobic bonding at positions 13 and 29 with perhaps a minor contribution by position 28; it follows that hydrophobic bonding does not occur at positions 27 and 28. That position 29 is hydrophobic and 30 is not is further supported by the binding *n*-butyl group (**21**) which is only twofold better than the *p*-ethyl group (**25**). That a flat interaction between areas A, C, and D and the enzyme is required as shown in Figure 1 is supported by the sevenfold loss in binding by the *t*-butyl group (**22**) compared to ethyl (**25**). This flat interaction is further supported by the similar binding of the *p*-ethyl (**25**) and *p*-isopropyl substituents (**23**) where the latter has one out-of-plane methyl group.

The 50-fold increment in binding[6] by the *p*-methoxy group (**11**) over **1** was then investigated. The *p*-hydroxy group (**12**) has now been synthesized and found to give a tenfold increment in binding over the parent **1**; the remaining fivefold increment of **11** is accountable by hydrophobic interaction of the methyl at position 15 (**24** *vs.* **25**). One anomaly with **11** must still be accountable; how can position 14 bind the methyl of **24** by hydrophobic bonding and the oxygen of **11** and **12** by a donor–acceptor complex?[12] This can be accounted for if position 14 on the enzyme is part of a phenyl group of a phenylalanine; the methyl could then interact by hydrophobic bonding and the oxygen as an electron donor to the π cloud of the underlying phenyl group on the enzyme. That such a donor–acceptor complex with the amide oxygen of **19** can also occur in this area at position 13 will be discussed later.

The binding by the remaining ethers (**13**–**15**) can now be explained. The twofold increment of the *p*-ethoxy group (**13**) over *p*-methoxy (**11**) is accountable by hydrophobic bonding of the terminal methyl group of **13** at either position 16 or 29. The *p*-phenylpropyloxy group (**14**) gives only a twofold increment in binding over the *p*-ethoxy group (**13**) and **14** is of the two best reversible inhibitors of guanine deaminase in Table I. Thus, only one more carbon of **14** than **13** binds to the enzyme. Since position 17 has been shown to be hydrophobic and position 30 not, it follows that the propyloxy moiety of **14** binds at positions 14–17 and the terminal phenyl of **14** gives no additional hydrophobic interaction at positions 18, 19, or 34.

The dibenzofuran (**15**) and the *m*-biphenyl (**28**) groups bind to the enzyme about the same, the major difference in structure being the ether bridge of **15**. If the ether bridge of **15** could complex at position **14**, then **15** could be expected to be a tenfold better inhibitor than **28** (compare **1**, **11**, and **13**). However, the ether oxygen does not reside at position 14, but resides inside area D between positions 4 and 16. The oxygen interaction appears to be between positions 14 and 13 since the oxygen of either **11** or **19** can complex to the enzyme; thus it would appear that the ether oxygen of **15** is not close enough to the oxygen-donor binding area.

The 60-fold increment in binding[3] by the *m*-bromoacetamido group (**18**) over the parent **1** was then investigated. Three possibilities were considered: (a) the carboxamido part of **18** might complex to the enzyme by a donor–acceptor complex, (b) the bromomethyl might interact hydrophobically with the enzyme, and (c) the bromine atom might interact in a donor–acceptor complex.[12] In order to separate out possibility a, the *m*-formamido analog (**19**) was synthesized; **19** showed a 22-fold increment in binding over the parent **1** and a 13-fold increment over the *m*-amino group (**17**). This 13-fold increment can only be due to a donor interaction of the amide oxygen with an acceptor group on the enzyme; this amide oxygen can reside at either position 13 or adjacent to position 2. Since a donor atom on an inhibitor (**11**, **12**) can interact with the enzyme as position 14, it is logical to assume that the amide oxygen of **19** can interact similarly at position 13 with the hydrophobic electron acceptor on the enzyme.[13] It follows that the remaining 2.6-fold difference between the bromoacetamido (**18**) and formamido (**19**) groups is due to interaction with the enzyme at positions 20, 21, or 25 by either hydrophobic bonding or donor–acceptor interaction.

A suitable working hypothesis for the 2.6-fold increment observed between **18** and **19** is the hydrophobic interaction of the bromine atom at position 25; this is derived from comparison of **18**–**20** and **31**–**34**. Replacement of formyl group (**19**) by benzoyl (**20**) give a further ninefold enhancement in binding by the benzene ring which must reside in area F if the amide oxygen resides at position 13; this increment is probably too large to be due to a donor–acceptor complex with the enzyme, but could be readily accountable by a hydrophobic interaction of two of the carbons in area F. Note that the benzamido (**31**) and phenylureido (**32**) groups bearing a sulfonyl fluoride give identical fourfold increments in binding over the formamido group (**19**). These results are accounted for if hydrophobic bonding occurs at positions 24 and 25 by the

(12) For a discussion of the modes of complexing between inhibitors and enzymes see ref 7 Chapter II.

(13) The ground-state conformation of acetanilide has its carbonyl plane 38° from coplanarity from the benzene ring. In this discussion the assumption has been made that the carbonyl group of **18**, **19**, **31**, and **32** gives maximum interaction with the enzyme at position 13 when the carbonyl approaches coplanarity to the 9-phenyl group; the energy needed can arise from the greater binding energy of the C=O at position 13 when the C=O is coplanar. This coplanarity would cause the benzene ring of **31** in area F and **32** in area K also to approach coplanarity to the 9-phenyl group. See B. F. Pederson and B. Pederson, *Tetrahedron Letters*, 2995 (1965) for the ground-state conformation of acetanilide.

phenyl group of **31**[14] residing in area F and the phenyl group of **32**[14] in area K. Furthermore, one NH of **32** resides at position 20 without repulsion indicating that this position is not hydrophobic; it also follows that positions 21–23, 2, 27, and 35–37 are not hydrophobic.[13]

Note that the *p*-fluorosulfonylbenzamido group of **33**[14] gives no appreciable change in binding compared to the parent **1**; since positions 14–16 are hydrophobic, it is logical for the carboxamido function of **33** to reside at positions 12–14 which positions the phenyl group in area L without hydrophobic bonding. Similarly, the *p*-bromoacetamido group (**34**)[3] should not give an increment in binding over the parent **1** since the carboxamido would reside at positions 12–14 and the bromine atom at position 26 or 28.

Although no evidence is available, it is probable that positions 32, 33, 40, and 41 are not hydrophobic since the adjacent positions are not. It is also interesting to note that the *p*-CF_3 group of **26** gives no increment in binding over the *p*-CH_3 (**24**), indicating that a fluorine atom between positions 13 and 14 does not form a donor–acceptor complex with the enzyme.

In a study of the binding of the $C_6H_5(CH_2)_n$ group (**1**–**5**), it is clear that the best binding occurred when $n = 0$; apparently the phenylalkyl groups do not have the proper conformation within their enzyme–inhibitor complexes to give appreciable hydrophobic bonding in areas A, B, D, E, and I.

Xanthine Oxidase.—In Table I are listed the results of inhibition of xanthine oxidase with the same 34 compounds evaluated on guanine deaminase; some of these 9-phenylguanines were previously shown to be good inhibitors of xanthine oxidase.[3] Thus the topography of the hydrophobic bonding region of xanthine oxidase could also be studied by these compounds and is presented in Figure 2; again each position has been numbered and each area lettered in the same manner as Figure 1 for guanine deaminase.

The 9-H of guanine is probably not complexed to xanthine oxidase since guanine and 9-methylguanine (**10**) are nearly equally effective inhibitors.[11] Since the 9-methyl group of **10** gives no increment in binding over guanine, it is clear that position 1 (Figure 2) is not in a hydrophobic region; it is less likely that the 9-H is complexed to the enzyme and the loss in binding when this group is replaced by methyl is equally compensated for by hydrophobic bonding by the 9-methyl group since a large loss in binding occurs when hypoxanthine is substituted by a 9-methyl group. However, 9-phenylguanine (**1**) gives a 140-fold increment in binding over 9-methylguanine (**10**); that this interaction by the 9-phenyl group is due to hydrophobic bonding was previously demonstrated.[6] A 140-fold increment in hydrophobic bonding requires a minimum of three carbons interacting in this manner,[12] but may involve even four or five carbons. Since one side of the phenyl ring is in a polar region and one side is in a hydrophobic region,[6] the hydrophobic side is arbitrarily assigned to the left; the guanine may then complex with either its pyrimidine moiety to the left or the right, but not necessarily in the same direction as in guanine deaminase (Figure 1). Thus positions 3–6 are in a hydrophobic area and position 2 may or may not be. That position 3 is in a hydrophobic area is also supported by the slight loss in binding caused by an *m*-amino group (**17**) in position 11.

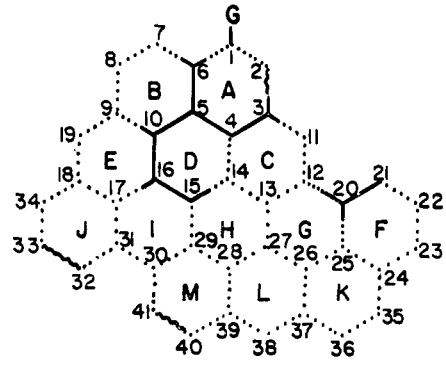


Figure 2.—A proposed map of the hydrophobic bonding region of bovine milk xanthine oxidase; G = 9-guanyl, —— = hydrophobic, ····· = not hydrophobic, ~~ = unknown.

Introduction of an *o*-chloro (**7**) or *o*-bromo (**8**) group causes a 20-fold loss in binding compared to the parent **1**. Since the *o*-fluoro (**6**) group gives little change in binding, the effect of the halogen of **7** and **8** is not electronic, but steric. These results indicate that the phenyl ring of **1** should be coplanar to the purine ring for optimum binding; thus the 20-fold loss in binding with **7** and **8** could be due to the energy required to bring the rings into coplanarity. The α-naphthyl group (**9**) gives only a twofold increment in binding over **7**; this increment is accountable by the hydrophobic bonding of only one carbon in area B. Since the *m*-methyl group (**29**) also gives a twofold increment in binding over the parent **1**, the difference in binding between **7** and **9** is due to a hydrophobic interaction at position 10; it follows that positions 7–9 are not in a hydrophobic area.

In contrast to guanine deaminase, introduction of a methyl group (**24**) gives a fourfold loss in binding with xanthine oxidase, indicating that in the latter enzyme position 14 is in a polar region. That position 14 is polar is further supported by the fourfold increment in binding by the *p*-carboxylate group (**16**). Actually, a larger increment in binding by the COO^- group could be expected if it is interacting with the enzyme in a donor–acceptor complex; thus some repulsion of the COO^- group at position 15 might be occurring since position 15 is in a hydrophobic area and position 13 is not, as will be shown later.

That position 15 is hydrophobic is indicated by the twofold increment in binding by the *p*-ethyl group (**25**) compared to *p*-methyl (**24**); this increment is not due to an interaction at position 13, which will later be shown to be nonhydrophobic. A nearly threefold increment in binding by *p*-isopropyl (**23**) compared to *p*-ethyl (**25**) could be interpreted to mean that hydrophobic bonding occurs at both positions 13 and 15; however, since position 13 is not hydrophobic, the effect of chain branching could be a more favorable ground-state conformation of **23** for complexing to position 15. That a flat interaction near coplanarity with the phenyl group is necessary for **23** and **25** to complex with xanthine oxidase is indicated by the sevenfold loss in binding by the *t*-butyl group (**22**) compared to isopropyl (**23**).

(14) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **11**, 650 (1968), paper CXXIII of this series.

The β-naphthyl group (**30**) gives no increment in binding over the parent 9-phenyl group (**1**). However some loss in binding must occur at position 14; therefore, a more valid comparison taking into account this repulsion would be β-naphthyl (**30**) and *p*-ethylphenyl (**25**) where the former is 1.5-fold better. This difference can be accounted for by the twofold increment in binding at position 10 by the *m*-methyl group (**29**). These increments between **28** and **30** may not be strictly additive; therefore, position 16 could also be hydrophobic since there is one strong line of evidence with **28** that it is. The *m*-phenyl group (**28**) gives a fivefold increment in binding over the parent **1**; of this, twofold is accountable by binding at position 10 and the remainder must be by either position 9 or 16. Since position 9 is not hydrophobic, then position 16 should be; it is unlikely that hydrophobic bonding would reappear at position 19 adjacent to the nonhydrophobic position 9, which is supported by the binding of **14** to be discussed later.

The *p*-phenyl group (**27**) gives a sixfold increment in binding over the *p*-methyl group (**24**); part of this increment is due to hydrophobic bonding at position 15 and the remainder may be due to some donor–acceptor character between the *p*-phenyl and the polar group(s) on the enzyme that can complex a *p*-carboxylate (**16**) and can be covalently linked from position 27 by the *p*-bromoacetamido derivative (**34**).[3] Thus positions 27–29 are not likely hydrophobic. That position 29 is not hydrophobic (nor is 30) is further supported by no increment in binding between *p*-ethyl (**25**) and *p*-butyl (**21**) where the butyl group would occupy positions 14, 15, 29, and 30.

The *p*-hydroxy group (**12**) gives a twofold increment in binding; this is probably due to a donor–acceptor interaction with the same group on the enzyme that complexes the *p*-carboxyl of **16**. When the hydroxyl group of **12** is methylated to give **11** some loss in binding should occur due to the loss of binding of the acidic hydrogen of **12**, but some gain in binding should occur by hydrophobic interaction at position 15; however, it is somewhat surprising that the net difference between these forces results in **12** being equal to the parent **1**. When the methoxyl (**11**) is increased to ethoxyl (**13**), a fourfold increment in binding occurs at position 16, since position 29 is not hydrophobic. Further extension of the ether chain to phenylpropyl (**14**) gives only a slight increment in binding over **13**, indicating positions 17–19 and 34 are not hydrophobic.

The major structural difference between the dibenzofuran (**15**) and the *m*-biphenyl (**28**) is the ether bridge in **15**, yet **15** is a fourfold less effective inhibitor than **28**; this result might be due to a repulsion of the ether bridge which resides inside of ring D of Figure 2 and not at position 14.

The eightfold increment in binding[3] by the *m*-bromoacetamido group (**18**) over the *m*-amino group (**17**) was then investigated. Threefold of this increment was due to the carboxamide oxygen of **19** interacting at position 13 in a donor–acceptor complex;[13] the remaining threefold increment of **18** must then be due to an interaction of the bromomethyl group at positions 20, 21, or 25 either by a donor–acceptor complex or a hydrophobic interaction. Replacement of the bromomethyl group of **18** with phenyl (**20**) gave no change in binding, indicating that the bromomethyl and phenyl groups bind in the same way. A hydrophobic interaction between positions 20 and 21 is the preferred explanation since **31** is a 16-fold better inhibitor than **32**; the latter comparison indicates that position 20 is hydrophobic (repelling the NH of **32**) and positions 24–26 and 35–37 are not hydrophobic. Since the binding by the phenyl ring of **20** and **31** is accountable at positions 20 and 21, it follows that positions 22–25 are not hydrophobic.[13]

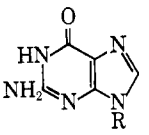
There are two possible conformations for the *p*-bromoacetamido group of **34**, the first placing the amide oxygen at position 16 and the second at position 12; the latter is preferred for positioning the polar amide oxygen since position 15 is hydrophobic and position 12 is not. Such a binding conformation of the amide oxygen would place the bromomethyl group at positions 27 and 26 or 28. It is then at position 27 where covalent bond formation between **34** and the enzyme occurs when **34** shows its irreversible inhibition;[3] therefore position 27 is reconfirmed as a polar position. The threefold loss in binding between the *p*-bromoacetamido (**34**) group and the *p*-amino group (I_{50} = 0.6 μM)[6] is accountable by repulsion of the bromomethyl group from positions 27 and 26 or 28, all of which are not hydrophobic. The threefold increment between the bromomethyl group of **34** and the *p*-fluorosulfonylphenyl group of **33** is probably due to a donor–acceptor interaction between position 27 and this benzene ring in area L; note also that position 38 is immediately adjacent to a polar region since the sulfonyl fluoride of **33** attached to position 38 can rapidly form a covalent bond with the enzyme.[14] It follows that positions 26–28 and 37–39 are not hydrophobic.

The small increments in binding observed with the 9-phenylalkylguanines (**2**–**5**) can now be rationalized from Figure 2. The benzyl group (**2**) would have to have its phenyl residing in area B in order to give hydrophobic bonding at positions 5, 6, and 10; however, a 20-fold loss in binding due to steric interaction would occur (compare **1** and **7**). Since the maximum hydrophobic interaction thermodynamically possible by two carbons (positions 5 and 6) is 100-fold and position 10 gives a twofold increment (**1** *vs.* **28**), the maximum interaction expected by the benzyl substituent would be $2 \times 100/20 = 10$. Since only a twofold increment between **2** and **10** is observed, it follows that carbons at position 5 and 6 give only a $100/5 = 20$-fold increment. If the phenethyl group (**3**) could interact with its benzene ring residing in area D, then a hydrophobic interaction should occur at positions 4–6, 10, 15, and 16; however, repulsion will occur from position 14 (**1** *vs.* **24**). Since the β-naphthyl group (**30**) and phenyl group (**1**) are equally effective, the net gain in binding at positions 10, 15, and 16 is lost by repulsion at position 14. Therefore, any net hydrophobic bonding would have to occur at positions 4–6. Since a >20-fold increment in binding should occur, the observed fourfold increment shows that this ideal conformation for **3** is not achieved in the enzyme–inhibitor complex. Similar arguments can be advanced for **4** and **5**. In summary, the best binding to xanthine oxidase occurs with a $C_6H_5(CH_2)_n$ group when $n = 0$.

Comparison of the Hydrophobic Bonding Region of Xanthine Oxidase and Guanine Deaminase.—The dif-

TABLE II

PHYSICAL PROPERTIES[a] OF



No.	R	% yield	Formula	λ_{max}, mμ pH 1	λ_{max}, mμ pH 13
4	$C_6H_5(CH_2)_3$	14	$C_{14}H_{15}N_5O \cdot 0.5H_2O$	251, 279	259,[f] 266
5	$C_6H_5(CH_2)_4$	17	$C_{15}H_{17}N_5O$	254, 281	258,[f] 269
6	*o*-FC_6H_4	18	$C_{11}H_8FN_5O$	260, 273[f]	266
7	*o*-ClC_6H_4	24	$C_{11}H_8ClN_5O$	259, 270[f]	268
8	*o*-BrC_6H_4	8	$C_{11}H_8BrN_5O$	259, 271[f]	268
9	α-Naphthyl	16[b]	$C_{15}H_{11}N_5O$	260,[f] 280	275
12	*p*-HOC_6H_4	26	$C_{11}H_9N_5O_2$	230, 269[f]	251, 263[f]
14	*p*-$C_6H_5(CH_2)_3OC_6H_4$	35[c]	$C_{20}H_{19}N_5O_2$	233, 270[f]	268
15	Dibenzofuran-3-yl	31[c]	$C_{17}H_{11}N_5O_2$	254, 288	255, 288, 300[f]
19	C_6H_4NHCHO-*m*	45[d]	$C_{12}H_{10}N_6O_2$	245,[f] 277[f]	268[f]
20	$C_6H_4NHCOC_6H_5$-*m*	35[e]	$C_{18}H_{14}N_6O_2 \cdot 0.75H_2O$	264	269
21	$C_6H_4(C_4H_9$-*n*$)$-*p*	27	$C_{15}H_{17}N_5O$	264, 271[f]	268
22	$C_6H_4(C_4H_9$-*t*$)$-*p*	13	$C_{15}H_{17}N_5O$	262, 280[f]	269
23	$C_6H_4(C_3H_7$-*i*$)$-*p*	16	$C_{14}H_{15}N_5O$	260, 265[f]	268
26	$C_6H_4CF_3$-*p*	23	$C_{12}H_8F_3N_5O$	270	238, 278
27	$C_6H_4C_6H_5$-*p*	34[c]	$C_{17}H_{13}N_5O$	262	268
28	$C_6H_4C_6H_5$-*m*	43[c]	$C_{17}H_{13}N_5O$	255	250[f]

[a] All compounds were prepared from 2-amino-6-chloro-5-phenylazo-4-pyrimidinol and purified by the previously described methods,[6] unless otherwise indicated; each analytical sample gave combustion values for C, H, and N within 0.4 of the theoretical percentage and each moved as a single spot on tlc on silica gel with EtOH–$CHCl_3$ (3:5). All of the compounds had uv and ir spectra in agreement with their assigned structures; since all but two compounds (**4**, mp 262–265°, and **5**, mp 283–284°) did not melt below 300°, uv data is included. [b] An unstated positional isomer of 9-naphthylguanine has been described.[16a] [c] The purification step by solution in NH_4OH was omitted since it was insoluble. [d] Prepared by reaction of **17** with 99% HCOOH at 100°, then recrystallization from DMF–H_2O. [e] Prepared by reaction of **17** with benzoic anhydride in DMF, then recrystallization from MeOEtOH. [f] Inflection.

ferences in these hydrophobic bonding regions (Figures 1 and 2) will be discussed first.

(1) Area A gives 50-fold better hydrophobic bonding to xanthine oxidase than guanase; the converse is true with the adjacent area D. The total hydrophobic bonding in areas A and D is quite similar for both enzymes.

(2) Guanine deaminase shows additional hydrophobic bonding in area E, but xanthine oxidase does not.

(3) In area F, xanthine oxidase shows hydrophobic bonding at positions 20 and 21, but guanine deaminase shows hydrophobic bonding at positions 24 and 25.[13]

(4) Position 14 on xanthine oxidase repulses a methyl group, but attracts this group on guanine deaminase. Conversely, the polar carboxylate of **16** is attracted to position 14 or xanthine oxidase, but is repulsed on guanine deaminase. However, the ether oxygen at position 14 that is attracted to guanine deaminase is not attracted to xanthine oxidase.

(5) The *p*-bromoacetamido group of **34** can form a covalent bond with xanthine oxidase at position 27, but guanine deaminase cannot.[3] Similarly, the sulfonyl fluoride of **33** can form a covalent bond with xanthine oxidase just adjacent to position 38, but does not irreversibly inactivate guanine deaminase.[14]

The remainder of the hydrophobic bonding region on the two enzymes is remarkedly similar. Each hydrophobic region could serve the biological function of repulsing the more polar nucleosides and nucleotides from the respective enzymes so that these are not substrates or inhibitors.[15]

Construction of candidate irreversible inhibitors that project a covalent forming group such as bromoacetamido or sulfonyl fluoride into a nonhydrophobic region can now be done on a more rational basis. Such studies are continuing and initial studies leading to a new irreversible inhibitor of xanthine oxidase is reported in the following paper.[14]

Chemistry.—Of the new compounds in Table I, all except **19** and **20** were prepared by condensation of the appropriate amine with 2-amino-6-chloro-5-phenylazo-4-pyrimidino followed by reductive formylation[6] and ring closure;[6,16] the remaining two compounds were synthesized by acylation of the corresponding amine (**17**). Data on these new compounds are compiled in Table II.

(15) Reference 7, p 121.

(16) (a) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 3046 (1959); (b) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).